Developed Galantamine Therapy for Alzheimer's Disease by Introducing Nano-Drug Delivery Systems

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Abstract: The major cause of dementia, a major public health problem, is Alzheimer's disease (AD). A reliable method for the diagnosis and follow-up of Alzheimer's disease is needed together with a specific biological marker. Galantamine, an acetylcholinesterase inhibitor for AD therapy has several reported side effects. One approach to reduce dosing amounts, frequency of administration, and adverse side effects while maintaining the drug efficiency, is the development of drug delivery systems using nanoparticles. Presently Galantamine (Gal) coated with either Cerium/ Calcium hydroxyapatite (Ce/Ca-HAp) or carboxymethyl chitosan/ Ceria/ Calcium hydroxapatite (CMCS/Ce/Ca-HAP) was *i.p*. injected at a dose of 2.5 mg/kg b.wt. for 2 and 4 weeks. 86 female adult albino Wistar rats (189- 200 gm weight) were used. Alzheimer was induced in ovariectomized rats by Aluminium chloride (AlCl3) oral treatment at doses of 17mg/kg b.wt. daily for 2 months. Rats were divided into six groups: Group (1) normal control; Group (2) Galantamine *i.p*. injected at a dose of 2.5 mg/kg b.wt; Group (3) Alzheimer ; Group (4) Alzheimer's induced rats treated *i.p.* with Gal; Group (5) Alzheimer's induced rats treated with Gal. coated with Ce/Ca-HAp; Group (6) Alzheimer's induced rats treated with Gal. coated with Gal coated with CMCS/Ce/Ca-HAp for 2 and 4 weeks. In the concurrent study, AD induced histological alterations manifested as amyloid plaque formation of different sizes; congestion with perivascular edema; degenerated neurons with diffused gliosis; loss of pyramidal cells; separation of cortical tissue and formation of fibrous glial scar. Several tests may be cumulatively used for early detection as decreased Ach; Bcl2; Tpl; GSH; SOD; CAT; Cyto P450 and increased Ab; Chol; B-FABP; NO; MDA; GSSH. Treatment with Gal coated by Ce/Ca-HAp imposed highly significant improvement to near to normal levels in both histological and biochemical parameters. Gal coated by CMCS/Ce/Ca-HAp failed to encounter obvious ameliorations. In conclusion, brain markers (Ach; Bcl-2; Aβ; TPL; Chol; B-FABP; NO; MDA) together with brain antioxidants (GSH; SOD; CAT; CytoP450) may impose progressive laboratory testing method besides well known imaging techniques. Galantamine therapy may impose limited improvements thus drug delivery systems Gal coated by Ce/Ca-HAp may aid to minimize dosing amounts, frequency of administration, and adverse side effects of drug while increasing its therapeutic efficacy.

Keywords: Alzheimer (Alz), Ceria-doped calcium hydroxyapatite (Ce/Ca-HAp), Carboxymethyl Chitosan/Ceria/hydroxyapatite composite (CMCS/Ce/Ca-HAp), Galantamine, Rat, Histology and Biochemistry.

INTRODUCTION

Dementia is one of the major public health problems, where increasing numbers of patients impose a major financial burden on health care systems. It is majorly related to aging, and with the increasing numbers of elderly people in the population, the number of patients with dementia is growing rapidly. The major cause of dementia is Alzheimer's disease (AD). It is a progressive neurodegenerative disorder of the brain that is characterized by loss of neurons because of extracellular accumulation of amyloid beta (Ab) and intracellular hyperphos-phorylation of the tau protein [1].

Risk factors for AD are age and a positive family history of dementia, since more than one-third of AD patients have one or more affected first degree relatives [2]. Other risk factors that may be associated with the development of AD include severe head trauma, low levels of education, female gender, previous depression, and vascular factors [3, 4].

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Neuropsychological tests are often influenced by education [5], practice [6], and sociocultural or ethnic factors [7]. As a screening test for dementia, the sensitivity of MMSE (mini-mental state examination) test has been 56-90 % and specificity has varied from 85 % to 95 % [8].

The two major microscopic lesions for AD are amyloid plaques and neurofibrillary tangles (NFT), which are found significantly more often in AD compared to normal aging. Computed tomography (CT) does not differentiate early AD from normal aging with high diagnostic accuracy [9]. Single photon emission computed tomography (SPECT) may be useful in the differential diagnosis of dementia [10]. Magnetic resonance imaging (MRI) offers a superior anatomic discrimination power and permits accurate imaging of the affected regions.

Thus, a reliable method for the diagnosis and follow-up of AD is needed.

An ideal biomarker should detect a fundamental feature of the neuropathology of AD, be able to detect AD early in the course of the disease with high

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sensitivity, distinguish it from other dementias, and be validated in neuropathologically confirmed AD cases.

A biomarker reflecting neuropathological changes at the molecular level in AD brain would be very useful in the differential diagnosis of dementia, and in distinguishing AD patients from those individuals with mild cognitive impairment who do not develop AD, and from patients with depression [11].

Studies on biochemical markers for the diagnosis of AD in cerebrospinal fluid (CSF) and blood are based on the detection of inflammatory proteins, markers of cholesterol homeostasis, oxidative stress, or related to characteristic pathological alterations in AD [12].

Cognitive deficits in AD have been widely associated with dysfunction of the cholinergic system [13] consequent upon degeneration of cholinergic cell bodies in the basal forebrain [14]. In post-mortem frontal cortex from AD patients, acetylcholine (ACh) levels, cholinacetyltransferase (ChAT) and acetylcholinesterase (AChE) activity were all reduced compared to controls [15]. Consequent to the cholinergic hypothesis of dementia, animal studies have been used to evaluate the role of cholinergic neurotransmission in learning and memory and selective lesion of cholinergic neurones remains nowadays as a widely used method to mimic some aspects of neurodegeneration in AD [16].

Bcl-2 (B-cell lymphoma 2), is a regulator protein that regulates cell death (apoptosis), by either inducing (pro-apoptotic) or inhibiting (anti-apoptotic) apoptosis. That Bcl-2 is expressed in neurons in the aged human brain complements observations suggesting neuronal death by apoptosis in AD patients [17]. A critical role of the β-amyloid (Aβ) in the pathogenesis of AD has been supported by human, animal, and *in vitro* studies [18]. Intracellular and membrane-associated Aβ, especially Aβ 42 in the temporal neocortex, may be more closely related to AD symptoms than other measured Aβ species [19].

Tissue factor thromboplastin TPL is the primary initiator of the extrinsic coagulation pathway. In contrast to other coagulation factors, it is an integral membrane glycoprotein that does not circulate as a plasma protein [20]. Although tissue factor might potentially contribute to the formation of senile plaques, it could also accumulate in the plaques as a secondary response to other biochemical perturbations [21].

Cholesterol plays an important role in promoting the deposition of amyloid in the brain [22]. They reported that participants who had higher levels of LDL cholesterol and lower levels HDL cholesterol had higher levels of amyloid in the brain. Brain-derived metabolites of cholesterol also appear to be elevated in the early stages of disease prior to the onset of cognitive impairment rendering them an important marker of underlying cerebrovascular disease preceding cognitive impairment and risk for developing cognitive impairment [23].

Brain-type fatty acid binding proteins (B- FABP] [24] may be suitable markers for the detection of brain injury. B-FABP is a member of a family of nine distinct FABP types, each named after the tissue in which it was first detected [25]. They are rapidly released from damaged cells into the circulation and are cleared from the circulation by the kidney with a plasma half-life of 20 min [26]. In adult-stage mice, B-FABP is produced in very low concentrations and is detected only in glial cells (presumptive astrocytes) of the white matter [27].

Nitric oxide NO is an atypical neurotransmitter formed from L-arginine by the enzyme nitric oxide synthase (NOS) [28]. NO is a well-known physiological signaling agent, and a pleiotropic regulator in various pathologies including tumor growth and AD [29]. (NO-) dependent oxidative stress results in mitochondrial ultrastructural alterations and DNA damage in cases of AD [30]. In fact, it plays a crucial role in mitochondrial respiration [31], since even low (nanomolar) concentrations of NO were found to reversibly inhibit the mitochondrial respiratory chain enzyme cytochrome oxidase (complex IV) and compete with molecular oxygen.

MDA arises largely from the peroxidation of PUFA. A number of studies document elevated levels of MDA in AD in the plasma/serum [32, 33]. The most prevalent antioxidant in most brain cells is GSH. It can react with ROS and oxidized products forming glutathione disulphide (GSSG), either catalysed by Glutathione Peroxidase (GPx) or independently. The GSSG can then be converted back to reduced GSH by Glutathione Reductase (GR). Studies of human brains have indicated that the ratio between reduced and oxidized glutathione (GSH/GSSG) is decreased in affected brain regions of AD patients compared to controls [34]. Superoxide dismutase (SOD) is part of the initial defense against ROS and catalyses the conversion of $O2\cdot-$ to H2O2 and oxygen (O2). The activity of SOD in serum was reduced in AD patients compared to controls and was negatively correlated to the lipid peroxidation marker MDA [35].

Galantamine, is an acetylcholinesterase inhibitor that may serve to prevent hypertension from precipitating vascular dementia, which is the second most common cause for dementia after AD. The cerebrovascular injury caused by hypertension has been associated with compromised cognitive function, mainly memory dysfunction and decreased speed of information processing. It was reported that galantamine works by enhancing cholinergic function by increasing the concentration of acetylcholine in the brain and enhancing cholinergic neuro-transmission in the brain [36]. Yet side effects from the drug have been thoroughly reported where higher doses are gradually required.

One approach to reduce dosing amounts, frequency of administration, and adverse side effects while maintaining the drug efficiency, is the development of new drug delivery systems with inflammatory site targeting and long circulating time [37].

Hydroxyapatite (HAp) has been extensively used in medicine for implant fabrication owing to its similarity with mineral constituents found in hard tissues [38], which leads to formation of bonds between the bone and the implanted materials being acted as biocompatible phase reinforcement in composites as well coatings to metal implants and granular fillers for direct incorporation into human tissues. Implementation of new species in the HAp lattice offer fundamentally new possibilities and areas of their practical applications in biology and medicine

Again Ceria (CeO2) nanoparticles exhibit high catalytic activity and a regenerative capacity to neutralize ROS. Ceria was found to protect cells against oxidative stress, inflammation, or damage caused by radiation. The particles are small and can cross the blood brain barrier [39].

Carboxymethyl chitosan (CMCS) is one of the most investigated water-soluble derivatives of chitosan (CS) that own specific biological activities such as antitumor activity, immune-stimulating effects, enhancing pro-tective effects against infection with some pathogens in mice, antimicrobial activity and radical scavenging activity. Because of the carboxymethylation, CMCS possesses negative charges when dissolved in water, the CMCS hydrogels seem to be successfully prepared by physical crosslink with calcium-based biopolymers [40].

Successful therapeutic regimens for incurring favourable pathological influence for AD necessitate reliable earlier diagnosis for the onset of clinical settings. They should be highly specific to AD especially in the early stages of the disease [1].

Thus the aim of the presented study was to identify several sensitive biological markers to detect fundamental features of the neuropathology of Alzheimer's disease in its earlier stages. As Galantamine seems to impose serious side effects besides its poor therapeutic capabilities new delivery systems using nanoparticles are given.

MATERIAL AND METHODS

86 female adult albino Wistar rats (189- 200 gm weight) from the Animal breeding colony of the Medical Research Centre Ain Shams University were presently used. After a one week pre-experimentation adaptation (food and water ad libitum) period animals were ovariectomized surgically as several lines of evidence suggest that changes in hormones after menopause may play an important role in the incidence of cognitive dysfunction & also in the development of the AD [41]. Alzheimer induced rats were ovariectomized prior to Aluminium chloride (AlCl3) oral treatment at doses of 17mg/kg b.wt. [42] daily for 2 months after one month post-operative procedure.

Galantamine was *i.p*. injected at a dose of 2.5 mg/kg b.wt. for 2 and 4 weeks [43].

Galantamine was coated with either Cerium/ Calcium hydroxyapatite (Ce/Ca-HAp) or carboxymethyl chitosan/ Ceria/ Calcium hydroxapatite (CMCS/Ce/Ca-HAP) and *i.p*. injected at a dose of 2.5 mg/kg b.wt. for 2 and 4 weeks.

Animals were allotted as follows:-

a) *Group 1 control (C):* Gonad intact animals (8)

b) *Group 2 Galantamine (Gal):* Gonad intact animals (8) treated with galantamine.

c) *Group 3 Alzheimer (Alz)*: Ovariectomized animals (22) treated orally with $AlCl_3$ (17 mg/kg b.wt) daily for 2 months following one month surgery.

d) *Group 4 (Alz + Gal):* Alzheimer's disease-induced rats (16) treated *i.p.* with Gal (2.5 mg/kg b.wt.).

e) *Group 5 (Alz + Gal. + Ce/Ca-HAp):* Alzheimer's disease-induced animals (16) treated (i.p) with Gal. coated with Ce/Ca-HAp (2.5 mg/kg b.wt.).

f) *Group 6 (Alz +Gal. + CMCS/Ce/Ca-HAP):* Alzheimer's disease-induced animals (16) treated *i.p* with Gal coated with CMCS/Ce/Ca-HAp (2.5 mg/kg b.wt.).

After 2 and 4 weeks rats were ether inhalation anaesthetized where brains were collected from all groups. One half of each brain was homogenized immediately to give 10% (w/v) homogenate in ice-cold medium containing 50 mM Tris–Hcl (pH 7.4) and 300 mM sucrose. The homogenate was centrifuged at 3000 rpm for 10 min at 4 ◦C. The supernatant (10%) was separated for biochemical estimations.

The quantitative measurement of brain markers included Ach levels [44]; $Bcl\text{-}_2$, [45]; Aβ [46]; TPL [47]; Chol [48]; B-FABP [49]; NO [50] and MDA [51]. Brain antioxidants included GSH [52], GSSH, SOD [53], CAT [54] and $CytoP_{450}$ reductase [55].

The other half was washed in saline (0.9% NaCl) and placed accordingly in 10% neutral buffered formalin for fixation and processed for histological analysis by Haematoxylin and Eosin for general histological examination [56].

RESULTS

Histological Investigations

Hx&E staining data are shown in Figure **1** in control (Figure **1A**) and experimental groups (Figure **1B-1H**). Sections of rat brain from Gal animals manifested no alterations from control (**1B**). On the other hand following Alz induction, amyloid plaques of different sizes, congestion with perivascular edema, degenerated neurons with diffused gliosis, loss of pyramidal cells, separation of cortical tissue and formation of fibrous glial scar were manifested (Figure **1C** & **1D**). Following Gal therapy rat brain sections presented mild attenuation in amyloid plaques which persisted in a less aggressive manner with the progress of time; peri-vascular edema accompanied by diffused gliosis and degeneration in some of the hippocampal neurons (Figure **1E**). Using Gal coated by Ce/Ca-HAp designated near to normal patterns following 4 weeks of treatment (Figure **1F**). While Gal coated by CMCS/Ce/Ca-HAp failed to induce ameliorative levels of recovery (Figure **1G** & **1H**).

Biochemical Investigations

Brain Marker Profile

Concurrent results signify significant decreases in Ach, $Bcl\text{-}_2$ and TPL in brain tissue from Alz-induced rats as compared to normal control animals. On the contrary, significant increases in Ab, Chol, BFABP NO and MDA were recorded in the same group of rats. All parameters were significantly ameliorated to near to normal figures following treatment with Gal coated by Ce/Ca-HAp and not Gal coated by CMCS/Ce/Ca-HAp which failed to impose ameliorative figures (Table **1**).

Brain Enzymes Antioxidants Profile

Presently, significant depletion was obvious in GSH, SOD, CAT and CytoP450reductase in brain tissue of Alz-induced rats as compared with normal control. Partial amendment in above parameters was reached following Galantamine treatment. Treatment with Gal coated by Ce/Ca-HAp imposed highly significant improvement to near to normal levels in the previous parameters. On the contrary, Gal coated by CMCS/Ce/Ca-HAp failed to encounter any obvious ameliorations. These results validate fore mentioned histopathological alterations (Tables **1** & **2**).

DISCUSSION

Alzheimer's disease (AD) is a progressive neurodegenerative disorder that is the most common form of dementia, accounting for approximately 50-60% of all cases [57]. The disorder usually starts in a mild form and progressively gets worse because of late discovery of onset together with the lack of specific efficient therapeutic regimens and due to its progressive course.

Many studies of putative biomarkers have been conducted, but none of the antemortem tests has fulfilled all the criteria for an ideal biomarker. This necessitates the use of several methodology for the early identification together with the different diagnostic imaging methods as photon emission computed tomography (SPECT) and Magnetic resonance imaging (MRI)). Such biomarkers include Ach, Bcl_2, amyloid peptide, TPL, Chol, BFABP, NO and MDA in addition to the brain antioxidant enzymes GSH, GSSH, SOD, CAT and $CyoP_{450}$. Biomarkers seem to be more sensitive to Alzheimer changes in its earlier stages as presently designated that would impose a reliable method for early diagnosis.

In the concurrent study, AD was reported to induce serious histological alterations manifested as amyloid plaque formation of different sizes; congestion with perivascular edema; degenerated neurons with diffused gliosis; loss of pyramidal cells; separation of cortical tissue and formation of fibrous glial scar.

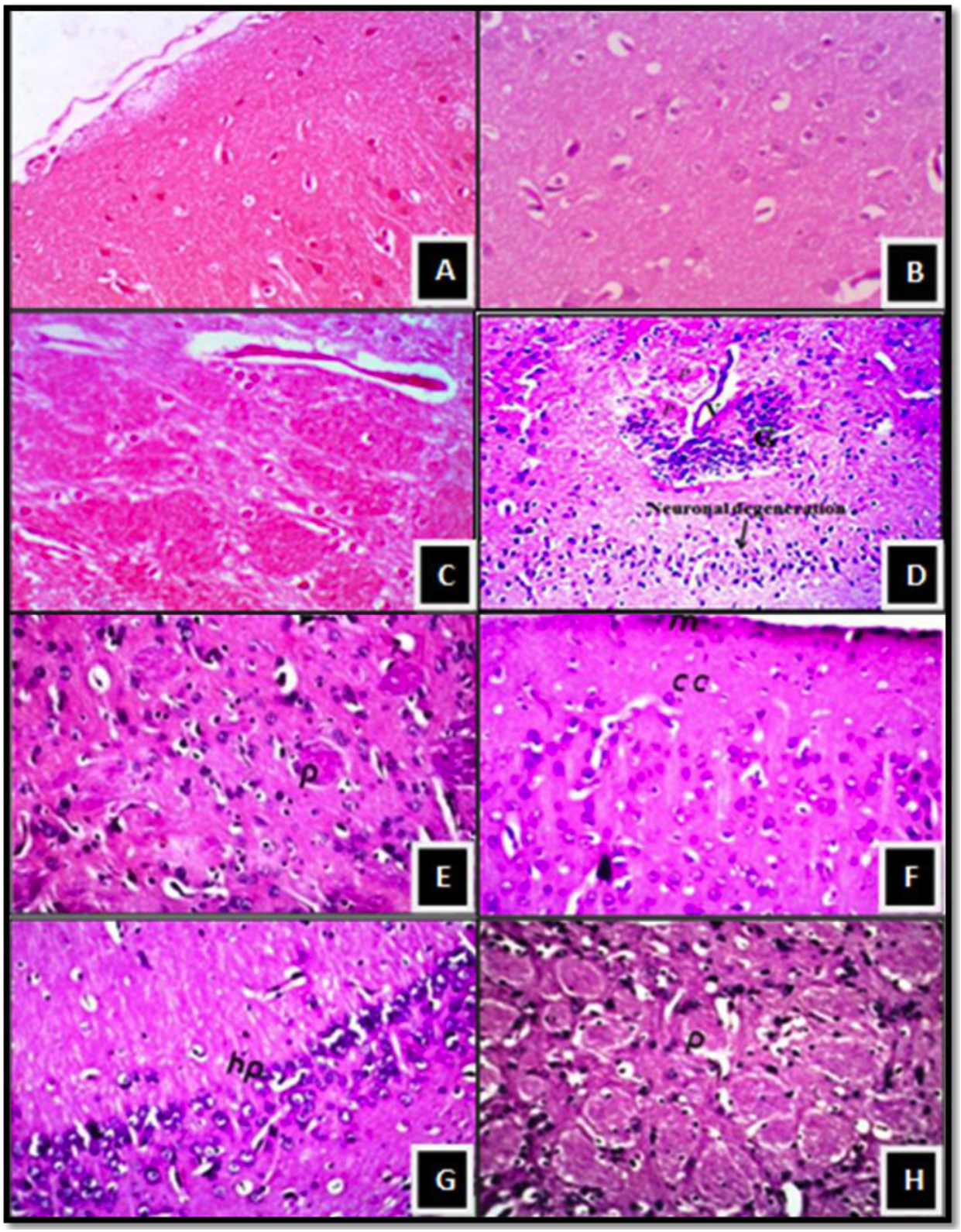

Figure 1: Photomicrograph of brain sections of rats from control and experimental groups. (**A**): normal control. (**B**): Gal treated showing no alterations from control. (**C** & **D**): Alz induced showing amyloid plaques of different sizes, perivascular edema and diffused gliosis. (**E**): Alz induced after Gal therapy showing persistence of amyloid plagues and diffused gliosis. (**F** & **G**): Alz induced treated with Gal coated by Ce/Ca-HAp showing near to normal patterns in cerebral cortex and hippocampus. (**H**): Alz induced treated with Gal coated by CMCS/Ce/Ca-HAp showing persistence of lesions. (**H E**; X 200) (p) amyloid plaques; (cc)cerebral cortex; (hp) hippocampus.

Table 1: Concentration of Different Brain Markers in Brain Tissue Samples from Alz-Induced and Treated Rats with Galantamine, Gal+Ce/Ca-HAp and Gal+CMCS/Ce/Ca-HAp

		Control	ALZ	ALZ+ Gal	Alz+Gal+Ce/ Ca-HAp	Alz+Gal+CMCS /Ce/Ca-HAp
Ach(µmol/ mg protein)	2 w	$100.67^{A}_{a} \pm 3.22$	$71.68^{B}_{a} \pm 2.24$	$83.29^{C}_{a} \pm 2.84$	$90.54^{D}_{a} \pm 2.93$	$84.01^{E}_{a} \pm 2.87$
	4 w	$101.42^{A}_{a} \pm 3.32$	$67.55^{B}_{b} \pm 2.16$	$94.68^{C}_{b} \pm 3.09$	$97.76^{D}_{b} \pm 3.23$	$94.88^{E}_{b} \pm 3.11$
Bcl-2 (pg/mg protein)	2 w	$92.33^{A}_{a} \pm 2.91$	$58.93^{B}_{a} \pm 1.78$	$71.45^{C}_{a} \pm 2.31$	$77.31^{D}_{a} \pm 2.47$	$72.04^{E}_{a} \pm 2.35$
	4 w	$92.42^{A}_{a} \pm 2.94$	$54.27^{B}_{b} \pm 1.49$	$80.73^{C}_{b} \pm 2.55$	$86.55^{D}_{b} \pm 2.69$	$81.26^{E}_{b} \pm 2.58$
Amyloid peptide (pg/mg protein)	2 w	$13.26^{A}_{a} \pm 0.53$	$25.77^{B}_{a} \pm 0.93$	$19.05^{C}_{a} \pm 0.76$	$17.25^{D}_{a} \pm 0.69$	$18.79^{E}_{a} \pm 0.71$
	4 w	$13.21^{A}_{a} \pm 0.51$	$29.02^{B}_{b} \pm 1.12$	$15.82^{C}_{b} \pm 0.62$	$14.44^{D}_{b} \pm 0.58$	$15.63^{E}_{b} \pm 0.64$
TPL (µg/mg protein)	2 w	1277.4±21.59	785.41±15.78	953.68±19.25	1077.43±21.03	961.22±19.43
	4 w	1259.8±20.87	694.33±14.25	1104.55±22.17	1269.55±22.86	1127.38±22.06
Chol (µg/mg protein)	2 w	$648.66^{A}_{a} \pm 13.16$	$1008.29^{B}_{a} \pm 23.56$	$870.56^{C}_{a} \pm 18.82$	$801.21^{D}_{a} \pm 17.41$	$863.98^{E}_{a} \pm 18.77$
	4 w	$652.51^{A}_{a} \pm 13.19$	$1152.65^{B}_{b} \pm 25.78$	$749.91^{C}_{b} \pm 15.33$	$682.32^{D}_{b} \pm 14.62$	$736.72^{E}_{b} \pm 15.18$
B-FABP (pg/mg protein)	2 w	$39.91^{A}_{a} \pm 2.14$	$75.99^{B}_{a} \pm 3.86$	$58.54^{C}_{a} \pm 2.78$	$53.76^{D}_{a} \pm 2.52$	$57.88^{E}_{a} \pm 2.69$
	4 w	$40.52^{A}_{a} \pm 2.23$	$81.31^{B}_{b} \pm 4.09$	$50.27^{C}_{b} \pm 2.39$	$46.56^{D}_{b} \pm 2.28$	$49.55^{E}_{b} \pm 2.37$
NO (pg/mg protein)	2 w	$36.43^{A}_{a} \pm 1.97$	$70.67^{B}_{a} \pm 2.94$	$51.31^{C}_{a} \pm 2.28$	$47.22^{D}_{a} \pm 2.11$	$50.82^{E}_{a} \pm 2.23$
	4 w	$35.99^{A}_{b} \pm 1.95$	$76.55^{B}_{b} \pm 3.18$	$43.77^{C}_{b} \pm 2.04$	$39.81^{D}_{b} \pm 1.96$	$43.49^{E}_{b} \pm 2.02$
MDA (nmol/mg protein)	2 w	$4.07^{A}_{a} \pm 0.101$	$8.02^{B}_{a} \pm 0.206$	$6.28^{C}_{a} \pm 0.154$	$5.79^{D}_{a} \pm 0.146$	$6.08^{E}_{a} \pm 0.151$
	4 w	$4.13^{A}_{a} \pm 0.103$	$8.61^{B}_{b} \pm 0.273$	$5.51^{C}_{b} \pm 0.138$	$5.05^{D}_{b} \pm 0.123$	$5.39^{E}_{b} \pm 0.134$

Values are expressed as mean ± SE.
^{A,B,C,D}Means with a common superscript within a row are statically significantly different (P<0.05).
$^{a, b}$Means with a common subscript within a column are statically significantly different (P<0.05).

Table 2: Concentration of Different Brain Enzyme Antioxidants in Brain Tissue Samples from Alz-Induced and Treated Rats with Galantamine, Gal+Ce/Ca-HAp and Gal+CMCS/Ce/Ca-HAp

		Control	ALZ	ALZ+ Gal	Alz+Gal+Ce/ Ca-HAp	Alz+Gal+CMCS /Ce/Ca-HAp
GSH (U/mg protein)	2 w	$41.17^{A}_{a} \pm 1.423$	$26.89^{B}_{a} \pm 0.832$	$33.26^{C}_{a} \pm 0.957$	$35.07^{D}_{a} \pm 0.988$	$33.79^{E}_{a} \pm 0.972$
	4 w	$40.93^{A}_{a} \pm 1.418$	$23.47^{B}_{b} \pm 0.795$	$37.91^{C}_{b} \pm 1.086$	$40.54^{D}_{b} \pm 1.302$	$38.11^{E}_{b} \pm 1.117$
GSSG (µM/mg protein)	2 w	$0.63^{A}_{a} \pm 0.008$	$1.16^{B}_{a} \pm 0.018$	$0.82^{C}_{a} \pm 0.012$	$0.74^{D}_{a} \pm 0.011$	$0.81^{E}_{a} \pm 0.013$
	4 w	$0.64^{A}_{a} \pm 0.008$	$1.42^{B}_{b} \pm 0.024$	$0.74^{C}_{b} \pm 0.011$	$0.65^{D}_{b} \pm 0.009$	$0.74^{E}_{b} \pm 0.012$
SOD (U/mg protein)	2 w	$3.08^{A}_{a} \pm 0.071$	$1.69^{B}_{a} \pm 0.034$	$2.55^{C}_{a} \pm 0.045$	$2.79^{D}_{a} \pm 0.052$	$2.55^{E}_{a} \pm 0.045$
	4 w	2.9^{A}_{a} 3±0.068	$1.48^{B}_{b} \pm 0.029$	$2.87^{C}_{b} \pm 0.057$	$2.95^{D}_{b} \pm 0.071$	$2.91^{E}_{b} \pm 0.069$
CAT (U/mg protein)	2 w	6.50^{A}_{a} 2±0.173	$5.09^{B}_{a} \pm 0.101$	$5.78^{C}_{a} \pm 0.136$	$6.08^{D}_{a} \pm 0.148$	$5.69^{E}_{a} \pm 0.133$
	4 w	$6.49^{A}_{a} \pm 0.168$	$4.78^{B}_{b} \pm 0.092$	$6.03^{C}_{b} \pm 0.149$	$6.43^{D}_{b} \pm 0.171$	$5.97^{E}_{b} \pm 0.152$
Cyto P_{450} reductase (ng/mg protein)	2 w	$2.19^{A}_{a} \pm 0.023$	$1.23^{B}_{a} \pm 0.017$	$1.82^{C}_{a} \pm 0.021$	$2.11^{D}_{a} \pm 0.029$	$1.96^{E}_{a} \pm 0.025$
	4 w	$2.11^{A}_{a} \pm 0.024$	$0.958^{B}_{b} \pm 0.012$	$2.06^{C}_{b} \pm 0.024$	$2.34^{D}_{b} \pm 0.034$	$2.16^{E}_{b} \pm 0.028$

Values are expressed as mean ± SE.
^{A,B,C,D}Means with a common superscript within a row are statically significantly different (P<0.05).
^{a,b}Means with a common subscript within a column are statically significantly different (P<0.05).

Similarly, neuropathological examination of the AD rat brain showed extensive neuronal loss, accumulation of fibrillary amyloid β plagues, and neurofibrillary tangles within neurons [58]. Mahdy *et al.* [59] and Yassin *et al.* [60] observed necrosis of the brain, spongy appearance, plaques, and loss of normal structure, various sizes of amyloid plaques in the hippocampus, neurofibrillary tangles and fatty changes in Alzheimer induced rat brain tissue.

Aluminum salt has been found to induce the overexpression of APP. Present manifestations may be primarily a sequel to inflammatory responses that are known to play an important role in neurodegenerative disease. Vallés *et al*. [61] demonstrated that Aβ peptide causes oxidative stress in the neurons and inflammation in the astrocytes indicating that this toxic peptide can affect not only neuronal cells but astrocytes too. Wu *et al*. [62] also suggested that, the amyloid plaques caused by Al administration plays a role in the pathology of AD by directly inducing neuronal cytotoxicity and stimulating microglia to secrete cytokines and reactive oxygen species (ROS) which also damage neurons. Amyloid plaques could activate astrocytes and oligodendrocytes to produce chemokines, in particular Monocytes chemotactic protein (MCP-1), which serves as potent *in vitro* microglial and macrophage chemoattractants.

The challenges for establishing an early diagnosis of Alzheimer's disease (AD) have created a need for biomarkers that reflect the core pathology of the disease making earlier diagnosis possible in a clinical setting. They should be highly specific to AD and sensitive to changes, especially in the early stages of the disease [1].

Recorded results signify a considerable decrease in Ach content and a significant increase in Bcl_{-2} and TPL in Alzheimer induced rats. Contrary to that a limited increase in Ab was recorded and a significant increase in Chol, BFABP, NO and MDA.

Cognitive deficits in neuropsychiatric disorders have been closely related to cholinergic deficits where total Ach levels in both human and rats were reduced [15]. The cholinergic hypothesis suggests that a dysfunction of acetylcholine containing neurons in the brain contributes substantially to the cognitive decline observed in those with advanced age and AD [63].

Decreased expression of bcl-2 is observed in Alzheimer's dementia in neurons with neurofibrillary tangles [64]. Studies undertaken from Bcl-2 localization (mitochondria and endoplasmic reticulum) suggest that it inhibits generation of reactive oxygen species or lipid peroxidation [65]. It is also reported to modulate calcium fluxes from intracellular stores in hippocampal neurons *in vitro* [66].

Overproduction of amyloidogenic forms of the amyloid-(Aβ), a peptide that is generated by a sequential cleavage of APP by the β-and γ-secretases [67] is the hallmark of AD. Similarly, present studies have signified significant increase in A β in AD rats. Aβ is a complex biological molecule which interacts with many types of receptors and/or forms insoluble assemblies and, eventually, its nonphysiological depositions alternate with the normal neuronal conditions [68]. In aging and pathological conditions,the formation and clearance of Aβ are disturbed leading to an accumulation of Aβ and senile plaque formation. Neurodegeneration in AD is mediated in part through soluble forms of Aβ. Increased soluble Aβ concentration correlates with cognitive decline in AD-affected individuals. Hence, it may be considered as a reliable predictor of AD [68].

TPL is an integral membrane protein that is normally sequestered from circulating factor VII and other coagulation factors. Tissue injury exposes TPL to circulating factor VII resulting in complex formation and initiation of blotting cascade via the extrinsic pathway [21] In the concurrent study marked decrease in TPL has been recorded.

It has been suggested that increased levels of free cholesterol in neuronal cell membranes may provoke Aβ formation [60]. Highly significant augmented levels for cholesterol have been presently well documented. Several related studies have reported concomitant findings as Hughes [23] and Reed *et al.* [22]. Experimental *in vitro* and *in vivo* data indicate that brain cholesterol homeostasis is coupled with brain amyloid metabolism [70]. Cell culture studies demonstrate that membrane cholesterol controls the direction of the processing of the APP *in vitro* [70]. Again, under *in vitro* conditions, cholesterol has been shown to influence a number of processes involved in the generation of Aβ peptide of residues and neurofibrillary tangles [71].

In a study of patients B-FABP levels were elevated in serum of 29% of the patients with AD. B-FABP expression was observed in reactive astrocytes in brain sections. Thus it could be used in an attempt to reveal a sensitive marker for various neurodegenerative diseases and can therefore have importance for defining subgroups of these patients [72].

NO (produced by endothelial cells of blood vessels) is a protective molecule that maintains immune, cardiovascular, nervous, kidney, stomach and intestinal, skin and other beneficial effects. Accordingly, lack of NO production by endothelial cells due to increase in amyloid beta peptide aggregate formation and/or free radical generation only contributes to

pathology of AD. It was verified by [73] that Aβ produced nitric oxide which resulted in a change to the protein Drp1, called SNO-Drp1. This change, which was the attachment of NO to the protein, assisted by cysteine at the 644th position, caused an increase in mitochondrial fragmentation and cell death in Alzheimer's brains [74].

Increased levels of MDA in the brain in AD have been confirmed by several studies [1]. One of the main reasons for high malondialdehyde (lipid peroxidation) in elder patients could be melatonin deficiency as decrease in melatonin seems to be related to aging. The authors emphasized that low melatonin levels could be explained not only by a decrease in melatonin due to aging, but also by a decrease in melatonin due to AD.

Oxidative stress is a significant element in AD pathogenesis. Possibly, lack of protection against ROS production in the aging brain could be one triggering cause of AD. Oxidative stress is one of the first consequences of Aβ overproduction in the brain. The pressure from oxidative stress in the aging brain in combination with a lowered antioxidant defense creates a harmful combination that could disturb functions and damage organelles such as mitochondria. This would eventually lead to loss of synapses and cell death that give rise to the clinical symptoms associated with AD [75].

The activity of SOD is a sensitive index in oxidative damage as it scavenges the superoxide anion to form hydrogen peroxide leading to diminished toxic effects. Also, Glutathione reduced, glutathione reductase and glutathione-S-transferase are closely related to the direct elimination of reactive oxygen species. Therefore, the reduction in the activity of these enzymes may result in a number of deleterious effects due to the accumulation of superoxide radicals and hydrogen peroxide, linked with neurodegenerative diseases [76]

Galantamine the acetylcholinesterase inhibitor, has long been the drug of choice for treatment of AD patients for its recorded beneficial role in the slowing down of the degenerative outcomes on the brain that cause impaired memory. Pathways of Galantamine therapy is through preventing acetylcholinesterase from breaking down acetylcholine in the brain. As a result, an increased concentration of acetylcholine leads to increased communication between nerve cells. This may temporarily alleviate or stabilize some symptoms of Alzheimer's disease. In the present study therapeutic doses of Galantamine to AD rats signified limited ameliorations in the studied parameters of brain markers and antioxidant. Similarly, Galantamine coated by CMCS/Ce/Ca-HAp failed to encounter any obvious improvement parameters. Nevertheless, on coating Galantamine by Ce/Ca-HAp all previous parameters retreated to near to normal levels. These results were simultaneously confirmed by pre-mentioned histological parameters.

CONCLUSION

According to the present findings multiple biomarkers would indeed improve diagnostic tools for the early detection of the onset of AD. Brain markers (Ach; Bcl-2; Aβ; TPL; Chol; B-FABP; NO; MDA) together with brain antioxidants (GSH; SOD; CAT; CytoP450) may impose a progressive laboratory testing method besides well known imaging techniques. In addition, as Galantamine therapy may impose limited improvements thus the necessity for novel drug delivery systems to minimize dosing amounts, frequency of administration, and adverse side effects of drug while increasing its therapeutic efficacy is needed.

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CONFLICTS OF INTEREST

No conflict of interest.

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