Neuroprotective effect of Egyptian *Hyphaene thebaica* fruit extract against Alzheimer's disease in an animal model

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Background/aim
Alzheimer's disease (AD) is a chronic neurodegenerative disease. The AD incidence is rising and is becoming a heavy economic burden on society and patients' families. The present study was directed to investigate the protective and therapeutic effects of *Hyphaene thebaica* fruit extract (HTE) on AD in an animal model.

Materials and methods
A total of 90 adult albino male rats were involved in this study and were classified into six groups (15 each). Two negative control groups were used: one did not take any supplementation (G1) and a second negative control group in which normal rats were supplemented with HTE (G2). AD induction was performed by orally injecting the animals with 50 mg $AlCl_3$/kg body weight for 6 weeks. Four groups took $AlCl_3$. First group served as a positive control (G3). Two groups were used to investigate the effect of the orally injected HTE on AD rats either as a therapeutic (G4) or preventive (G5) agent. The last group was treated with the reference drug donepezil (G6). After treatment with HTE for 4 weeks, biochemical analyses of acetylcholinesterase activity, lipid peroxidation (malondialdehyde), and reduced glutathione were done in the brain tissue homogenate. Lipid profile was determined in sera. Expression level assessments of genes encoding microtubule-associated protein tau and amyloid precursor protein were carried out as well as DNA adducts were measured in brain tissues. Histopathological investigations of brain tissues were also performed.

Results
The results showed that brain samples of AD group exhibited significant changes in biochemical, molecular, and histopathological parameters. The samples of the groups treated with HTE showed improvements in the studied parameters. The treatment of induced AD group with HTE showed better results than supplementing the extract after occurrence of the signs of Alzheimer.

Conclusion
It was concluded that HTE acts as a promising candidate natural product to ameliorate and protect brain injury in AD-induced rats.

Keywords:
Alzheimer's disease, antioxidants, DNA adducts, gene expression, *Hyphaene thebaica*, tau protein

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Introduction

The world is experiencing an increasing number of patients with Alzheimer's disease (AD), resulting in a heavy burden on the national economy. Overall, 70% of all dementia cases are patients with AD [1]. The characteristics of such chronic neurodegenerative disorders are progressive memory loss, affecting social, physical activities as well as the quality of life. In 2012, ~35.6 million people were experiencing dementia worldwide, which will double every 20 years, reaching 115.4 million in 2050 as reported by the WHO [2]. Accordingly, the health and social burden of these populations will balloon dramatically.

Some biomarkers are common for diagnosing the disease, which help in the identification of definitive therapeutic targets and represent the most important targets for discovering a safe treatment for AD. One of the markers detected in AD diagnosis was the reduction of neurotransmitter acetylcholine synthesis. This suggestion was based on the dysfunction of neurons that contain acetylcholine which substantially contributes to the observation of the cognitive decline in patients with AD [3]. Earlier studies showed deterioration in acetylcholinesterase activity (AChE) in the AD-affected brain [4].

Reactive oxygen species (ROS) plays a very important target in all neurodegenerative diseases in parallel with AChE as a measure for AD incidence [5]. The reason for focusing on ROS as a target is that it induces damage in mitochondria DNA and neuronal cell nuclei [6,7]. Moreover, it increases lipid peroxidation products and decreases antioxidant enzymes activities [8]. Two major neuropathological features are, till now, the most specific markers for AD diagnosis: extracellular and intracellular. The plaque formation (the amyloid-β protein) is the extracellular one, whereas the intracellular one is represented by neurofibrillary tangles, which consist of hyperphosphorylated tau protein in the form of paired helical filaments. Both the amyloid-β protein and the tau protein are mainly closed to the brain's hippocampus region and in the cerebral cortex. The two brain parts are responsible for memory and other higher cognitive functions [9].

DNA and RNA might be damaged by formation of ROS or reactive nitrogen species (RNS), leading to the loss of the nitrogen bases in AD cells. The association of ROS or RNS-mediated DNA damage and over-expression of nitrotyrosine is considered as a molecular marker for the damage of DNA by ROS or RNS [10]. Moreover, DNA damage is associated with lipid peroxidation end products (ALEs), for example, trans-4-hydroxy-2-nonenal, during the DNA-trans-4-hydroxy-2-nonenal adduct generation [11].

Up to our knowledge, there is no therapeutic treatment for AD, but some drugs are used in the relief of symptoms accompanying the disease. The present study is to investigate a new source of natural agent aiming to achieve an AD treatment which may increase the relief of symptoms of the disease. Herbal medicine is always considered as a safer drug rather than pharmaceutical preparations. The plant *Hyphaene thebaica* is a desert palm native to India, Egypt, and Africa. It is known as Doum or gingerbread palm. The fruit exhibits antioxidant activity because of the water-soluble phenolics' presence [12]. It also possesses anti-inflammatory, antihypertensive, and antimicrobial activities owing to the presence of flavonoids [13–15].

This study aims to investigate both the protective and therapeutic effects of *Hyphaene thebaica* fruit extract (HTE) on AD in an animal model by measurements of AChE, lipid peroxidation [malondialdehyde (MDA)], and reduced glutathione (GSH). Moreover, expression level assessments of genes encoding microtubule-associated protein tau (MAPT) and amyloid precursor protein (APP) were carried out. The DNA adducts were measured in brain tissues, and histopathological investigations of brain tissues were also performed.

Materials and methods

Chemicals

The chemicals used were of high analytical grade (Sigma-Aldrich, St Louis, Missouri, USA). The kits used for the quantitative determination were purchased from Biodiagnostic (Giza, Egypt) and Greiner Diagnostic GmbH (Germany). TRIzol solution was purchased from Invitrogen (Germany). The PCR and reverse transcription kits were purchased from Fermentas (Waltham, Massachusetts, USA). SYBR Green Mix was purchased from Stratagene (San Diego, California, USA).

Hyphaene thebaica

Dry Doum fruit (*H. thebaica*) was collected from the local market (Dokki, Giza, Egypt). The plant was documented by Dr Mona Kassem, professor at Phytochemistry and Plant Systematics Department, National Research Center, Egypt.

Plant extraction

Overall, 500 g of dry *H. thebaica* fruit was suspended in 70% ethanol, and then the ethanol was evaporated under vacuum at 40°C, yielding a semisolid free plant extract residue.

Ethical consideration

All procedures described were reviewed and approved by the Animals Ethical Committee of the National Research Centre, under the ethical number 181/61, and conformed to the ethical guidelines of the 1975 Helsinki Declaration.

Experimental design

A total of 90 adult albino male rats were kindly provided by the Animal House of National Research Centre, Egypt, weighing from 150 to 200 g. Rats were preserved under the same temperature and air conditions with diet and water access. The animals were divided into sex groups as follows (15 each):

Group 1: it included normal healthy rats that received 0.5 ml of 0.9% saline which was considered as a negative control group.

Group 2: it included animals that were daily supplemented with the plant extract (HTE) with the rate of 1.0 g/kg body weight [16] for 4 weeks

and considered as plant extract-treated normal group.

Group 3: it included animals that received aluminum chloride ($AlCl_3$) for 6 weeks with the rate of 50 mg/kg body weight, intermediate dose to cause AD in rats and considered as supportive control group [17,18].

Group 4: it included animals that received $AlCl_3$ for 6 weeks then treated with HTE for another 4 weeks and considered as therapeutic group.

Group 5: it included animals received HTE and $AlCl_3$ at the same time for 6 weeks and considered as protective group.

Group 6: it included animals that received $AlCl_3$ for 6 weeks and then treated with the reference drug (donepezil) with the rate of 1.5 mg/kg body weight [19] for another 4 weeks.

All doses were orally administered once daily in the morning. At the end of the experiment period, the blood was collected and centrifuged for the separation of serum for 10 min at 3000 rpm and 4°C. The serum was stored at −80°C for further biochemical investigations. The animals were cervically decapitated, and the brain was immediately removed and washed with saline solution. The brain was dissected and divided into two parts. For lipid peroxidation (MDA) determination, a small piece of the first part of the brain was homogenized in 10% trichloroacetic acid or ice-cold bidistilled water for reduced GSH and AChE. The other piece of the first part of the brain was used for molecular analysis as it stored at −80°C. The second part of the brain was kept immediately in 10% formalin buffer for histopathological examination.

Biochemical studies

Lipid peroxidation (MDA) and reduced GSH were measured according to Ohkawa *et al.* [20] and Moron *et al.* [21] respectively, using a Biodiagnostic Kit (Cairo, Egypt). Lipid profile [triglycerides, cholesterol, and low-density lipoprotein (LDL)-levels] was determined colorimetrically in sera according to the methods of Rifai *et al.* [22] for triglycerides and Deeg and Ziegenhorn [23] for cholesterol and LDL-levels, using biochemical kits of Greiner Diagnostic GmbH. AChE was estimated spectrophotometrically according to Ellman *et al.* [24], which was modified by Gorun *et al.* [25].

The percentage of improvement or protection was calculated as follows:.

$$\%\text{improvement} = [(T - P)/(P - C)] \times 100$$

Where T is the mean of the treated group, P is the mean of the positive control group, and C is the mean of the negative control [26].

Gene expression analysis

Extraction of total RNA and cDNA synthesis

Total RNA was extracted from male rat brain tissues using TRIzol Reagent (Invitrogen) kit. The isolation method was executed in accordance with the instructions given by the manufacturer in the aforementioned kit. The extracted RNA was dissolved in diethylpyrocarbonate water and stored at −80°C till use [27]. Reverse transcription was done to synthesize cDNA [27,28]. The reaction was prepared as per RevertAid First Strand cDNA Synthesis Kit (MBI Fermentas). The cDNA-containing PCR products were preserved at −20°C till use for the amplification of the DNA [28].

Quantitative real-time-PCR

The expression value assessments of the tested genes were done using Step One Real-Time-PCR system (Applied Biosystem, USA), and the cDNA copy number of the male rats was determined. A 25 µl reaction mixture contained 12.5 µl of SYBR green (TaKaRa' Biotech Co. Ltd., Mountain View, Santa Clara, California, USA), 0.5 µl of 0.2 µM forward and reverse primers each, 6.5 µl of DNAs-RNAs free water, and 2.5 µl of synthesized cDNA. Specific primer sequences of the genes used are listed in Table 1. The target genes were quantified related to the reference gene (β-actin) using the $2^{-\Delta\Delta CT}$ method [29].

Table 1 Primer sequences used for real time quantative-PCR

Genes	Primer sequence (5′–3′)	References
APP	F: ACT GGC TGA AGA AAG TGA CAA T	Stein and Johnson [30]
	R: AGA GGT GGT TCG AGT TCC TAC A	
MAPT	F: CCC TGG AGG AGG GAA TAA GAA G′	Miyazaki *et al.* [31]
	R: AGG TGC CGT GGA GAT GTG T	
β-actin	F:GGAGATTACTGCCCTGGCTCCTA	Deng *et al.* [32]
	R: GACTCATCGTACTCCTGCTGCTG	

APP, amyloid precursor protein; F, forward primer; *MAPT*, microtubule-associated protein tau; R, reverse primer.

Determination of DNA adducts using high-performance liquid chromatography

DNA from rat brain was extracted according to Ahmed *et al.* [29], then digested, and the 8-hydroxy-2-deoxyguanosine (8-OhdG) adduct was measured using CoulArray-equipped high-performance liquid chromatography (Model 5600). The analytes were revealed in two modules of the coulometric array; four sensors of electrochemical were attached in series for each, enabling targets to be identified based on the potential for reduction [29]. The ultraviolet detection had been set to 260 nm. Using CoulArray software, the high-performance liquid chromatography was controlled, and then the data were collected and analyzed. The mobile phase was 5% methanol at pH 5.2 in 50 mmol/l sodium acetate. The potentials of electrochemical detector for 2-deoxy guanosine (2-dG) and 8-OHdG were 120/230/280/420/600/750/840/900 mV and the flow rate was 1 ml/min.

Histopathology

Brain sections from each group were preserved immediately in buffer 10% formalin for staining using hematoxylin and eosin as described by the method of Hirsch *et al.* [33].

Statistical analysis

The data were analyzed through comparison of values for various treatment groups with the individual control values. For each group, all data were expressed as a mean±standard deviation with a 5% rate error. Significant differences between groups were analyzed statistically using an analysis of one-way variances, analysis of variance, using the computer program SPSS, version 16.0 (233 South Wacker Drive, 11th Floor, Chicago, Illinois 60606-6307, USA). The variations were considered significant at P value less than or equal to 0.05.

Results

Biochemical results

In Table 2, the GSH level was significantly decreased ($P \leq 0.05$) in group 3 as compared with control group. Groups 4 and 5 showed significant change ($P \leq 0.05$) in GSH level compared with group 3. The level of GSH was increased by 39.49 and 78.97% in groups 4 and 5, respectively.

Significant increase ($P \leq 0.05$) in MDA level was observed in group 3 brain tissue as compared with group 1. In group 4, MDA levels were significantly decreased ($P \leq 0.05$) compared with group 3 and showed improvement by 66.98%. The coadministration of an extract with $AlCl_3$ (group 5) prevents the hazard effect by 99.04%. Meanwhile, the reference drug showed improvement only by 48.32%.

For lipid profile, all studied parameters were increased in group 3. The two supplemented groups with plant extract (groups 4 and 5) showed significant decrease. The improvement in total cholesterol reached 96.59 and 91.10% in groups 4 and 5,

Table 2 Effect of Plant extract and donepezil on, glutathione, malondialdehyde, lipid profile, and acetylcholinesterase activity in brain homogenate

Groups	Parameters					
	GSH (mean±SD) (mg/g tissue)	MDA (mean±SD) (μmol/g tissue)	TC (mean ±SD) (mg/dl)	LDL-C (mean ±SD) (mg/dl)	TG (mean ±SD) (mg/dl)	AChE (mean±SD) (μMSH/min/mg protein)
Control group (G1)	332.03±50.95a	1.84±0.22c	72.27 ±5.51bc	24.23±6.03b	55.61±9.99c	61.37±6.33c
Control extract group (G2)	344.33±56.91a	1.8±0.53c	69.71±5.61c	23.96±4.13b	57.73 ±12.19bc	55.55±3.53c
AD group (G3) (AlCl3 group)	114.1±25.12c	3.93±0.58a	130.41 ± 11.08a	55.88±7.02a	127.69 ±13.97a	195.76±19.74a
Extract therapeutic group (G4)	200.18 ±51.03b	2.53±0.21b	70.29±9.49c	26.33±6.14b	71.51±8.35b	117.14±18b
% improvement	39.49	66.98	96.59	93.36	77.94	58.50
AlCl3+Plant (G5) (prevention)	286.2±59.1a	1.86±0.29c	67.10±7.53c	24.06±5.67b	47.94±8.79c	68.96±12.37c
% improvement	78.97	99.04	91.10	99.46	89.35	94.35
Therapeutic donepezil (G6)	296.25±37.85a	2.92±0.67b	82.78 ±10.76b	28.46±4.36b	53.84 ± 12.16c	71.78±8.79c
% improvement	83.59	48.32	81.92	86.63	97.54	92.25

All data are expressed as mean±SD. AChE, acetylcholinesterase activity; AD, Alzheimer's disease; GSH, glutathione; LDL-C, low-density lipoprotein cholesterol; MDA, malondialdehyde; TC, total cholesterol; TG, triglycerides. Different letters a, b, c, d, in the same column are significant at P value less than or equal to 0.05, using analysis of variance test.

respectively. Donepezil showed improvement in total cholesterol only by 81.92%. The % improvement in LDL or triglycerides in the prevented group was ranked former to that in the posttreatment group.

AChE levels increased significantly in rats' brains upon receiving $AlCl_3$ only for 6 successive weeks compared with group 1 (Table 2). However, group 4 showed that AChE level decreased significantly as compared with group 3 (58% improvement). However, rats treated with donepezil exhibited significant decrease in AChE levels when compared with those in the group 3 (92.25% improvement). Interestingly, group 5 showed a significant decrease in AChE (94.34% improvement). The use of plant extract in normal-treated group did not exhibit noticeable changes in all parameters, as seen in Table 2.

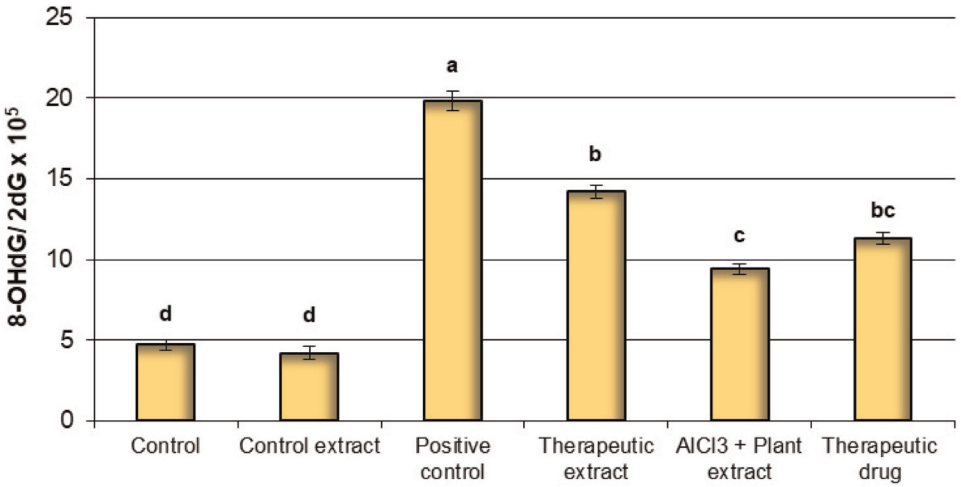
Levels of the 8-OHdG generation in brain tissues of AD-induced rat genome following *H. thebaica* extract treatment are summarized in Fig. 1. It was showed that AD induction in rats revealed a significant increase in the 8-OHdG/2-dG ratio compared with those in group 1. The increase in 8-OHdG/2-dG ratio was partially improved owing to supplementation with *H. thebaica* extract. However, group 5 induced better improvement in 8-OHdG/2-dG ratio than either group 5 or group 6 as compared with group 3 (Figs 2 and 3).

The expression levels of the genes related to AD pathways, including MAPT and APP, in brain tissues of AD rats were quantified by real-time-PCR (Figs 2 and 3). AD induction in rats significantly increased the expression of the *APP* gene in brain tissue when compared with that in groups 1 and 2. The percentage of the mRNA expression of the *APP* gene in group 3 was higher than those in group 1 (Fig. 2). This increase was ameliorated by using HTE either as groups 4 and 5. The elevated level of *APP* gene expression was reduced in group 5 greater than that in either group 4 or group 6 (Fig. 2). In group 3, there was a markable increase in *APP* gene expression compared with group 1. The decrease in expression levels of *MAPT* gene was ameliorated in the groups 4, 5, and 6 (Fig. 3).

Histopathological results

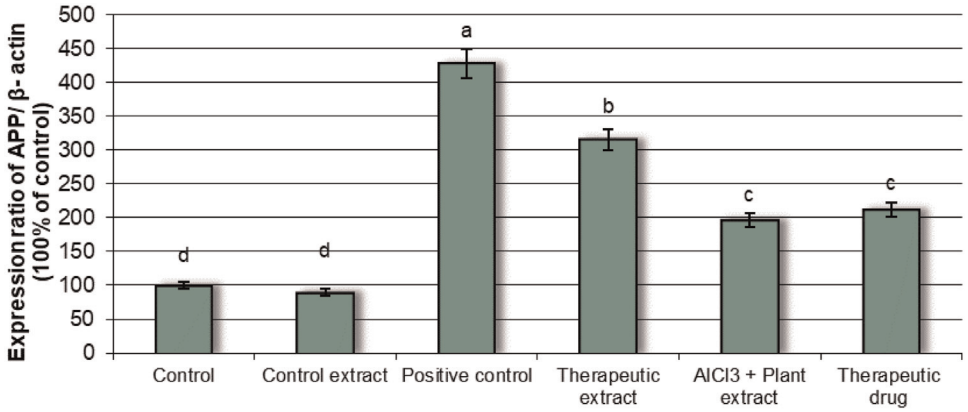
Brain sections from different groups are shown in Figs 4 and 5. Group 1 showed normal histological features (Fig. 4a–c), a well-defined molecular layer, a large number of small cells in the granular layer, and large Purkinje cells that present in the cell layer of Purkinje, as well as almost normal neuronal cells, no edema, no interstitial neuronal atrophy, and no shrinkage. Group 2 declared normal histological picture (Fig. 4d–f). Group 3 illustrated marked distortion in granular cell layer, sparse cell distribution of Purkinje layer, and a marked reduction in cellular size of the molecular layer; there is moderate edema and interstitial neuronal atrophy and shrinkage (Fig. 4g–i). Group 4 showed a well-defined molecular layer of small cells carefully packed into the granular layer and large Purkinje cells (Fig. 5a–c). Group 5 displayed the cerebellum has normal histological features, showing a very clear molecular layer and the presence of numerous carefully packed small cells in the granular layer, and large Purkinje cells (Fig. 5d–f). Group 6 declared

Figure 1



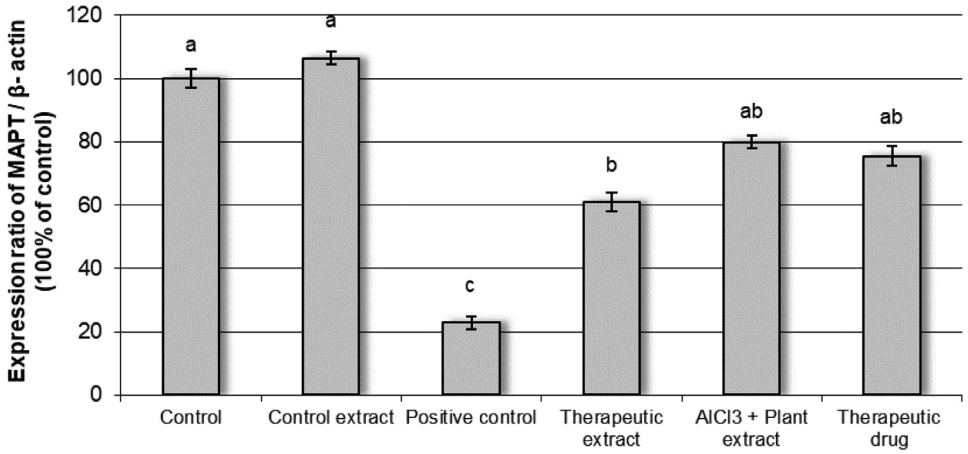
Generation of 8-OHdG in the brain tissues of male rats treated with *Hyphaene thebaica* extract with/or after AD induction. DNA damage was expressed as the ratio of oxidized DNA base (8-OHdG) to nonoxidized base (2-dG) in brain DNA. Data are presented as mean±SEM. a, b, c: mean values within column with unlike superscript letters were significantly different ($^aP<0.001$, $^bP<0.01$, $^dP<0.05$). AD, Alzheimer's disease; 2-dG, 2-deoxy guanosine; 8-OHdG, 8-hydroxy-2-deoxyguanosine.

Figure 2



Expression levels of *APP* gene in brain tissues of AD-induced male rats treated with *Hyphaene thebaica* extract with/or after AD induction. Data are presented as mean±SEM. a, b, c: followed by different superscripts are significantly different ($P \leq 0.05$). a, b, c: percentage values within column with unlike superscript letters were significantly different ($^{a}P<0.001$, $^{b}P<0.01$, $^{c,\ d}P<0.05$). AD, Alzheimer's disease; APP, amyloid precursor protein.

Figure 3



Expression levels of *MAPT* gene in brain tissues of AD-induced male rats treated with *Hyphaene thebaica* extract with/or after AD induction. Data are presented as mean±SEM. a, b, c: percentage values within column with unlike superscript letters were significantly different ($^{a}P<0.001$, $^{b}P<0.01$, $^{c}P<0.05$, $^{ab}P>0.05$ compared with a or b). AD, Alzheimer's disease; MAPT, microtubule-associated protein tau.

seminormal histological features with edema and interstitial neuronal atrophy and shrinkage (Fig. 5g–i).

Discussion

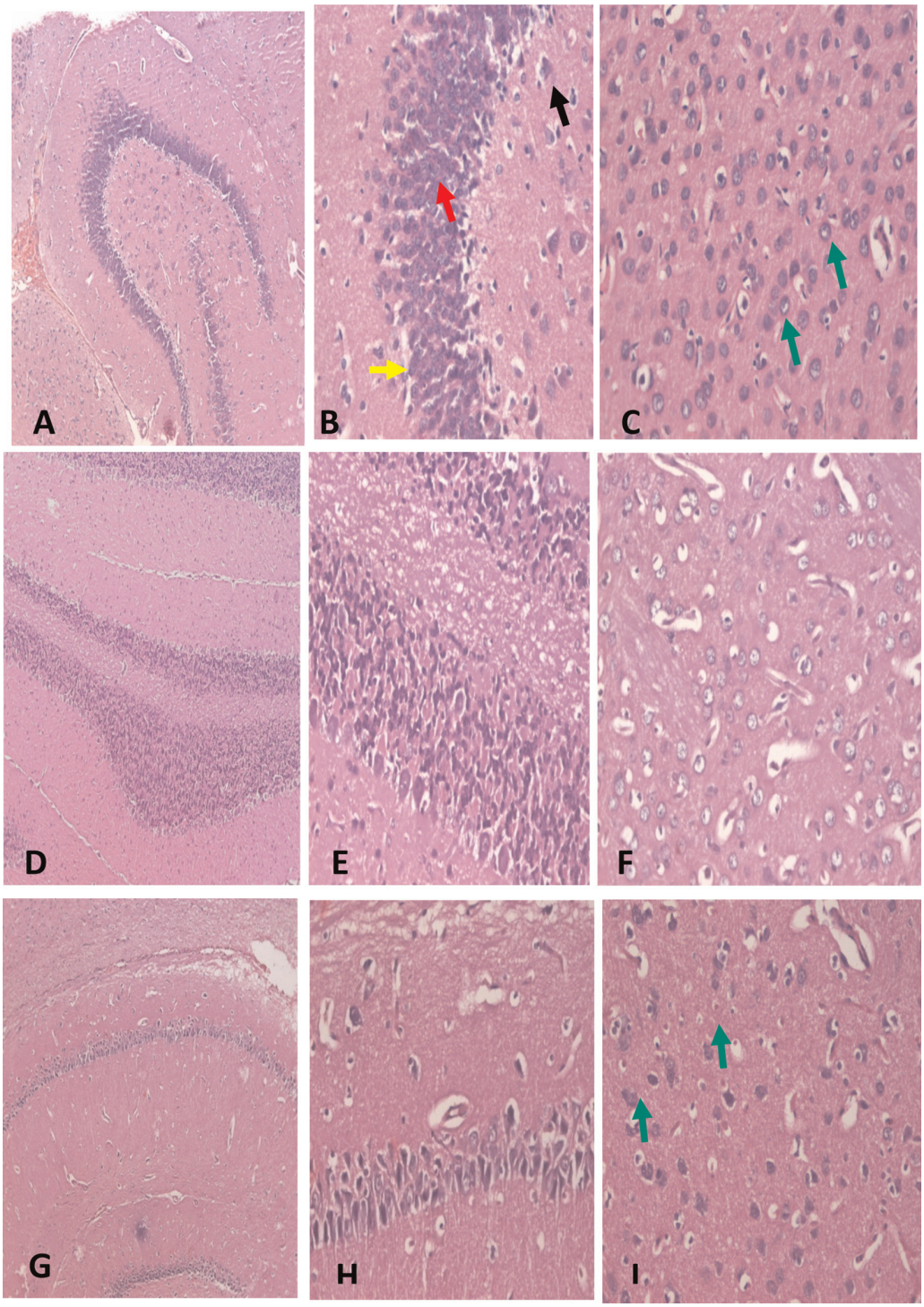
AD is a multifactorial disease. Hence, one target therapy is not effective [34]. Drug discovery from natural products to control AD is a current trend. Although *H. thebaica* (Doum) has been used in folk medicine in Egypt since a long time, there is no single publication concerning its effect on AD. In this study, the antioxidant capacity of Doum extract caused amelioration in GSH and MDA levels in rats injected with $AlCl_3$ for AD induction.

As a fact, the antioxidant defense mechanisms decrease in neurodegenerative diseases and during aging, causing increase in oxidative stress [35]. Free radicals evolved through oxidative stress injure the abundant polyunsaturated fatty acids in brain [36,37]. Elimination of ROS essentially depends on GSH redox cycle, as it represents the first defense against oxidative stress [38].

One of the helpful strategy in controlling AD is the elimination of free radicals by supplemented antioxidant [39,40]. Flavonoids that are rich in HTE [41,42] can be one of the reasons in controlling the redox state. Sureda *et al.* [43] proved that flavonoids increase GSH content and reduce MDA level. The increased GSH level in AD rats treated with HTE is a good sign for the effect of HTE as an antioxidant.

Pure flavonoids or total extracts can minimize pro-inflammatory cytokines (IL-6, TNF-α, IL-1β, and

Figure 4

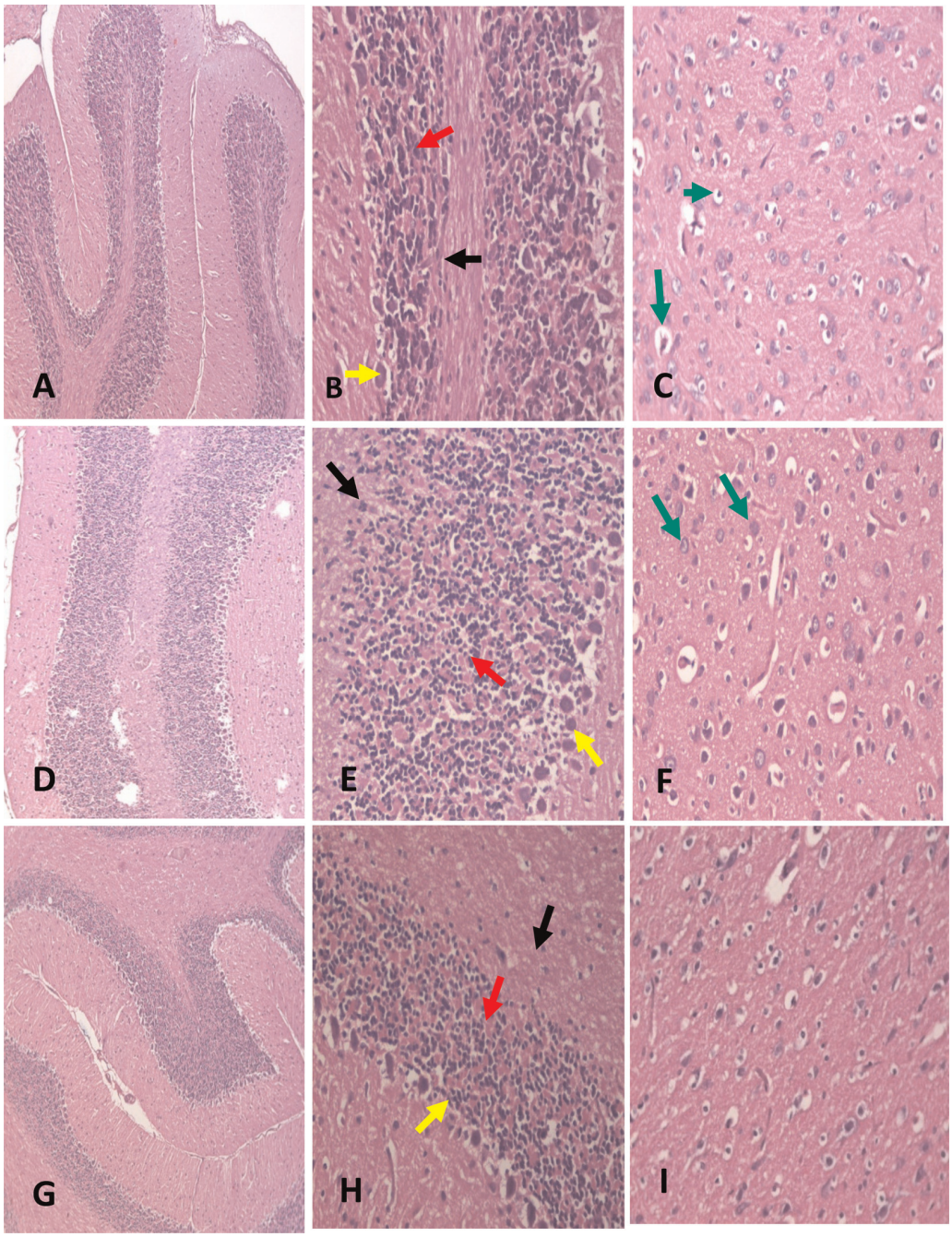


Photomicrographs of brain sections in control, control extract, and AD group. (a, b, c) Group 1: brain section of normal control rat showing the cerebellum with normal histological features, illustrating a well-defined molecular layer (black arrow), presence of numerous closely packed small cells in the granular layer (red arrow), and large Purkinje cells in the Purkinje cell layer (yellow arrow). (d, e, f) Group 2: control extract showing the cerebellum with normal histological features: a well-defined molecular layer, numerous closely packed small cells in the granular layer, and large Purkinje cells in the Purkinje cell layer. (g, h, i) Group 3: brain section of AD group, showing cerebellum with marked distortion in granular cell layer (sparse cell distribution), sparse cell distribution of Purkinje layers, and marked reduction in cellular size of the molecular layer, as well as moderate edema and interstitial neuronal atrophy and shrinkage (green arrow) (hematoxylin and eosin stain, ×100, ×400, ×400). AD, Alzheimer's disease.

COX-2) expression, and consequently, prevent neural damage and diminish inflammatory markers [44].

Tannin, one of the HTE constituents [41,42] possesses strong anti-inflammatory and antioxidant properties. It also shows a neuroprotective effect

Figure 5



Photomicrographs of brain sections in therapeutic, prevention, and reference drug groups. (a, b, c) Group 4: brain section of rat with extract therapeutic group showed the cerebellum with normal histological features: a well-defined molecular layer (black arrow), presence of numerous closely packed small cells in the granular layer (red arrow), and large Purkinje cells in Purkinje cell layer (yellow arrow), as well as edema and interstitial neuronal atrophy and shrinkage (green arrow). (d, e, f) Group 5: brain section of rat with $ALCl_3$+ plant (prevention) showed the cerebellum with normal histological features: a well-defined molecular (black arrow), presence of numerous closely packed small cells in the granular layer (red arrow), and large Purkinje cells in Purkinje cell layer (yellow arrow), and almost normal neuronal cells (green arrow). (g, h, i) Group 6: brain section of rat with $AlCl_3$+reference drug showed the cerebellum with normal histological features, illustrating a well-defined molecular (black arrow), presence of numerous closely packed small cells in the granular layer (red arrow), and large Purkinje cells in the Purkinje cell layer (yellow arrow), as well as edema and interstitial neuronal atrophy and shrinkage (green arrow) (hematoxylin and eosin stain, ×100, ×400, ×400).

against AD in several in vitro and in vivo models of AD [45].

The increases in LDL, cholesterol, and triglycerides levels in AD model are parallel with different authors [46–48]. These increases are strong AD pathogenesis risk factor, inducing brain deposition of amyloid-beta precursor protein and the increase in amyloid-beta (Aβ) accumulation in neuronal endolysosomes, leading to neuroinflammation [49].

The total LDL, cholesterol, and triglycerides levels in brain were ameliorated in rat treated with oral HTE. Three therapeutics approved by FDA, are donepezil, rivastigmine, and galantamine. These drugs act as choline esterase inhibitors. However, their adverse effects lead to cholinergic activation in other central and peripheral pathways, some of which are severe [50]. *H. thebaica* extract succeeded to inhibit the enzyme AChE activity in AD rats. Its effect on choline esterase can be attributed to the presence of phenolic acids and flavonoids [51,52].

Aβ accumulation in the brain is commonly assumed to be the starting trigger of a pathological cascade that ultimately results in synaptic dysfunction and its loss, neuronal death, and ultimately cognitive dysfunction [53]. Accordingly, the APP expression and the MAPT expression were assessed. The results revealed significant increase in the *APP* gene expression and decrease in MAPT expression in AD rats. High generation of DNA adduct levels in the form of OHdG/2-dG ratio was marked in AD rats.

The present results showed that HTE could be able to regulate the expression of *APP* and *MAPT* genes as well as decrease the generation of DNA adducts compared with those in AD rats. These findings coincide with Mohebali *et al*. [54], who declared that flavonoids and phenolic compounds extracted from medicinal plants regulated gene expression of Alzheimer-related disease.

All measured parameters in this study were assured with the histological results, where they showed an accumulation of fibrillar, extensive neuronal loss proteins such as extracellular Aβ plaques, and neurofibrillary tangles within neurons in AD brain, which coincides with Clark [55]. These pieces of evidence lead to impairment of mechanisms of axonal integrity and neurodegenerative disorders. Neurite degeneration through the destabilization of microtubule proteins could be the key factor that induces impairment and memory loss in patients with AD. The administration of HTE to AD rats improved AD pathogenesis as shown by a decreased rate of AChE and increased antioxidant parameters in the brain, which was further supported by an improvement in the brain tissue characteristics of histopathological analysis. Increase in the size of neuronal bodies and layer in HTE-treated AD rats relative to that found in AD untreated rats (positive control) provides a neuroanatomical basis that may enhance learning and memory abilities, as found in previous studies [56].

Conclusion

In conclusion, the administration of HTE to AD rats improved the pathogenesis of $AlCl_3$. This was proved by the amelioration of the studied biochemical, gene expression, and histological parameters.

The treatment of AD rats with HTE on day one of $AlCl_3$ induction showed better results than supplementing the extract after occurrence of the signs of Alzheimer.

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Nil.

Conflicts of interest

There are no conflicts of interest.

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