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Neuroprotective Effect of Cardamom Essential Oil Encapsulated Nanoparticles and Swimming in Aluminum Induced Alzheimer's disease in Rats

Magdi N. Ashour [1], Tahany R. Elias [1], Wafaa I. Rasheed [1], Safinaz E. El-Toukhy [1], Eman R Youness [1], Amany A. Sleem [2], Manal M. Ramadan [3], Khaled F. Mahmoud [4], Noha N. Yassen [5], Mahmoud A. Abdel-Monem[1], Omnia Aly [1*]

[1] Department of Medical Biochemistry, National Research Centre, Cairo, Egypt
[2] Department of pharmacology, National Research Centre, Cairo, Egypt
[3] Department of Chemistry of Flavour and Aroma, National Research Centre, Cairo, Egypt
[4] Department of Food Science and Technology, National Research Centre, Cairo, Egypt
[5] Department of Pathology, National Research Centre, Cairo, Egypt

In Loving Memory of Late Professor Doctor ""Mohamed Refaat Hussein Mahran""

Abstract
As there is currently no cure for Alzheimer's disease (AD), considerable research is required to create plans to block the processes that cause memory loss. Functionalized nanomedicines that are nanoparticles contain central core structures that guarantee the encapsulation to offer protection and longer blood circulation. The purpose of this study was to investigate the neuroprotective effects of swimming exercise and nanoparticle-encapsulated cardamom oil (NCEO) on aluminum chloride-induced oxidative damage in experimental rats used as models for Alzheimer's disease. Experimental rats were induced for AD then groups were divided into different treatment groups; dopenzil, NCEO and swimming training. Different biochemical and pathological parameters were evaluated in both serum and muscles. Results showed and improvement in the concerned parameters with different treatment strategies. It can be concluded that both swimming and NCEO can be advised for AD patients.
Keywords: Alzheimer's disease- swimming- nanoparticles encapsulated cardamom oil- Irisin- Aβ- PGC-1α- FNDC5.

1. Introduction

As the global population ages, so does the incidence of Alzheimer's disease (AD), the most prevalent type of dementia among the elderly, which currently affects over 35 million people globally [1]. Today, an estimated 6.2 million Americans 65 years of age and older suffer with Alzheimer's disease. If medical advancements are not made to prevent, halt, or cure AD, this figure may rise to 13.8 million by 2060. In 2019, the most recent year for which data are available, official death certificates reported 121,499 deaths from AD, making the disease the sixth most common cause of death in the US and the fifth most common among Americans 65 and older [2].

Since there is currently no proven cure for AD, major efforts are being made to find ways to block the processes that cause memory impairment, synapse failure, and neuronal damage [3]. A substantial body of research suggests that synapse loss and failure are the root causes of memory impairment in AD. Treatments targeted at maintaining or regaining synaptic function and cognitive abilities are therefore strongly recommended [4].

Improved motor function, including increased walking speed, balance, and coordination, as well as neurodegenerative disorders like Alzheimer's, have been related to physical activity and exercise therapies [5;6].

The central nervous system (CNS) may be significantly impacted by the effects of peripheral hormones, including insulin, leptin, glucagon-like peptide 1 (GLP-1), glucocorticoids, and others, according to a body of research [7]. Hormone-induced disruption of the signaling pathway has been connected to brain illnesses like AD [8].

It was discovered that irisin, a myokine released into the bloodstream during physical activity, can induce thermogenesis and adipocyte browning in both humans and mice [9]. The transmembrane precursor protein fibronectin type III domain

* Corresponding author: onnyashraf@live.com
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containing 5 (FNDC5), which is expressed in muscle and regulated by peroxisome proliferator-activated receptor-γ coactivator 1α (PGC-1α), is cleaved from irisin. In the hippocampus, FNDC5/irisin increases the expression of brain-derived neurotrophic factor (BDNF) [10]. a part of the brain that is crucial to memory and learning. This suggests that in brain illnesses like AD, FNDC5/irisin may have a neuroprotective function.

The current knowledge in the field of AD strongly indicates that Irisin could be considered and explored as a biomarker of unhealthy aging and neurodegeneration [11].

Amyloid beta (Aβ) is a glycoprotein that is naturally present in the brain as a byproduct of signal tr222ransduction and is produced by the breakdown of amyloid precursor protein (APP) [12]. Alzheimer's disease patients experience cognitive impairments as a result of the brain's dysregulation of Aβ levels, which results in the deposition of Aβ and the development of senile plaque [13].

An imbalance occurs between the clearance and creation of these peptides from the different areas of the brain, which leads to the accumulation of insoluble Aβ in AD. The bulk of AD is occasionally brought on by ineffective Aβ peptide elimination, while familial AD, which is less prevalent, is brought on by changes in genes related to Aβ metabolism [14].

Reducing the amount of Aβ in the brain is one potential therapeutic approach for treating AD. This can be done in a few different ways, such as by developing mechanisms that balance the production and clearance of Aβ peptide in the brain, triggering mechanisms that clear Aβ species, or inhibiting the generation of Aβ by targeting APP or secretases [15].

The development of neurofibrillary tangles (NFTs), which are aberrant accumulations of phosphorylated tau protein, and amyloid beta-induced senile plaque development in the hippocampus are two of the disease's pathogenic causes [16]. By means of proteolytic cleavage, an amyloid precursor protein is converted into the peptide Aβ. Glycoprotein APP is important for processes like signaling, neuronal growth, and intracellular transport that are involved in preserving neuronal homeostasis [17]. In contrast, tau protein is a microtubule-associated protein that is widely distributed in central nervous system (CNS) neurons. It is primarily involved in maintaining the stability of microtubules in axons, but it is also crucial for cell signaling, synaptic plasticity, and the regulation of genomic stability [18].

Because of their favorability to the brain, increased stability, biocompatibility, and biodegradability, protection from enzymatic degradation, increased half-life, improved bioavailability, and controlled release, nanomedicines may be more advantageous than other conventional methods of drug delivery to the brain for the treatment of AD [19].

The conjugation or encapsulation of drugs, together with their protection and prolonged blood circulation, are ensured by the fundamental core structures of NP-functionalized nanomedicines [20; 21]. Because it is tailored to target specific cells or even an intracellular compartment such as Aβ in cells, it can also transport the medication directly to the affected area at a predetermined dosage [22].

FNDC5/irisin levels in AD-affected rats will be investigated in this study in order to test the theory that FNDC5/irisin may be a crucial mediator of the beneficial effects of exercise on synaptic plasticity and memory in AD models. This implies that irisin/FNDC5 might be a good target for medical intervention. Investigating the neuroprotective benefits of swimming exercise and cardamom oil-encapsulated nanoparticles in aluminum chloride-induced oxidative damage in experimental rats utilized as Alzheimer's disease models was the aim of this work.

2. Materials and methods

2.1. Experimental design

Animals: Three to five-week-old male albino rats weighing 90 to 100 grams each were utilized from the National Research Center's animal house in Cairo, Egypt. The National Research Centre in Cairo, Egypt's Ethical Committee and the National Institutes of Health in the United States' Guide for Care and Use of Laboratory Animals were consulted before making decisions on animal procedures.

Fifty male albino rats weighing between 110 and 170 grams were randomly assigned to five groups, each with ten animals. Rats in **Group I (Swimming Control)** were given oral 5% Tween 80 in addition to swimming instruction. **Group II (AD):** rats were intraperitoneally injected with 100 mg/kg of aluminum chloride solution to induce AD, which acted as a disease control. **Group III (AD+DP):** After receiving an intraperitoneal injection of an aluminum chloride solution, rats were given an oral dose of donepezil hydrochloride (marketed as Arecipt) at a dose of 1 mg/kg one hour later. **Group IV (AD + NCEO):** After intraperitoneal injection of an aluminum chloride solution, rats were given oral doses of 1/20 of encapsulated cardamom (EC) oil nanoparticles one hour later. **Group V (AD+Swimming):** the rats underwent intraperitoneal injection of a 100 mg/kg aluminum chloride solution to induce AD, and later that day, they engaged in swimming exercise.

After the end of the study duration **(42 days**), animals were fasted overnight, three ml of blood was aspirated under formalin anesthetized from the

peripheral orbital sinus, divided into two parts; one was added to an anticoagulant vacutainer for whole blood collection and the another one on a plain tube then centrifuged for 15 min at 3000 rpm to get the clear serum that was stored at -80°C till the evaluation day.

Serum was assayed for reduced glutathione, Amyloid beta 1-40 (Aβ 1-40), Irisin and interleukin -10 (IL-10). Peroxisome proliferator-activated receptor-gamma coactivator (PGC1-α) and Fibronectin type III domain-containing protein 5 (FNDC5) expression in muscle tissues were reverse transcribed quantitatively using real time polymerase chain reaction (qRT-PCR),

Following death, muscle samples from every animal were dissected and preserved for at least 12 hours in a 10% neutral buffered formalin solution. After a half-hour soak in tap water, each specimen was cleaned in xylene, dehydrated using increasing alcohol grades, and then embedded in paraffin. For histological analysis, serial slices 3 µm thick were cut and stained with hematoxylin and eosin [23].

Images were captured at the pathology lab, National Research Centre – Egypt using image analysis system with microscope Olympus CX41and SC100 video camera attached to computer system. Photomicrographs were taken at different magnifications and processed using Adobe Photoshop version 8.0

2.2. Induction of Alzheimer's disease

According to **Kandimalla et al. [24]** in distilled water, an aluminum chloride solution was prepared and injected intraperitoneally into rats with aluminum chloride solution (100 mg/kg) for 42 days for AD induction.

2.3. Routine Swimming Exercise

The animals were kept in a plastic tank with water kept at 35 ± 1 °C, measuring 80 cm in circumference, 100 cm in height, and 40 cm in water depth. Over the first week, the rats acclimated to the swimming practice. Rats were first trained to swim for 15 minutes, and their swimming time was increased by 15 minutes every day until they could swim for an hour. After that, for six weeks, a daily swimming session lasting one hour was maintained five times a week. After every workout, the animals were dried and housed in a warm atmosphere. To reduce the immediate effects of exercise, rats were euthanized 48 hours after their last workout [25].

2.4. Preparation of nano-encapsulated cardamom essential oil (NCEO)

The stabilized nano-capsule was prepared by chloride octahydrate -Gum Arabic according to the methodology of Klinkesorn et al. **[26]** with some modifications as follows:

Gum Arabic 35 g was gradually dissolved in 1000 ml of double distilled water in various concentrations (3.5 %), under magnetic stirring for 24 h in order to completely hydration, the solution is kept in the refrigerator at 4°C for 24 hours until the gelatinous state occurs.

Then followed by chloride octahydrate layer as follows:

3.116 g of ZrOCl2·8H2O (Aldrich) was dissolved with 200 ml of nanopure water in a beaker. In a round bottom flask, we mixed 82.19 ml of H3PO4 (85%, Fisher) with 117.8 ml of nanopure water. The round bottom flask containing the H3PO4 solution was placed into an oil bath at a constant temperature of 94ºC with constant stirring. When the temperature of the solution equilibrated to 94˚C we added dropwise the ZrOCl2·8H2O solution into the round bottom flask. The resulting solution was maintained at a constant temperature of 94ºC and constant stirring for 5 days. Then the solution was filtered and the solid washed with abundant nanopure water to remove salts an artificial membrane (dialysis bag) is used **[27].**

After preparing the coating solutions, cardamom seed oil encapsulation was prepared as follows:

First, 510 ml of cardamom seed oil extracted was added to the previously prepared buffered zirconium chloride wall material solution gradually and homogenized in the homogenizing rotor for approximately 15 minutes at 18,000 rpm. The solution was dispersed under magnetic stirring and stirred overnight to ensure complete dispersion.

Second, after adding the first layer, the system has become stable and therefore only needs to add the second layer of the previously prepared Gum Arabic solution to the resulting emulsion after 24 hours to ensure its complete stability and almost oil droplets merge after the homogenization process in the first path inside the first capsule, then add Tween 20 to the resulting emulsion with stirring for 20 minutes, followed by the addition of the second layer of Gum Arabic gradually with continuous stirring and passing the mixture through a high pressure valve homogenizer at 280 bar with some modifications. Finally, the size of the nano-capsules reached 1275 ml containing 510 ml of oil at a ratio of 0.4 / ml, and the nano-capsules were stored in dark containers to protect from light and at a temperature of 4 ° C until use.

2.5. Preparation of nano-capsulate of cardamom seed oil

2.5.1. Preparation of polymers used in packaging

2.5.1.1. First layer (Gum Arabic):

Gum Arabic 35 g was gradually dissolved in 1000 ml of double distilled water in various concentrations (3.5 %), under magnetic stirring for 24 h in order to completely hydration, the solution is kept in the refrigerator at 4°C for 24 hours until the gelatinous state occurs.

2.5.1.2. Second layer (chloride octahydrate)

3.116 g of $ZrOCl_2 \cdot 8H_2O$ (Aldrich) was dissolved with 200 mL of nanopure water in a beaker. In a round bottom flask, we mixed 82.19 mL of H_3PO_4 (85%, Fisher) with 117.8 mL of nanopure water. The round bottom flask containing the H_3PO_4 solution was placed into an oil bath at a constant temperature of 94ºC with constant stirring. When the temperature of the solution equilibrated to 94˚C we added dropwise the $ZrOCl_2 \cdot 8H_2O$ solution into the round bottom flask. The resulting solution was maintained at a constant temperature of 94ºC and constant stirring for 5 days. Then the solution was filtered and the solid washed with abundant nanopure water to remove salts an artificial membrane (dialysis bag) is used **[28].**

2.6. Preparation of nano-emulsion cardamom seed oil

Tween 20 was dissolved in 315.0 ml double-distillated water 2% (v/v) at room temperature. The mixture was shaken with a magnetic type stirrer for 10 minutes to get a homogeneous solution. The cardamom seed oil extract (126.0 ml oil) was added slowly and mixed with a direct driven homogenization by high speed homogenizer at 18000 rpm for 15 minutes. The resulting emulsion was sonicated using a 25 kHz ultrasonic. The sonication time was fixed for 20 minutes. Ultrasonification generates intensive and disruptive forces in order to minimize nano-emulsion droplets **[29].** The resultant nano-emulsions were stored in the laboratory condition (4°C).

2.7. Preparation of nano-capsulate cardamom seed oil

The stabilized nano-capsule was prepared by chloride octahydrate -Gum Arabic according to the methodology of Klinkesorn et al. **[26]** with some modifications. The coating solutions were prepared as previously and used in cardamom seed oil encapsulation by two layers.

First, the extracted 510 mL cardamom seed oil was added to the previously prepared buffered zirconium chloride wall material solution gradually and homogenized in the homogenizing rotor for approximately 15 minutes at 18,000 rpm. The solution was dispersed under magnetic stirring and stirred overnight to ensure complete dispersion.

Second, after adding the first layer, the system has become stable and therefore only needs to add the second layer of the previously prepared Gum Arabic solution to the resulting emulsion after 24 hours to ensure its complete stability and almost oil droplets merge after the homogenization process in the first path inside the first capsule, then add Tween 20 to the resulting emulsion with stirring for 20 minutes, followed by the addition of the second layer of Gum Arabic gradually with continuous stirring and passing the mixture through a high pressure valve homogenizer at 280 bar with some modifications.

Finally, the size of the nano-capsules reached 1275 ml containing 510 ml of oil at a ratio of 0.4 / ml, and the nano-capsules were stored in brown containers away from light and at a temperature of 4 ° C until use.

2.8. Chemical properties

2.8.1. Determination of total phenolic content (TPC)

The total phenolic content in cardamom oil extract, and micro-capsule samples was quantified using the Folin-Ciocalteu reagent **[30].** Briefly, 50 mg of crude extract was mixed with 0.5 mL of Folin-Ciocalteu reagent and 7.5 mL of deionized water. The mixture was kept at room temperature for 10 min and then 1.5 mL of sodium carbonate (2 %) solution was added. The mixture was heated in a water bath at 40 ºC for 20 min and then cooled in an ice bath; absorbance was measured at 755 nm using a spectrophotometer. Amounts of total phenolic contents were calculated using Gallic acid calibration curve within range of 10-100 ppm ($R^2 = 0.9952$). The results were expressed as Gallic acid equivalents (GAE) g/100 g of dry plant matter.

2.8.2. Determination of total flavonoid content (TFC)

The total flavonoids in cardamom oil extract, and micro-capsule were measured by a spectrophotometric method following a previously reported method **[31].** Briefly, plant extract of each material (1 mL containing 0.1 mg/mL) was diluted with 4 mL water in a 10 mL volumetric flask. Initially, 0.3 mL of 5 % $NaNO_2$ was added to each volumetric flask, at 5 min, 0.3 mL of 10 % $AlCl_3$ was added and at 6 min, 2 mL of 1 M NaOH was added. Water (2.4 mL) was then added to the reaction flask and mixed well. Absorbance of the reaction mixture was measured at 510 nm. Total flavonoids were determined as catechin equivalents (g/100 g of dry plant matter).

2.8.3. DPPH free radical scavenging assay

The DPPH assay in cardamom oil extract, and micro-capsule were performed as described by Bozin *et al.* **[32].** The samples (from 0.2-500 µg/mL) were mixed with 1 mL of 90 µM DPPH solution and filled up with 95 % methanol, to a final volume of 4 mL. The absorbance of the resulting solutions and the blank were recorded after 1 h at room temperature. For each sample, three replicates were recorded. The disappearance of DPPH was read spectrophotometrically at 515 nm using a spectrophotometer. Inhibition of free radical by DPPH in percent (%) was calculated in the following way:

Antiradical activity (%) =

$$100 - (A_{blank} - A_{sample})/A_{blank}$$

Where, A_{blank} is the absorbance of the control reaction mixture excluding the test compounds and A_{sample} is the absorbance of the test compounds. Extract concentration providing 50 % inhibition (IC_{50}) was calculated from the plot of inhibition percentage against extract concentration.

2.9. Physical properties

2.9.1. Encapsulation efficiency (EE %)

Encapsulation efficiency (EE) was estimated spectrophotometrically following the procedure of Benzie and Strain, **[33].** by FRAP assay. Fifteen milliliters of hexane were added to 1.0 g of cardamom oil extract, and micro-capsule in a glass jar with a lid, and was shaken by hand for the oil forms, during 2 min, at room temperature. The solvent mixture was filtered through a Whatman filter paper no.1 and the solvent was left to evaporate at room temperature, until constant weight. Encapsulation efficiency (EE) was calculated as follows:

$$EE\ \% = \frac{W_T}{Wi} \times 100$$

Where W_T is the total amount oil the extract in the capsule and Wi is the total quantity of the extract added initially during preparation.

2.9.2. Thermal stability (DSC)

Thermal stability of cardamom oil and micro-capsule was measured using a differential scanning calorimeter (DSC) (Mettler Toledo, SWITZERLAND) according to Hazra *et al.* **[34]** as follows. Ten milligrams samples were placed in aluminium crucibles under a flow of nitrogen gas (40 ml/ min). A dynamic scan was performed at a heating rate of 10 °C/min over a temperature range of -150 to 300°C. Evaporation enthalpies were calculated by peak area integration of DSC profiles, and the results were compared with the estimated vaporization enthalpy of major components.

2.9.3. Transmission Electron Microscopy (TEM)

Cardamom oil extract, and micro-capsule morphological characteristics were tested by TEM (1400, JEOL, Japan) using Saloko *et al.,* **[35]** method. The nano-particles suspension was dripped onto a 400-mesh copper grid coated with Forvar and stained with 2% phosphotungstic acid **[36].** The samples were air-dried at room temperature for more than 2 hrs before analyzing on the TEM.

2.10. Biochemical parameters

The levels of amyloid beta 1-40 (Aβ 1-40), irisin, and interleukin -10 (IL-10) were measured using an ELISA kit that was acquired from Sunlong Biotech Co. (Zhejiang, China) and is sandwich-type. Using Ellman's protocol, reduced glutathione in muscle tissue homogenates was identified [37]. The Ellman's reagent (DTNB; 5, 5'-dithiobis (2-nitrobenzoic acid) is reduced by a free sulfhydryl group on GSH to produce 5-thio-2-nitrobenzoic acid, which has a yellow hue and can be measured with a spectrophotometer at 412 nm. This is the basis of the assay.

2.10.1. Reverse transcription-quantitative polymerase chain reaction (RT-qPCR) analysis of muscle PGC-1α mRNA and FNDC5 mRNA expression

TRIzol reagents (Invitrogen, Carlsbad, CA, USA) were used to extract total RNA from muscles in accordance with the manufacturer's instructions. Using an RNeasy mini Kit (Qiagen, USA) for purification and DNase treatment, RNA was then measured spectrophotometrically in a NanoPhotometer (NanoDrop 2000, Implen, Germany). Using a high capacity RNA to cDNA kit (Applied Biosystems, USA), the extracted RNA was reverse transcribed into cDNA. It was then inactivated at 95 °C for five minutes after being incubated at 37 °C for one hour, and it was stored at –20 °C until needed. Using Quantitect SYBR Green PCR reagents, real-time PCR was carried out using an Agilent Mx3000P light cycler. The following primers were acquired from the Qiagen QuantiTect collection: Rn_Fndc5_2_SG Quanti Tect Primer Assay, Cat #QT02383276; peroxisome proliferator activated receptor-gamma coactivator1 alpha (PGC-1α) (Rn_Ppargc1a_1_SG Quanti Tect Primer Assay, Cat #QT00189196); and Rn_Actb_1_ SG QuantiTect Primer Assay, Cat #QT00193473). The cycling conditions were as follows: 15 minutes at 95 °C, 45 cycles of 15 s at 95 °C, 30 s at 60 °C, and 30 s at 72 °C. The 2−ΔΔCt technique [38] was employed

to examine the data, with β-actin serving as the internal reference gene.

2.11. Immunohistochemical study:

Prior to being stained with immunostain, the sections were deparaffinized and rehydrated using a progressive series of alcohol. The endogenous peroxidase activity was suppressed for 20 minutes by freshly produced 0.3% hydrogen peroxide in methanol. After using microwave antigen retrieval, β-Amyloid and PGC-1α antibody were then incubated. The primary antibodies used were: Beta Amyloid Polyclonal Antibody (E-AB-15509, Rabbit Monoclonal Primary Antibody, concentrated dilution; 1:100) (Elabscience, 14780 Memorial Drive, Suite 108, Houston, Texas, 77079, USA) & PGC-1 Alpha polyclonal antibody (E-AB-65852, Rabbit Monoclonal Primary Antibody, Concentrated dilution; 1:100) (Elabscience, 14780 Memorial Drive, Suite 108, Houston, Texas, 77079, USA). An HRP conjugated goat anti-mouse/rabbit antibody was used as secondary. The chromogen diaminobenzidine and the Ultravision LP polymer system (Lab Vision, California, United States) were used to amplify and see the antigen-antibody combination. When a marker completely loses its immunohistochemical stain, it is regarded as negative expression. Positive markers stain the cell membrane and cytoplasm. Over the whole region, the expression of PGC-1α and β-Amyloid was assessed at a ×200 magnification.

2.12. Morphometric analysis:

Using the Leica Qwin 500 Image Analyzer (LEICA Imaging Systems Ltd, Cambridge, UK), which comprises of a Leica DM-LB microscope with a JVC color video camera coupled to a computer system, LeicaQ500IW, the morphometric analysis was carried out at the Pathology Department of the National Research Center.

Slides were placed on the stage of the microscope. The light source's level was changed as necessary. The successful adjustment of the illumination was confirmed using the video monitor. The morphometric analysis was performed on β-Amyloid and PGC-1α-stained slides to ascertain the percentage of positively stained cells in the area. The areas that needed to be measured were automatically covered by a blue mask, also called a binary picture. Subsequently, the area of this binary picture was calculated, representing the area of the object that needed to be measured. The results of each measurement are shown in micrometers, and the mean area over all fields under study was eventually computed.

2.13. Statistical analysis

All data were analyzed using SPSS version 25 (IBM corporation, Armonk, NY, USA). Data were represented as the mean± standard deviation (SD). Tukey's post hoc test was performed if the p value was < 0.05 in one-way analysis of variance (ANOVA). Pearson´s correlation was done to correlate the evaluated parameters in the studied groups. Statistical significance was defined as a p value less than 0.05.

3. Results

3.1. Physical properties

3.1.1. Transmission Electron Microscopy (TEM)

The morphology and particle size of the two-layer cardamom oil capsules were measured using TEM. These results are shown in Figure 1. Cardamom oil, encapsulated in capsules of two different layers, had a large particle size in the micrometer range, with an average particle size ranging from 0.15 to 1.13 µm. The layers are clearly shown in Figure 1. Cardamom oil coated in two layers had the shape of homogeneous and round particles, and had no aggregation, and had a circular shape and smooth surface and were separate.

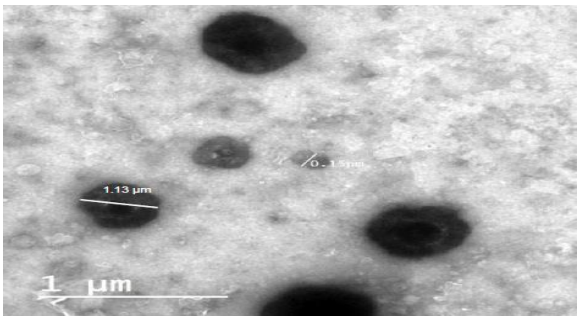

Fig. 1. A TEM image of ca cardamom oil capsule

3.1.2. Differential Scanning Calorimetry (DSC)

Two steep sorbents at temperatures representing melting points of 32.15 and 100°C are shown in the DSC thermal diagram of the cardamom oil nano-emulsion (Fig. 2A). The bio-compounds of cardamom oil represent a large area starting from 20 and ending at about 80 degrees Celsius. This sharp heat absorption peak starts from the melting point of the weak melt transition at 32.15°C. Therefore, cardamom oil un-encapsulated in its free form cannot be used in food applications as an additive due to its low thermal stability.

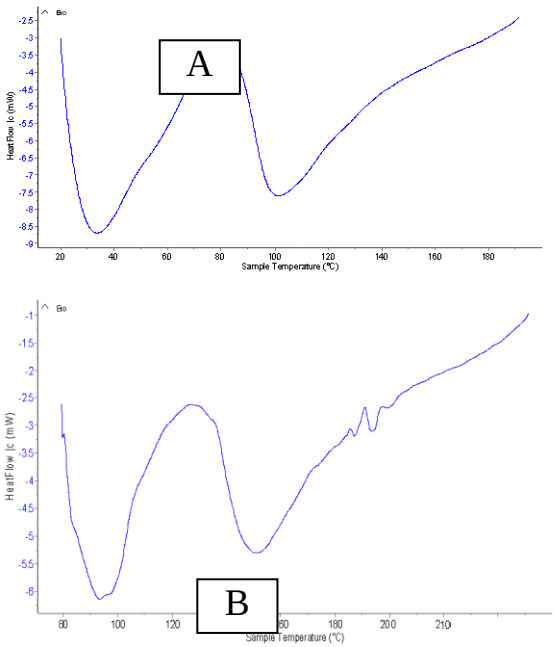

Fig. 2 The DSC thermogram of cardamom oil coated in two different layers

The DSC thermogram of cardamom oil coated in two different layers has three melting points (Fig. 2B) (i.e. 93.32, 132.24 and 191.55°C), the tolerance of free cardamom oil ranged from approximately 80 to 120 °C, which is higher than before. It was more resistant to exposure to high temperatures than the microcapsule oil sample inside the capsule to 191.55 degrees Celsius, where the crystalline state was formed around the cardamom oil with two layers of packaging materials that gave the ability to protect it from exposure to high temperatures. This proved the success of the microcapsule encapsulation process of cardamom oil with thermal stability against temperature. Through these results, high temperatures can be tolerated for use in food applications, especially in bakery products.

3.1.3. Encapsulation efficiency (EE %)

The results in Figure (3) showed that the packaging efficiency of the cardamom oil nano-emulsion sample was 92.57%, while the packaging efficiency of the cardamom oil sample in a microcapsule with two different layers was 97.18%, which gave good results in packaging, on the contrary, the nano-emulsion of cardamom oil gave an efficiency Less packaging due to the presence of a thin layer of emulsion that prevents the presence of all the added oil inside the emulsion, but its arrival to reduce the active roots was higher compared to cardamom oil coated with two layers of micr-ocapsules, the process of reaching the vital active ingredients inside it is less to reach the target.

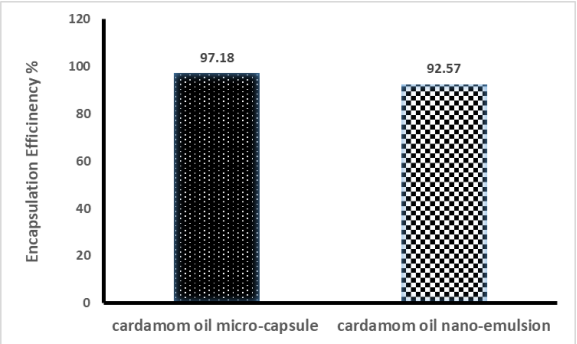

Fig. 3. Encapsulation efficiency of Cardamom oil forms by FRAP assay

3.2. Chemical properties

3.2.1. Total phenolic (TPC) and total flavonoids (TFC) of cardamom oil forms

The optimal content of phenol in cardamom oil extract, and microcapsules varies depending on the method of preparation and the extent of its bioactive compounds being released. The most important feature of cardamom oil extract is the polyphenols in its structure. Total polyphenol content (TPC) is the most important determinant of cardamom, and a high polyphenol value indicates high biological activity. Table (1) showed the total contents of polyphenols and flavonoids in the two forms of cardamom oil extracted and micro-capsule. The cardamom oil sample that was encapsulated in two layers of micro-capsules (2.31 g GAE/100 g oil), and the untreated crude oil sample had the lowest activity (1.77 g GAE/100 g oil). It was found that the cardamom oil sample coated with two microcapsules and contained 5.18 g catechins / 100 g oil. The untreated crude oil contained a lower concentration of flavonoids compared to the other two methods.

Table 1: Total phenolic and flavonoids contents of cardamom oil extract, and micro-capsule

Types of Extraction	Total phenolic (g GAE/100g oil)	Total flavonoids (g catechin / 100g oil)
Cardamom oil extract	1.77±0.05	4.52±0.81
Cardamom oil micro-capsule	2.31±0.11	5.03±0.72

3.2.2. DPPH free radical scavenging

The free radical scavenging abilities of cardamom oil extract and cardamom oil two microcapsule layers were measured by DPPH assay and the results were compared in the form of IC_{50} value (Fig. 4). The IC50 values of untreated cardamom oil were calculated and compared to other forms microcapsule in order to investigate the free radical removal activity. The IC_{50} of cardamom oil

extracted with a maximum of 30% (22.85 µg/ml) was examined, which indicates the least activity in removing free radicals, and thus DPPH inhibition will be more effective. The antioxidant power of cardamom oil encapsulated in a microcapsule showed higher activity than the extracted cardamom oil in removing DPPH radicals.

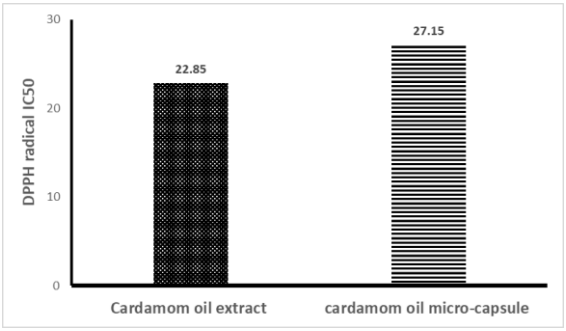

Fig.4. DPPH free radical scavenging activity (IC_{50} µg/mL) of oil cardamom extract and micro-capsule

After the end of the study duration (42 days), histopathological sections from the brain of both the control group and those injected intraperitoneally with aluminum chloride were stained with haematoxylin and eosin for microscopical examination. It shows normal cell arrangement and morphology as represented in figure (5). Alzheimer's disease was induced using aluminum chloride as shown in figure (6)

The mean level of copy numbers ± SD of Peroxisome proliferator-activated receptor-gamma coactivator (PGC1-α) & Fibronectin type III domain-containing protein 5 (FNDC5) of the studied groups are shown in table (2). It revealed a highly statistically significant decrease in the level of PGC1-α on comparing Alzheimer induced group, Alzheimer induced treated donepezil group, Alzheimer induced treated donepezil & NCEO group and Alzheimer induced + swimming group with the control group ($p< 0.001$). On comparing with Alzheimer induced group, Alzheimer induced treated donepezil group showed a statistically significant increase ($p< 0.05$).When these results were compared to Alzheimer induced treated donepezil group, there was a statistical significant decrease with Alzheimer induced group, Alzheimer induced treated donepezil & NCEO group and Alzheimer induced + swimming group ($p< 0.05$). Regarding (FNDC5), also it shows same results as PGC1-α however with less improvement in G3, 4&5.

The mean level ± SD of Amyloid beta 1-40 (Aβ 1-40), Irisin, interleukin -10 (IL-10) & glutatione (GSH) are shown in table (2). It showed a statistically significant increase in Aβ 1-40 level in AD induced group; this level was reduced in G3, 4&5 revealed by a statistically significant decrease ($p< 0.05$). For Irisin, interleukin -10 (IL-10) & glutatione (GSH), there was a statistically significant decrease in AD induced group that was improved in G3, 4&5 revealed by a statistical significant decrease ($p< 0.05$).

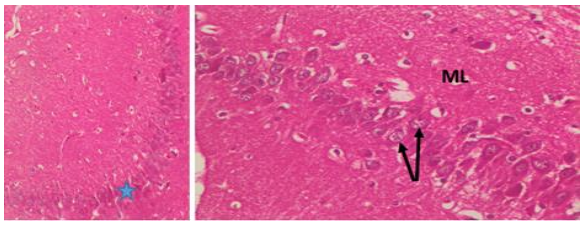

Figure (5): Photomicrography of the hippocampal region during control swimming reveals 4-6 compact layers of pyramidal cells, both large and small (blue star); the majority of these cells have vesicular nuclei (black arrow). Many glial cells can be seen among the neuronal processes in the molecular layer (ML). (H & E 200x & 100x).

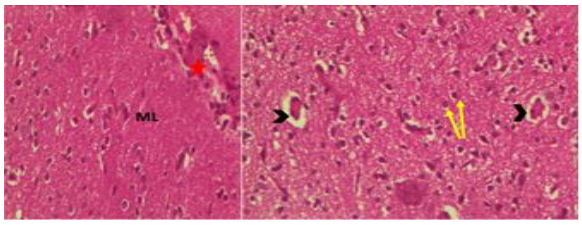

Figure (6): A positive control photomicrograph of the hippocampal region in Alzheimer's disease (AD) reveals disarray and loss of tiny pyramidal cells (red star), some of which have pale nuclei and others have black nuclei. The size of the big pyramidal cells (black head arrows) had been significantly shrunk; the outer layer had been more severely impacted, with darker nuclei. While the molecular layer (ML) displayed more glial cells and larger neurons (yellow arrows), the granular layer also displayed noticeable vacuolation. (H & E 200x&100x)

Table (2) Effect of swimming on the levels of PGC1-α and FNDC5 in muscles in studied groups

	PGC1-α	**FNDC5**
G1	15.3 ± 5.8	3.5 ± 0.7
G2	2.78 ± 0.28 ^{a,c,e}	1.2 ± 0.28 ^{a,c,d}
G3	4.2 ± 1.07 ^{a,b,d,e}	1.64 ± 0.21 ^{a,b}
G4	2.57± 0.27 ^{a,c,e}	1.74 ± 0.23 ^{a,b}
G5	3.2 ± 0.73 ^{a,c,d}	1.39 ± 0.32 ^{a}

G1= Control swimming (CONT-SWIM.); G2=AD (Alzheimer disease); G3= AD+DP (Alzheimer disease+ donepezil); G4= AD+ 1/20th NCEO; G5=(AD-SWIM) Alzheimer disease + swimming, a; is the statistical significant difference when compared to G1; b, is the statistical significant difference when compared to G2, c; is the statistical significant difference when compared to G3,d; is the statistical significant difference when compared to G4 and e; is the statistical significant difference when compared to G5.

Table (3) Effect of swimming on the levels of PGC1-α and FNDC5 in muscles in studied groups

	Aβ 1-40(Pg/ml)	**Irisin (Pg/ml)**	**IL-10 (Pg/ml)**	**GSH Pg/ml**
G1	34.92 ± 4.84	179.49± 8.48	62.7 ± 5.14	56.0 ± 3.3
G2	76.0 ± 3.9 ^{a,c,d,e}	37.18 ± 2.33 ^{a,c,d,e}	29.04 ± 2.95 ^{a,c,d}	34.71 ± 4.01 ^{a,c,d,e}
G3	47.7 ± 4.1 ^{a,b,d,e}	61.91 ± 4.79 ^{a,b,d,e}	43.4 ± 5.0 ^{a,b,d,e}	60.2 ± 3.37 ^{a,b,e}
G4	63.15± 4.80 ^{a,b,c,e}	80.20 ± 4.90 ^{a,b,c}	35.8 ± 2.47 ^{a,b,c,e}	65.18 ± 12.02 ^{a,b,e}
G5	66.34 ± 5.78 ^{a,b,c,d}	88.0 ± 7.17 ^{a,b,c}	27.4 ± 1.38 ^{a,c,d}	56.0 ± 3.30 ^{a,b,c,d}

AB: Amyloid beta; IL-10: interleukin-10; GSH: glutatione .G1= Control swimming (CONT-SWIM.); G2=AD (Alzheimer disease); G3= AD+DP (Alzheimer disease+ donepezil); G4= AD+ 1/20th NCEO; G5=(AD-SWIM) Alzheimer disease + swimming, a; is the statistical significant difference when compared to G1; b, is the statistical significant difference when compared to G2, c; I the statistical significant difference when compared to G3,d; is the statistical significant difference when compared to G4 and e; is the statistical significant difference when compared to G5.

Table (4) Pearson´s correlation of the evaluated parameters in the studied groups

	Aβ 1-40 (Pg/ml)	Irisin (Pg/ml)	IL-10 (Pg/ml)	GSH (Pg/ml)	Muscle PGC1-α	Muscle FNDC5
Aβ 1-40(Pg/ml)	----------	-.675**	.064	-.712**	-.723**	-.574**
Irisin (Pg/ml)		----------	-.177	.718**	.880**	.621*
IL-10 (Pg/ml)			----------	-.136	-.366	-.269
GSH (Pg/ml)				----------	.681**	.447*
Muscle PGC1-α					----------	.838**
Muscle FNDC5						----------

* p<0.05 - ** p<0.001

For confirming our results, Pearson´s correlation was calculated for the concerned parameters along the studied groups as shown in table 3. It showed a highly statistically negative correlation in Aβ 1-40 when correlated with Irisin, GSH and both muscle PGC1-α & FNDC5, r was -0.675, -.712, -0.723 & -.574 respectively. However, a highly statistically significant positive correlation was observed in irisin when correlated with GSH and muscle PGC1-α, r was 0 .718 & 0.880 respectively. Moreover, a statistically significant positive correlation was observed when correlating muscle FNDC5 with both Irisin & GSH, r was 0 .621 & 0.447 respectively.

3.2.3. Immunomorphometric Assessment of β-Amyloid staining in muscle tissue:

The results presented in Table 4 showed the percentage area of β-Amyloid expression in muscle tissue for different groups. This first group; Control swimming group (Fig 7.A) and the second group; Alzheimer's disease (Fig 7.B) used to evaluate the other studied groups, they had mean percentage area of β-Amyloid expression which were 10.39±0.9 & 26.67±2.88 respectively.

The third and the fifth groups; Alzheimer's disease - donepezil (Fig 7.C) & Alzheimer's disease swimming (Fig 7.E) showed improvement as the mean percentage area of β-Amyloid expression were 17.58±1.4, 19.33±1.8 respectively, which represented the statistical significance difference of the studied groups when compared with Control swimming and Alzheimer's disease.

The best result has been showed by the fourth group; Alzheimer's disease + DP-1 20th NCEO 11.64±1.04, with statistical significance difference with Alzheimer's disease only (Fig 7.D).

Results of Area% for immunohistochemical expression of β-Amyloid & PGC-1α in Muscle tissue were expressed as mean ± SE as shown in table 4.

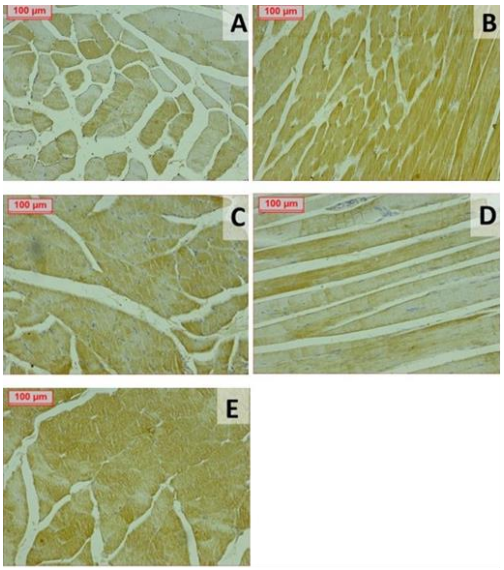

Figure (7): A photomicrography of Muscle tissue for all tested groups showing their immunohistochemical expression for **β-Amyloid**;

(A) G1: Control swimming, (B) G2: Alzheimer disease, (C) G3: Alzheimer disease- donepezil, (D) G4: Alzheimer disease +DP-1 20th NCEO, (E) G5: Alzheimer disease swimming (β-Amyloid; 200x).

3.2.4. Immunomorphometric Assessment of PGC-1α staining in muscle tissue:

The results presented in Table 3 depict the percentage area of PGC-1α expression in muscle tissue for different groups. This first group; Control swimming group (Fig 8.A) and the second group; Alzheimer's disease (Fig 8.B) used to evaluate the other studied groups, they had mean percentage area of β-Amyloid expression which were; 26.63±1.16 &15.21±1.05 respectively. The third group; Alzheimer's disease - donepezil (Fig 8.C), had mean percentage area of PGC-1α expression 21.73±1.18.

The best result has been showed by the fourth group; Alzheimer's disease + DP-1 20th NCEO 25.55±1.82, with statistical significance difference with Alzheimer's disease only (Fig 8.D).On the other hand the fifth group; Alzheimer's disease swimming (Fig 8.E) had mean percentage area of PGC-1α expression in this group was 18.99±0.85, which was with statistical significance difference with control swimming group only.

T able (5): Percentage area of the studied immunomorphometric markers

Parameters / Groups	Area % of β-Amyloid	Area % of PGC-1α
G1: Control swimming	10.39±0.9[b]	26.63±1.16[b]
G2: Alzheimer disease	26.67±2.88[a]	15.21±1.05[a]
G3: Alzheimer disease- donepezil	17.58±1.4[a,b]	21.73±1.18[a,b]
G4:Alzheimer disease +DP-1 20th NCEO	11.64±1.04[b]	25.55±1.82[b]
G5: Alzheimer disease swimming	19.33±1.8[a,b]	18.99±0.85[a]

a P value of studied groups compared with G1; Control swimming.

b P value of studied groups compared G2; Alzheimer disease.

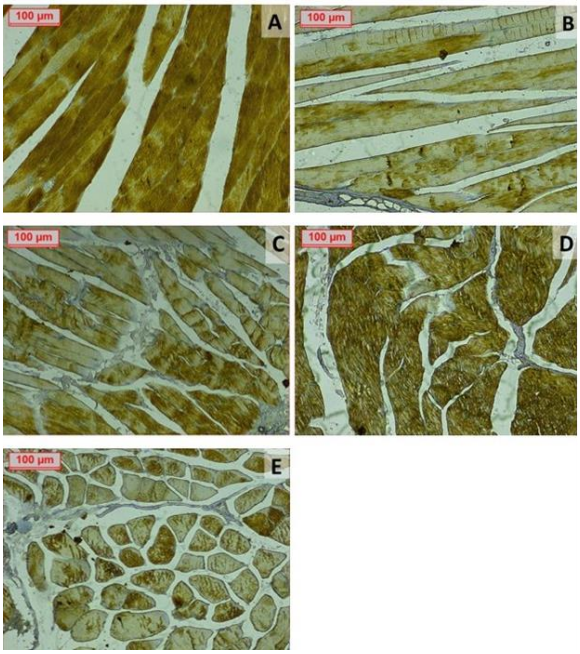

Figure (8): A photomicrography of Muscle tissue for all tested groups showing their immunohistochemical expression for **PGC-1α**;

A) G1: Control swimming, (B) G2: Alzheimer disease, (C) G3: Alzheimer disease- donepezil, (D) G4: Alzheimer disease +DP-1 20th NCEO, (E) G5: Alzheimer disease swimming (PGC-1α; 200x).

4. Discussion

Alzheimer's disease (AD) is a distinctive neurodegenerative disorder caused by excessive loss of neurons, mostly in the hippocampus and cerebral cortex primarily affecting memory. This pathology is characterized by cognitive impairment, memory loss, and behavioral abnormalities. The respective symptoms start to progress with massive loss of cholinergic neurons that synthesize acetylcholine (ACh) and are responsible, to a great extent, for coordinating memory functions. A variety of potential underlying mechanisms have been proposed to explain the pathogenesis of AD **[8;39].**

It is commonly acknowledged that endurance training improves cognitive performance and brain health. It is linked to de novo neurogenesis in the dentate gyrus, as well as enhanced blood flow and synaptic plasticity in the hippocampal regions [40]. Exercise has been shown to improve insulin signaling, tau hyperphosphorylation, and spatial memory [41].

The purpose of this study is to investigate the effectiveness of swimming training in reducing Aβ plaques and inflammation associated with AD, as well as the efficacy of cardamom oil in nano-encapsulated form (NCEO) targeting the AD brain.

One of the key signs of oxidative stress is lipid peroxidation. Aluminum has been shown to activate and accelerate lipid peroxidation in the brain when iron is present [42]. By promoting iron-based oxidation in the brain, which primarily modifies iron homeostasis through the Fenton reaction, aluminum exacerbates iron-induced oxidative damage [43].

Aluminum has been implicated in modulating brain amyloidosis through oxidative damage, according to reports [44]. A rise in oxidative stress caused a redox reaction that inhibited several natural antioxidant enzymes, including glutathione, catalase, and superoxide dismutase, which are essential in

preventing damage from free radicals [45]. In our investigation, an injection of aluminum chloride caused a significant disruption in the brain's oxidative stress, as evidenced by a drop in the amount of reduced glutathione, which is accountable for damaging neurons. This outcome is consistent with [46].

Improvements in neurobehavioral measures, such as cognitive abilities and anxiety-reducing effects, were observed after cardamom oil administration. Additionally, it reduced the activity of acetylcholinesterase in the cortex and hippocampal regions and enhanced brain levels of glutathione, catalase, superoxide dismutase, and lipid peroxidation, all of which indicate a decrease in oxidative damage. Cardamom oil treatment also decreased neuronal degeneration by raising BDNF levels and preventing the development of amyloid β in the cortex and hippocampal regions. Cardamom oil therapy prevented the development of amyloid βplaque [47]. In this study, administering an injection of aluminum chloride causes an increase in amyloid β levels and a decrease in glutathione levels in the groups under investigation. On the other hand, administering cardamom oil encapsulated nanoparticles decreased oxidative damage by elevating Aβ and GSH levels. This outcome is consistent with [47].

Besides, routine swimming exercise was confirmed to be neuroprotective against cognitive impairments in different animal models by increasing hippocampal BDNF levels **[48;49].** Bashiri and his colleagues found a significant increase in BDNF levels in the hippocampus of STZ-treated mice followed by swimming exercise **[50].**

Irisin secreted during exercise could control energy expenditure through PGC-1α and moreover, improves the production of BDNF in AD. Though, irisin may enhance the neurological problems in patients with AD through the activation of PGC-1α and BDNF pathways **[51].**

In the present study, injection of aluminum chloride leads to the decrease of IL-5, PGC1-α & FNDC5 levels and elevation of irisin levels in the studied groups and treatment with cardamom oil improved these levels. These results are in line with the work of Jo & Song, **[51].**

To the best of our knowledge cardamom oil encapsulated nanoparticles presented in this work was applied for the first time as it combined the advantages of cardamom oil as a natural product as well as that of nanoparticles in improving the adverse symptoms of AD.

By slowing the development of white matter lesions and the loss of gray and white matter volume, as well as by promoting neuroplasticity, which in turn encourages motor learning or relearning during rehabilitation, exercise can both prevent and mitigate the effects of aging and disease on the brain [52;53].

As obvious from our results there was a statistically significant improvement in the evaluated parameters in both serum and muscle, however this improvement was more significant in rats trained swimming for 42 days in PGC1-α, Aβ 1-40 and irisin. This can support the hypothesis that AD could be treated or to a leaser extent minimize its worse symptoms by physical exercise, especially swimming.

The type of exercise performed determines the amount of irisin released into the bloodstream after exercise; aerobic exercise training is known to induce larger levels of serum irisin than resistance exercise [54].

Another significant molecular mediator for the advantageous reactions to exercise in the hippocampal regions is the induction of BDNF [55]. Research has shown that exercise has a stimulatory effect on both peripheral and central irisin in certain animal models of memory impairment, and this upregulation may represent a potential neuroprotective mechanism of exercise in Alzheimer's disease [56, 57].

Souzaa and his collaborators concluded that swimming exercise prevented behavioural and neurobiological deficits and suggested that these neuroprotective effects are likely to involve the inhibition of inflammation and upregulation of BDNF in brain of mice. Thus, it is possible that physical exercise can be used as a non-pharmacological approach to alleviate both cognitive and non-cognitive symptoms of AD **[58].**

Alzheimer’s disease (AD) is characterized by several pathological features, one of which is the accumulation of aggregated proteins like β-Amyloid and tau. The exact roles of these proteins are still unresolved, but researchers suggested that they have an early and crucial role in muscle degeneration. However, it was revealed that different forms of intracellular β-Amyloid assemblies also contribute to the pathophysiological alterations observed in AD **[59].**

For AD, current medication only provides symptomatic alleviation. NMDA (N-methyl-D-aspartate) receptor antagonists, such as memantine, cause adverse effects like nausea, vomiting, and diarrhea. Acetylcholinesterase (AChE) inhibitors, such as galantamine, rivastigmine, and donepezil hydrochloride, are frequently used, but they have unfavorable side effects like bronchoconstriction and hypotension [60].

Though they should be taken with caution as synthetic active items, herbal pharmaceuticals have become more popular because of the belief that they are safer than synthetic drugs. For instance, down-regulating acetylcholinesterase (AChE) and increasing brain-derived neurotrophic factor (BDNF) production are two ways that essential oils may demonstrate their neuroprotective effect [47].

Previous research indicates that there are considerable differences in the expression of β-Amyloid in muscle tissue among the different groups under investigation. Moderate multifocal Aβ plaque deposition was noted in the disease control group. On the other hand, cardamom oil therapy reduced the amount of Aβ plaques that were deposited. The results of our investigation align with Immunohistochemistry (IHC) analysis, which revealed a considerable rise in Aβ expression in the disease control group [47]. On the other hand, therapy with cardamom oil encapsulated nanoparticles dramatically reduced Aβ expression.

The pathogenesis of neurodegenerative diseases like Alzheimer's disease (AD), Huntington's disease (HD), Parkinson's disease (PD), and amyotrophic lateral sclerosis (ALS) involves a decrease in the transcription and/or protein expression of PGC-1α. Mice lacking PGC-1α demonstrate a decrease in the number of mitochondria, increased locomotor activity, and develop striatal pathology **[61].**

The goal of the current study is to investigate how changes in the expression of PGC-1α coactivator 1α (PPAR-γ) coactivator 1α (PGC-1α), a crucial regulator of glucose homeostasis in the liver and muscle during fasting or insulin resistance conditions through the activation of gluconeogenic metabolic pathways, may affect the amyloid neuropathology of AD. Additionally, the study aims to test the hypothesis that promoting PGC-1α expression in neurons could be developed as a novel therapeutic approach for AD [62].

According to our findings, PGC-1α expression is different in Alzheimer's patients than in the control group. Additionally, there seems to be an effect on PGC-1α expression in the Alzheimer's disease group (G3) following the introduction of the medication donepezil. PGC-1α expression is also affected by the combination of Alzheimer's disease and DP-1 20th NCEO therapy (G4). Furthermore, swimming's impact on the Alzheimer's disease group (G5) is apparent.

Given the close relationship between mental health and aerobic exercise, it has been noted that aerobic exercise plays a vital role in maintaining brain plasticity and cognitive reserve as well as boosting resilience against neurodegeneration. However, due to age-related comorbidities, cognitive decline, and the requirement for help to enhance safety, engaging in regular physical activity is becoming increasingly difficult and risky for individuals with Alzheimer's [63].

The current investigation found that, in comparison to the AD-induced model, regular swimming exercise or a 6-week course of dopenzil and nano-encapsulated cardamom essential oil (NCEO) equally improved the insulin signaling cascade. This improvement was linked to increased muscle FNDC5 and serum irisin. A highly statistically significant positive association ($r = 0.88$) supported this conclusion. It's interesting to note that NCEO and swimming improved more.

Ex vivo human adult cortical slices have been shown by Lourenco and colleagues to express FNDC5/irisin, suggesting a physiological role for FNDC5/irisin in memory formation and brain function [48]. Furthermore, studies on AD patients and animals intoxicated with Aβ have shown decreased levels of CSF and brain irisin [56; 57]. As our work has shown, frequent exercise and/or supplementing with cardamom essential oil may have neuroprotective effects on the brain, which may be related to the elevation of FNDC5/irisin expression.

Because AD is a CNS neurodegenerative disease, medications used to treat it need to be able to cross the blood brain barrier (BBB). Therefore, therapeutic agents need to be able to pass through the BBB in order to be used to treat AD. medications used to treat AD confront various obstacles, which are addressed by nanoparticles (NPs) designed to transport medications across the blood-brain barrier [64]. Because of the special qualities of the nanoparticles, which allow them to be adaptable to immunological response and metabolic processes, nanoscale formulations can be employed for efficient targeting and distribution of therapeutic agents [65].

Using engineered nanoparticles (NPs) with unique physicochemical properties and the ability to cross the blood–brain barrier (BBB) is one possible strategy to address biological and pharmacological challenges in the treatment of brain illnesses like AD [66]. These NPs may enhance the pharmacokinetic and pharmacodynamic characteristics of low-toxicity drugs by controlling AD disorders and distributing medication to the brain [67].

Since the main targets of the drugs currently available on the market are tau proteins, neuroinflammation, and Aβ proteins, it is critical to develop new drug targets that will not only treat symptoms but also halt the disease's progression in its early stages, potentially improving quality of life [68].

The combined use of neuronal stem cells and nanotechnology-based multimodal drug delivery systems, in conjunction with a stress-free, physically active lifestyle and a healthy diet, may, in the majority of cases, prevent AD pathologies and

enhance brain synaptic transmission, which could lead to future therapeutic research advancements in the fight against AD [69].

5. Conclusion

Conclusively, the present work highlighted the field of nanotechnology in improving the adverse effects of AD as well as swimming and clarified the improvements in both biochemical and pathological parameters. Though it is recommended to use cardamom essential oil encapsulated nanoparticles in addition to swimming to gain the ultimate benefit. Further analysis and interpretation would be necessary to understand the implications of these findings in the context of Alzheimer's disease and the effects of swimming and medication interventions.

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