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Exploring the Potential of Nano-Encapsulated Elettaria Cardamom Oil as Anti-Alzheimer Agent

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Abstract

Alzheimer's disease (AD) is a progressive neurodegenerative disease that contributes significantly to the global prevalence of dementia in the elderly population worldwide. The current study's goal is to examine the promising effects of cardamom oil in nano-encapsulated form, as a neuroprotective agent in rats suffering from AD like symptoms induced by aluminum chloride ($AlCl_3$). Sixty male albino rats classified as follows: Group (I) =Control; Group (II)=AD; Group (III)=AD+donepezil; Group (IV)=AD+1/10th crude cardamom oil; Group (V)=AD+1/10th nano-encapsulated cardamom oil; Group (VI)=AD+ donepezil +1/10th nano-encapsulated cardamom oil. The experiment period was 42 days. Amyloid-*β* (A*β*), acetylcholine (ACh), acetylcholinesterase (AChE), interleukin-17 (IL-17), fibronectin type III domain-containing protein 5 (FNDC5), Peroxisome proliferator-activated receptor gamma coactivator 1-α (PGC-1α) and brain-derived neurotrophic factor (BDNF) were measured. Also, histopathology for brain tissues was done. Our results showed that there is a significant decrease in BDNF, Ach, PGC-1α and FND C5 while a significant increase in IL-17, Aβ and AChE in AD rats when compared with control group. Treatment with donepezil is associated with a significant increase in BDNF and Ach and significant decrease in IL-17, Aβ and AChE when compared with Alzheimer group. All parameters were improved significantly when the rats were treated with nano-encapsulated cardamom oil when compared with Alzheimer group. This study demonstrated that cardamom oil protects against the neurotoxicity caused by aluminum chloride and histopathological studies support these biochemical results. We conclude that the cardamom oil in nano form in a dose of 1/10th is a promising natural protecting agent of neural cell during Alzheimer's disease.

Keywords: Nano-encapsulated Cardamom oil, Brain-derived neurotrophic factor, Peroxisome proliferator-activated receptor gamma coactivator 1-α, Alzheimer's disease, Rats.

1. Introduction

Alzheimer's disease (AD) is a multifactorial neurodegenerative disorder characterized by cellular and molecular dysregulation. Because of the complexity of Alzheimer's disease, developing therapeutics is notoriously difficult **[1].**

The current pharmacotherapy for Alzheimer's disease only provides symptomatic relief. Acetylcholinesterase (AChE) inhibitors, including galantamine, rivastigmine, and donepezil hydrochloride, induce bronchoconstriction and hypotension. Herbal or alternative medicine systems are gaining more attention in global therapeutic research. Several disease pathways involved in the progression of Alzheimer's disease have been shown to be affected by neuroprotective effects of natural products **[2].** Some plant-based essential oils (EOs), like cardamom oil from Elettaria cardamom, have been shown to have anticholinesterase activity and neuroprotective effects by down-regulating AChE and increasing brain-derived neurotrophic factor (BDNF) levels in the brain **[3].** Cardamom oil's major chemical constituents are 1,8 cineole (25-45%), a-terpinyl acetate (20-53%), limonene (5.6%), linalyl acetate (8.2%), and linalool (5.4 percent). Numerous pharmacological and biological properties, such as anticonvulsant, gastroprotective, antioxidant, anti-inflammatory, antimicrobial, chemopreventive, anti-anxiety, and antihypercholesterolemic, have been attributed to cardamom oil. 1,8 cineole, the principal phytoconstituent of cardamom oil, inhibited AChE **[3].**

Nanotechnology based techniques like nano-encapsulation have been developed as one such technology that involves the use of delivery vessels, also referred to as nano carriers to encapsulate EOs bioactive molecules. It also offers different advantages to EOs such as protection from degradation, enhanced bioactivity targeted delivery and controlled release. Moreover,

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encapsulation of EOs improves the bioactive properties of essential oils and can be used in food and pharmaceutical industries **[4].**

Extracellular amyloid-β (Aβ) plaques and intraneuronal neurofibrillary tangles caused by phosphorylated tau proteins have been identified as important pathologic features of Alzheimer's disease (AD). Furthermore, a number of factors such as glucose metabolism, mitochondrial dysfunction, synaptic transmission failure, oxidative stress, and cell apoptosis all played a role in the development of Alzheimer's disease **[5].**

One of the most striking biochemical changes in people with Alzheimer's disease is a decrease in acetylcholine levels in the hippocampus and cortex of the brain. Acetylcholinesterase, the primary enzyme in acetylcholine breakdown and hydrolysis, controls acetylcholine concentration. Acetylcholine is essential for proper brain operation. Due to a lack of acetylcholine, the cognitive and behavioral abilities of Alzheimer's patients deteriorate over time. Therefore, preventing Alzheimer's disease by blocking acetylcholinesterase is being considered as a treatment option **[6].**

High levels of inflammatory mediators (e.g., pro-inflammatory cytokines and chemokines) in Alzheimer's disease patients are evidence of the activation of inflammatory pathways in pathologically vulnerable regions of their brains. These cytokines and complement factor accumulations have been associated with Alzheimer's disease-related memory loss, as well as declines in learning ability and mental activity **[7].**

Enhanced levels of IL-17 have been found in the cerebrospinal fluid and serum of Alzheimer's disease patients, suggesting that T helper 17/ regulatory T cells (Th17 cells) may be involved in the inflammatory responses associated with this disease, as suggested by previous in vivo and in vitro studies **[7].**

The expression of brain-derived neurotrophic factor (BDNF) in the hippocampus, a brain region important for learning and memory, is stimulated by fibronectin type III domain-containing protein 5 (FNDC5). This suggests that FNDC5 may have neuroprotective properties in brain disorders like Alzheimer's **[8].** BDNFis mainly produced by cerebral cortex and hippocampus, participates in the growth, differentiation and development of neurons, and has a good role in promoting and protecting the nervous system., It is found that the level of BDNF in many patients with nervous system diseases is significantly reduced, and its level is closely related to the learning and memory function of patients; In addition, BDNF is conducive to the survival of neurons in the brain of patients with AD, which is considered to be one of the important targets for the treatment of AD **[9].** BDNF plays a role in maintaining forebrain cholinergic cells' health and overall memory performance, both of which suffer from decreased signalling with AD, it is critical to investigate treatment mechanisms that can mitigate these physiological changes **[10].**

Peroxisome proliferator-activated receptor gamma coactivator 1-α (PGC-1α) is found in tissues that require a lot of energy, such as brown adipose tissue, skeletal muscle, and the brain. In the brain, decreased PGC-1 activity causes neurodegeneration by inducing mitochondrial dysfunction. PGC1 may be important in maintaining brain function in Alzheimer's disease, according to clinical research, because its levels in Alzheimer's patients are lower than in normal subjects. According to this evidence, considering PGC-1's role may help us figure out what causes Alzheimer's disease and how it progresses, according to this evidence. on PGC-1's role may result in a deeper comprehension of the pathological processes underlying Alzheimer's disease **[11].** A production of Aβ was reduced by increasing PGC1-α **[12].**

Our aim in this study to investigate the promising effect of nano-encapsulated form of cardamom oil, as a neuroprotective agent in rats suffering from AD like symptoms induced by aluminum chloride ($AlCl_3$).

2. Materials and methods

Drugs and reagents

1-Extraction and phytochemical analysis of cardamom oil

a) Isolation of essential oil

Cardamom essential oil was extracted for 3 hours using Clevenger's apparatus and the hydro-distillation method. The volatile oil yield was weighed and calculated in g100 g dry plant **[13].**

Essential oils analysis

Gas chromatographic-mass spectrometric analysis (GC/MS)

Hewlett-Packard model (5890) coupled gas chromatography / mass spectrometry Hewlett-Packard-MS (5970) was used for the analysis. Ionization voltage was 70 eV, and the mass range was 39-400 a.m.u. The isolated peaks were identified by comparing them to those of authentic compounds and published data in a mass spectral library (Table 1.) (National Institute of Standards and Technology, NIST). Peak area integration was used to perform the quantitative determination. The identification of the GC components was also confirmed using NIST mass spectra library data and a comparison of their retention indices to those of genuine compounds **[14].**

2- Preparation of polymers used in packaging

a) First layer (Arabic Gum):

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.

b) 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 **[15].**

3- 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.* [16]** with some modifications (The solution was dispersed using a high-speed homogenizer at 18,000 rpm then add the second layer of the previously prepared gum arabic solution to the resulting emulsion after 24 hours to ensure its complete stability and add the second layer of gum arabic gradually and stir constantly). The coating solutions were prepared as previously and used in cardamom seed oil encapsulation by two layers.

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 (0.1 %) 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.

4-Determination of Antioxidant Activity, by

a) ABTS assay

For 2,2- azinobis (3- ethyl-benzothiazoline - 6- sulfonic acid) (ABTS) assay, the procedure followed the method of **Arnao *et al.* [17].** Results are expressed in mM Trolox equivalents (TE)/ ml extract. Additional dilution was needed if the ABTS value measured was over the linear range of the standard curve.

b) FRAP assay

The ferric reducing antioxidant power (FRAP) assay was done according to method of **Benzie and Strain [18].** Results are expressed in mM TE/ml extract. Additional dilution was needed if the FRAP value measured was over the linear range of the standard curve.

c) DPPH assays

The 2,2'-diphenyl-1-picrylhydrazyl (DPPH) assay was done as reported by **Thaipong *et al.* [19]**, the antioxidant activity was determined by means of a calibration curve prepared with ascorbic acid, and expressed as mg of ascorbic acid equivalent (AAE) per ml of sample.

5-Determination of phenolic content

The phenolic content was determined using the Folin-Ciocalteu method. According to **Thaipong *et al.* [19]** it was determined using a calibration curve prepared with gallic acid and expressed as mg of gallic acid equivalent (GAE) per ml of sample.

6- Determination of flavonoid content

The total flavonoid content was determined as reported by **Thaipong *et al.* [19]** It was expressed as mg of catechin equivalent (CE) per ml of sample.

7- Acute oral toxicity study

Acute toxicity test is carried out on five different groups of rats (n=5). Each group was given 10, 15, 20, 25, and 30 times from the highest tested dose of EC extract (400 mg/kg); i.e., 4, 6, 8, 10, and 12 g/kg) orally by gavage. The animals were monitored for the next 24 hours to see if they died, and the LD50 was calculated according to **Gomaa *et al.* [20].**

Animals and ethical approval

The animals were kept in a 12/12 hr light/dark cycle at room temperature and relative humidity of 40-60%. Prior to the experiment, the animals were acclimatised and fed on standard pellet diet and had unlimited access to water for seven days. 60 rats were divided into six groups after a week of acclimatisation (each containing ten animals). The National Research Centre's Ethics Committee approved all experimental protocols under number 19-229.

Experimental design

Sixty albino male rats (110- 170 g) were randomized divided into six groups / 10 animals each.

Group I (normal control): rats were received 5% Tween 80 orally.

Group II (AD): rats were injected intraperitoneal with $AlCl_3$ solution (100 mg/kg) for 42 days for AD induction then serve as disease control.

Group III (AD+DP): rats were injected intraperitoneal with $AlCl_3$ solution, then treated with donepezil hydrochloride (commercial name; Arecipt) (1 mg/kg) orally for 42 days, 1 hour after administration of $AlCl_3$.

Group IV (AD +ECO): rats were injected intraperitoneal with $AlCl_3$ solution and treated with crude form of EC oil (1/10 LD50) orally for 42 days, 1 h after $AlCl_3$ administration.

Group V (AD + NCEO): rats were injected intraperitoneal with $AlCl_3$ solution, then treated by capsulated nanoparticles of cardamom (EC) oil at dose 1/10 orally for 42 days, 1 h after $AlCl_3$ administration.

Group VI (AD +DP+NCEO): rats were injected intraperitoneal with $AlCl_3$ solution, then received encapsulated nanoparticle of EC oil (1/10 LD50) plus donepezil hydrochloride orally for 42 days, 1 h after $AlCl_3$ administration [3].

Induction Alzheimer's disease

According to **Kandimalla *et al.* [21],** In distilled water, an $AlCl_3$ solution was prepared and injected intraperitoneally into rats with $AlCl_3$ solution (100 mg/kg) for 42 days for AD induction.

Collection of samples

Serum was separated from blood samples taken from the posterior vena cava and stored at 20 °C until further use. All animals were sacrificed under ether 2% anesthesia after an overnight fast. The hippocampi of individual rats were removed, placed on dry ice, dissected, dried, weighed, and stored at 80 °C before being homogenized in phosphate-buffered saline (pH 7.4) and centrifuged for 10 minutes to remove debris. The samples' supernatants were snap-frozen in liquid nitrogen and kept at -20 °C until ELISA, spectrophotometric, and qRT-PCR analyses were carried out. Protein concentration was calculated using Lowry's method **[22].**

Biochemical measurements

Pro-inflammatory cytokines such as

Interleukin-17 (IL-17) was measured by ELISA according to the method described by **Numasaki *et al.* [23]** following the manufacturer's protocol (R&D systems).

Cholinergic Parameters

Acetylcholine (Ach) was measured by ELISA kit according to the method described previously **[24]** following the manufacturer's protocol (R&D systems).

Acetylcholinesterase (ACHE), was measured by ELISA Kit according to Ellman's assay **[25]** following the manufacturer's protocol (R&D systems).

Ellman's assay

Amyloid beta (Aβ)

Amyloid precursor protein was measured by ELISA Kit according to the method described previously **[26]** following the manufacturer's protocol (R&D systems).

Brain-derived neurotrophic factor (BDNF)

Brain-derived neurotrophic factor (BDNF) was measured by ELISA Kit using the procedure outlined earlier **[27]** following the manufacturer's protocol (R&D systems).

Expression of PGC-1α mRNA and FNDC5 mRNA in brain by reverse transcription-quantitative polymerase chain reaction (RT-qPCR)

Total RNA was extracted from the brain using TRIzol reagents (Invitrogen, Carlsbad, CA, USA) per the manufacturer's instructions. RNA was quantified spectrophotometrically in a NanoPhotometer after being purified and DNase-treated with the RNeasy mini Kit (Qiagen, USA) (NanoDrop 2000, Implen, Germany). The extracted RNA was reverse transcribed into cDNA using a high-capacity RNA to cDNA kit (Applied Biosystems, USA), with incubation at 37 °C for 1 h, inactivation at 95 °C for 5 minutes, and storage at - 20 °C until use. Real-time PCR was carried out on an Agilent Mx3000P Light Cycler with Quantitect SYBR Green PCR reagents. The following primers were purchased from the Qiagen Quantitect collection: fibronectin type III domain containing 5 (FNDC5) (Rn Fndc5 2 SG Quanti Tect Primer Assay, Cat #QT02383276), peroxisome proliferator activated receptor-gamma coactivator1 alpha (PGC-1) (Rn Ppargc1a 1 SG QuantiTect Primer Assay, Cat # Cycling conditions were 15 minutes at 95 degrees Celsius, followed by 45 cycles of 15 seconds at 95 degrees Celsius, 30 seconds at 60 degrees Celsius, and 30 seconds at 72 degrees Celsius. The $2^{-\Delta\Delta Ct}$ method was used to analyse the data, with β-actin serving as the internal reference gene **[28].**

Statistical analysis

SPSS version 25 was used to analyse all data (IBM corporation, Armonk, NY, USA). Statistical significance was defined as a p value 0.05. Data was presented as mean standard deviation (SD). Tukey's post hoc test was used (ANOVA) if the p value in one-way analysis of variance was 0.05.

3. Results

Data in table (1) showed that crude form of cardamom oil having high content of alpha. -Terpinyl acetate (50.24 %) and Eucalyptol (39%) when analyzed by gas chromatography-mass spectrometry analysis.

The results in Table (2) showed that the total contents of polyphenols and flavonoids in the form of extracted cardamom oil as well as the nano-capsule. The cardamom oil sample that was coated with two layers of showed the highest value of TP (2.31 g GAE/100 g), while the untreated crude oil sample with the lowest activity of 1.77 g GAE/100 g oil. Also, it was found that the cardamom oil sample encapsulated in a nano-capsule had the highest value of flavonoids, which was 5.03 gm of catechins/100 gm of oil, followed by a sample of cardamom oil extracted and it contained 4.52 gm of catechins/100 gm of oil.

The data in Table (4) showed that there is a significant decrease in BDNF and Ach a significant increase in IL-17, Aβ and AChE in AD group when compared with control group. It has been established that donepezil treatment is associated with a significant increase in BDNF and Ach and significant decrease in IL-17, Aβ and AChE when compared with Alzheimer group while treatment with cardamom oil in the two forms (crude & nano) showed significant increase in BDNF and significant decrease in

IL-17, Aβ and AChE when compared with Alzheimer group. Based on the findings reported here we find that 1/10th nano-encapsulated cardamom oil gives promising results similar to the group treated with donepezil which is the first choice treatment of Alzheimer but treated with nanoencapsulated cardamom oil is more safe and without side effects of chemical products. Nanoe-ncapsulation could be a promising technique for addressing these limitations because it prevents essential oil exposure and degradation by forming a physical barrier that protects the bioactive constituents.

Table (1) Chemical components of cardamom (*Cardamum Elettaria*) essential oil (CEO) by gas chromatography-mass spectrometry analysis (GC-MS)

Peak	RT	Name	Formula	Area	Area Sum %
1	11.545	.alpha.-Pinene	C10H16	7150622.1	0.84
2	12.997	Sabinene	C10H16	8500688.2	1
3	15.346	Eucalyptol	C10H18O	330336110	39
4	17.7	1,6-Octadien-3-ol, 3,7-dimethyl-	C10H18O	15360998	1.81
5	20.504	Terpinen-4-ol	C10H18O	6649294.6	0.78
6	21.034	.alpha.-Terpineol	C10H18O	16839489	1.99
7	23.104	Linalyl acetate	C12H20O2	27256136	3.22
8	25.709	.BETA.-TERPINYL ACETATE	C12H22O2	4769894.4	0.56
9	26.583	.alpha.-Terpinyl acetate	C12H20O2	425610688	50.24

Table (2): Total phenolic and flavonoids contents of cardamom oil extract and nano-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 nano-capsule	2.31±0.11	5.03±0.72

Table (3) Effect of treatment with cardamom oil (crude and nano-encapsulated forms) on the levels of PGC1-α and FNDC5 in brains of different groups using real time PCR technique

	PGC1-α	FNDC5
Group I	1.85 ± 0.08[a,c]	1.73 ± 0.2[b]
Group II	1.13 ± 0.07[b]	0.78 ± 0.17[a]
Group III	1.57 ± 0.2[c]	1.45 ± 0.26[b]
Group IV	1.1 ± 0.51[b]	1.66 ± 0.25[b]
Group V	1.65 ± 0.4[c]	2.99 ± 0.44[c]
Group VI	1.08 ± 0.21[b]	1.04 ± 0.14[a]

Group I = Control; Group II= Alzheimer's disease; Group III= Alzheimer's disease + Donepezil; Group IV= Alzheimer's disease + 1/10th crude cardamom essential oil; Group V= Alzheimer's disease +1/10th Nano-encapsulated cardamom essential oil; Group VI= Alzheimer's disease + Donepezil +1/10th Nano-encapsulated cardamom essential oil. Data sharing the same superscript= not significant (N.S); sharing different superscript = significant where $p< 0.05$.

Table (4) Effect of treatment with cardamom oil (crude and nano-encapsulated forms) on the levels of BDNF, IL-17, Aβ, ACh and AChE in brains of different groups

Group No	BDNF (Pg/ml)	IL-17 (Pg/ml)	Aβ (1-40) (Pg/ml)	AChE (ng/ml)	ACh (U/ml)
Group I	176.0 ± 2 .7[a,b]	67.4 ± 5.6[a,b]	34.0± 2.9[a,b]	3.64 ± 0.5 [a,b]	8.21 ± 1.0 [a,b]
Group II	127.32 ± 4.48[c]	114.5 ± 6.5[c]	76.0 ± 3.9[c]	6.69 ± 0.59[c]	3.01 ± 0.55[c]
Group III	174.0 ± 4.37[a,b,c]	58.70 ± 5.42 [a,b,d]	47.7 ± 4.1 [a,b,c]	3.2 ± 3.04[a,d,e]	5.71 ±0.81 [a,b]
Group IV	166 ± 2.66[a,b,d]	66.0 ± 5.0 [a,b,d]	42.0 ± 3.6 [a,b,d]	2.81 ± 0.43 [a,b,e]	3.8 ± 0.75[e]
Group V	165.0± 11.26 [a,b,d]	63.23 ± 7.93 [a,b,d]	35.0 ± 3.4 [a,b,d]	2.4 ± 0.4[a,c,d]	5.85 ± 0.78 [a,b]
Group VI	170.0 ± 5.1 [a,b,d]	56.9 ± 5.7 [a,b,d]	63.15± 4.80 [a,b,d]	3.72 ± 0.62[a,d,e]	5.71± 0.58 [a,b]

Group I = Control; Group II= Alzheimer's disease; Group III= Alzheimer's disease + Donepezil; Group IV= Alzheimer's disease + 1/10th crude cardamom essential oil; Group V= Alzheimer's disease +1/10th Nano-encapsulated cardamom essential oil; Group VI= Alzheimer's disease + Donepezil +1/10th Nano-encapsulated cardamom essential oil. Data sharing the same superscript= not significant (N.S); sharing different superscript = significant where $p< 0.05$.

4. Discussion

It is still debatable whether aluminum chloride levels are significantly elevated in Alzheimer's disease patients. A recent analysis of 37 studies involving 1227 participants (621 AD patients) found that the level of aluminum chloride is significantly higher in

the brain tissues, serum, and cerebrospinal fluid (CSF) of people with Alzheimer's disease (AD), implying that elevated aluminum chloride levels may be a marker of AD or play a critical role in AD development **[29].**
The current study found that rats exposed to aluminum chloride for 42 days displayed cognitive deficits, abnormal biochemistry, and histological changes in the brain resembling to dementia associated with Alzheimer's disease as shown in table (4) & fig (2). Treatment with CEO in crude or in nano-encapsulated form can improve cognition, biochemistry, and histology. Our results are in agreement with **Auti and Kulkarni, [3]; Gomaa *et al.* [20]; Weng *et al* [29].**
Disruption in cholinergic neurotransmission strongly correlates with the severity of neuropathological changes associated with Alzheimer's disease **[30]. Xie *et al.* [31] & Justin *et al.* [32]** reported that $AlCl_3$ disrupts cholinergic neurotransmission by inducing AChE activity and thus increasing ACh breakdown in the brain. The present study's findings suggested an association between an elevated serum level of acetylcholinesterase (AChE) and a decreased serum level of acetylcholine (ACh) in AlCl3-intoxicated rats (table 4). The direct effect of $AlCl_3$ may be responsible for the elevated AChE activity that was observed in $AlCl_3$-treated rats **[33].** It has been reported that $AlCl_3$) can interact with the peripheral sites of AChE, modifying its secondary structure and, as a result, increasing its activity **[34].** Inhibiting AChE is an important beneficial strategy in the treatment of AD. AChE inhibitors improve acetylcholine accessibility by preventing its destruction, which aids in the management of Alzheimer's disease symptoms by improving cholinergic transmission in the brain **[35; 36].** In the current study, cardamom oil provid neuroprotection by decreasing AChE activity in aluminum-treated rats, possibly by lowering aluminum loading in the hippocampus and cortex. Our results were in agreement with **Lin *et al.* [37]; Auti and Kulkarni, [3]; Mohamed *et al.* [38].**
It has been demonstrated that natural substances, such as essential oils, possess potent AChE inhibitory activity **[3].** Compared to normal control rats, aluminum chloride treatment increased AChE activity in our study. It has been demonstrated in vitro that 1,8 cineole, the primary chemical constituent of cardamom oil, inhibits the AChE enzyme.
Chronic $AlCl_3$ exposure increased Aβ formation while decreasing degradation **[39].** Similarly, results in the present study showing a significant increase in the serum level Aβ in rats with AD as compared to their control counterparts which are in agreement also with **Gomaa *et al.* [20]** who postulated that the positive effect of CEO on the rate Aβ formation is mediated by CEO antioxidant property and inflammatory suppression. Also, CEO lignans have been shown to inhibit Aβ oligomerization and fibril formation in AD animal models, implying a potential neuroprotective effect.
Aβ aggregation also stimulates IL-17 production as reported by **Dubenko *et al.* [40].** The main role of IL-17 in AD pathogenesis appears to be the attraction of neutrophils and the stimulation of their function. Furthermore, higher levels of IL-17 were found in the serum of Alzheimer's disease patients than in healthy controls **[41].** Similarly, the current study revealing a significant increase in rats' serum IL-17 levels associated with AD (group II). Administration of CEO as well as DP solely or combined with each other resulting in a significant reduction in IL-17 and Aβ serum levels (table 4).
However, the impact of IL-17A on AD pathogenesis is controversial. A study found that IL-17 played a protective role in an animal model of Alzheimer's disease. Overexpression of IL-17 in the brain reduced cerebral amyloid angiopathy and improved anxiety and learning deficits **[42].** Another co-player in AD is BDNF which is one neurotrophic factor that has been linked with AD since it is positively correlated with learning and memory. BDNF plays an important role in neuronal cell differentiation and growth, as well as protecting neurons from injury and inhibiting neuroinflammation **[43].** As a result, it appears reasonable to speculate that there may be a link between BDNF levels in the brain and serum **[44].**
It has been noted that Alzheimer's patients have lower levels of BDNF. BDNF enhances central cholinergic neurotransmission in vitro and in vivo studies. It has also been noted that BDNF modulates and regulates synaptic neuronal plasticity, resulting in increased synaptic transmission and neuroprotection against brain insults **[3].** In the current investigation, treatment with cardamom oil in nano-encapsulated form showed significant increase in BDNF level.
Donepezil is a mixed competitive, reversible, centrally acting, and highly selective acetylcholinesterase inhibitor (AChE). Because donepezil is a lipophilic substance, it accumulates in lipid tissue such as the brain and thus inhibits AChE activity in the brain for a longer period of time than in the blood **[45].** In the present study, results revealed a significant reduction in the serum level of AChE and IL-17 in rats associated with AD treated with DPZ (group III) as compared to their levels in rats with AD without treatment (group II) as shown in (table 4). **Jian *et al.* [46]** revealed that donepezil also reduces neurodegeneration in the cortex and hippocampus by increasing BDNF expression. Donepezil is a traditional acetylcholinesterase inhibitor that can also increase BDNF expression in the CNS, inproving neuronal activity and plasticity while also protecting neuronal cells. The current study's findings agreed with those of the previous authors.
Cardamom oil doses between 400 and 600 mg/kg were found to be safe for the animals in studies by **Gomaa et al. [20]** and **Yudhani et al. [47].** The highest extract dose considered to be safe was 400 mg/kg. The dose of oil extract was used 1/10 th 400 mg/kg as a therapeutic dose and no mortality was noticed during the period of the experiment.
CEO demonstrated anti-inflammatory activity in a variety of inflammatory disorders **[48],** in a diabetic retinopathy model **[49],** and in a neuron and microglia culture **[50].** As far as we know, no study until now is carried out to evaluate the effect of CEO in nano-encapsulated form on the level of Ach, AchE, and IL-17 in rat intoxicant with aluminum chloride.
In agreement with **Auti & Kulkarni [3]; Daneshi-Maskooni *et al.* [51]** the current study showed a significant elevated in FNDC5 levels in the brain tissues of rats with AD when treated with cardamom oil in crude form (group IV) as shown in (table 3). Also, using NCEO at concentration 1/10th of the safe dose (400 mg) (group V) showing significant upgrading of PGC-1α level as well as FNDC5 level in brain tissues of rats comparing to rats treated with DP (group III), crude oil (group IV) and mixture of NCEO plus DP (VI) as shown in table 3.
In agreement with **Kaur *et al.* [52]; Amidfar *et al.* [53]; Peng and Wu. [54],** the present study revealed a significant decrease in the brain tissues levels of PGC-1α & FNDC5 in rats with AD (group II) as compared to their control counterparts (group I)

as shown in table 3. There is evidence that the PGC-1/FNDC5/BDNF axis is important for FNDC5/irisin-induced neuroprotection.
The current study sought to investigate the neuroprotective effects of cardamom oil in Alzheimer's disease-induced rats in form of depletion of pyramidal layer and granular layer, also there are amyloid plaque, neurofibrially tangles as a long-pink-filaments in the cytoplasm with granulovacuolar degeneration of neuronal cells congested cerebral vessels.
Our results also, were in agree with **Auti and Kulkarni, [3]** who postulated that cardamom oil treatment showed significant inhibition of AChE activity. In addition, hematoxylin and eosin (H. & E.) and congo red staining of the hippocampus and cortex revealed inhibition of neuronal damage and amyloid β plaque formation as they used cardamom oil in crude form for treatment rat with AD **[2].** Our study revealed that the groups treated by cardamom of nano form showed an improvement of neuronal degenerative changes caused by Alzheimer disease as decreased number of amyloid plaque and neurofibrially tangles in the cytoplasm **(Fig. 5).**

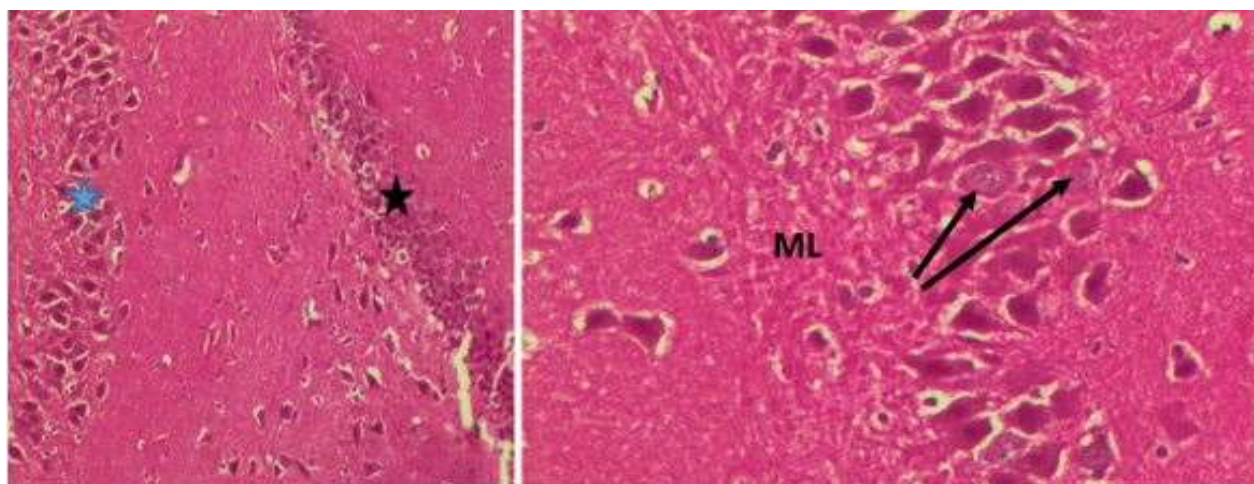

Figure (1): A photomicrography of hippocampus region of (**Group I; control**) showing shows layers of compact granular cells with dark nuclei **(black star)** alternating with 5–6 compact layers of small and large pyramidal cells **(blue star)**, most have vesicular nuclei **(black arrow).** Molecular layer **(ML)** reveals numerous glial cells among neuronal processes **(H & E 100x&200x).**

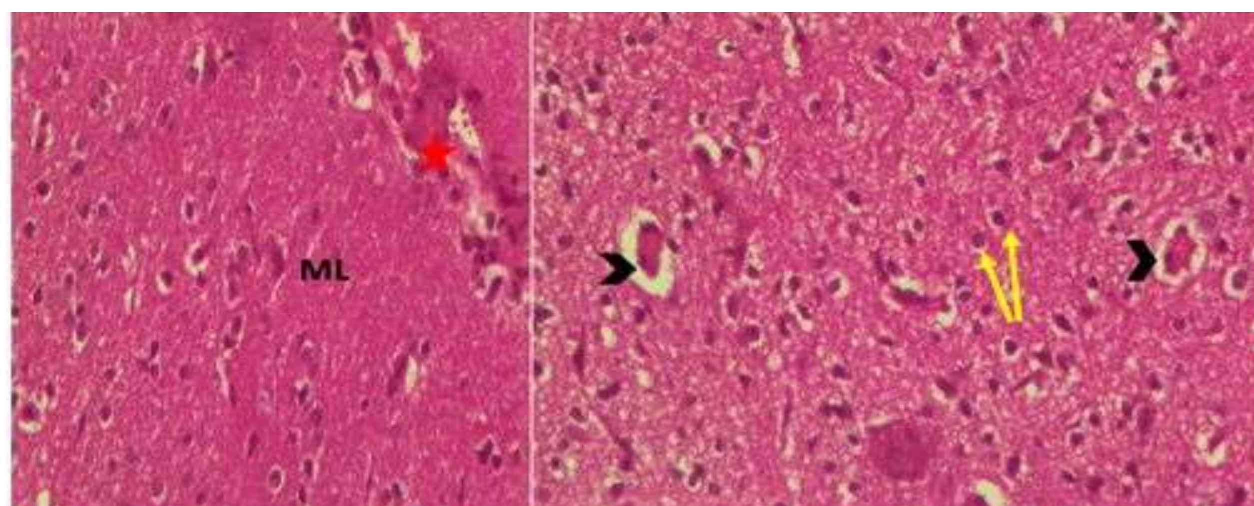

Figure (2): A photomicrography of hippocampus region of **Group II; Alzheimer's disease (AD)** demonstrates disorganisation and cell loss in small pyramidal cells **(red star)**, some of which had pale nuclei while others had dark nuclei. The size of the large pyramidal cells shrank significantly **(black head arrows),** with the outer layer being more affected, with darkened nuclei. The granular layer also had significant vacuolation, whereas the molecular layer **(ML)** had enlarged neurons **(yellow arrows)** and an excess of glial cells **(H & E 100x&200x).**

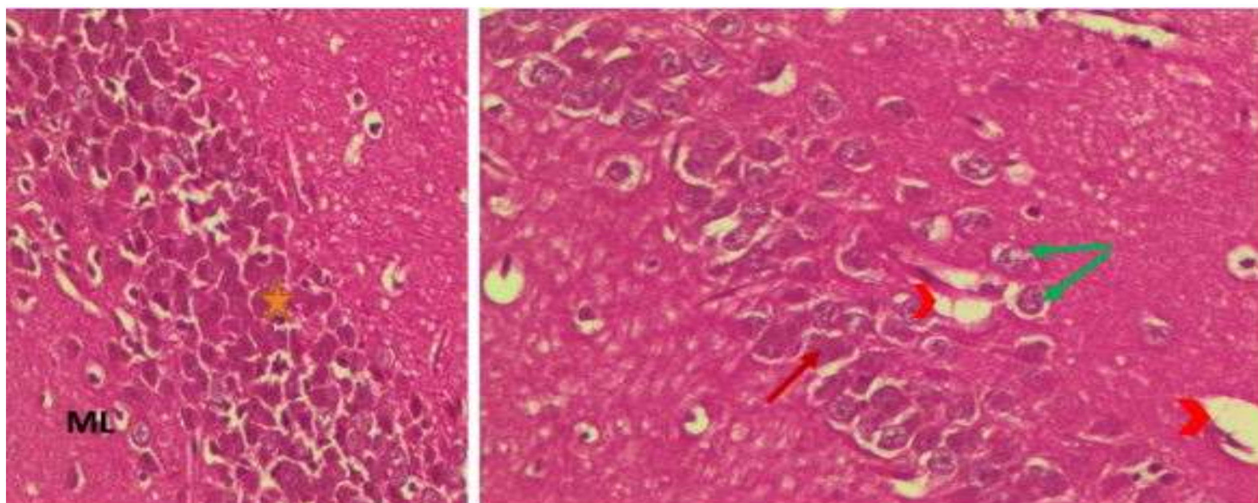

Figure (3): A photomicrography of hippocampus region of (**Group III; Alzehimer + Donepezil (DP)**) showing small pyramidal cells are preserved **(green arrows)**; but with large pyramidal cell apoptosis **(red arrow)**, not well organized cell layers **(light brown star)**, Granular cell population is average. The Molecular Layer (ML) primarily displays normal cells & fibers, with widened capillaries **(red head arrows) (H & E ×200).**

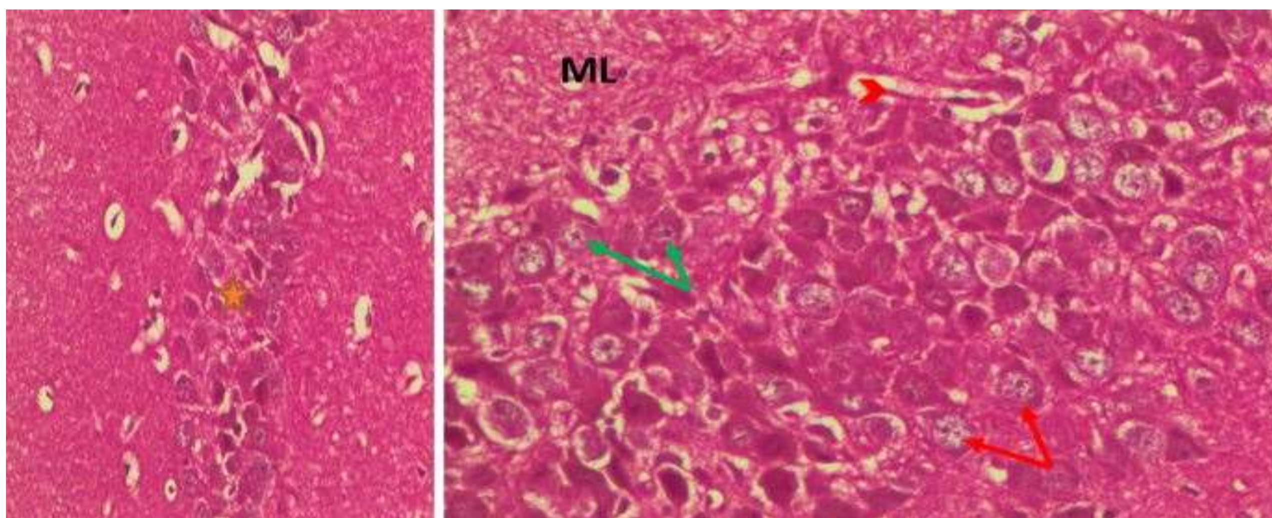

Figure (4): A photomicrography of hippocampus region of (**Group IV; Alzehimer + 1/10 Crude cardamom oil**) showing preservation of small pyramidal cells **(green arrows)** and large pyramidal cells **(red arrow)**, not well organized cell layers **(light brown star)**, average Granular cell population The Molecular Layer **(ML)** primarily displays normal cells & fibers, with widened capillaries **(red head arrows) (H & E 100x,200x).**

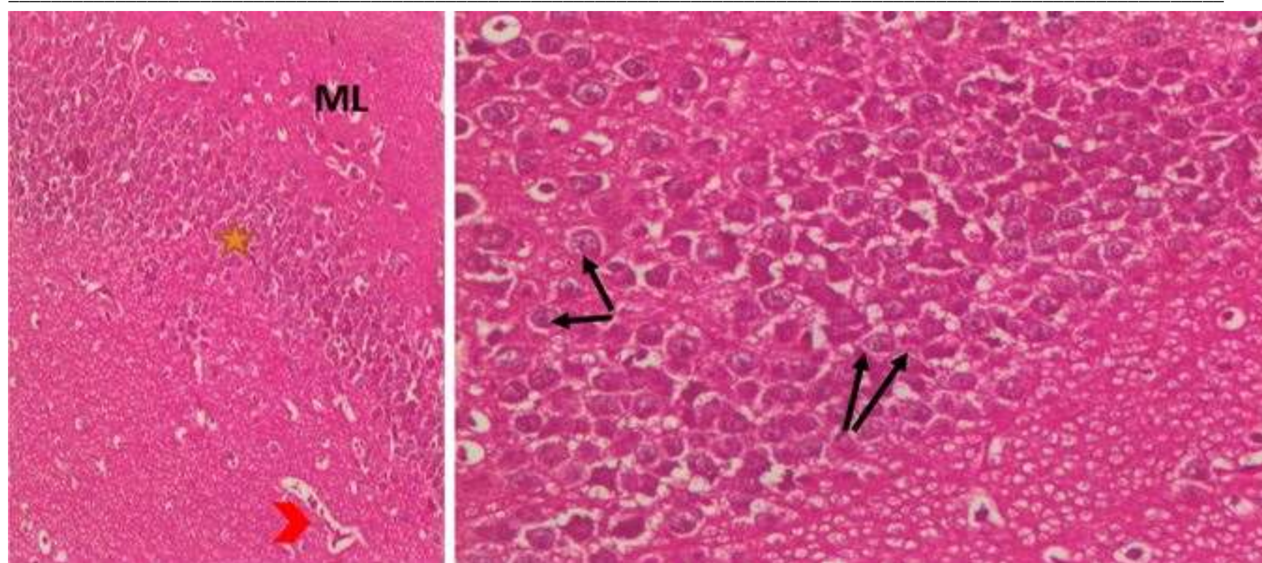

Figure (5): A photomicrography of hippocampus region of (**Group V; Alzehimer 1 /10 Nano-encapsulated cardamom essential oil)** showing well persevered pyramidal cells with vesicular nuclei **(black arrows)**, well organized cell layers **(light brown star)**, average population of granular cells. Molecular layer **(ML)** mostly shows normal cells & fibres, with widened capillaries **(red head arrows) (H & E 100x,200x).**

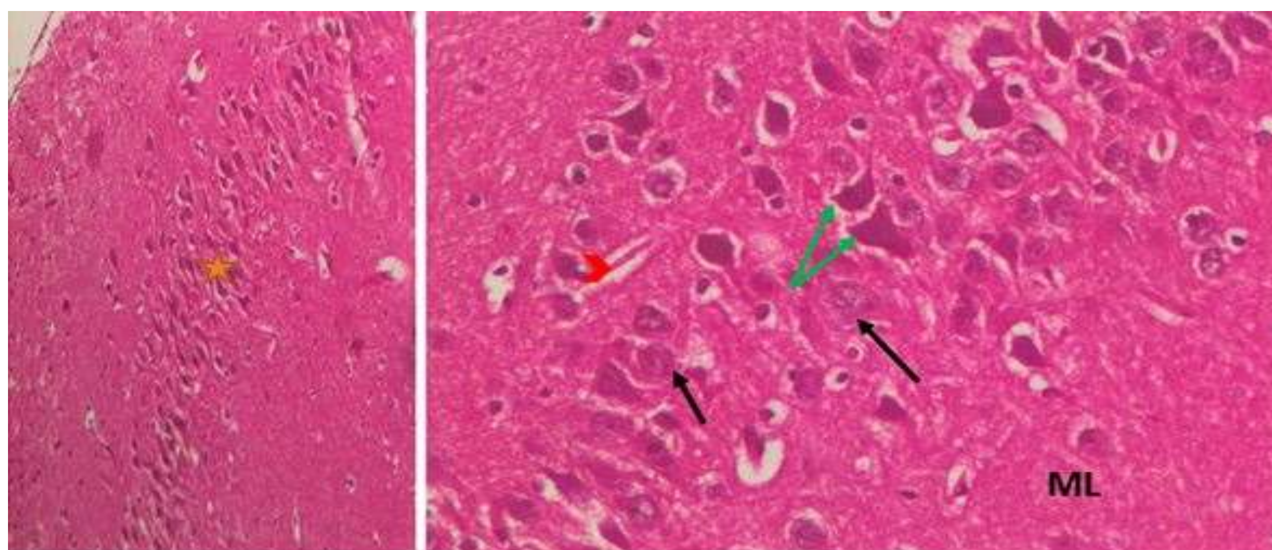

Figure (6): A photomicrography of hippocampus region of (**Group VI; Alzehimer + 1/10 Nano-encapsulated cardamom essential oil + Donepezil)** showing well persevered pyramidal cells **(green arrows)** with vesicular nuclei **(black arrows)**, well organized cell layers **(light brown star)**, average granular cell population The Molecular Layer **(ML)** is primarily composed of normal cells & fibers, with widened capillaries **(red head arrows) (H & E 100x,200x).**

Conclusion

The present study discussed the favorable and promising effects of cardamom oil in nano-encapsulated form (NCEO) as a natural product which has anti-inflammatory effects as well as biochemical effects on cholinergic system, to prevent or delay the development of Alzheimer's disease. To achieve this goal, we used donepezil (DP), as a classical drug used for AD treatment, for comparing both forms of cardamom oil, crude (ECO) and nano-encapsulated (NCEO). The present study investigated that cardamom oil is acting as a natural neuroprotective agent. We conclude that cardamom oil in nano form may become a promising management of Alzheimer's disease in the future.

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Authors' contributions
MNA, TRE, KFM and MMR put the design of this study. AAS, OA, NNY and MAA carry out the experimental and practical parts. MHA contributed in the interpretation of the data. All participated in the manuscript writing and revision. And finally, all authors have read and approved the manuscript.

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There are no conflicts of interest.

Ethics approval
The National Research Centre's Ethics Committee approved all experimental protocols under number 19-229.

Consent for publication
All the authors listed in this article have approved the final version to be published.

Availability of data and materials
All data generated or analyzed during this study are included in this published article.

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