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The protective effects of nano-selenium particles and thymoquinone against rats' lipopolysaccharide-induced Alzheimer's disease

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Abstract: Neurodegenerative dementia most commonly manifests as Alzheimer's disease, with a highly complex pathophysiology. Nano-selenium can decrease the neuronal damage that occurs in neuronal diseases; also, thymoquinone has significant antioxidant and anti-inflammatory effects, so it could be used as an effective neuroprotective agent as an additive treatment option for Alzheimer's disease. This study aims to evaluate the effect of nano-selenium and thymoquinone (TQ) on neurodegeneration and neuroinflammation in Alzheimer's disease. Fifty male rats with albinism were classified into five equivalent groups each of ten rats, in which a group of them was used as a control (group 1). In contrast, the other forty rats were injected with lipopolysaccharides to induce Alzheimer's model and then were divided into positive control rats (group 2), rats given nano-selenium (group 3), rats given thymoquinone (group 4), and rats given both nano-selenium and thymoquinone (group 5). Amyloid β-42 (Aβ-42), tumor necrosis factor-α (TNF-α), and interleukin-6 (IL-6) were estimated by ELISA, whereas glutathione (GSH) and malondialdehyde (MDA) were estimated by colorimetry. The mean expression levels of Aβ-42, TNF-α, IL-6, and MDA were significantly decreased, and the mean expression level of GSH was significantly increased in rats given treatments compared to the group of Alzheimer rats with no treatment. This study demonstrated that nano-selenium and thymoquinone can be additive therapies for Alzheimer's disease.

Keywords: Neurodegeneration, selenium nanoparticles, Alzheimer's, thymoquinone, rats, neuroprotection.

1. INTRODUCTION

A persistent deterioration in cognition is the outcome of Alzheimer's disease (AD), the most frequent cause of dementia[1]. Alzheimer's disease is a long-term, crippling neurological condition that gradually impairs behavior, memory, and cognitive function[2].

Cognitive capacities gradually declining is the hallmark of Alzheimer's disease (AD), which has been connected to a substantial decrease in brain volume in AD patients relative to healthy individuals. The atrophy is caused by the loss of neurons, the deterioration of synapses, and the disruption of neural connections, especially in the hippocampus, a portion of the brain involved in memory and spatial orientation[3,4].

The accumulation of amyloid β-42 (Aβ-42) outside of cells is a neuropathological sign of Alzheimer's disease. An increasing body of research indicates that improved neuroinflammatory processes play a critical role in the onset of AD[5]. Even though Aβ-42 is often only produced in trace levels, it is the primary Aβ type that is deposited in plaques. Studies conducted in vitro and in vivo have shown that Aβ42 is required for the initial vascular Aβ deposition seeding [6,7]. These cytokines function as biomarkers to assess biological responses to therapeutic intervention as well as pathologic and normal biological processes [8].

Cytokine storm is a potentially fatal inflammatory reaction that is typified by an overreactive immune system. It may result from different treatments, autoimmune diseases, or

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infections [9]. A cytokine storm is ultimately caused by the release of interleukin-6 (IL-6), IL-10, and tumor necrosis factor-alpha as a result of neuroinflammation[10]. Although interleukin (IL-10) is considered the ultimate anti-inflammatory cytokine, it has been demonstrated that T-cell-derived IL-10 acts on effector T cells during neuroinflammation, promoting their survival and escalating inflammation and CNS autoimmunity [11]. Because it can penetrate the blood-brain barrier, tumor necrosis factor-alpha is known to activate astrocytes and microglia, escalating inflammation [10]. Studies have repeatedly recommended that TNFα should be pharmaceutically targeted to minimize systemic inflammation and avoid neurotoxicity in AD. TNFα has been identified as an inflammatory biomarker highly related to AD [12].

In several neurovascular disorders, oxidative stress has been identified as a well-known pathogenic state. The process begins with an increase in the production of highly oxidizing free radicals (such as reactive oxygen species, or ROS, and reactive nitrogen species, or RNS), which build up to a point where the endogenous antioxidant system is unable to neutralize them. This leads to a seriously disrupted balance between the levels of antioxidants and free radicals, which damages cells. Numerous studies have demonstrated the crucial role that oxidative stress plays in initiating and progressing certain cell signaling pathways linked to neurological disorders, including Alzheimer's disease (AD) [13]. It has been proposed that high MDA and low GSH levels play a crucial role in the pathophysiology and neuronal damage experienced by AD patients [14].

The condition is not effectively treated by currently available medicines, including monotherapies and combination therapies, due to the disease's complicated pathological process [15].

A perennial blooming plant native to Mediterranean and Asian regions, Nigella sativa (N. sativa) is a member of the Ranunculaceae family and thrives in Afghanistan, India, Indonesia, Pakistan, and Italy. Most people refer to it as black cumin or black seed. The well-known properties of this herbal plant include antibacterial, antiviral, anti-inflammatory, wound healing, and dermatological [16]. Thymoquinone (TQ), a bioactive volatile oil component obtained from black cumin (Nigella sativa) seeds, exhibits strong antioxidant and anti-inflammatory properties, suggesting that it may be used as a neuroprotective drug [17].

TQ was administered as an anti-neurodegenerative medication to AD patients to shield hippocampus neurons from the neurodegenerative effects of Aβ. TQ offers exceptional protection to brain cells against damage caused by oxidative stress. TQ is also used to treat drug dependence and abuse in cases of cognitive and memory impairments. Because of its antioxidant and anti-inflammatory properties, TQ slows the progression of AD[18].

In tiny amounts, selenium (Se) is a naturally occurring metalloid element that is vital to both human and animal health, but excess selenium can be detrimental. Se is essential to the proper operation of the human body. Because it is integrated into selenoproteins, antioxidant defense systems are supported. Selenoproteins have neuroprotective properties, regulate reproductive processes, and take a role in the metabolism of thyroid hormones. Se has one of the tightest gaps between hazardous levels and nutritional deficits among all the elements. Because inorganic and organic species have different biological properties, the toxicity of a substance may vary depending on its chemical type [19].

Recent developments in nanotechnology have the potential to provide mass-market screening and treatment solutions at low cost. Nanoparticles (NPs) can effectively penetrate the blood-brain barrier (BBB) for targeted drug delivery with low toxicity and few side effects [20].

The aim of the study is to evaluate the effect of nano-selenium and thymoquinone treatments on AD rat models through the estimation of brain tissue levels of amyloid beta-42 and serum levels of TNF-α and IL-6 by ELISA technique. Also, the estimation of Glutathione (GSH) and Malondialdehyde (MDA) by colorimetric method to investigate the possibility of using nano-selenium particles and thymoquinone as additive therapy in AD.

2. METHODS

2.1. Animals

From the experimental animal section at Cairo University's Faculty of Medicine, 50 albino male rats weighing between 150 and 200 g were purchased. A semi-purified diet consisting of (g/kg): 100g Casein, 750g Sucrose, 50g Cellulose, 50g Fat blends, 10g Vitamin mix, and 40g Mineral mix was provided to the rats in their pathogen-free, sterile, and temperature-regulated housing. This formulation satisfies the nutritional requirements for the growth of rats. The rats also had free access to water at 22 degrees Celsius and a 12-hour light/dark cycle.

Randomly chosen rats were divided into five equal groups, each with ten rats. The intended design of the experiment was as follows:

•Ten rats served as the **group 1** healthy control rats that received nothing.

• Alzheimer's disease was induced among all rats in the successive four groups by intraperitoneal (IP) injection with 0.8 mg/kg body weight of lipopolysaccharides (LPS) for 1 week [21]. 10 rats were positive AD control **(group 2),** and the remaining 3 groups (each with 10 rats) received the following therapy after the first week:

•**Group 3**: received a daily injection of nano-selenium at a 5 mg/kg dose (referring to the previous studies on this compound by Gudkov *et al*. [22]) throughout four weeks.

•**Group 4**: received a daily injection of thymoquinone at a 2 mg/kg dose (referring to the previous studies on this compound by Tabeshpour *et al*.[23] and Fadishei *et al*.[24]) throughout four weeks.

•**Group 5**: received a daily injection of nano-selenium and thymoquinone at a dose of (5 mg/kg and 2mg/kg respectively) throughout four weeks.

The thymoquinone solution was prepared by dissolving it in 0.5% dimethyl sulphoxide and adding olive oil. At the same time, the fermented soy's aqueous portion served as a precursor for the creation of SeNPs, in which the stabilization of the nanoparticles was achieved by the proteins presented in the fermented soy extract, which were created by the surface-bound proteins by adsorbing as a coating over the synthetic SeNPs.

2.2. Sampling and preparation

Rats' venous blood was drawn from the retro-orbital vein before beheading, left to clot for thirty minutes, and then centrifuged at 10,000 × g for twenty minutes. The serum was frozen until the measurement of IL-6 and TNF-α levels using the ELISA technique. Animals were then anesthetized with sodium phenobarbital (60 mg/kg) and killed by decapitation followed by brain tissue extraction.

The brains were quickly removed and homogenized in PBS (phosphate buffered saline) solution for estimation of amyloid beta-42 by ELISA technique in addition to GSH and MDA by colorimetric method.

2.3. Biochemical Tests

2.3.1. Estimation of amyloid beta-42 interleukin-6, tumour necrosis factor-alpha and by ELISA technique

That test used the quantitative sandwich enzyme immunoassay technique. An antibody specific to the measured marker (either IL-6, TNF-α, or Aβ-42) had been pre-coated on a microplate. The immobilized antibody bound any detectable marker (IL-6, TNF-α, or Aβ-42) that was present in the wells where samples and standards were pipetted. After removing any unbound materials, the wells were supplied with a biotin-conjugated antibody that was targeted for the marker being examined (IL-6, TNF-α, or Aβ-42). Following the wells' cleaning, Horseradish Peroxidase (HRP) coupled with Avidin was added. The wells were then cleaned to remove any remaining enzyme-avidin reagent and then filled with a substrate solution. The quantity of detected marker (IL-6, TNF-α, or Aβ-42) bound during the first phase was correlated with the amount of color produced. The color's intensity was measured at 450 nm after the color development had stopped. The supplier of the kit was (Cusabio, China).

2.3.2. Estimation of Glutathione and Malondialdehyde by colorimetric method

A Bio diagnostic colorimetric test kit was used to measure GSH. GSH was used to reduce 5, 5'-Dithiobis (2-nitrobenzoic acid) (DNTB) to a yellow chemical product. It was shown that the lowered chromogen's absorbance at 405 nm was directly correlated with the GSH concentration.

A Bio diagnostic colorimetric assay kit was used to measure MDA. Thiobarbituric acid (TBA) and lipid peroxidation products (MDA) reacted in an acidic media, and the colored complex that resulted was measured as the basis for the procedure. The color that was acquired was measured at 534 nm.

2.3. Statistical Analysis

Utilizing the statistical package for social science (SPSS Inc., Chicago, IL, USA, version 21), the statistical analysis was carried out. The following tests were performed to evaluate the central tendency of the data as well as their distribution concerning the mean values: X mean and SD standard deviation. The biological variables were compared using a one-way analysis of variance (ANOVA). Using the Pearson correlation coefficient test (r), it was possible to ascertain whether two numerical variables had a linear relationship. For a two-tailed test, statistical significance was determined by using $P < 0.05$.

3. RESULTS

3.1. Biochemical traits of the subject under study

According to **Table (1):**

Amyloid beta-42 level (pg/mg protein) in brain tissue:

As shown in **Figure 1**, In comparison to the group of Alzheimer's rats receiving no treatment (11.21 ± 2.13 pg/mg protein), the mean brain tissue level of amyloid beta-42 in rats treated with nano-selenium, rats treated with thymoquinone, and rats

treated with both nano-selenium and thymoquinone (4.86 ± 2.37, 5.80 ± 2.03 and 3.91 ± 1.85 pg/mg protein, respectively) were significantly lower. The mean brain tissue level of amyloid beta-42 did not differ significantly between the rats treated with thymoquinone (5.80 ± 2.03 pg/mg protein) and nano-selenium (4.86 ± 2.37 pg/mg protein) at $p > 0.05$. Additionally, there was no statistically significant difference observed in the mean brain tissue levels of amyloid beta-42 between the rats treated with nano-selenium (4.86 ± 2.37 pg/mg protein) and the rats treated with thymoquinone (5.80 ± 2.03 pg/mg protein) when compared to the rats treated with both treatments (3.91 ± 1.85 pg/mg protein $p > 0.05$).

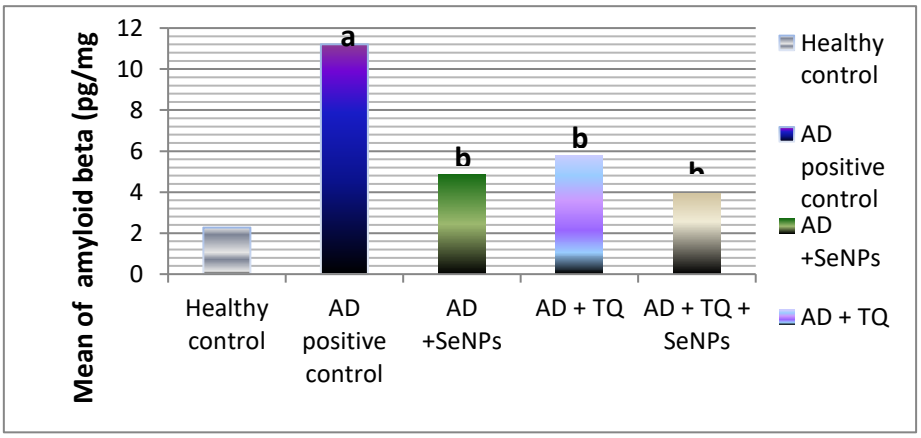

Figure 1. The mean of amyloid beta-42 in all the studied groups (a. Significant from the healthy control group at $p < 0.05$. b. Significant from AD positive control group at $p < 0.05$. c. Significant from AD + SeNPs group at $p < 0.05$. d. Significant from AD + TQ at $p < 0.05$.

Tumor necrosis factor-alpha levels (pg/ml) in serum:

As shown in **Figure 2**, In comparison to the group of Alzheimer rats receiving no treatment (105.65 ± 10.90 pg/ml), the mean serum levels of TNF-α in rats treated with nano-selenium, rats treated with thymoquinone, and rats treated with both nano-selenium and thymoquinone (63.03 ± 11.07, 66.05 ± 9.96, and 36.41 ± 10.53 pg/ml, respectively) were significantly lower. Between the rats treated with thymoquinone (66.05 ± 9.96 pg/ml) and nano-selenium (63.03 ± 11.07 pg/ml), there was no discernible difference in the mean serum level of TNF-α ($p > 0.05$). However, the mean serum TNF-α level in the group of rats treated with nano-selenium and thymoquinone was significantly lower (36.41 ± 10.53 pg/ml) than in the groups treated with nano-selenium (63.03 ± 11.07 pg/ml) and thymoquinone (66.05 ± 9.96 pg/ml) at $p < 0.05$.

Interleukin- 6 levels (pg/ml) in serum:

As shown in Figure 3, In comparison to the group of Alzheimer rats receiving no treatment (99.73 ± 13.72 pg/ml), the mean serum levels of IL-6 in rats treated with nano-selenium, rats treated with thymoquinone, and rats treated with both nano-selenium and thymoquinone (61.43 ± 11.58, 68.06 ± 6.39, and 45.30 ± 10.30 pg/ml, respectively) were significantly lower. The mean blood level of IL-6 did not differ significantly at $p > 0.05$ between the rats treated with nano-selenium (61.43 ± 11.58 pg/ml) and thymoquinone (68.06 ± 6.39 pg/ml) groups. On the other hand, the mean serum level of IL-6 in the rats treated with thymoquinone and nano-selenium was significantly lower (45.30 ± 10.30 pg/ml) than in the rats administered nano-selenium (61.43 ± 11.58 pg/ml) and thymoquinone (68.06 ± 6.39 pg/ml) at $p < 0.05$.

Glutathione level (mmol/mg protein) in brain tissue:

As shown in **Figure 4,** in comparison to the group of Alzheimer rats receiving no treatment (21.22 ± 4.62 mmol/mg protein), the mean brain tissue level of GSH in rats treated with nano-selenium, rats treated with thymoquinone, and rats treated with both nano-selenium and thymoquinone (41.16 ± 5.87, 32.26 ± 9.14, and 46.37 ± 11.64 mmol/mg protein, respectively) were significantly higher. Between the rats treated with thymoquinone (32.26 ± 9.14 mmol/mg protein) and nano-selenium (41.16 ± 5.87 mmol/mg protein), there was no discernible variation in the mean GSH level in brain tissue ($p > 0.05$). The group of rats treated with nano-selenium (41.16 ± 5.87 mmol/mg protein) and the group of rats treated with both nano-selenium and thymoquinone (46.37 ± 11.64 mmol/mg protein) did not significantly vary in terms of the mean brain tissue level of GSH at $p > 0.05$. On the other hand, the mean brain tissue GSH level in the rats treated with nano-selenium plus thymoquinone was significantly higher (46.37 ± 11.64 mmol/mg protein) than in the rats treated with thymoquinone.

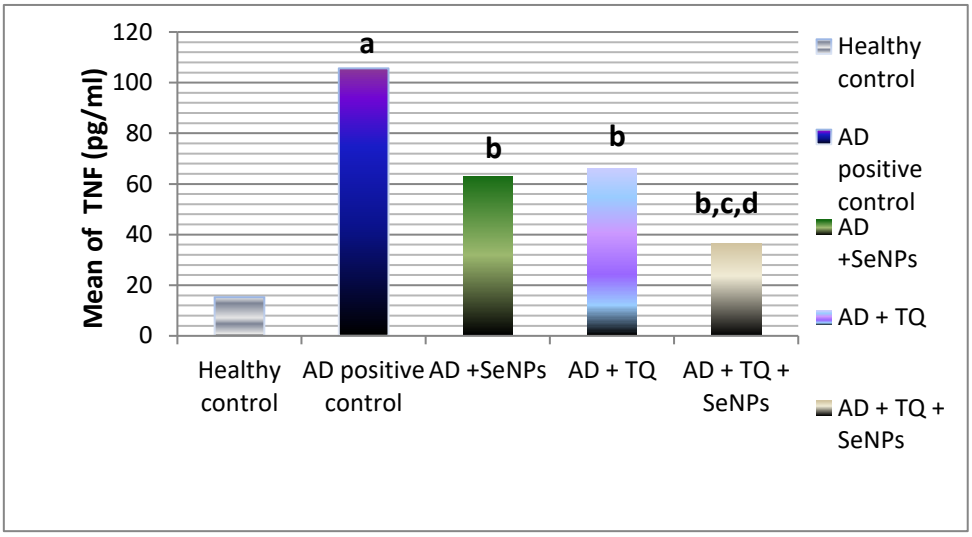

Figure 2. The mean of Tumor necrosis factor-alpha in all the studied groups (a. Significant from the healthy control group at $p < 0.05$. b. Significant from AD positive control group at $p < 0.05$. c. Significant from AD + SeNPs group at $p < 0.05$. d. Significant from AD + TQ at $p < 0.05$)

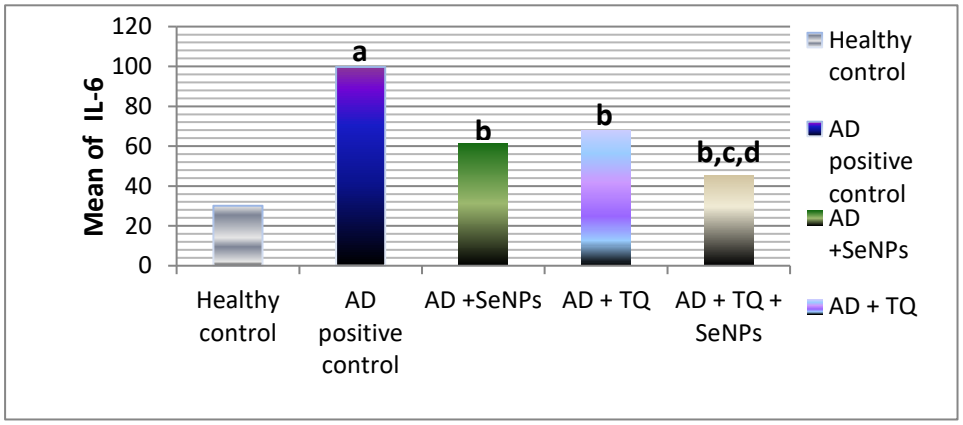

Figure 3. The mean of Interleukin -6 in all the studied groups (a. Significant from the healthy control group at $p < 0.05$. b. Significant from AD positive control group at $p < 0.05$. c. Significant from AD + SeNPs group at $p < 0.05$. d. Significant from AD + TQ at $p < 0.05$.)

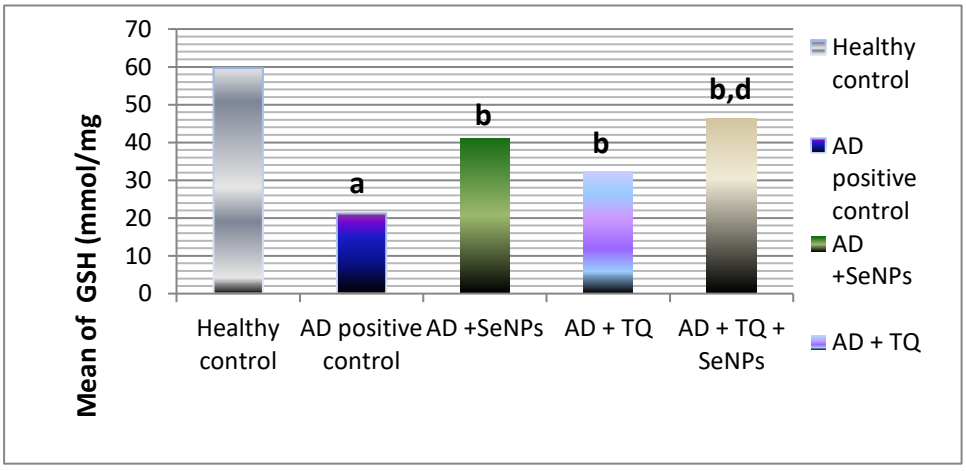

Figure 4. The mean of Glutathione in all the studied groups (a. Significant from the healthy control group at $p < 0.05$. b. Significant from AD positive control group at $p < 0.05$. c. Significant from AD + SeNPs group at $p < 0.05$. d. Significant from AD + TQ at $p < 0.05$.

Malondialdehyde level (nmol/mg protein) in brain tissue:

As Shown in **Figure 5**, in comparison to the group of Alzheimer rats receiving no treatment (73.23 ± 18.94 nmol/mg protein), there was a significant decrease in the mean brain tissue level of MDA in rats treated with nano-selenium, rats treated with thymoquinone, and rats treated with both nano-selenium and thymoquinone (27.37 ± 9.42, 29.23 ± 12.18, and 23.28 ± 4.89 nmol/mg protein), respectively. The mean brain tissue MDA level did not significantly differ between the rats treated with thymoquinone (29.23 ± 12.18 nmol/mg protein) and nano-selenium (27.37 ± 9.42 nmol/mg protein) at p > 0.05. Additionally, the mean brain tissue MDA level did not differ significantly at p > 0.05 between the rats treated with both nano-selenium and thymoquinone (23.28 ± 4.89 nmol/mg protein), nano-selenium (27.37 ± 9.42 nmol/mg protein), and thymoquinone (29.23 ± 12.18 nmol/mg protein).

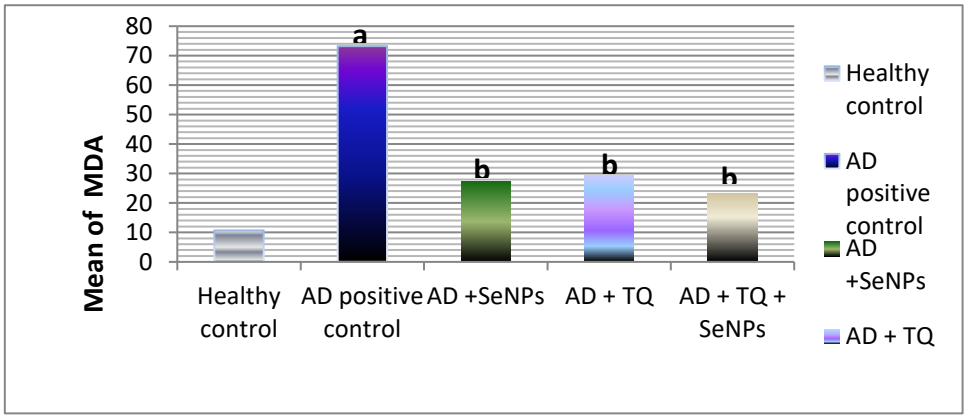

Figure 5. The mean of Malondialdehyde in all the studied groups (a. Significant from the healthy control group at p < 0.05. b. Significant from AD positive control group at p < 0.05. c. Significant from AD + SeNPs group at p < 0.05. d. Significant from AD + TQ at p < 0.05.)

Table (1): Comparison of laboratory data statistics between the control group, Alzheimer disease positive control group, AD rats received nano-selenium group, AD rats received thymoquinone group, and AD rats received both nano-selenium and thymoquinone group

Variables	Healthy control Group N = 10	AD positive control Group N = 10	AD+SeNPs Group N = 10	AD+TQ Group N = 10	AD+SeNPs+TQ Group N = 10
	Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD
Amyloid beta (pg/mg protein)	2.27 ± 0.87	11.21 ± 2.13[a]	4.86 ± 2.37[b]	5.80 ± 2.03[b]	3.91 ± 1.85[b]
TNF-α (pg/ml)	15.26 ± 2.29	105.65 ± 10.90[a]	63.03 ± 11.07[b]	66.05 ± 9.96[b]	36.41 ± 10.53[b,c,d]
IL-6 (pg/ml)	30.08 ± 3.35	99.73 ± 13.72[a]	61.43 ± 11.58[b]	68.06 ± 6.39[b]	45.30 ± 10.30[b,c,d]
GSH (mmol/mg protein)	59.67 ± 6.71	21.22 ± 4.62[a]	41.16 ± 5.87[b]	32.26 ± 9.14[b]	46.37 ± 11.64[b,d]
MDA (nmol/mg protein)	10.68 ± 3.19	73.23 ± 18.94[a]	27.37 ± 9.42[b]	29.23 ± 12.18[b]	23.28 ± 4.89[b]

a. Significant from the healthy control group at p < 0.05.
b. Significant from AD positive control group at p < 0.05.
c. Significant from AD + SeNPs group at p < 0.05.
d. Significant from AD + TQ at p < 0.05

3.2. Correlations between different parameters in the group of rats given nano-selenium treatment:

As shown in **Table (2)** and **Figures (6, 7, and 8)**: There is a significant positive correlation between amyloid beta and MDA (r = 0.184) at p < 0.05, also there are positive correlations between IL-6 and both amyloid beta and MDA (r = 0.634 and r = 0.842, respectively) at p < 0.05. Whereas there is a significant negative correlation between MDA and IL-6 (r = -0.779) at p < 0.05.

3.3. Correlations between different parameters in the group of rats given thymoquinone treatment:

As shown in **Table (3)** and **Figures (9)**: There is a significant positive correlation between IL-6 and amyloid beta (r = 0.878) at P < 0.05.

Table (2): Correlations in the Alzheimer's group treated with nano-selenium

	Amyloid beta	TNF-α	IL-6	GSH	MDA
Amyloid beta (r)	1.000	0.032	0.634*	0.166	0.814*
(P value)	0.000	0.931	0.049	0.648	0.004
TNF -α (r)	0.032	1.000	-0.383	-0.574	-0.093
(P value)	0.931	0.000	0.275	0.083	0.799
IL-6 (r)	0.634*	-0.383	1.000	0.128	0.842**
(P value)	0.049	0.275	0.000	0.724	0.002
GSH (r)	0.166	-0.574	0.128	1.000	-0.163
(P value)	0.648	0.083	0.724	0.000	0.654
MDA (r)	0.814*	-0.093	0.842**	-0.163	1.000
(P value)	0.004	0.799	0.002	0.654	0.000

*: Statistically significant difference at $p < 0.05$.

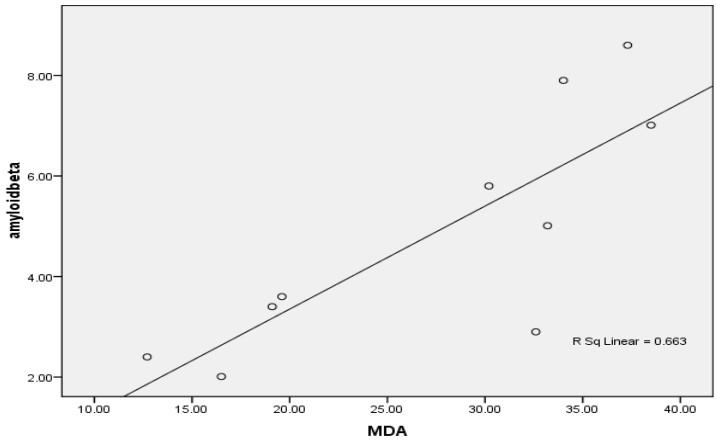

Figure 6. Correlation between MDA and amyloid beta in the group of rats with Alzheimer's disease treated with nano-selenium

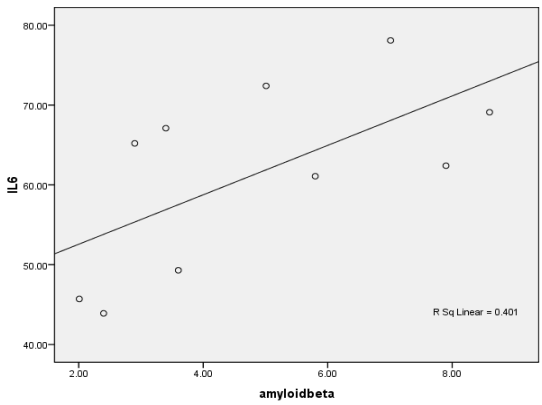

Figure 7. Correlation between amyloid beta and IL-6 in the group of rats with Alzheimer's disease treated with nano-selenium

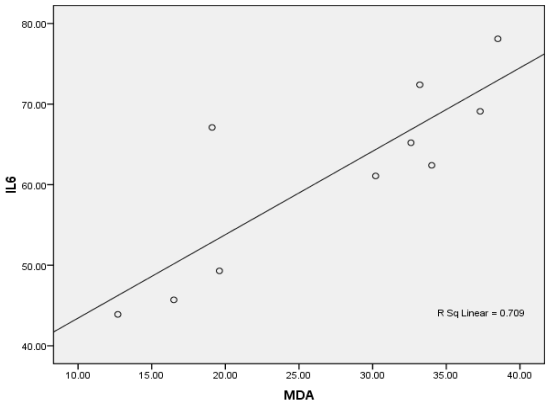

Figure 8. Correlation between MDA and IL-6 in the group of rats with Alzheimer's disease treated with nano-selenium

3.4. Correlations between different parameters in the group of rats given both nano-selenium and thymoquinone treatments:

As shown in **Table (4)** and **Figure (10)**: There is a significant negative correlation between GSH and TNF-α (r = -0.651) at $P < 0.05$.

Table (3): Correlations in the Alzheimer's group treated with thymoquinone

	Amyloid beta	TNF-α	IL-6	GSH	MDA
Amyloid beta (r)	1.000	-0.164	0.878*	-0.130	0.080
(P value)	0.000	0.650	0.001	0.720	0.827
TNF -α (r)	-0.164	1.000	0.065	-0.088	-0.113
(P value)	0.650	0.000	0.858	0.808	0.755
IL-6 (r)	0.878*	0.065	1.000	-0.285	-0.059
(P value)	0.001	0.858	0.000	0.425	0.872
GSH (r)	-0.130	-0.088	-0.285	1.000	0.276
(P value)	0.720	0.808	0.425	0.000	0.441
MDA (r)	0.080	-0.113	-0.059	0.276	1.000
(P value)	0.827	0.755	0.872	0.441	0.000

*: Statistically significant difference at $p < 0.05$.

Table (4): Correlations in the Alzheimer's group treated with nano-selenium and thymoquinone

	Amyloid beta	TNF-α	IL-6	GSH	MDA
Amyloid beta (r)	1.000	-0.336	0.163	0.203	-0.117
(P value)	0.000	0.342	0.652	0.574	0.749
TNF-α (r)	-0.336	1.000	0.142	-0.651*	-0.050
(P value)	0.342	0.000	0.695	0.042	0.891
IL-6 (r)	0.163	0.142	1.000	-0.150	-0.052
(P value)	0.652	0.695	0.000	0.679	0.887
GSH (r)	0.203	-0.651*	-0.150	1.000	0.088
(P value)	0.574	0.042	0.679	0.000	0.809
MDA (r)	-0.117	-0.050	-0.052	0.088	1.000
(P value)	0.749	0.891	0.887	0.809	0.000

*: Statistically significant difference at $p < 0.05$.

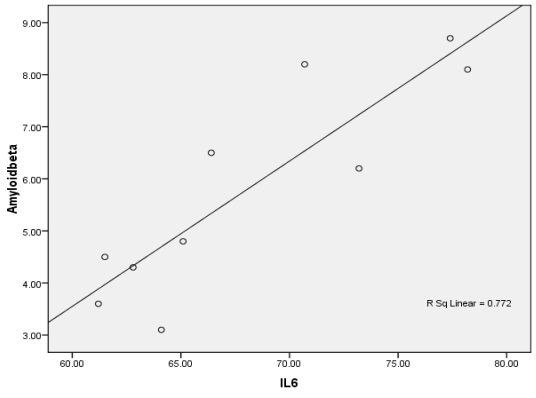

Figure 9. Correlation between IL-6 and amyloid beta in the group of rats with Alzheimer's disease treated with thymoquinone

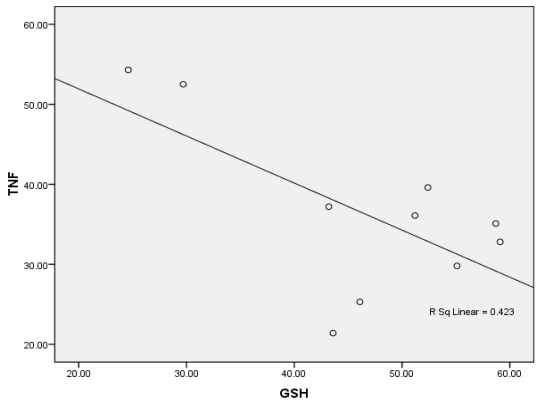

Figure 10. Correlation between GSH and TNF-α in the group of rats with Alzheimer's disease treated with nano-selenium and thymoquinone

3.5. Histopathological examination of brain tissue:

As shown in **Figure (11)**, Hematoxylin and eosin-stained slices of the dorsal striatum (caudate/putamen) (H&E x400, scale bar 50 μm) were extracted from:

(i) the healthy control group which showed normal glial cells (g), normal capillaries (c), and normal neurons (N). **(ii) Alzheimer disease positive control group** in which several darkly degenerated neurons are seen. The neurons' peri-neuronal vacuolations (V) and darkly pigmented, shrunken, pyknotic nuclei were either deformed or irregular. Additionally, the perivascular gap (Virchow-Robin space), which separated the vessel from the neuropil

(spiral arrow), markedly widened. **(iii) The AD rats that were given the nano-selenium treatment group** displayed a healthy structure of capillaries (c) and striatal neurons (N), with the majority of neurons (N) in a healthy neuropil (neu) exhibiting noticeable nucleoli and vesicular nuclei. **(iv) The AD rats who were given the thymoquinone therapy group** showed preserved striatal neuronal and glial cell architecture, with just a small number of shrunken neurons visible. **(v) In the group of AD rats treated with both nano-selenium and thymoquinone**, the striatum exhibited hypocellularity, as evidenced by an astrogliosis (As) picture showing pencil fibers of Wilson (W) and a small number of regenerated neurons (N). Certain capillaries consisted of a thin, delicate tube lined with a single layer of endothelial cells (c). Vascular congestion and dilatation were clearly visible (arrowhead).

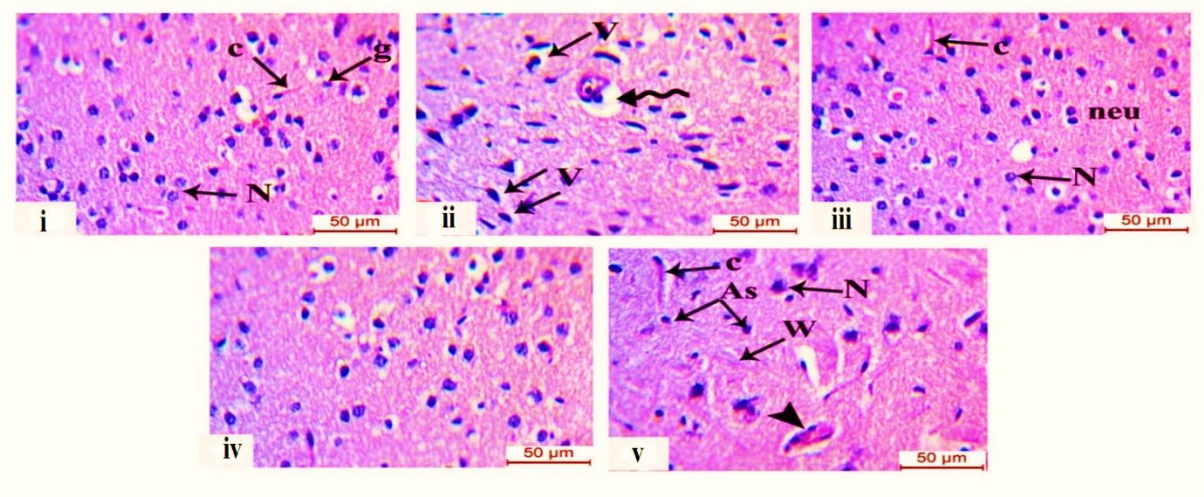

Figure 11: Histopathological examination of rat's brain tissue in (i) healthy control group (ii) AD positive control group (iii) AD rats received nano-selenium group (iv) AD rats received TQ group (v) AD rats received both nano-selenium and TQ group.

4. DISCUSSION

Elderly people who have dementia primarily suffer from Alzheimer's disease, which is the most common type of neurodegenerative sickness. A patient's memory and cognitive impairment that substantially interferes with daily activities is the clinical definition of Alzheimer's disease (AD). Because AD is so common and persistent, there is presently no recognized cure, which puts people's health and the healthcare system at risk. Current AD medications cannot stop dementia from progressing or reversing its course; they can only momentarily delay cognitive decline.

Se is an essential micronutrient that has many complex effects on human health. Se is essential for regulating synaptic neuronal activity. Se serves as an antioxidant as well, and when it is deficient, oxidative stress increases, hastening the onset of Alzheimer's disease. Thymoquinone is a bioactive volatile oil component that has strong anti-inflammatory and antioxidant properties. This oil has the potential to be an effective neuroprotective agent. The purpose of the study is to assess how thymoquinone and nano-selenium affect Alzheimer's disease.

In the current study, the mean brain tissue level of amyloid-beta in rats treated with thymoquinone was significantly lower compared to the group of Alzheimer rats with no treatment at $p < 0.05$.

Elibol *et al.* investigated the molecular restorative effects of TQ in the hippocampal region of AD rats injected with amyloid-beta 1-42 (Aβ1-42). It was discovered that TQ injection reduced the expressions of Aβ, allowing TQ to reverse the neuropathology by eliminating Aβ plaques and preserving neuron viability [25]. It was also noted by Alasmari *et al.* that TQ could lessen the neuroinflammatory characteristics and behavioral abnormalities in AD models. TQ demonstrated its ability to regulate β-amyloid neurotoxicity, a result linked to lessened behavioral signs of AD [26]. Furthermore, as shown by Alhibshi *et al.*, TQ may have neuroprotective effects on neurons because of its capacity to degrade Aβ accumulation, inhibit its neurotoxic effect, and shield neuronal cells from Aβ-induced neurotoxicity in cholinergic neurons derived from cultured hiPSCs [27]. Also, consistent with the current investigation Abulfadl *et al.*, thymoquinone treatment at all dosages was successful in reducing the buildup of Aβ plaques in the brains of our AD model rats and enhancing their memory and learning capacities [28].

Rats given nano-selenium in the current study demonstrated a significant decrease in the mean brain

tissue level of amyloid beta compared to the group of Alzheimer rats receiving no therapy ($p < 0.05$).

These outcomes were consistent with those of Al Kahtani, who found that the antioxidant properties of Se and SeN particles may be responsible for their ability to lower Aβ and, consequently, amyloidosis in the brain. Therefore, treating neurotoxicity and subsequently enhancing cognitive function may benefit from using additional antioxidant compounds like Se and SeNPs [29]. Additionally, Gholamigerav *et al.* looked into how Selenium nanoparticles (SeNPs) therapy affected the neurotoxicity in an animal model of AD, and the findings indicated a decrease in Aβ deposition [30]. Furthermore, Guo *et al.* synthesized ultrasmall selenium quantum dots (SeQDs) having a multitarget therapeutic effect that was easy to obtain and could swiftly pass through the blood-brain barrier. The outcomes of the experiment demonstrated that SeQDs exhibited broad-spectrum antioxidant activity, robust free-radical scavenging activity, and the ability to shield cells from oxidative stress brought on by various stressors. In addition to effectively stopping the AD cascade reaction by inhibiting Aβ aggregation and considerably reducing Aβ-mediated cytotoxicity, the SeQDs also prevented tau protein activation, preserved nerve cell stability, and shielded nerve cells from oxidative stress [31].

The results of this investigation showed that rats treated with thymoquinone had significantly lower mean blood levels of TNF-α and IL-6 as well as mean brain tissue levels of MDA when compared to the group of Alzheimer rats who received no therapy ($p < 0.05$).

These results were in line with those of Abbas *et al.*, who found that treatment with thymoquinone significantly reduced the increase in levels of MDA, TNF-α, and IL-6 in animals exposed to aluminum chloride (AlCl3), which caused progressive multiregional neurodegeneration and encouraged oxidative stress and neuroinflammation. TQ may therefore be a viable therapeutic for oxidative stress- and neuroinflammatory-related neurodegenerative disorders as well as neurotoxicity [32]. Furthermore, the findings of Nemati *et al.* showed a reduction in TNF-α, IL-6, and MDA in the model of male Wistar rats given thymoquinone, a drug with pharmacological effects including anti-inflammatory and antioxidant properties, who had brain ischemia [33]. Additionally, TQ was shown by Bargi *et al.* to be able to ameliorate rats' LPS-induced memory deficits, albeit at lower doses. These behavioral outcomes were linked to a drop in the levels of oxidative damage markers in brain regions, such as malondialdehyde (MDA), and a reduction in the hippocampal neuroinflammatory cytokines TNF-α and IL6 [34]. Also, the results were consistent with the findings of Abdulfadl *et al.*, who reported a decrease in the levels of the ensuing proinflammatory cytokines, TNF-α and IL-1β, as well as a release in protein levels following thymoquinone treatment. Additionally, the results were consistent with the findings of Jakaria *et al*. who reported a significant decrease in MDA levels, an improvement in turning behavior, and prevention of SNpc neuron loss following thymoquinone treatment [28].

Rats treated with nano-selenium in the current study had significantly reduced mean serum concentrations of IL-6 and TNF-α, as well as mean brain tissue levels of MDA, as compared to the group of rats with Alzheimer's disease who did not receive treatment ($p < 0.05$).

The results aligned with those of Ye *et al.* who observed that due to their exceptional biological activities, nano-selenium particles (SeNP) have garnered a lot of attention recently. Consequently, they employed modified SeNP in their investigation to examine its impact on colitis induced by 3% dextran sulfate sodium (DSS). The findings demonstrated a noteworthy reduction in TNF, IL-6, and MDA in colon tissue, which mitigated DSS-induced colitis by diminishing inflammation and enhancing antioxidant capacity [35]. Zeghloul *et al.* also observed that selenium nanoparticles (SeNPs) decreased TNF, IL-6, and MDA levels in rats with STZ-induced nephropathy, which is consistent with these findings [36]. Furthermore, El-Badry *et al.* found that rabbits' consumption of both organic and nano sources of selenium significantly increased their blood's total antioxidant capacity and lessened the harmful effects of heat stress by lowering their blood's malondialdehyde level [37].

In the current investigation, the mean GSH level in brain tissue was shown to be significantly greater ($p < 0.05$) in the rats treated with thymoquinone than in the Alzheimer's rats that were not treated.

These outcomes were in line with those of Krewenka *et al.* who looked at how TQ affected models of oxidative stress and mitochondrial impairment brought on by rotenone in primary mesencephalic cells and N18TG2 neuroblastoma cells, as well as rotenone/MPP+ in those cells. They discovered that TQ treatment greatly raised the cell's reduced glutathione content [38]. Additionally, D-galactose was given to rats in a study by Oksouei *et al.* that resulted in memory losses comparable to those caused by aging naturally while also raising oxidative stress by lowering GSH. The outcomes demonstrated that TQ therapy might reverse the d-gal-induced memory loss in rats by raising GSH levels and averting oxidative hippocampal damage [39].

In this investigation, the mean GSH level in brain tissue was likewise considerably higher in the nano-selenium-treated rats than in the untreated Alzheimer's group of rats ($p < 0.05$).

That was in line with the findings of Ye *et al.* who employed modified SeNPs to examine their impact on colitis induced by 3% dextran sulfate sodium (DSS). The study's results revealed a noteworthy rise in the GSH/GSSG ratio in colon tissue, an important redox state indicator that lowers DSS-induced colitis through antioxidant function [35]. These outcomes were consistent with those of Gao *et al*. who found that Se could lower MDA and ROS levels, raise GSH levels, and lessen cell damage brought on by oxidative stress [40]. Furthermore, our outcomes corroborated those of Al Kahtani who discovered that an increase in GSH content in groups treated with Se and SeNPs is suggestive of the antioxidant function of Se and SeN particles. The development of selenoproteins like GPx, which boosts antioxidant capacity and may be used as a brain chemotherapeutic drug, could be the cause of improvement [29].

5. CONCLUSIONS

Thymoquinone and nano-selenium particles showed improvement in histopathology study in AD rats' model. Also, this study demonstrated that nano-selenium and thymoquinone reversed levels of different biomarkers (Aβ-42, TNF-α, IL-6, GSH, and MDA) towards normal levels decreasing the inflammation and oxidative stress which played an important role in the progression of AD. The combination of SeNPs and TQ showed more improvement than each of them as a single treatment. Nano-selenium and thymoquinone can be used as additive therapies for Alzheimer's disease.

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Conflicts of Interest: This study has not received any funding.

Ethical Statement: The protocol for treating animals was done following the moral guidelines for animal facilities, Faculty of Pharmacy, Girls, Al-Azhar University, Cairo, Egypt (REC number: 257).

Author Contribution: This work was carried out in collaboration between all authors. **Noha El-Sabbagh** worked on the methodology, data collection analysis, and paper writing. **Doha Ellakwa** contributed to the methodology, data collecting, writing, and editing of the work.

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