

NEURORESTORATIVE MULBERRIES POTENTIAL OF ALZHEIMER'S DISEASE IN ANIMAL MODEL

FAROUK K EL-BAZ¹, HANAN F ALY², HOWAIDA I ABD-ALLA^{3*}, SANAA A ALI²¹Department of Plant Biochemistry, National Research Centre, 33 El Bohouth St. (former El Tahrir St.), Dokki-Giza, P.O.12622, Egypt.²Department of Therapeutic Chemistry, National Research Centre, 33 El Bohouth St. (former El Tahrir St.), Dokki-Giza, P.O.12622, Egypt.³Department of Chemistry of Natural Compounds, National Research Centre, 33 El Bohouth St. (former El Tahrir St.), Dokki-Giza, P.O.12622, Egypt. Email: howaida_nrc@yahoo.com

Received: 07 May 2018, Revised and Accepted: 21 June 2018

ABSTRACT

Objective: Alzheimer's disease (AD) is one of the most common neurodegenerative disorders. The present study aimed to identify the potential neurorestorative of *Morus alba* and *Morus rubra* fruits extract on aluminum chloride (AlCl₃)-induced AD rats.

Methods: Each *Morus* species extract *M. alba* and *M. rubra* at a dose 300 mg/kg body weight (b.wt.) was orally administered, daily for 6 weeks post AlCl₃ induction (100 mg/kg b.wt./day/6 weeks, orally). The polyphenols content was determined according to the colorimetric method of Folin-Ciocalteu using gallic acid as a standard. In addition, brain neurotransmitters, total protein content (TPC), and antioxidant biomarkers were determined. Histopathological assessment was performed on the brains of rats.

Results: Administration of AlCl₃ neurotoxicity induced a significant increment of brain acetylcholinesterase, and nitric oxide, while significant decrease in four monoamines (norepinephrine, epinephrine, 5-hydroxytryptamine serotonin, and dopamin), along with brain tissue ATPase, and TPC were determined. AlCl₃-induced changes in peroxidative products, such as protein carbonyl, catalase, and superoxide dismutase activity. AlCl₃ induction resulted in significant decrease in antioxidant enzymes, serum level of total antioxidant capacity, and caused histological alteration for brain tissue. Treatment of AD-rats with *M. alba* and *M. rubra* (300 mg/kg b.wt./day) causes modulation of most biochemical markers brain function and confirmed by brain histopathological study.

Conclusion: Collectively, the present results indicated that *M. rubra* and *M. alba* exerts neuroprotection against AlCl₃-induced brain pathology. To the best of our knowledge, none of the literature studies have demonstrated the effect of these *Morus* species against Al-neurotoxicity at biochemical and electron microscopical levels.

Keywords: Neurorestorative, Brain neurotransmitters, Alzheimer's disease, *Morus*, Polyphenols content, Histopathological assessment.

© 2018 The Authors. Published by Innovare Academic Sciences Pvt Ltd. This is an open access article under the CC BY license (<http://creativecommons.org/licenses/by/4.0/>) DOI: <http://dx.doi.org/10.22159/ajpcr.2018.v11i10.27155>

INTRODUCTION

Alzheimer's disease (AD) continues to be a growing health concern worldwide as it is considered one of the most chronic neurodegenerative diseases [1]. It is characterized by progressive and slow neurodegeneration in different regions of brain [2]. Aluminum (Al) is a neurotoxin that suppresses many of the biological functions in humans and animals causing various adverse effects [3]. On exposure to Al, the relationship between amyotrophic lateral sclerosis and dialysis encephalopathy in the neurodegenerative disorders as Alzheimer has been suggested [3]. Abnormal levels of oxidative stress have been reported in an AD in bloodstream and brain [1,2]. Biomarkers of oxidative stress in AD are classified as an oxidative attack on proteins leads to the formation of protein carbonyls (PCs) and protein nitration as well as superoxide dismutase (SOD) and lipid peroxidation [4]. On the other hand, polyphenols have gained tremendous interest as the candidates for the therapeutic and/or preventive of neurodegenerative disorders [5,6]. Neurodegenerative diseases are related to deficiency of antioxidants that could be prevented by radical scavengers [7,8]. *Morus* genus, a member of the Moraceae family, is a fruit that has a high content of radical scavengers including the polyphenols, prenylated flavonoids, stilbenes, and benzoic acid derivatives [5,9]. Phenolic acids and flavonols support the potential preventive health benefits of fruit-derived products' intake in relation to many diseases associated with oxidative stress [5,9]. These compounds have shown a wide range of bioactive features including neuroprotective functions and immunomodulatory activities [6,10].

A major phenolic, syringic acid, isolated from *M. niger* have been reported to have a neuroprotective effect [11]. This effect was suggested to include the nitro-oxidative system modulation in brain and serum of mice. Zheng *et al.* [12] reported that flavanone C-glycoside, prenylated 2-arylbenzofuran derivatives (moracin glycosides), and benzoic acid derivatives were isolated from *M. alba*. These compounds showed protective influence against doxorubicin-induced cell death cardiomyopathy in H9c2 cells. The protective effect of HaCaT cells by the typical anthocyanin, cyanidin-3-O-glucoside (C3G), against ultraviolet radiation has been explained by the suppression of COX-2 expression and by the scavenging of reactive oxygen species (ROS). Regulation of hippocampal ERK expression has been demonstrated by C3G, and it also showed cognitive influence on spatial memory [13]. Furthermore, a clinical trial on the efficiency of mulberry extract in enhancing cognitive ability has been conducted [14]. A study reported an antiaging effect of herbal cream loaded with phyto extracts including *M. alba* [14]. Previous work showed the identification of many unsaturated fatty acids (FAs) involving omega-3 and -6 FAs in the total extract of *M. alba* [5]. These acids have previously been linked to both protective and pathogenic effects in AD [15]. The metabolism of unsaturated FAs (as *cis*-linoleic and linolenic acids) is altered and significantly dysregulated in the brain of AD patient. Other acids (chlorogenic, caffeic, and syringic) were previously reported in *Morus* species. Phenolic acids of chlorogenic and caffeic were reported to have neuroprotective activity [16]. These properties were exerted by preventing neurodegeneration induced by oxidative

stress in rats' brain and by inhibiting acetylcholinesterase (AChE) and butyrylcholinesterase, the key enzymes linked to AD [1,2]. Other classes of polyphenols including anthocyanins, flavanols, flavones, triterpenes, and stilbenes are found in *Morus* species in appropriate amount. The potential effects of compounds related to these classes in reducing cerebral injury and improving cognitive function were reported [1,2,6]. *Morus* species has been approved as effective against metabolic deteriorations and Al-induced developmental retinopathy [5,9]. None of these studies have demonstrated the effect of *M. rubra* and *M. alba* against Al-neurotoxicity at biochemical and electron microscopic levels. Therefore, the present search aimed to evaluate the therapeutic impact of both *Morus* species fruits on biochemical detritions in aluminum chloride (AlCl₃)-induced AD rats.

METHODS

Chemicals

Donepezil, reagents, and kits were purchased from the Sigma Chemical Company (USA), whereas AlCl₃ was purchased from CDH, India.

Preparation of fruits extract

M. alba and *M. rubra* fruits were obtained from the local market, Egypt, during July 2017. The plant was kindly authenticated by Mrs. Treas Labib, Herbarium section, El-Orman, Egypt, with voucher specimen of 0111236, 01112235 for *M. rubra* and *M. alba*, respectively. The fresh fruits of each species (2.5 kg) were extracted separately with 70% aqueous ethanol for 3 h, on an orbital shaker in the dark at room temperature. Each extract was then concentrated under vacuum using rotary evaporator (Heidolph-Germany) till dryness to get 32.89 and 31.27% of crude ethanol extract of *M. alba* and *M. rubra*, respectively. The crude extracts were stored at -4°C for further investigations.

Determination of total polyphenols

The total polyphenols in extracts were determined according to Folin-Ciocalteu procedure [17]. 400 µL of the sample (two replicates) were taken in test tubes; 1.0 mL of Folin-Ciocalteu reagent (diluted 10-fold with distilled water) and 0.8 mL of 7.5% sodium carbonate were added. The tubes were mixed and allowed to stand for 30 min, and the absorption at 765 nm was measured against a blank, which contained 400 µL of ethanol in place of sample. The total polyphenols content was expressed as gallic acid equivalent in mg/g of ethanol extract.

Biological experiment

Animals

The male Wistar albino rats (150±10 g) were provided by the Animal House of the National Research Centre (NRC) and housed in group of 10 rats per cage, maintained in controlled environment condition at 26–29°C. They were provided with a fixed light/dark cycle for 1 week (w) as an adaptation period to acclimatize under normal combination with free access to water and food. The study was approved (approval no: 1456) by the Ethical Committee of the NRC, Egypt, provided that the animals will not suffer at any stage of the experiment.

Induction of AlCl₃ induced-AD

AlCl₃ solutions were made freshly at the beginning of each experiment. For oral administration, AlCl₃ was dissolved in drinking water and administered orally in a dose of 100 mg/kg, to rats daily for 6 weeks 0.5 mL/100 g b.wt. [3]. Donepezil drug was daily administered post-induction of AlCl₃ for 6 weeks in a dose 10 mg/kg b.wt. parallel with berries extract [18].

Experimental design

Seventy rats were divided randomly into six groups of 10 rats each. Group 1: Normal of healthy control rats. Groups 2 and 3: *M. alba* and *M. rubra* extract-treated control rats. Group 4: serving as AD-rats, where rats were orally administered with AlCl₃. Groups 5 and 6 were the AD-treated rats with ethanolic extract of *M. alba* and *M. rubra* (300 mg/kg b.wt.), daily for 6 weeks, respectively [16]. Group 7 was the AD-treated rats with standard drug mg/kg b.wt. daily for 6 weeks.

Blood samples preparation

Overnight fasted animals were sacrificed under slight diethyl ether anesthesia. The blood was collected by puncture of the sublingual vein in clean and dry test tube. Blood was left 10 min to clot and centrifuged at 3000 rpm to obtain serum. The separated serum was used for biochemical analysis of total antioxidant capacity (TAC) and PC. The other biomarkers were estimated in brain tissue.

Brain tissue sampling and preparation

At the end of the experiment, the rats were fasted overnight, subjected to anesthesia and sacrificed. The whole brain of each rat was rapidly dissected, washed with isotonic saline and dried on filter paper. The brain was weighed and homogenized in ice-cold medium containing 50 mM Tris/HCl and 300 mM sucrose at pH 7.4 to give a 10% (w/v) homogenate [3,6]. This homogenate was centrifuged at 1400× g for 10 minutes at 4°C. The supernatant was stored at -80°C and used for neurotransmitters (AChE, epinephrine [Ep], norepinephrine [Nep], dopamine [DA], and 5-hydroxytryptamine [5-HT]) determination, biochemical analyses that included oxidative stress biomarker enzymatic antioxidants, ATPase, and TPC. The animals were disposed of in bags provided by the Committee of Safety and Environmental Health, NRC.

Estimation of brain neurotransmitters

Serum AChE and Ep were measured by a quantitative enzyme-linked immunosorbent assay according to the manufacturer's instructions [19]. The concentrations of brain NEp, DA, and serotonin (5-HT) were determined using high-performance liquid chromatography with electrochemical detection technique according to Zagrodzka *et al.* [20]. ATPase in brain was estimated according to the method of Matteucci *et al.* [21].

Estimation of brain TPC

TPC was estimated according to the method of Bradford [22].

Estimation of antioxidant stress biomarkers

TAC was assayed according to the method of Koracevic *et al.* [18]. SOD was assayed according to Nishikimi *et al.* [23]. CAT enzyme was determined in tissue homogenate by colorimetric assay method according to Aebi [24]. PC was determined in tissue homogenate by colorimetric assay method [24]. NO level was assayed in liver tissue by the spectrophotometric method according to Berkels *et al.* [25].

Histological examination of brain tissue

Histopathological assessment was performed on the brains of rats selected from each group. Brains were immediately fixed in 10% formaldehyde, embedded in paraffin, and 5 µm sections were performed, then stained with hematoxylin and eosin (H and E) and examined microscopically.

Statistical analysis

All data sets were expressed as the mean±standard deviation (SD). The data were analyzed statistically for normal distribution using SPSS software and Co-State for Windows, version 8. Values of unshared letters are statistically significant at p<0.05.

RESULTS

Total polyphenols content

M. rubra showed a higher level of total polyphenols as gallic acid equivalents of ethanol extract (mg/g of ethanol extract). Total polyphenols were 622.4±24.2 and 308.4±17.6 for *M. alba* and *M. rubra*, respectively. The data performed the average values±SD of three samples.

Effects of *Morus* species on catecholamines and AChE neurotransmitters levels

The present results declared an insignificant change in catecholamines (NEp and Ep) and AChE levels in control rats administered *M. alba* and *M. rubra* as compared to normal untreated rats. Significant reduction in NEp and Ep neurotransmitters level in AD induced rats with percentages

49.73% and 44.66%, respectively while a significant increase in AChE (47.52%) was recorded. On the other hand, treatment of AD rats with *M. alba* and *M. rubra* exhibited a significant increase in NEp and Ep levels compared to AD-induced rats with amelioration percentages 30.96 and 33.69%, respectively, for *M. rubra* and 31.54 and 33.00%, respectively, for *M. alba*. However, a significant reduction in AChE level was noticed with improvement percentage 78.56 and 74.26%, respectively, for *M. alba* and *M. rubra* compared to anti-Alzheimer reference drug which recorded 34.18, 42.98 and 64.18%, respectively (Table 1).

Effects *Morus* species on monoamines neurotransmitters levels

Insignificant change was recorded in monoamines; serotonin 5-HT and DA DA levels in control rats treated with either *M. alba* and *M. rubra* as compared to control rats while there were significant reductions in 5-HT and DA levels detected in AD-induced rats by 48.74 and 50.74%, respectively. Noticeable amelioration in 5-HT and DA levels on treated AD rats with *M. alba* and *M. rubra* with amelioration percentages 23.45 and 29.89%, respectively, for *M. rubra* and 24.69 and 25.14%, respectively, for *M. alba*, compared to reference drug which recorded 34.68 and 31.59%, respectively (Table 2).

Effects *Morus* species on brain ATPase and total protein content (TPC) in brain tissue of AD-induced rats

Regarding ATPase and TPC (Table 3), insignificant change was recorded in control rats treated with *M. alba* and *M. rubra* compared to untreated control rats while AD-induced rats declared significant decrease in ATPase level and TPC (TPC) by 44.44 and 58.07%, respectively. Marked amelioration was noticed in ATPase and TPC on treated AD rats with *M. alba* and *M. rubra* with percentages 25.00 and 37.17%, respectively, for *M. alba* and 33.33 and 40.80%, respectively, for *M. rubra* compared to reference drug (36.11 and 50.93%, respectively).

Effects of *Morus* species on brain NO and PC levels in brain tissue of AD-induced rats

Table 4 demonstrated an insignificant change in NO and PC levels in control rats treated with *M. alba* and *M. rubra* as compared to control untreated rats. Significant increase by 30.12 and 98.75%, in NO and PC levels were noticed in AD-induced rats as compared to control one. Remediation of AD rats with *M. alba* and *M. rubra* showed obvious improvement in NO and PC levels with amelioration percentages 24.76 and 51.00%, respectively, for *M. rubra*, while the percentages reached 28.67 and 53.25%, respectively, for *M. alba* compared to standard drug

which exhibited percentages of improvement by 22.26 and 80.00%, respectively.

Considering antioxidant enzymes as well as TAC (Table 5), there was insignificant change in CAT enzyme activity in control rats treated with *M. alba* and *M. rubra* as compared to normal control rats while a significant increase in SOD and TAC was observed in control rats treated with either *M. rubra* (57.16 and 30.45%, respectively) or *M. alba* (31.49 and 43.47%, respectively). However, a significant decrease in SOD, CAT activities, and TAC level was detected in AD-induced rats by 52.59, 77.77, and 53.62%, respectively. Treatment of AD rats with *M. alba* and *M. rubra* showed a significant increase in antioxidant enzyme activities and TAC by percentages of improvement 79.45, 63.33, and 84.06% of SOD, CAT, and TAC, respectively, for *M. rubra* while *M. alba* recorded 78.43, 62.22, and 69.56%, respectively, compared to the reference drug which showed percentages 95.42, 68.89, and 63.77%, respectively.

Histological examination

The histological examination confirms the biochemical studies. The biochemical study showed that both *M. alba* and *M. rubra* fruits did not have any side effects when given to control groups. The cerebellum of the control rats has shown granular and Purkinje layers. Moreover, Purkinje cells were observed in the Purkinje layer and large numbers of closely packed small cells in the granular layer (Fig. 1a and b). Group administered with AlCl₃ revealed focal gliosis in the cerebral cortex and pyramidal cells in the hippocampus, separated from each other; with irregular outline-enlarged pyramidal cells, binucleated nuclei, vacuolation, necrosis, and aggregation of blood cells with marked distortion in granular cell layer (Fig. 1c and d). Groups had given *M. alba* showed the cerebellum with normal histopathological features. In the Purkinje cell layer, there were large Purkinje cells. In the granular layer, there were closely packed good-defined granular small cells (Fig. 1e and f). Group treated with *M. rubra* was characterized by defined molecular and granular Purkinje layers (Fig. 1g and h). The histopathological characters of the cerebellum in the group given the standard drug were in the normal case of well-defined molecular and granular layers (Fig. 1i and j).

DISCUSSION

The present results clearly indicated a significant rise in oxidative stress biomarkers as NO levels associated with significant decrease in antioxidant enzymes; SOD, CAT activities, as well as TAC [22]. The

Table 1: Effect of *Morus* species on NEp, Ep, and AChE neurotransmitters levels in brain tissue of AD-induced rats

Group	Biomarker		
	NEp (ng/g tissue)	Ep (ng/g tissue)	AChE (ng/mL)
Normal control	190.95±10.10 ^b	300.12±5.10 ^e	90.22±2.10 ^b
<i>M. alba</i> +Control	199.90±8.20 ^b	293.65±5.10 ^e	88.20±2.10 ^b
% change	+4.69	-2.16	2.24
% of improvement	-	-	-
<i>M. rubra</i> +Control	199.61±21.10 ^b	295.55±6.20 ^e	80.10±3.10 ^b
% change	+4.45	1.52	1.24
% of improvement	-	-	-
AD induced rats	95.99±5.90 ^a	66.10±10.50 ^f	133.10±10.1 ^h
% change	-49.73	-44.66	+47.52
% of improvement	-	-	-
<i>M. alba</i> +AD rats	156.22±8.90 ^c	265.10±5.90 ^g	62.22±5.99 ^e
% change	-18.19	-11.66	-31.04
% of improvement	31.54	33.00	78.56
<i>M. rubra</i> +AD rats	155.10±7.10 ^c	267.20±11.90 ^g	66.10±2.11 ^e
% change	-18.77	-10.99	-26.73
% of improvement	30.96	33.69	74.26
Reference drug+AD rats	161.25±12.90 ^c	295.10±7.25 ^e	75.20±10.10 ^f
% change	-15.55	-1.67	-16.65
% of improvement	34.18	42.98	64.18

NEp: Norepinephrine, Ep: Epinephrine, AChE: Acetylcholinesterase, SD: Standard deviation, AD: Alzheimer's disease, *M. alba*: *Morus alba*, *M. rubra*: *Morus rubra*. All data were expressed as mean±SD of 10 rats in each group. Statistical analysis is carried out using SPSS computer program (version 8) combined with Co-state computer program, where unshared letters are significant at p≤0.05

Table 2: Effects *Morus* species on 5-HT and DA neurotransmitters levels in brain tissue of AD-induced rats

Group	Biomarker	
	5-HT (ng/g tissue)	DA (ng/g tissue)
Normal control	88.20±1.59 ^a	62.10±2.10 ^{ef}
<i>M. alba</i> +Control	87.20±5.20 ^a	65.10±6.10 ^e
% change	-1.13	+4.83
<i>M. rubra</i> +Control	89.10±2.10 ^a	63.20±7.20 ^e
% change	+1.02	+1.77
AD induced rats	45.21±2.90 ^b	30.59±1.90
% change	-48.74	-50.74
<i>M. alba</i> +AD rats	66.99±0.95 ^s	46.20±3.20 ^c
% change	-24.05	-25.60
% of improvement	24.69	25.14
<i>M. rubra</i> +AD rats	65.89±0.55 ^s	49.15±2.90 ^b
% change	-25.29	-20.85
% of improvement	23.45	29.89
Reference Drug+AD rats	75.80±6.20 ^f	50.20±3.10 ^b
% change	-14.06	-19.16
% of improvement	34.68	31.59

5-HT: 5-Hydroxytryptamine (serotonin), DA: Dopamine, AD: *Alzheimer's disease*, SD: Standard deviation, *M. alba*: *Morus alba*, *M. rubra*: *Morus rubra*. Mean±SD of 10 rats in each group. Statistical analysis is carried out using SPSS computer program (version 8) combined with Costate computer program, where unshared letters are significant at p<0.05

Table 3: Effects *Morus* species on brain ATPase and TPC in brain tissue of AD-induced rats

Group	Biomarker	
	ATPase (μmoles/g tissue)	TPC (mg/g tissue)
Normal control	9.00±0.69 ^a	82.10±3.90 ^b
<i>M. alba</i> + Control	8.59±0.62 ^{ab}	80.10±6.90 ^b
% change	-4.55	-1.66
<i>M. rubra</i> + Control	8.99±0.50 ^{ab}	81.23±5.60 ^b
% change	-0.1	-2.75
AD induced rats	5.00±0.39 ^c	33.50±4.60 ^c
% change	-44.44	-58.07
<i>M. alba</i> + AD rats	7.25±0.69 ^c	63.20±3.25 ^d
% change	-19.44	-20.90
% improvement	25.00	37.17
<i>M. rubra</i> + AD rats	8.00±0.55 ^{ab}	66.10±5.20 ^d
% change	-11.11	-19.49
% improvement	33.33	40.80
Reference Drug + AD rats	8.25±0.53 ^{ab}	74.20±6.10 ^e
% change	-8.33	-7.13
% improvement	36.11	50.93

TPC: Total protein content, AD: *Alzheimer's disease*, SD: Standard deviation, *M. alba*: *Morus alba*, *M. rubra*: *Morus rubra*. All data were expressed as mean±SD of 10 rats in each group. Statistical analysis is carried out using SPSS computer program (version 8) combined with Co-state computer program, where unshared letters are significant at p<0.05

reported value of plasma/serum and erythrocytes red blood cells levels in the AD were varied from some studies to another [4,26,27]. The level of SOD is enhanced by oxidative stress in early stages of AD and the brain SOD and CAT, the first line defense antioxidants were inhibited after oral administration of Al sulfate [11].

Nitrated protein and PCs were produced as a result of protein peroxidation, and oxidation has been suggested as the biomarkers of free-radical damage against protein [4,27]. High PCs level in serum/plasma of AD was demonstrated as the marker of oxidative stress in the brain, and it is capable of destroying neurons [4,27]. The present results declared that a significant increase in PC tissue of AD-induced rats. PC level in cerebrospinal fluid is universally significantly higher in the AD than in controls and in other brain regions and hippocampus [4,6].

Table 4: Effects of *Morus* species on brain NO and PC level in brain tissue of AD-induced rats

Group	Biomarker	
	NO (U mol/g tissue)	PC (nmol/mg protein)
Control	76.20±9.10 ^a	4.00±0.10 ^e
<i>M. alba</i> +Control	72.90±1.20 ^a	3.95±0.11 ^e
% change	-4.33	-1.25
% improvement	-	-
<i>M. rubra</i> +Control	73.10±2.90 ^a	3.90±0.20 ^e
% change	-4.00	-2.5
% improvement	-	-
AD induced rats	99.10±2.90 ^b	7.95±1.20 ^b
% change	+30.12	98.75
% improvement	-	-
<i>M. alba</i> +AD rats	77.25±0.10 ^c	5.82±0.03 ^c
% change	+1.38	+45.50
% improvement	28.67	53.25
<i>M. rubra</i> +AD rats	80.23±5.10 ^c	5.91±2.10 ^c
% change	+5.38	+47.75
% improvement	24.76	51.00
Reference drug+AD rats	82.19±6.13 ^d	4.75±0.66 ^d
% change	+7.86	+18.75
% improvement	22.26	80.00

NO: Nitric oxide, PC: Protein carbonyl, AD: *Alzheimer's disease*, SD: Standard deviation, *M. alba*: *Morus alba*, *M. rubra*: *Morus rubra*. All data were expressed as mean±SD of 10 rats in each group. Statistical analysis is carried out using SPSS computer program (version 8) combined with Costate computer program, where unshared letters are significant at p<0.05

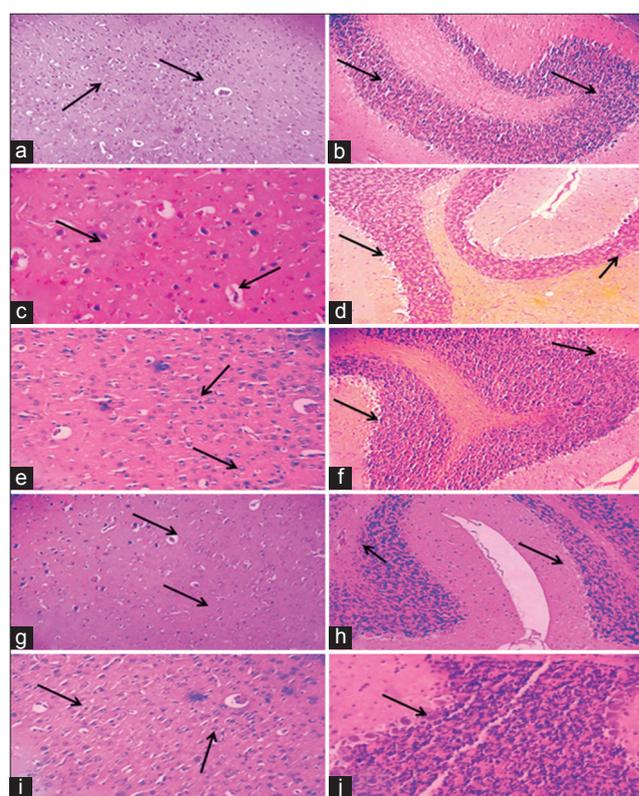


Fig. 1: (a and b) Purkinje layer and granular layer; (c and d) group administered aluminum chloride; (e and f) groups treated with *Morus alba*; (g and h) group treated with *Morus rubra*; and (i and j) group treated with the standard drug

Decreased levels of AChE activity and gamma-aminobutyric acid concentrations have been observed with senile plaques in brains of Alzheimer's. Administration of AlCl₃ caused histological aberrations, elevated activity of AChE while the reduction in NEp, Ep, DA, and 5-HT

Table 5: Effects of *Morus* species on brain antioxidant enzymes, SOD, and CAT activities as well as serum TAC of AD-induced rats

Group	Biomarker		
	SOD (U/g tissue)	CAT (U/g tissue)	TAC (mmol/mg protein)
Control	63.50±11.20 ^c	0.90±0.11 ^b	0.69±0.01 ^a
<i>M. alba</i> +Control	83.50±11.20 ^e	1.00±0.10 ^b	0.99±0.09 ^b
% change	+31.49	+11.11	+43.47
% improvement	-	-	-
<i>M. rubra</i> +Control	99.80±13.10 ^{ab}	0.99±0.13 ^b	0.90±0.13 ^b
% change	+57.16	+10.00	+30.45
% improvement	-	-	-
AD-induced rats	30.10±1.50 ^d	0.20±0.01 ^d	0.32±0.01 ^e
% change	-52.59	-77.77	-53.62
% improvement	-	-	-
<i>M. alba</i> +AD rats	79.90±15.10 ^e	0.76±0.11 ^e	0.80±0.03 ^d
% change	+25.83	-15.55	+15.94
% improvement	78.43	62.22	69.56
<i>M. rubra</i> +AD rats	80.55±13.10 ^e	0.77±0.20 ^e	0.90±0.05 ^b
% change	+26.85	-14.44	+30.43
% improvement	79.45	63.33	84.06
Reference Drug+AD rats	90.69±9.60 ^{ab}	0.82±0.11 ^b	0.76±0.04 ^d
% change	+42.82	-8.89	10.14
% improvement	95.42	68.89	63.77

SOD: Superoxide dismutase, CAT: Catalase, TAC: Total antioxidant capacity, AD: Alzheimer's disease, SD: Standard deviation, *M. alba*: *Morus alba*, *M. rubra*: *Morus rubra*. All data are expressed as mean±SD of 10 rats in each group. Statistical analysis is carried out using SPSS computer program (version 8) combined with Costate computer program, where unshared letters are significant at $P \leq 0.05$

in AD induced rats was detected. AD is involved the deficiency of the neurotransmitters AChE and DA that are linked to malfunctions in the catecholaminergic and cholinergic systems. Memory and cognitive function are affected by the acetylcholine-containing neurons. In the current study, the Alzheimer' group recorded low DA, NEp, and 5-HT than those of controls. In the previous study, the level of NEp is depressed in the cortex and cerebellum of animals has administered low copper or high Al [11]. The neuroprotective effect of *Morus* species, especially the *M. rubra*, may be exerted by reducing intracellular ROS level and protection of neuronal cells against oxidative injury, the most important neurodegeneration features [28,29].

In our present study, the neuroprotective activities of the investigated *Morus* species fruits may be related to polyphenols. *Morus*' polyphenols represent a diverse group of compounds such as hydroxyl cinnamates, catechins, flavonols, flavanols, stilbenoids, and tannins. Tannins are divided into hydrolyzable and condensed (proanthocyanidins) tannins. A proanthocyanidins-standardized cranberry extract was reported to alleviate β -amyloid peptide toxicity in *Caenorhabditis elegans* model of AD by improving proteostasis through heat shock transcription factor-1 [30]. Oligomeric condensed tannins (proanthocyanidins), naturally occurring antioxidants, are widely available in *Morus* species. An oligomeric proanthocyanidin isolated from grape seeds has been provided as antiradical scavenging agent exerts protection against DNA damage and lipid peroxidation [31]. In our current study, other *Morus*' polyphenols such as flavonoids (quercetin derivatives and kaempferol derivatives), phenolic acids (hydroxybenzoic and hydroxycinnamic acids) may exert the healing potency on neurotoxicity [1,4,6].

In AD group, the antioxidant enzymes such as SOD and CAT are decreased in their activities by oxidative stress as declared in the current results [4,32]. In our present study, the presence of polyphenols in *M. rubra* and *M. alba* with AChE inhibiting, antioxidant properties and/or their effect on neuronal signal transduction and neuroinflammation may be attributed to the current reported data of the extract-treated group compared with the control [7,32,33].

Other essential micronutrients of trace elements and vitamins such as carotenoid (A), riboflavin (B₂), niacin (B₃), ascorbic acid (C), and tocopherol (E) were reported in high amount in the colored mulberries as purple *M. rubra* fruits [4,5]. Micronutrients have attracted increasing attention as potential agents for neuroprotection as many of them have

antioxidant properties [4,33]. These compounds and fruit colorants (anthocyanins and carotenoids) play a central part in optimizing health and in the maintenance of tissue function through the interaction of hydroxyl group of anthraquinones with the active site residues of β -site amyloid precursor protein cleaving enzyme 1 [34]. Certain anthocyanins-enriched extract was reported to alleviate behavioral abnormalities of AD in the mouse model [13,35]. C3G was the major anthocyanin in many species of *Morus*. It has reported as an antioxidant agent and showed a protective role against vascular failure induced by peroxynitrite and endothelial dysfunction [13]. Thus, C3G may be involved in the neuroprotective effect of *M. alba* extract. A benzenoid (Z)-1-hydroxy-4-(2-nitrophenyl) benzene, isolated and characterized from the root bark of *M. australis* was reported to display inhibitory effects on adenosine diphosphate (ADP)-aggregating factor and inhibited production of NO in murine cell line [28].

The current findings demonstrated that administration of *M. rubra* (300 mg/kg) to rats displayed brain selective AChE inhibition. They also increase DA level, serotonin (5-HT) and NEp and prevented the metabolism of DA and 5-HT.

The role of essential FAs for the function and the integrity of nervous cell membranes and brain are well established. Mulberry fruit has essential FAs which humans cannot synthesize and must be obtained through diet. Essential FAs are polyunsaturated FAs (UFAs) with long-chain derived from a linoleic, linolenic, and oleic acid, where they are important for the formation of healthy cell membranes, the development and function of the nervous system and brain [4,5]. A previous work showed the GC-MS of organic extracts of the *M. rubra* fruits established their unique constituents including unsaturated FAs [5]. UFAs including omega-3 and -6 FAs have previously been attributed to the protective activity against cell damage of the brain at various ages and during aging [15]. El-Baz et al. [5] reported the identification of a phenanthrene derivative (10-methoxy-9-phenyl-phenanthrene) in the crude alcohol extract and methylene chloride fraction of *M. alba*. These derivatives were reported as important pharmacological agents with diverse biological effects including NO production inhibition, radical-scavenging, and protective effect on cerebral ischemic stroke [36].

The noticeable amelioration in monoamines; 5-HT, DA, as well as NEp, which is detected on treated AD-induced rats with *M. alba*, may be explained, *M. alba* fruits extract is considered a potent protector against

glyphosate-induced toxicity and has protected the hippocampal neurons against amyloid β -peptide (1-42)-induced cell death in a concentration-dependent manner. Its protective effect could result from synergism or antagonism between the various bioactive polyphenols in the fruits extract [36]. These fruits extracts have a potent neuroprotective profile and also have a modulatory effect on cellular and biomolecular processes in preventing redox status to arrest apoptotic and necrotic processes underlying focal cerebral ischemia [4,5]. Polyphenols, including flavonoids as isoprenylated flavonoids (morustralins A and B), and a prenylated flavanone, named morflavanone A were isolated from *M. alba*. Quercetin, kaempferol-3-rutinoside, and rutin have been isolated from many *Morus* species. Modulation of P-glycoprotein, rather than anti-inflammatory and antioxidant affects of *Morus* species could be the suggested the mechanism of polyphenols in the protection of neurons and prevention of AD [33]. Many of these compounds may cross the blood-brain barrier [33], as indicated in the present histopathological amelioration of brain tissue (E-H) as compared to standard drug (I and J).

Chronic neuroinflammation may contribute to the neuroanatomy of the pathology or the appearance of activated microglia [33]. This is ascertained in the present histopathological examination (Photomicrographs C and D), which revealed focal gliosis in the cerebral cortex and pyramidal cells in the hippocampus marked distortion in the granular cell layer. Photomicrograph 1 showed the examination of brain sections of control rats with the normal structure of the cerebral cortex (A) and hippocampus (B) (H and E, $\times 200$). Group administered with $AlCl_3$ revealed focal gliosis in the cerebral cortex (C), and pyramidal cells in the hippocampus marked distortion in granular cell layer (D) (H and E, $\times 200$). Group treated with *M. alba* showed the cerebellum having well-defined molecular, granular, large Purkinje cell in the Purkinje cell layer (E and F) (H and E, $\times 200$). Furthermore, group treated with *M. rubra* showed marked defined molecular, granular, Purkinje layers (G and H) (H and E, $\times 200$). The cerebellum of the group received the standard drug showed well-defined molecular and granular layers and other normal histopathological features (I and J) (H and E, $\times 200$).

CONCLUSION

It could be concluded that *Morus* can effectively protect against $AlCl_3$ -induced neurotoxicity and the mechanisms underlying the neuroprotective effect are potentially associated with its antioxidant, anti-inflammatory, and antiapoptotic properties. Both *Morus* species significantly improved $AlCl_3$ -induced biochemical and neurochemical abnormalities, reversed the abnormal alterations of neurotransmitters in the striatum, and alleviated oxidative stress.

AUTHOR'S CONTRIBUTIONS

Farouk K. El-Baz: Plan of work, writing and revision. Hanan F. Aly: Biochemical measurements, statistical analysis, and writing. Howaida I. Abd-Alla: Samples, biochemical measurements discussion, and corresponding author. Sanaa A. Ali: Histological investigation.

CONFLICTS OF INTEREST

Authors declare that they have no conflicts of interest.

REFERENCES

- Jiang Y, Gao H, Turdu G. Traditional Chinese medicinal herbs as potential AChE inhibitors for anti-Alzheimer's disease: A review. *Bioorg Chem* 2017;75:50-61.
- Yang Y, Wang JZ. Nature of tau-associated neurodegeneration and the molecular mechanisms. *J Alzheimer's Dis* 2018;62:1305-17.
- Kawahara M, Kato-Negishi M. Link between aluminum and the pathogenesis of Alzheimer's disease: The integration of the aluminum and amyloid cascade hypotheses. *Int J Alzheimers Dis* 2011;2011:1-17.
- Chang YT, Chang WN, Tsai NW, Huang CC, Kung CT, Su YJ, et al. The roles of biomarkers of oxidative stress and antioxidant in Alzheimer's disease: A systematic review. *Bio Med Res Int* 2014;2014:1-14.
- El-Baz FK, Hassan AZ, Abd-Alla HI, Aly HF, Mahmoud K.

- Phytochemical analysis, assessment of ant proliferative and free radical scavenging activity of *Morus alba* and *Morus rubra* fruits. *Asian J Pharm Clin Res* 2017;10:189-99.
- Awad HM, Abd-Alla HI, Ibrahim MA, El-Sawy ER, Abd-Alla MM. Flavones from heavenly blue as modulators of alzheimer's amyloid-beta peptide (A β) production. *Med Chem Res* 2018;27:768-76.
- Awad HM, Abd-Alla HI, Mahmoud KH, El-Toumy SA. *In vitro* anti-nitrosative, antioxidant, and cytotoxicity activities of plant flavonoids: A comparative study. *Med Chem Res* 2014;23:3298-307.
- Mohamed NZ, Abd-Alla HI, Aly HF, Mantawy M, Ibrahim N, Hassan SA. CCL4-induced hepatonephrotoxicity: Protective effect of nutraceuticals on inflammatory factors and ant oxidative status in rat. *J Appl Pharm Sci* 2016;4:87-100.
- El-Baz FK, Aly HF, Abd-Alla HI. Berries supplementation modulates body weight and metabolic deteriorations in obese rats. *Asian J Pharm Clin Res* 2018;11:322-8.
- Abd-Alla HI, Sweelam HM, Mohamed TA, Gabr MM, El-Safty MM, Hegazy MF. Efficacy of extracts and iridoid glucosides from *Pentas lanceolata* on humoral and cell-mediated immune response of viral vaccine. *Med Chem Res* 2017;26:2196-204.
- Phukan P, Bawari M, Sengupta M. Promising neuroprotective plants from north-east India. *Int J Pharm Pharm Sci* 2015;7:28-39.
- Zheng XK, Cao YG, Ke YY, Zhang YL, Li F, Gong JH, et al. Phenolic constituents from the root bark of *Morus alba* L. and their cardioprotective activity *in vitro*. *Phytochemistry* 2017;135:128-34.
- Tan L, Yang HP, Pang W, Lu H, Hu YD, Li J, et al. Cyanidin-3-O-galactoside and blueberry extracts supplementation improves spatial memory and regulates hippocampal ERK expression in senescence-accelerated mice. *Biomed Environ Sci* 2014;27:186-96.
- Chan EW, Lye PY, Wong SK. Phytochemistry, pharmacology, and clinical trials of *Morus alba*. *Chin J Nat Med* 2016;14:17-30.
- Bourre JM. Roles of unsaturated fatty acids (especially omega-3 fatty acids) in the brain at various ages and during ageing. *J Nutr Health Aging* 2004;8:163-74.
- Nag G, De B. Acetylcholinesterase inhibitory activity of *Terminalia chebula*, *Terminalia bellerica* and *Emblca officinalis* and some phenolic compounds. *Int J Pharm Pharm Sci* 2011;3:121-4.
- Singleton, VL, Rossi, JA. Colorimetry of total phenolics with phosphomolybdc-phosphotungstic acid reagents. *Am J Enol Viticult* 1995;16:144-58.
- Koracevic D, Koracevic G, Djordjevic V, Andrejevic S, Cosic V. Method for the measurement of antioxidant activity in human fluids. *J Clin Pathol* 2001;54:356-61.
- Fernando HA, Chin HF, Ton SH, Kadir KA. Stress and its effects on glucose metabolism and 11 β -HSD activities in rats fed on a combination of high-fat and high-sucrose diet with glycyrrhizic acid. *Diabetes Res* 2013;95:1903-13.
- Zagrodzka J, Romaniuk A, Wiczorek M, Boguszewski P. Bicuculline administration into ventromedial hypothalamus: Effects on fear and regional brain monoamines and GABA concentrations in rats. *Acta Neurobiol Exp* 2000;60:333-43.
- Matteucci E, Cocci F, Pellegrini L, Gregori G, Giampietro O. Measurement of ATPases in red cells: Setting up and validation of a highly reproducible method. *Enzyme Protein* 1994;48:105-19.
- Bradford MM. A rapid and sensitive method for the quantization of microgram quantities of protein utilizing the principle of protein-dye binding. *Anal Biochem* 1976;72:248-54.
- Nishikimi M, Rae NA, Yagi K. The occurrence of superoxide anion in the action of reduced phenazine methosulphate and molecular oxygen. *Biochem Biophys Res Commun* 1972;46:849-53.
- Aebi H. Catalase *in vitro*. *Methods Enzymol* 1984;105:121-6.
- Berkels R, Purol-Schnabel S, Roosen R. Measurement of nitric oxide by reconversion of nitrate/nitrite to NO. *Methods Mol Biol* 2004;279:1-8.
- Padurariu M, Ciobica A, Hritcu L, Stoica B, Bild W, Stefanescu C. Changes of some oxidative stress markers in the serum of patients with mild cognitive impairment and Alzheimer's disease. *Neurosci Lett* 2010;469:6-10.
- Cristalli DO, Arnal N, Marra FA, De Alaniz MJ, Marra C. Peripheral markers in neurodegenerative patients and their first-degree relatives. *J Neurol Sci* 2012;314:48-56.
- Liao YR, Kuo PC, Tsai WJ, Huang GJ, Lee KH, Wu TS. Bioactive chemical constituents from the root bark of *Morus australis*. *Bioorg Med Chem Lett* 2017;27:309-13.
- Tavares L, Figueira I, McDougall GJ, Vieira HL, Stewart D, Alves PM, et al. Neuroprotective effects of digested polyphenols from wild blackberry species. *Eur J Nutr* 2013;52:225-36.
- Guo H, Cao M, Zou S, Ye B, Dong Y. Cranberry extract standardized for

- proanthocyanidins alleviates β -Amyloid peptide toxicity by improving proteostasis through HSF-1 in *Caenorhabditis elegans* model of Alzheimer's disease. *J Gerontol A Biol Sci Med Sci* 2016;71:1564-73.
31. Bagchi D, Bagchi M, Stohs SJ, Das DK, Ray SD, Kuszynski CA, et al. Mitochondrial- and endoplasmic reticulum-associated oxidative stress in Alzheimer's disease: From pathogenesis to biomarkers. *Int J Cell Biol* 2012;2012:1-23.
 32. Aly HF, Abd-Alla HI, Ali SA, Aba-Alez R, Abu-Krishna MT, Mohamed MM. Bioinformatics: Inflammatory cytokines and attenuation of diabetes hypercholesterolemia-induced renal injury using morning glory and necklace pod extracts. *Asian J Pharm Clin Res* 2017;10:347-55.
 33. Sawikr Y, Yarla NS, Peluso I, Kamal MA, Aliev G, Bishayee A. Neuroinflammation in alzheimer's disease: The preventive and therapeutic potential of polyphenolic nutraceuticals. *Adv Protein Chem Struct Biol* 2017;108:33-57.
 34. Jung HA, Ali MY, Jung HJ, Jeong HO, Chung HY, Choi JS. Inhibitory activities of major anthraquinones and other constituents from *Cassia obtusifolia* against β -secretase and cholinesterases. *J Ethnopharmacol* 2016;191:152-60.
 35. Vepsäläinen S, Koivisto H, Pekkarinen E, Mäkinen P, Dobson G, McDougall GJ, et al. Anthocyanin-enriched bilberry and blackcurrant extracts modulate amyloid precursor protein processing and alleviate behavioral abnormalities in the APP/PS1 mouse model of alzheimer's disease. *J Nutr Biochem* 2013;24:360-70.
 36. Wang J, Wang T, Xie P, Yin G, Li X. New phenanthrene derivatives with nitric oxide inhibitory and radical-scavenging activities from *Pholidota imbricata* Hook. *Nat Prod Res* 2014;28:251-6.