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SYNERGISTIC EFFECT OF GALANTAMINE CARRIED ON A NOVEL NANO-DRUG DELIVERY SYSTEM ON INDUCED HEART DISORDERS IN ALZHEIMER'S DISEASE

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ABSTRACT

Background: Alzheimer's disease AD, the most common form of dementia, usually presents with cardiac disorders including heart failure. The aim of the present study was to verify that the curative role of Galantamine (the major therapeutic drug for AD) coated with Ce/Ca-HAp (nano-drug delivery particle) on brain tissue is capable of inducing similar therapeutic capabilities in heart disorders besides its role in AD therapy. Presentation of multiple biomarkers (CK, LDH, AST, H-FABP, TAC, TOC, TNO, Na+/ATPase, Ca2+ATPase and Mg2+ ATPase) for heart disorders may induce early detection thus adding to the efficacy of the curative competence. ***Methods:*** 70 adult female albino Wistar rats (189- 200 gm) were allotted into 5 groups: Control: Gonad intact rats (8). b) Galantamine (Gal): Gonad intact rats (8) treated with galantamine. c) Alzheimer (Alz): Ovariectomized rats (22) treated orally with $AlCl_3$ (17 mg/kg b.wt) daily for 2 months following one month surgery. d) Alz + Gal rats (16) e) Alz + Gal. + Ce/Ca-HAp rats (16). ***Results:*** Results verified marked increases in levels of CK, LDH, AST enzymes, NO, H-FABP, TOC, Ca^{2+}ATPase and Mg^{2+} ATPase while TAC and TNO and Na^{+}/A^{T}Pase levels were significantly decreased in AD rats. Histologically, intramyocardial deposits of Aβ peptides; narrowing of cardiac valves; increase in inflammatory cells monocytes, leucocytes and lymphocytes were viewed. Histochemically increase in total protein content and Ca ions were recorded. Marked improvements were more pronounced following GAL therapy coated with Ce/Ca-HAp than GAL alone. ***Conclusion:*** In conclusion, as cardiac damage highly presents with AD thus early detection using different biomarkers as cardiac profile (CK, LDH, AST and H-FABP) antioxidant and oxidative status (TAC, TOC and TNO) and ATPase profile (NKA, Ca^{2+}-ATPase and Mg^{2+}-ATPase) is vital for curative competence. Again as Ce/Ca-HAp nanoparticle presented as coating for GAL drug therapy, more promising ameliorative measures could be achieved.

KEYWORDS: Alzheimer's disease, dementia, Galantamine, nano-drug delivery particles, cardiac disorders, cardiac enzymes, ATPase profile of heart.

INTRODUCTION

Dementia is a gradual decline of mental ability that affects intellectual and social skills to the point where daily life becomes difficult. Alzheimer's disease, the most common form of dementia affects about 5% of people over age 65. It occurs more often with advancing age, affecting 20% to 25% of people over the age of 80. About 5% to 10% of dementia is vascular dementia, also known as dementia caused by stroke. At least 10% of cases of dementia are due to a combination of Alzheimer's disease and multiple strokes. In 1991, it was proposed that cerebral amyloid deposition represents the key pathogenic mechanism of AD development. The amyloid hypothesis suggested that amyloid initiates a cascade of pathological events, including the overexpression of neurofibrillary tangles that lead to neurodegeneration and cognitive decline.[1] Vascular risk factors such as hypertension, dyslipidemia and diabetes are well known risk factors for AD.[2] Furthermore, individuals with multiple vascular risks have more than twice the risk of developing dementia associated with AD compared to elderly without vascular risk factors.[3]

Since 1977, when the term 'cardiogenic dementia' was introduced,[4] cardiovascular diseases and risks have been recognized as factors contributing to the development of and coexisting with dementia.[5,6] It has recently been proposed that heart failure is a risk factor for Alzheimer's disease. Decreased cerebral blood flow and neurohormonal activation due to heart failure may contribute to the dysfunction of the neurovascular unit and cause an energy crisis in neurons. This leads to the impaired clearance of amyloid beta and hyperphosphorylation of tau protein, resulting in the formation of amyloid beta plaques and neurofibrillary tangles.[4]

Half of the people who die from AD have vascular lesions resulting from stroke. Brain imaging studies show a close association between vascular injury and cognitive decline in patients. One hypothesis is that vascular impairment in the brain accelerates beta–amyloid deposition, enhancing the pathology and dementia in individuals with AD. In addition, any changes in blood flow can affect brain cells.[7] According to their theory, the origin of dementia lies in the cardiovascular system, where years of constant activity stiffens the walls of the great arteries and there is less elasticity to cushion the impact of the pulse on the brain's smallest blood vessels.

Over the last five to 10 years the vascular view has shifted from being a marginal view to being accepted as one of the major risk factors.[8]

Cardiac enzymes are usually released into the bloodstream when heart muscle is damaged, such as during a myocardial infarction. Measuring blood levels of cardiac enzymes can therefore be indicative of heart muscle damage. CK, LDH and AST are present in sufficiently high content in myocardial tissue so that the death of a relatively small amount of tissue results in a substantial increase in measured enzyme activity.

Creatine Kinase (CK) is an enzyme found in skeletal muscle, cardiac muscle and brain tissue. It helps to regenerate used ATP. Elevated levels are associated with myocardial infarction (MI) and various muscle disorders.[9] Since CK has been shown to play a fundamental role in cellular energetics of the brain, any disturbance of this enzyme may exasperate the AD disease process.[10]

Lactate dehydrogenase (LDH) is an enzyme present in every cell, it is a tetramere molecule which is a combination of two different tissue components, muscle and heart and helps in energy metabolism.

CK levels increase within 3-12 hours of onset of heart cell damage reaching peak values within 24 hours and return to baseline after 48-72 hours. Serum activity of AST is noticeably increased after about12 hours, peaks between 1-2 days and returns to normal by the 3rd to 5th day. LDH activity increases after about 18 hours post MI, peaks between the second and third day and remains elevated for about a week.

Heart-type Fatty Acid-Binding Protein (H-FABP) is involved in active fatty acid metabolism where it transports fatty acids from the cell membrane to mitochondria for oxidation. H-FABP is a sensitive biomarker for myocardial infarction[11] and necrosis. Its levels can distinguish between healthy controls and patients with AD dementia with high sensitivity.[12]

Total antioxidant capacity or status (TAC) is a test to measure the antioxidant capacity of all antioxidants in a biological sample.[13] According to the authors it may be used as a biomarker for disease in medicine including vascular and psychiatric disorders. Total oxidative capacity (TOC) or peroxides in Oxidative stress is caused by an imbalance between the production of reactive oxygen and a biological system's inability to readily detoxify the reactive intermediates or easily repair the resulting damage. Oxidative stress is involved in many diseases myocardial infarction, Alzheimer's disease.[14]

The Na^+, K^+ -ATPase (NKA) is an ubiquitous enzyme responsible for the creation and maintenance of the Na^+ and K^+ gradients across the cell membrane by transporting 3 Na^+ out and 2 K^+ into the cell.[15] The plasma membrane Ca^{2+} ATPase (PMCA) is a transport protein in the plasma membrane of cells that serves to remove calcium (Ca^{2+}) from the cell. It is vital for regulating the amount of Ca^{2+} within cells.[16] An important role in the regulation of these processes, via maintaining intracellular concentrations of Na^+, K^+ and Ca^{2+}, is ascribed to sarcolemma Na-pump and Ca-pump. Both systems use for the active transport of ions energy derived from ATP hydrolysis which, in the case of (Na, K)-ATPase and also (Ca, Mg)-ATPase, is dependent on the presence of Mg^2.[17]

Cardiac profile, TAC, TOC and ATPase profile could be used as a diagnostic tools for the incidence of cardiac disorders accompanying Alzheimer's disease. With newly emerging evidence showing direct links between cardiovascular disease and Alzheimer's, thus necessitates the use of preventive therapies that target both diseases.

One of the major therapeutic pathways is Galantamine, which is an acetylcholinesterase inhibitor commonly used for Ad treatment in particular those of vascular origin. It also acts on prevention of hypertension from precipitating vascular dementia, which is the forerunner of AD. Galantamine works by enhancing cholinergic function by increasing the concentration of acetylcholine in the brain and enhancing cholinergic neuro-transmission in the brain.[18] Nevertheless, side effects from the drug have been reported in different tissues including heart as higher doses and therapeutic time durations are gradually required. According to the above-mentioned recently recorded evidences of the direct link between AD and cardiovascular disorders thus a preventive therapy targeting both diseases might be necessary. In fact up to the present moment there is no approved drug that may accomplish such goal.

In order to attenuate the doses required, frequency of administration, and possible initiated side effects while preserving the drug efficacy, is the introduction of novel drug delivery systems with inflammatory site targeting and long circulating time.[19]

Hydroxyapatite (HAp) (HAp, $Ca_{10}(PO_4)_6(OH_2)$ has been extensively used in medicine and suggested to be one of the most promising drug delivery systems owing to its biocompatibility, bioactivity, elevated hydrophilic character, chemical stability against oxidative conditions and non-toxicity nature.[20] Implementation of new species in the HAp lattice offer fundamentally new possibilities and areas of their practical applications in biology and medicine.[21]

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.[22]

Several drug delivery systems for Galantamine therapy have been examined, yet Galantamine coated with Cerium/ Calcium hydroxyapatite (Ce/Ca-HAp) has been recorded to be the most efficient to incur curative measures in brain of AD.[20,21]

Thus the aim of the presented study was to elucidate whether the curative role of Galantamine coated with (Ce/Ca-HAp) on brain tissue is capable of inducing similar therapeutic capabilities in heart disorders besides its role in AD therapy. Again the presentation of multiple biomarkers for heart disorders may induce early detection thus adding to the efficacy of the curative competence.

MATERIAL AND METHODS

Adult female albino Wistar rats weighing 189- 200 gm (n=70) were obtained from the Medical Research Centre Ain Shams University. Experimental protocols followed the Guidelines for the Care and Use of Laboratory Animals approved by the Institutional Ethics Committee of Ain Shams University. Rats were housed in standard conditions of temperature (22–24°C), humidity (60%) and a 12-h light/dark cycle, with food and water offered *ad lib*. Following a one week adaptation period rats were randomly assigned to their experimental groups. AD was induced to ovariectomized rats by Aluminium chloride (17mg/kg b.wt.)[23] daily for 2 months after one month post-operative procedure. Both Galantamine[24] and Galantamine coated with Cerium/ Calcium hydroxyapatite (Ce/Ca-HAp) were *i.p*. injected at 2.5 mg/kg b.wt. for 2 and 4 weeks. Grouping was done according to:

a) Control: Gonad intact rats (8). b) Galantamine (Gal): Gonad intact rats (8) treated with galantamine. c) Alzheimer (Alz): *Ovariectomized rats (22) treated orally with $AlCl_3$ (17 mg/kg b.wt) daily for 2 months following one month surgery. d) Alz + Gal rats (16) e) Alz + Gal. + Ce/Ca-HAp rats (16).*

Following 4 weeks rats were anaesthetized by ether inhalation, hearts carefully excised, where half was washed in ice cold saline and 10% homogenate of the washed tissue were prepared in 0.1 Tris–Hcl (pH 7.4). After centrifugation at 3000rpm at 4%C the supernatant (10%) was separated for biochemical estimations. These included cardiac profile: CK[25]; LDH)[26]; AST[27] and H-FABP[28]; antioxidant and oxidative status; TAC[29]; TOC[30] and TNO[31] and ATPase profile: Na+/K+-ATPase[32]; Ca2+ATPase[33] and Mg2+ATPase.[34]

The second half was washed in sterile phosphate buffered saline fixed with10% formaldehyde and paraffin embedded for histology analysis by standard methods. Embedded tissues were sliced into 5 µm thin sections stained with hematoxylin and eosin[35] mounted on slides and examined for histological changes. Histochemical studies included total protein content by bromophenol blue technique[36] and Calcium by Von Kosa method.[37]

RESULTS

Biochemical investigations

1-Cardiac Profile

In the present study, marked increases in levels of CK, LDH, AST enzymes were recorded as a result of AD (Table 1). These were more or less ameliorated by Gal therapy and yet more improved as Gal was coated with Ce/Ca-HAp. Similar results were encountered in H-FABP figures following Gal and Gal coated with Ce/Ca-Hap (Table 1).

2-Antioxidant and Oxidative Status

Presently while TAC and TNO levels were significantly decreased in Alz-induced rats as compared to normal ones yet such figures retreated greatly after treatment especially as Gal was coated with Ce/Ca-HAp. On the other hand, marked increases were evidenced in TOC that again were partially improved following Gal treatment especially visualized with Gal coated with Ce/Ca-Hap (Table 2).

3-ATPase Profile

On presenting ATPase profile in the current study, results manifested significant upregulated records of both Ca2+ATPase and Mg2+ ATPase in Alz-induced rats. Contrary to that Na+/ATPase were significantly attenuated in the same group of animals. Marked improvements were more pronounced following GAL therapy coated with Ce/Ca-HAp than GAL alone (Table 3).

Histological investigations

Heart sections manifested normal cardiac architecture in both control and GAL treated rats exhibiting regular cardiac muscle presentation with cardiomyocytes, cardiac muscle fibres and loose endomysial connective tissue containing many capillaries in its middle layer (Fig. 1 a & b). In a section from heart of Alz-induced rats, muscle fibres appeared necrotic staining deeper than normal (Fig.2 a) that were occasionally replaced by fibrous tissue (fig. 2 b) Intramyocardial deposits of beta amyloid plagues were present in the myocardium and

cardiac interstitium (Fig 2 c). Narrowing of cardiac valves was also detected due to deposition of calcified material into the tissue. Also viewed is an increase in inflammatory cells mainly monocytes, leucocytes and lymphocytes fibroblasts and macrophages (Fig. 2 d) accompanied by degenerative alterations. Such alterations persisted in sections obtained from Alz-induced rats treated with GAL (Fig. 3 a) although with lower profiles. Cardiomyocytes replaced by fibrous tissue persisted (Fig. 3 a). Loss of striation with complete necrosis and fragmentation with persistence of beta amyloid plagues was still prominently observed (Fig. 3 b). Cardiac sections of Alz-induced rats treated with GAL coated with Ce/Ca-HAp designated ameliorated figures where beta amyloid aggregates regressed (Fig 3 c). Myocytes appeared more or less near to normal in pattern (Fig. 3 c).

On staining sections for the identification of total protein content normal distribution quality was viewed in sections of control and GAL treated rats (Fig. 4 a). Nevertheless, increase staining profiles were infused in sections from ALz-induced animals in the sites of amyloid plagues (Fig. 4 c) that was more or less attenuated as AD rats were treated with GAL (Fig. 4 b). More prominent normal cardiac proteinic profiles were designated as GAL was coated with Ce/Ca-HAp before injection to rats (Fig. 4 d). High presence of calcified deposits was predominantly initiated in interstitial cells of heart tissue in Alz-induced rat sections as compared to normal controls (Fig.5 a & b) and to a lesser existence after treating AD rats with GAL (Fig. 5 c). These highly subsided in cardiac sections of rats given GAL coated with Ce/Ca-HAp (Fig. 5 d).

Table (1): ***Cardiac enzymes and H-FABP levels in control, and Alz, Gal., Alz +Gal, Alz +Gal coated by Ce/Ca-HAp treated rats.***

		Control	ALZ	GAl	Alz+GAl	Alz+Gal+Ce/Ca-HAP
CK	**Mean± S.E**	154^{A}±2.16	340.66^{B}±1.64	157.66^{C}±2.91	280.33^{D}±2.52	185.10^{E}±1.62
LDH	**Mean±S.E.**	457.33^{A}±2.94	877.66^{B}±2.78	519.33^{C}±1.82	793.33^{D}±1.52	658.66^{E}±2.62
AST	**Mean±S.E**	40.33^{A}±0.24	76.20^{B} ±1.94	54.15^{C}±0.82	69.33^{D}±0.88	63.23^{E}±0.53
H-FABP	**Mean± S.E**	0.337^{A}±0.017	0.83^{B} ±0.014	0.438^{C}±0.010	0.772^{D}±0.09	0.670^{E}±0.015

Values are expressed as mean ± S.E. - n= number of rats.
- $^{A, B, C, D,E}$ Means with a common superscript within a row are significantly different (P<0.05).

Table (2): ***Antioxidant and oxidative status in control and Alz, Gal., Alz +Gal, Alz +Gal coated by Ce/Ca-HAp treated rats.***

		Control	ALZ	GAl	Alz+GAl	Alz+Gal+Ce/Ca-HAP
TAC	**Mean± S.E**	1.155^{A}±0.90	0.771^{B}±0.63	0.933^{C}±0.72	0.794^{D}±0.64	0.864^{E}±0.14
TOC	**Mean±S.E.**	0.430^{A}±0.70	1.080^{B}±0.50	0.612^{C}±0.90	0.930^{D}±0.20	0.870^{E}±0.40
TNO	**Mea.n±S.E**	22.46^{A}±0.40	16.60^{B} ±0.16	21.04^{C}±0.24	18.72^{D}±0.31	**19.38** ^{E}±0.29

Values are expressed as mean ± S.E. - n= number of rats.
-$^{A, B, C, D,E}$ Means with a common superscript within a row are significantly different (P<0.05).

Table (3): ***Na+/K+, Ca2+, Mg2+ATPase levels in control and Alz, Gal., Alz +Gal, Alz +Gal coated by Ce/Ca-HAp treated rats.***

		Control	ALZ	GAl	Alz+GAl	Alz+Gal+Ce/Ca-HAP
Na^{+}/K^{+} ATPase	**Mean± S.E**	0.634^{A}±1.30	0.355^{B}±0.57	0.595^{C}±0.53	0.46^{D}±0.70	0.521^{E}±1.38
Ca^{2+} ATPase	**Mean±S.E.**	1.58^{A}±0.61	2.77^{B}±1.46	1.80^{C}±1.50	2.51^{D}±2.01	2.20^{E}±1.53
Mg^{2+} ATPase	**Mea.n±S.E**	5.83^{A}±0.47	8.53^{B} ±1.94	6.39^{C}±1.62	7.68^{D}±1.21	7.05^{E}±1.64

Values are expressed as mean ± S.E. - n= number of rats.
- A, B, C, D,E Means with a common superscript within a row are significantly different (P<0.05).

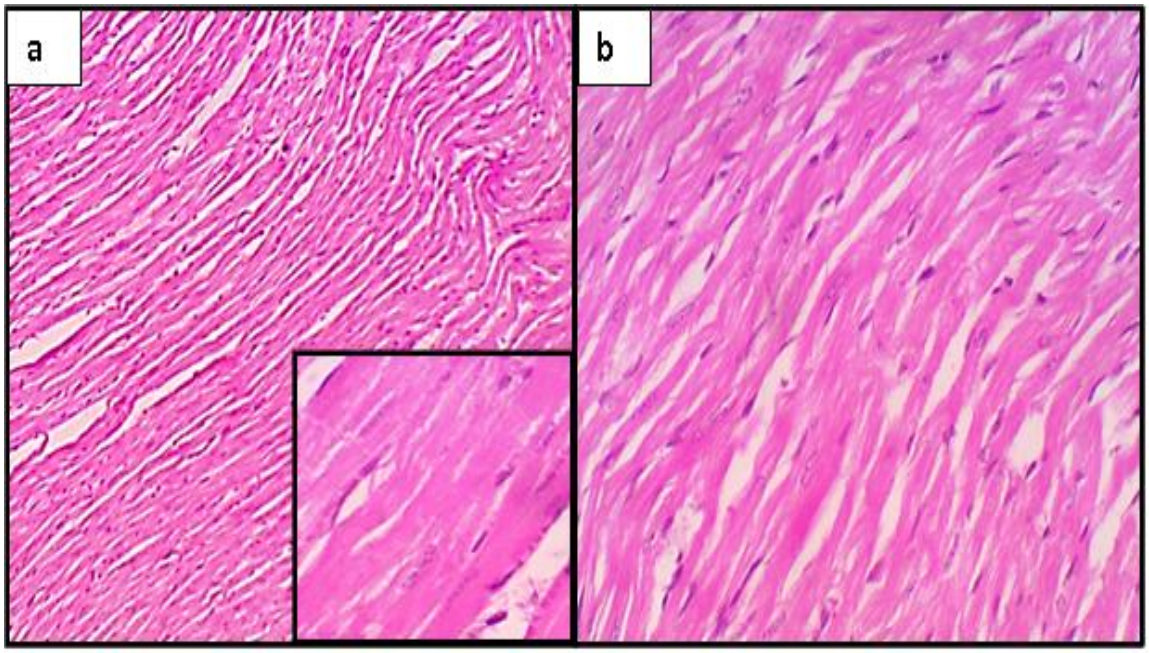

Figure 1: Photomicrographs of sections from control and treated rats (Hx & E.)

a – Heart section from control rat showing normal myocardial architecture (X 100)
bottom left: enlarged area of heart from control rat showing cardiomyocytes with dark and light bands (X 400)
b- Heart section from rat treated with Galantamine showing no alterations from normal myocardial sections (X 400)

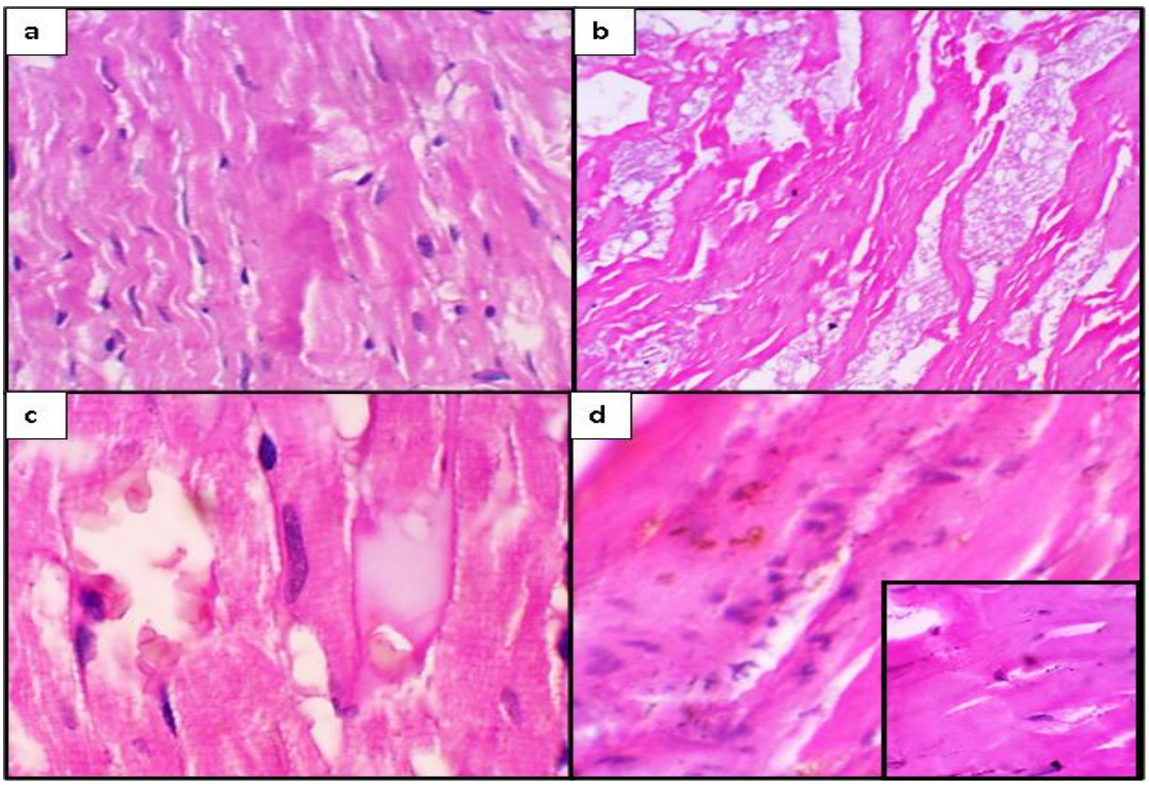

Figure 2: Photomicrographs of sections from Alz-induced rats (Hx & E.)

a- Showing deeper stained necrotic muscle fibres (X400)
b- Showing necrotic cardiomyocytes replaced by fibrous tissue(X100)
c- Showing beta amyloid plague deposits in cardiac interstitium (X 1000)
d- Showing inflammatory cell infiltration in interstitial tissue (X400) enlarged area showing degenerative alterations (X1000)

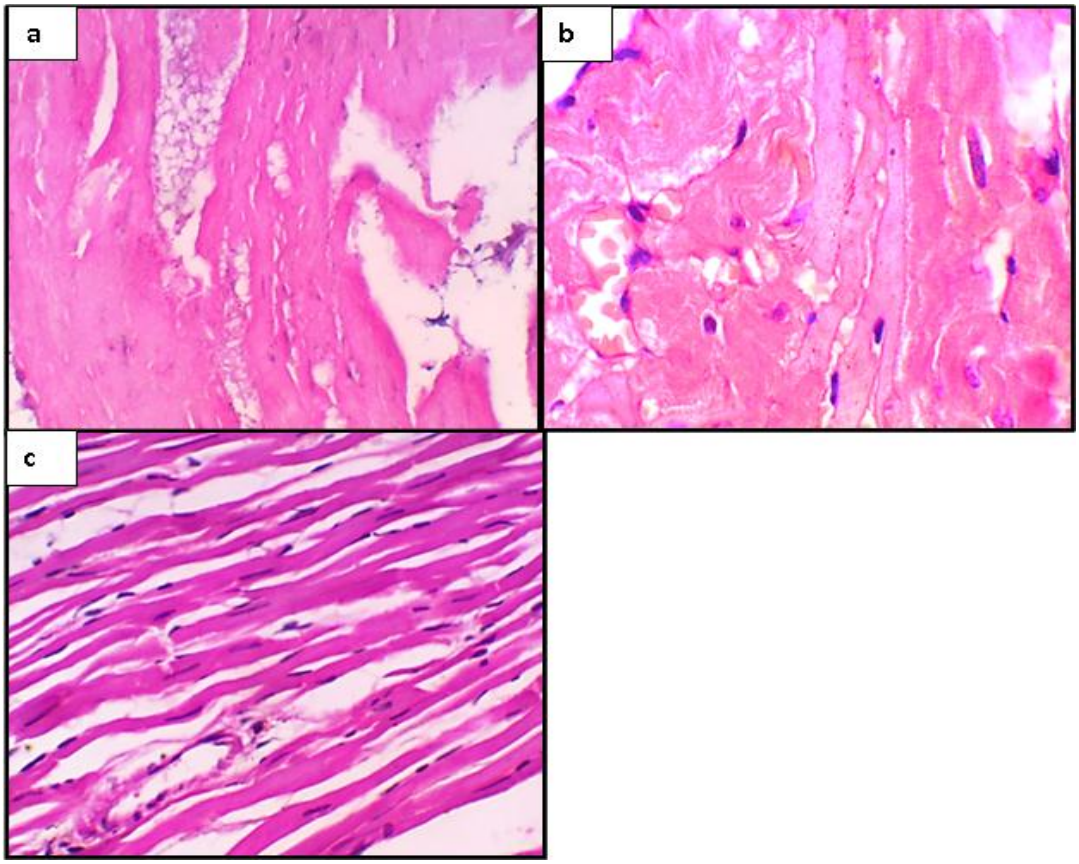

Figure 3: Photomicrographs of sections from Alz-induced rats treated with Gal and GAL coated with Ce/Ca-HAp (Hx & E.)

a- Alz +Gal showing persistence of cardiomyocytes replaced by fibrous tissue (X400)
b- Alz +Gal showing necrotic, fragmented cardiomyocytes with persistence of β amyloid plagues (X1000)
c-Alz +GAL coated with Ce/Ca-HAp showing near to normal cardiac pattern (X400)

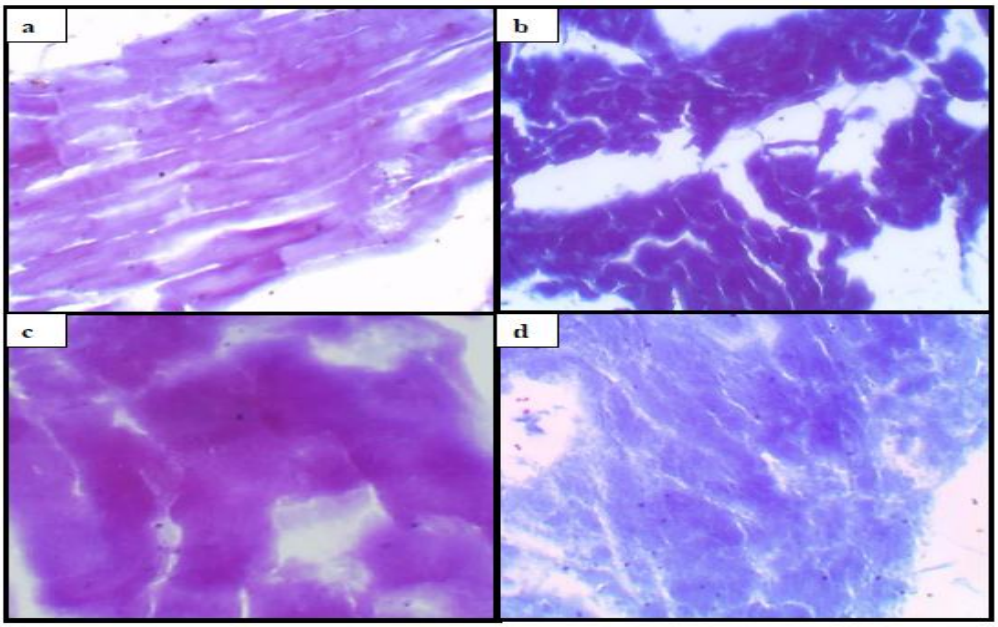

Figure 4: Sections from control and experimental rats showing total protein content in cardiac tissue (Bromophenol blue; X400)

a- Control rat
b- Alzheimer induced rat treated with Gal showing more or less attenuated staining quality
c- Alzheimer induced rat showing increase in staining profile in sites of amyloid plaques
d- Alzheimer induced rat treated with GAL coated with Ce/Ca-HAp showing near to normal proteinic profiles.

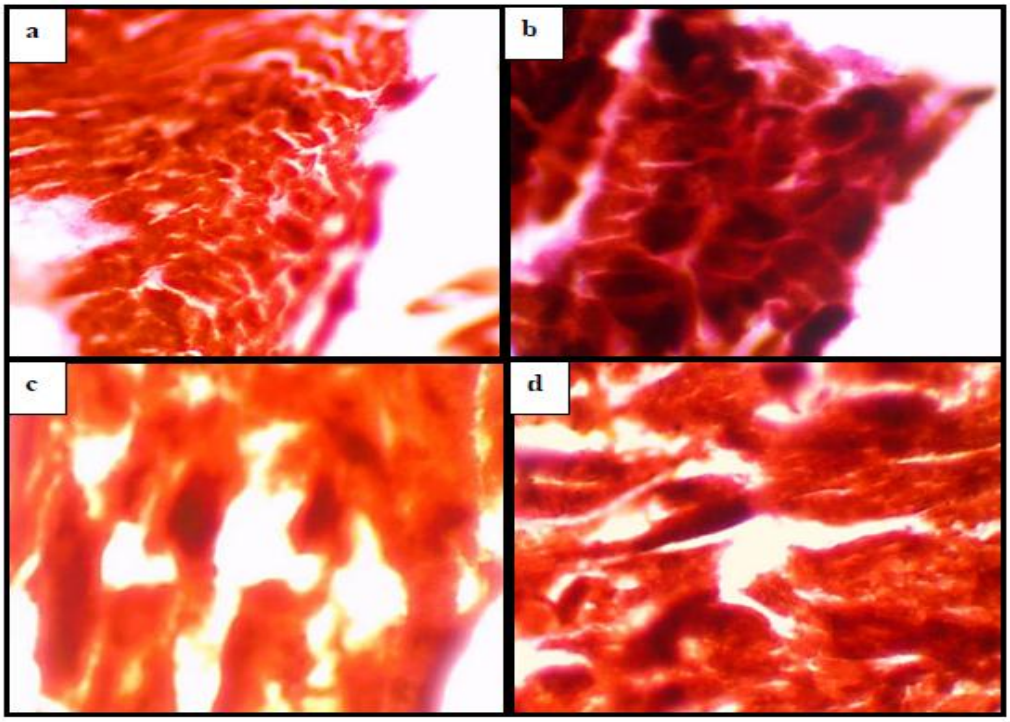

Figure 5: Sections from control and experimental rats showing calcium deposits in cardiac tissue (Von Kossa; X400)

a- Control rat
b- Alzheimer induced rat showing high presence of calcified deposits
c- Alzheimer induced rat treated with Gal
d- Alzheimer induced rat treated with GAL coated with Ce/Ca-HAp showing decrease in calcium deposits

DISCUSSION

In a recent study ***Mostapha and El-Bakry***[21] emphasized on the efficacy of coating GAL (effective remedy for AD) with drug delivery nano-particles (Ce/Ca-HAp) in incurring therapeutic measures for AD. Presently the efficacy of such regimens in inducing similar remedies for cardiac disorders that usually accompany AD is debated. Again on using synergic regimens for early diagnosis of both cardiac and AD might even present additional preventive roles to the progression of either diseases.

Accordingly, in the present study different biochemical tests were utilized to enhance early diagnostic measures for cardiac disorders namely cardiac profile, antioxidant and oxidative status and ATPase profile. Currently, marked increases in levels of CK, LDH, AST enzymes and H-FABP were recorded as a result of AD. As limited therapeutic levels were encountered with GAL therapy yet it was more improved as Gal was coated with Ce/Ca-HAp. Similar records were reported by ***Gallaraga et al and Teixeira and Borges.***[9,38] CK is responsible for the regeneration of ATP and its transfer from mitochondria to the cytosolic carrier. Creatine sarcomere-specific mitochondrial CK is only expressed in skeletal and heart muscle. As cardiac muscle is injured in cardiac disorders the enzymes and proteins leak out of damaged heart muscle cells and their levels rise in tissue and in the bloodstream. A high LDH and AST level often indicates high amounts of tissue damage and lysed cells and thus is a general indicator of both cellular and tissue disease.

Increase in H-FABP levels could be used as a reliable biomarker for cardiac disorders.[11,12,39] High H-FABP concentration worsen ventricular contractility, by disordering the splitting of mitochondrial proteins that impairs the production of energy by the mitochondria.[40]

Additional biochemical markers for heart disorders and AD was TAC, TOC and TNO. Presently TAC and TNO levels were significantly decreased in Alz-induced rats while TOC levels markedly increased as compared to normal ones. Similar records were recorded by several workers.[13,14,41,42,43] ***Nojiri et al.***[44] suggested an inverse association between TAC and the number of damaged vessels in brain or other tissues. Also, reports by ***Demirbag et al***[45] described increased DNA damage in the nucleus of coronary cells and decreased plasma TAC in coronary artery disease patients thus confirming present histological records. A well-established finding is the clear relationship between amyloid- β particles (Aβ) and oxidative stress where Aβ causes higher production of reactive oxygen species (ROS) which damages mitochondria thus leading to further enhanced production of ROS. In AD patients Aβ interacts with Aβ-binding alcohol dehydrogenase in the mitochondria.[46] Such interactive acts may cause still higher increase in ROS formation, mitochondrial

dysfunction and eventually apoptosis.[47] As oxidative stress is a significant element in AD pathogenesis thus attenuated protection against ROS production in elder patients might trigger and add to the onset of AD and its progression. Several studies have shown that oxidative stress can cause both increased production and accumulation of Aβ.[48] The pressure from oxidative stress in AD together with lowered antioxidant defense creates a harmful combination that could disturb functions and damage organelles specially mitochondria.

ATPase profile in the present study manifested significant upregulated records of both Ca^{2+}ATPase and Mg^{2+} ATPase in Alz-induced rats while levels of Na^{+}/ATPase were significantly attenuated. Na^{+}, K^{+}-ATPase (NKA) is responsible for the maintenance of Na^{+} and K^{+} gradients across the cell membrane by transporting 3 Na^{+} out and 2 K^{+} into the cell. NKA is also involved in neurological and cardiovascular disorders.[15] The cardiac myocyte glycoside ouabain acts by inhibiting the NKA pump thus increasing intracellular sodium that reduces activity of the sodium calcium exchanger that pumps one calcium out of the cell and three sodium ion into the cell. Thus inhibition in cardiac myocyte presently recorded NKA reduces the sodium calcium exchanger pump that in turn elevates intracellular calcium ions. This again is a normal sequel to the induced elevated ROS from generated Aβ in AD. Confirmatory data were also reported by ***Lui et al.***[49]

Plasma membrane Ca^{2+} ATPase (PMCA) serves to remove calcium (Ca^{2+}) from the cell. Calcium needed for cardiac muscle contraction entering via Ca^{2+} channels also triggers further release of Ca^{2+} from the major intracellular store, the sarcoplasmic reticulum and thus determining the extent of activation of the contractile proteins. For subsequent relaxation Ca^{2+} are lowered by their reuptake into the sarcoplasmic reticulum. Ca^{2+}-ATPase is responsible for pumping Ca^{2+} thus for the maintenance of cellular Ca^{2+} homeostasis Ca^{2+} influx must be compensated by Ca^{2+} removal from the cell via the Na^{+}–Ca^{2+} exchanger.[50] Accordingly any disturbances in the Ca2+-ATPase due to increased ROS or histological alterations may lead to unbalanced Ca^{2+} homeostasis and thus unstable cardiac functions.

Mg^{2+}-ATPase on the other hand, is an ATPase that pumps magnesium. Increasing concentrations of Mg activates the Mg-dependent ATPase in rat heart sarcolemma which is essential for normal cell membrane function and is the energy source for Na-K pump. Thus disruptions in the level of intracellular Mg^{+} may also cause an increase in intracellular Na and Ca and a loss of K. It was reported that Mg^{2+}-ATPase increases significantly in older rats.[51] One of the pathways for such increases might be the generation of free radicals as defined by the previous authors.

In the current study all the above mentioned biochemical biomarkers of cardiac profile (CK, LDH, AST and H-FABP) antioxidant and oxidative status (TAC, TOC and TNO) and ATPase profile (NKA, Ca^{2+}-ATPase and Mg^{2+}-ATPase) were partially improved following Gal treatment where near to normal levels were specially visualized with Gal coated with Ce/Ca-HAp. Among different therapeutic trials, cholinesterase inhibitors (ChEIs) as Galantamine are the first group of compounds that have produced considerable improvements in AD patients.[52,53] Thus it remains to be the major therapeutic drug of choice. Yet the ultimate benefits from of ChEIs remains an issue of debate.[52]

Nanotreatment methods for AD are categorized as neuroprotective from Aβ, oxidative stress of free radicles and nanocarriers for targeted drug delivery**. *Hosseini et al***[54] demonstrated that Cerium oxide nanoparticles have neuroprotective effects. CeO_2 doped calcium hydroxyapatite powder has been previously synthesized and used for its bioactivity towards AD by ***Wahba et al***[20] and has been proved to induce ameliorated measures in AD therapy on brain[20] and lung tissue.[21] It gains its bioactivity and effectiveness due its uniform rod-like shaped particles that enhance Ceria and drug-release towards infected zones. Such results were evidenced by the lower values of surface area, average pore radius and BET (Brunauer–Emmett–Teller theory) C-energetic constant.[20]

Histological assessment was followed to validate prementioned biochemical results. Heart sections from AD rats manifested intramyocardial deposits of beta amyloid plagues; narrowing of cardiac valves; increase in inflammatory cells mainly monocytes, leucocytes and lymphocytes and degenerative alterations. Although limited studies have discussed histological features in cardiac tissue of AD patients yet we have to suffice with that of ***Troncone et al***[55] who reported the presence of both Aβ_{40} and Aβ_{42} in the myocardium of AD patients associated with increased left ventricular thickness especially evidenced in elderly subjects which they attributed to increased aortic velocity and reduced diastolic function. In addition to the profound Aβ particles, deposition of amyloid fibres from circulating Aβ peptides may be also present that might eventually lead to cardiac dysfunction in AD. Necrotic alterations and inflammatory circulating cells may also affect myocardial function. In AD increase in free radical occurs circulating with blood flow and thus attacking several organs, in this case cardiac tissue causing disturbances in cardiac architecture. Additional risk factors would be the inadequate blood flow from AD that can damage and eventually kill different cell lines. As AD rats were administered GAL therapy alterations persisted although with lower profiles, whereas therapy with GAL coated with Ce/Ca-HAp evidenced regression in Aβ aggregates and near to normal patterns for cardiac tissue.

Among most of the metallic species, ceria (CeO_2) nanoparticles exhibit high catalytic activity and a

regenerative capacity to neutralize ROS. Ceria was found to protect cells against oxidative stress, inflammation and other cellular damage.[22] The particles are small and can cross the blood brain barrier enhancing the drug targeted release in infected areas

As Aβ are mainly proteins it was necessary to apply the Bromophenol blue technique for total proteinic distribution. Sections from AD rats designated deep staining quality at sites of amyloid plagues confirming their presence in cardiac tissue. Again, stains for Ca deposits validated the increased presence of Ca ions in intramyocardial sites. Similar records were presented by ***Turdi et al.***[56] Limited amelioration quality was perceived in AD rats treated with GAL while they highly subsided in cardiac sections of rats given GAL coated with Ce/Ca-HAp.

As far as the present authors are concerned this study is a new approach for histological coupled by histochemical investigation for AD in cardiac tissue authenticating biochemical measures. Thus further investigations need to be undertaken to authenticate present established findings.

CONCLUSION

Accordingly, evidences may be drawn that cardiovascular disease may greatly increase one's risk of developing AD and vice-versa. GAL therapy or other line of treatments cannot act alone to induce obvious remedies. On the other hand, a more promising method is the initiation of drug delivery systems as Ce/Ca-HAp that may increase the efficacy of the drug and induce a curative role in heart tissue also. As cardiac damage highly presents with AD thus early detection using different biomarkers as cardiac profile (CK, LDH, AST and H-FABP) antioxidant and oxidative status (TAC, TOC and TNO) and ATPase profile (NKA, Ca^{2+}-ATPase and Mg^{2+}-ATPase) is vital for curative competence.

CONFLICTS OF INTEREST

No conflict of interest.

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