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The co-administration of quercetin and gallic acid nanocapsules exhibits a protective effect against aluminium chloride in the brain of animal model

Reza Taghizadeh-Tabarsi [1], Alimohammad Madih [2], Mohammad Gilanifar [2], Fatemeh Zahra Gharib [3], Ali Fakhrtavoli [2], Amirhossein Esmaeilzadeh [2], Ali Taravati [4]*

[1] Faculty of Life Science and Biotechnology, Shahid Beheshti University, Tehran, 16589-53571, Iran

[2] Department of Veterinary Medicine, Islamic Azad University, Babol Branch, Tehran, Iran

[3] Department of Clinical Sciences, Bab.C., Islamic Azad University, Babol, Iran

[4] Department of Molecular and Cell Biology, Faculty of Basic Sciences, University of Mazandaran, Babolsar 47416-95447, Iran

Abstract

Introduction: Aluminum (Al) is associated with the development of various neurological disorders, including Alzheimer's disease (AD), highlighting the need for materials with protective effects. This study investigated the protective effect of quercetin and gallic acid nanocapsules on brain damage caused by aluminum chloride.

Materials and methods: Adult rats were chronically treated with aluminum chloride to generate a disease model. Gallic acid and quercetin were administered orally, both in free forms and as nanocapsules, to evaluate their protective effects. To assess oxidative stress, the levels of lipid peroxidation, total antioxidants, reduced glutathione, glutathione peroxidase, superoxide dismutase, catalase, and myeloperoxidase activity were measured. Brain tissue was also examined for structural abnormalities using hematoxylin and eosin staining.

Results: Aluminum chloride treatment significantly increased oxidative stress and brain damage. However, treatment with a combination of gallic acid and quercetin, both in free (20 mg/kg and 50 mg/kg, respectively) and nanocapsule forms, effectively reduced these effects. Histological evaluation showed that co-treatment with quercetin and gallic acid nanocapsules significantly reduced aluminum-induced toxicity and preserved normal brain structure. The nanocapsule forms were more effective at lower doses (10 mg/kg) compared to the free forms.

Conclusion: These findings suggest that quercetin and gallic acid nanocapsules can reduce the required therapeutic dose and limit the adverse effects of the free drugs. Nanocapsule formulations may enhance brain delivery and act as neuroprotective agents against aluminum-induced damage and the progression of Alzheimer's disease. The encapsulated form of quercetin and gallic acid appears to be a promising protective agent in preclinical evaluations.

Keywords: Alzheimer's disease, Aluminium chloride, Quercetin, Gallic acid, Nanocapsules

Corresponding Authors: Ali Taravati

✉ Email: a.taravati@umz.ac.ir

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Introduction

Alzheimer's disease is one of the neurodegenerative diseases that affected more than 55 million people in the world in 2020. This number will double approximately every 20 years, reaching 78 million in 2030 and 139 million in 2050 (1, 2). Alzheimer's often occurs in people over 65 years of age, but about 10% of patients develop early-onset Alzheimer's and develop this condition in their 30s to 60s (2). Also, women suffer from Alzheimer's more than men (3). So far, there is no known way to stop or prevent the progress of this disease, but some treatments help to improve the symptoms of the disease. Aluminum, as a metal that can cause Alzheimer's, puts humans at risk with different sources. The equipment is present in water, food, environment, medicinal compounds, etc., and it is placed in the soil due to acid rain. Disorders such as dementia (brain damage) in Alzheimer's disease as well as prevention of motor actions in Parkinson's disease in humans and animals are related to increasing consumption .Aluminum can cause inflammatory damage to brain tissue by causing oxidative stress (4, 5). Therefore, preventing oxidative stress and generating a protective effect using antioxidants has an important place. Gallic acid has effective antioxidant properties as a trihydroxybenzoic acid and a phenolic acid with a molecular weight of 170.12 g/mol. This compound is found in sumac, hazelnut, tea leaves, oak bark, and other plants. Gallic acid acts as an antioxidant and helps protect cells from oxidative damage. It has been observed that gallic acid has anti-cancer properties. It is also used in the treatment of internal bleeding. It is also used as medicine in the treatment of albuminemia and diabetes. Gallic acid plays a neuroprotective role in animal models that have the problem of nerve damage through pathways that include antioxidant and anti-inflammatory activity. Gallic acid has a spectacular protective effect on neurotoxicity and neurotoxicity that is caused after brain damage (6). Quercetin, as a flavonol from the group of flavonoid polyphenols, has antioxidant properties that can be found in many fruits, vegetables, leaves, seeds, and grains such as red onion and kale (1). This substance is one of the most abundant flavonoids in the diet with an average daily consumption of 50 mg (7). Quercetin, like other flavonoids, can cross the blood-brain barrier (BBB), which makes it a potential agent in preventing neurodegenerative disorders (8). Flavonoids widely have anti-inflammatory and antioxidant activity, both of which are effective in preventing Alzheimer's pathogenesis. Quercetin can treat many problems, including neurological disorders, and delay the process of nerve damage. This substance also has antioxidant and protective effects in preventing endothelial apoptosis caused by oxidants (9). The effectiveness of the drug in the central nervous system depends on the ability of the drug to cross the blood-brain barrier and reach therapeutic concentrations in the brain after administration. Therefore, failure in the treatment of central nervous system disorders is often not due to the lack of potential effect of the drug, but due to problems in the method of drug delivery (10). To overcome the problems and obstacles of drug delivery, nanoparticles and nanocarriers are being developed that can deliver drugs in a targeted manner and increase the effectiveness of drugs in a wide range of diseases from cancer to Alzheimer's (11, 12). Chitosan, as an alkaline polysaccharide that is biocompatible, can be effective in drug delivery because it can prevent enzymatic degradation. Since the nanocapsulation of the drug with chitosan can increase the passage through the blood-brain barrier, this carrier provides good conditions for drug delivery to brain cells (13, 14). Therefore, our hypothesis was that co administration of gallic acid and quercetin, particularly in a nano encapsulated form, would synergistically reduce oxidative stress and neuroinflammation in a rat model of aluminum chloride induced Alzheimer's model more effectively than either compound alone and that this combination can be effective even at lower doses due to improved bioavailability from nanocapsulation, based on this hypothesis, this study aimed to investigate the biochemical and histological effects of using chitosan-alginate nanocapsules with quercetin and gallic acid to treat or prevent aluminum chloride-induced brain damage and lesions. Through this research, we developed a combination therapy using quercetin and gallic acid nanocapsules by gavage to protect and prevent aluminum-induced brain damage in rat models.

Materials and methods

Nanocapsulation of gallic acid and quercetin

To generate of gallic acid and quercetin nanocapsules, gallic acid solution with a concentration of 50 mg/ml in ethanol and a solution of quercetin with a concentration of 6 mg/ml in DMSO and separately by chitosan with a concentration of 0.8 mg/ml and pH 5.4 were mixed (chitosan-drug) gently on stirrer (500 rpm). Separately, calcium chloride with a concentration of 3.35 mg/ml was slowly added to the 3 mg/ml alginate solution with a pH of 5.1, and then the chitosan-drug solution was slowly added to it (on stirrer 500 rpm). Then, the final drug nanocapsules solution was centrifuged at 13,000 rpm and the precipitate was dried with a freeze dryer. To evaluate the encapsulation efficiency, the presence of Quercetin and Gallic acid was checked by evaluating the supernatant absorption in 375nm for Quercetin and 270nm for Gallic acid.

Characteristics of quercetin and gallic acid nanocapsules

To determine the dimensions of the quercetin and gallic acid nanocapsules, the freeze-dried nanocapsules were dissolved in water. The size of the nanoparticles was then measured using a DLS device at a temperature of 25°C and a scattering angle of 90 degrees.

Hemolysis assay of nanocapsules

To evaluate the effect of gallic acid and quercetin nanocapsules on the lysis of red blood cells (RBC), human blood was centrifuged at 500 rpm and after washing with PBS, the RBCs were separated and incubated with gallic acid (50 mg/ml) and quercetin (20 mg/ml) for 3 h in 37C. After incubation, they were centrifuged at 1000 rpm and the absorbance of the supernatant was measured at 540 nm. Triton-X100 was used as a positive control and PBS buffer was used as a negative control.

$$\% \text{ Hemolysis} = [\ (A_{sample} - A_{blank}) / (A_{triton} - A_{blank})\ ] \times 100$$

Animals and study design

To investigate the protective effects of gallic acid and quercetin nanocapsules in rats, a total of 36 maturated male rats weighing between 250-300 grams were divided into 6 groups, each including 6 rats. The rats were subjected to a 12-hour light and dark cycle and were provided with unrestricted access to food and water (ethical approve code is IR.IAU.BABOL.REC.1400.028). Condition of each group shown below (Table 1).

Table1. Treatment groups.

The first group, as a control, was fed normal saline orally for 35 days.
The second group, as a positive control, rats were induced Alzheimer with aluminum chloride at a dose of 75 mg/kg by intraperitoneal injection for 35 days.
The third group was given gallic acid at a dose of 50 mg/kg and quercetin at a dose of 20 mg/kg was consumed as a daily cocktail for 35 days (by gavage).
Fourth group were fed gallic acid and quercetin nanocapsules with a dose of 10 mg/kg for 35 days (by gavage).
Fifth group received quercetin and gallic acid cocktail (in the form of gavage) with doses of 20 and 50 mg/kg, respectively, along with aluminum chloride (IP) with a dose of 75 mg/kg.
The sixth group of rats received the combined cocktail of quercetin and gallic acid nanocapsules (in gavage) at a dose of 10 mg/kg along with aluminum chloride (IP) at a dose of 75 mg/kg for 35 days.

The first group, as a control, was fed normal saline orally for 35 days. In the second group, as a positive control, rats were induced Alzheimer's with aluminum chloride at a dose of 75 mg/kg by intraperitoneal injection for 35 days. The third group was given gallic acid at a dose of 50 mg/kg and quercetin at a dose of 20 mg/kg was consumed as a daily cocktail for 35 days (by gavage). The fourth group was fed gallic acid and quercetin nanocapsules with a dose of 10 mg/kg for 35 days (by gavage). The fifth group received a quercetin and gallic acid cocktail (in the form of gavage) with doses of 20 and 50 mg/kg, respectively, along with aluminum chloride (IP) with a dose of 75 mg/kg. The sixth group of rats received the combined cocktail of quercetin and gallic acid nanocapsules by gavage at a dose of 10 mg/kg along with aluminum chloride (IP) at a dose of 75 mg/kg for 35 days.

Histopathological study

To evaluate the protective effect of quercetin and gallic acid nanocapsules by gavage on the cortex and hippocampal tissues, after the period of medication, the animals were deeply anesthetized with a high dose of ketamine (150 mg/kg) and xylazine (15 mg/kg), then they were prepared for tissue sampling. Then, the animal's brain were removed and after fixing the sample, tissue passage steps including dehydration, alcohol extraction, paraffin immersion, and molding were performed, and using a microtome, slices with a diameter of 5 to 7 µM were removed, and stained by Hematoxylin and Eosin. With an optical microscope, the structure and cellular morphology of the target tissue was examined.

Evaluation of oxidant and antioxidant parameters

Measurement of tissue stress markers such as MDA, TAC, SOD, catalase, glutathione peroxidase, reduced glutathione, and myeloperoxidase were measured and analyzed after the preparation of tissue homogenate, according to standard instructions. All the tissues were kept in a freezer at -80 degrees Celsius until the time of work. The frozen tissues were carefully weighed and homogenized in phosphate-buffered saline. After that, the samples were centrifuged at a temperature of 4 degrees Celsius for 15 minutes. The supernatant solution was used to measure the desired biochemical marker with commercially available kits (Navand Salamat, Iran).

Statistical analysis

All evaluations were done in three independent replications, and results analyzed by Graph pad prism 8 and SPSS with one way ANOVA method. The significance threshold was consider 0.05 for p-value.

Results

Size measurement of nanocapsules

The size of gallic acid and quercetin nanocapsules was evaluated by DLS device. As shown in Figure 1A, the sizes of quercetin and gallic acid nanocapsules were 135 and 161 nm, respectively.

Hemolysis assay of nanocapsules

The effect of generated nanocapsules on RBC, was investigated with a hemolysis test. As shown in Figure 1B, gallic acid and quercetin nanocapsules do not induce lysis of RBCs.

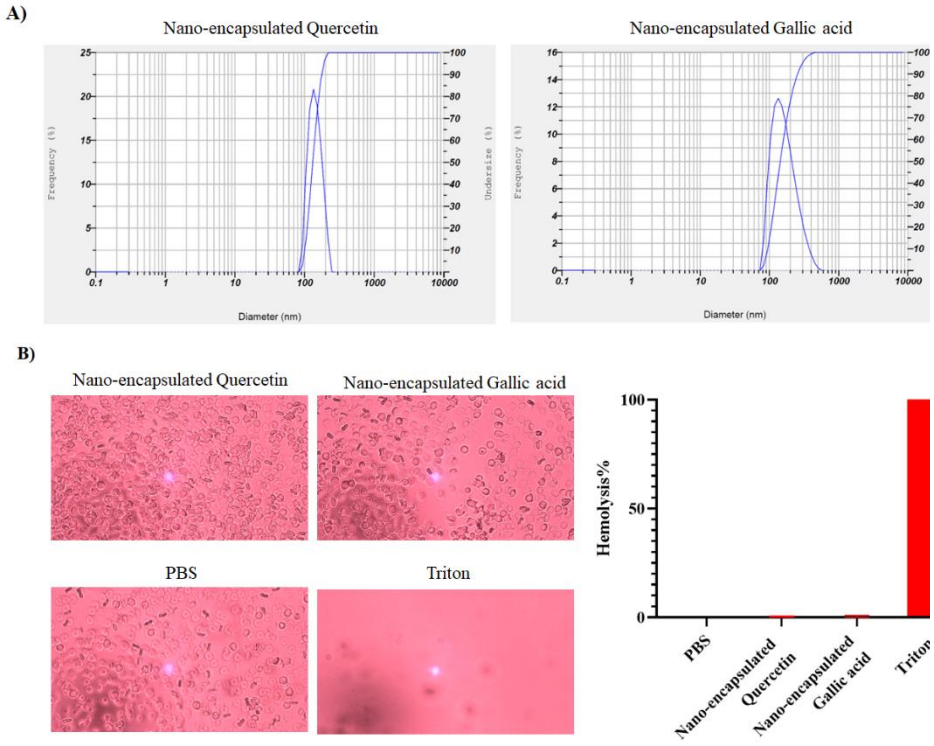
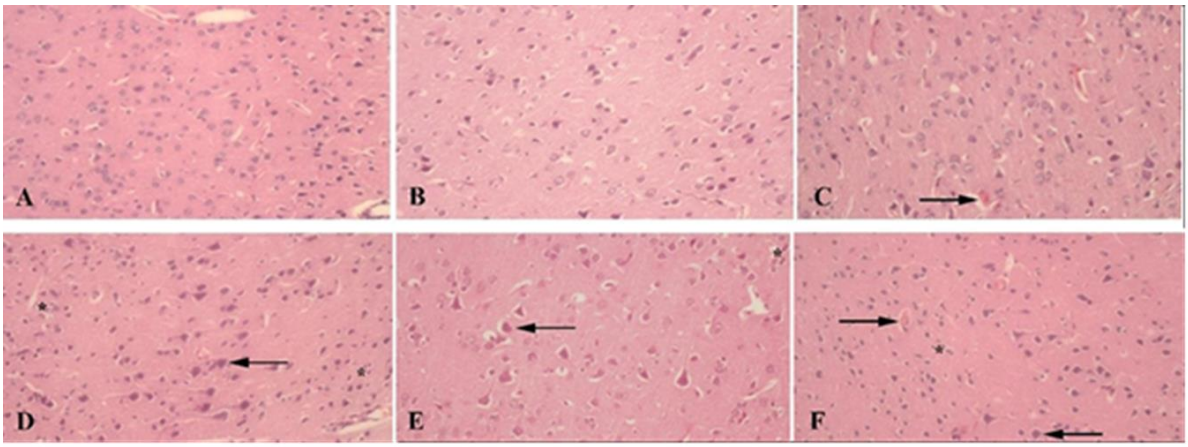


Figure 1. Characterizing the size and effect of hemolysis of drug nanocapsules. A) The size of nanocapsules was measured by DLS device, quercetin and gallic acid nanocapsules were 135 and 161 nm, respectively. B) Quercetin and gallic acid nanocapsules do not lyse RBCs, PBS was used as a positive control and Triton X100 was used as a positive control (Magnification 20X).

Histopathological assessment

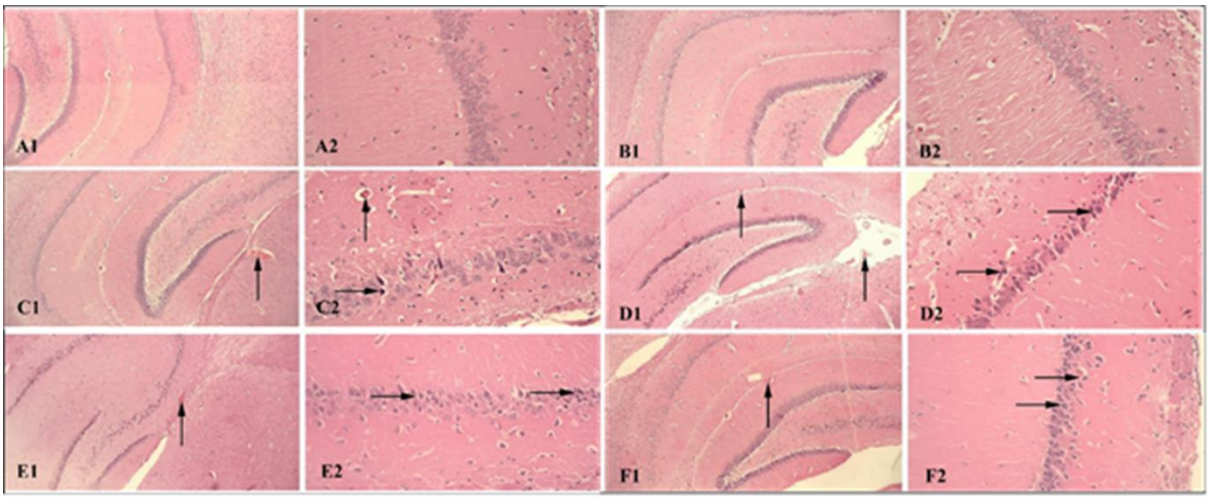
To investigate the protective effect of gallic acid and quercetin and their nanocapsules forms against the damage caused by aluminum chloride, the histopathology of the cortex and hippocampus tissue of the brain under normal conditions and after drug administration has been done with hematoxylin-eosin staining. As shown in Figure 2, the cortex tissue in the negative control group has normal conditions. In the group that received the cocktail of both gallic acid and quercetin nanocapsules by gavage, the conditions were normal, but the group that received the cocktail of gallic acid and quercetin by gavage had symptoms of hematuria. The group receiving aluminum chloride as a positive control group shows signs of necrosis and gliosis. The group that received the cocktail of gallic acid and quercetin nanocapsules, and aluminum chloride as a damage inducer, also has necrosis and gliosis, but hyperemia has not seen. The group that received both the gallic acid and quercetin cocktail by gavage, and aluminum chloride, showed necrosis, hyperemia, and gliosis, all three together. As shown in Figure 3, hippocampal tissue in the negative control group shows normal conditions. Also, normal tissue conditions without any necrosis and hyperemia can be seen in the group receiving the cocktail of both gallic acid and quercetin nanocapsules by gavage. The group receiving the gallic acid and quercetin cocktail generally has hyperemia and necrosis. The positive control group shows necrosis and hyperemia by receiving aluminum chloride. The group receiving quercetin and gallic acid nanocapsules cocktail and AlCl3 as a damage inducer shows reduced necrosis and hyperemia compared with the positive control group. The group receiving the gallic acid and quercetin cocktail by gavage, and aluminum chloride as a damage inducer, completely shows necrosis and hyperemia.



Gliosis	Necrosis	Hyperemia	Cortex
-	-	-	Control
-	-	+	Quercetin and Gallic acid
-	-	-	Nano-encapsulated Quercetin and Gallic acid
+ +	+ +	+	Aluminum chloride
+ +	+	+	Aluminum chloride + Quercetin and Gallic acid
+	+	-	Aluminum chloride + Nano-encapsulated Quercetin and Gallic acid

Figure 2. Investigating the protective effect of the drug in free and nanocapsule forms against aluminum chloride damage to the cortex. A) Normal conditions of the cortex tissue in the negative control group. B) Normal tissue

conditions in the group receiving quercetin and gallic acid nanocapsules by gavage, without necrosis and hyperemia. C) The group receiving gallic acid and quercetin cocktail is hyperemic. E) The group receiving the cocktail of both quercetin and gallic acid nanocapsules + aluminum chloride, flash shows necrosis and the star shows gliosis, F) The group receiving the cocktail of gallic acid, quercetin and aluminum chloride, The left flash shows necrosis, the right flash shows hyperemia and the star shows gliosis.



Necrosis	Hyperemia	Hypocamp
-	-	Control
+	+	Quercetin and Gallic acid
-	-	Nano-encapsulated Quercetin and Gallic acid
++	+	Aluminum chloride
++	+	Aluminum chloride + Quercetin and Gallic acid
+	+	Aluminum chloride + Nano-encapsulated Quercetin and Gallic acid

Figure 3. Investigating the protective effect of the drug in free and nanocapsule forms against aluminum chloride damage to the hippocampus. A) Normal tissue conditions in the negative control group. B) normal tissue conditions in the group receiving the cocktail of both quercetin and gallic acid nanocapsules by gavage, without necrosis and hyperemia, C) the group receiving the cocktail of gallic acid and quercetin by gavage in the free form, upper side flash shows blood and the right flash shows necrosis. D) The positive control group receiving aluminum chloride, the right flash shows necrosis and the upper side flash shows hyperemia, and it shows more normal tissue conditions than image F, F) group receiving quercetin, gallic acid and aluminium chloride, the right flash indicates necrosis and upper flash indicates hyperemia.

Oxidant and antioxidant

In order to investigate the protective effect of gallic acid and quercetin in free and nanocapsule form against the damage caused by aluminum chloride, the level of oxidative stress markers was investigated. As shown in Figure 4A, the amount of malondialdehyde (MDA) in the control group, free form of drug, nanocapsules drug, aluminum chloride, aluminium chloride + free form of drug, and aluminium chloride + nanocapsules drug were, 2.70±0.32, 2.36±0.24, 2.51±0.30, 8.48±0.56, 2.86±0.32, 2.71 ±0.28, respectively. MDA, in the group that received aluminum chloride increased significantly compared to the negative control group (p-value<0.001). Gallic acid and quercetin in both nanocapsules and free forms significantly reduced

MDA levels in rats treated with aluminum chloride (p-value<0.001). As shown in Figure 4B, the amount of superoxide dismutase (SOD) in the group of, control, free form of drug, nanocapsules drug, aluminum chloride, aluminium chloride + free form of drug and aluminium chloride + nanocapsule drug were, 2.27±0.39, 2.13±0.22, 2.12±0.19, 0.87±0.10, 1.52±0.26, 2.26 ±0.36, respectively. SOD in the group of rats that received aluminum chloride significantly decreased compared to the negative control group (p-value<0.001). Also, rats treated with aluminum chloride as a damage inducer, when they are treated with nanocapsules form of quercetin and gallic acid by gavage, have a significant increase compared to treatment with quercetin and gallic acid in free form and are closer to the negative control group (p-value= 0.001), which shows that the nanocapsules form is more effective than free quercetin and gallic acid. As shown in Figure 4C, the amount of TAC in the group of, control, free form of drug, nanocapsules drug, aluminum chloride, aluminium chloride + free form of drug and aluminium chloride + nanocapsule drug were, 29.45±1.56, 27.05±1.72, 27.02±2.00, 11.98±1.49, 23.90±1.91, 27.98 ±0.81 respectively. TAC in the group of rats receiving aluminum chloride has a significant decrease compared to the negative control group (p-value<0.001), The both gallic acid and quercetin nanocapsules can increase the TAC of rats induced with aluminum chloride more than the free form (p-value= 0.02). As shown in Figure 4D, the amount of myeloperoxidase in the group of, control, free form of drug, nanocapsules drug, aluminum chloride, aluminium chloride + free form of drug and aluminium chloride + nanocapsule drug were, 0.26±0.02, 0.27±0.04, 0.25±0.02, 1.10±0.06, 0.52±0.03, 0.27 ±0.04, respectively. Myeloperoxidase in a group of rats that received aluminum chloride has a significant increase compared to the negative control group (p-value<0.001), and treatment with both nanocapsules and free forms of gallic acid and quercetin by gavage causes a significant decrease (Figure 4D). As shown in Figure 4E, the amount of catalase enzyme in the group of, control, free form of drug, nanocapsules drug, aluminum chloride, aluminium chloride + free form of drug and aluminium chloride + nanocapsule drug were, 1.01±0.05, 0.94±0.08, 0.97±0.08, 0.41±0.05, 0.89±0.07, 0.89 ±0.07, respectively. Catalase enzyme in a group of rats receiving aluminum chloride has a significant decrease compared to the negative control group (p-value<0.001), and in all treatment groups with nanocapsules form and the free form of gallic acid and quercetin, an increase in its amount has been observed, but there is a significant difference between Treatment with nanocapsules form and free form of gallic acid and quercetin is not observed. As shown in Figure 4F-H, the amount of glutathione peroxidase in the group of control, free form of drug, nanocapsules drug, aluminum chloride, aluminium chloride + free form of drug and aluminium chloride + nanocapsule drug were 14.19±0.76, 13.53±0.55, 13.21±0.49, 4.52±0.38, 12.28±0.50, 13.79 ±0.85, respectively. The amount of GSH in the group of, control, free form of drug, nanocapsules drug, aluminum chloride, aluminium chloride + free form of drug and aluminium chloride + nanocapsule drug is, 14.19±0.76, 13.53±0.55, 13.21±0.49, 4.52±0.38, 12.28±0.50, 13.79 ±0.85, respectively. The amount of reduced glutathione, glutathione peroxidase, and glutathione reductase in all groups of rats receiving aluminum chloride has a significant decrease compared to the negative control group (p-value<0.001), on the other hand, in the groups treated with free-form and nanocapsules of both gallic acid and quercetin by gavage, there is a significant increase in all three markers. Note that the nanocapsule drugs at a lower dose (10 mg/kg) exhibit the same protective effect as the free drugs (50 mg/kg of gallic acid and 20 mg/kg of quercetin) (Table 2).

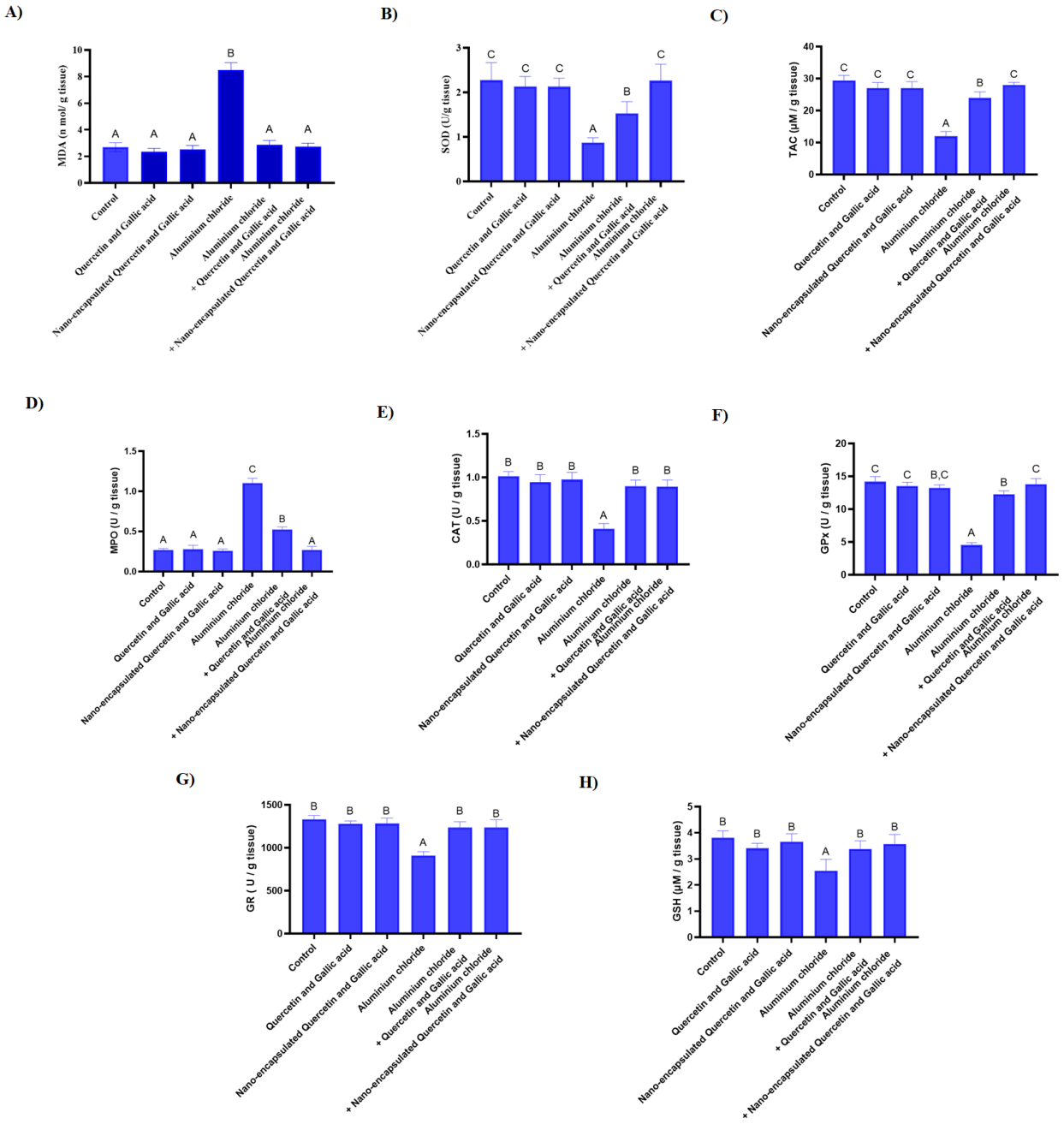


Figure 4. Investigating the protective effect of drugs in free forms and nanocapsules on oxidative stress. A) The amount of MDA increased following treatment with aluminium chloride, but it returned to its normal level when treated with quercetin and gallic acid by gavage in both free and nanocapsules form. B) Aluminium chloride reduces SOD, quercetin and gallic acid nanocapsules significantly increasing the amount of this enzyme more than the free form. C) Aluminium chloride reduces TAC, quercetin and gallic acid nanocapsules increase the amount of SOD and return to normal conditions. D) Aluminium chloride increases myeloperoxidase, quercetin and gallic acid nanocapsules decrease myeloperoxidase more significantly than the free form. E) The treatment of both forms increases catalase and compensates for the damage of aluminium chloride. F) The nanocapsules form more significantly compensates for the damage caused by aluminium chloride in the amount of GPx. G) Both forms of the drug compensate for the reduction of glutathione reductase. H) Aluminium chloride decreases GSH, quercetin and gallic acid free and nanocapsules compensate for the induced decrease.

Table 2. Investigating the protective effect of drugs in free forms and nanocapsules on oxidative stress (Data shown±SD).

Enzyme	Control	Quercetin and Gallic acid	Nano-encapsulate Quercetin and Gallic acid	Aluminium Chloride	Aluminium Chloride + Quercetin and Gallic acid	Aluminium Chloride + Nano-encapsulated Quercetin and Gallic acid
MDA	2.70±0.32	2.36±0.24	2.51±0.30	8.48±0.56	2.86±0.32	2.71±0.28
SOD	2.27±0.39	2.13±0.22	2.12±0.19	0.87±0.10	1.52±0.26	2.26±0.36
TAC	29.45±1.56	27.05±1.72	27.02±2.00	11.98±1.49	23.90±1.91	27.98±0.81
MPO	0.26±0.02	0.27±0.04	0.25±0.02	1.10±0.06	0.52±0.03	0.27±0.04
CAT	1.01±0.05	0.94±0.08	0.97±0.08	0.41±0.05	0.89±0.07	0.89±0.07
GPx	14.19±0.76	13.53±0.55	13.21±0.49	4.52±0.38	12.28±0.50	13.79±0.85
GSH	14.19±0.76	13.53±0.55	13.21±0.49	4.52±0.38	12.28±0.50	13.79±0.85

Discussion

Based on pre-clinical and laboratory observations, it can be stated that aluminum chloride causes Alzheimer-like complications through the formation of an alkylated product and the accumulation of this compound in tissues. Additionally, it disrupts the balance between oxidants and antioxidants in the tissue (15). However the mechanism of action of Quercetin and Gallic acid is not completely clear, gallic acid and quercetin both have been used as antioxidant substances and anti-inflammatory and protective indicators in many studies. Aluminum chloride has also been used as a substance to induce Alzheimer-like lesions in studies. In a study by Li Yuping et al. on the effect of quercetin against Alzheimer's-inducing beta-amyloid, quercetin was administered by gavage to mice with Alzheimer's disease, and the behavioral and histopathological results of the brain showed positive effects (16). Current FDA-approved treatments for Alzheimer's disease, such as cholinesterase inhibitors and NMDA receptor antagonists like memantine, provide only symptomatic relief and are often associated with limited efficacy and adverse side effects (17). In contrast, the encapsulated form of quercetin and gallic acid, as demonstrated in our study, shows potential not only in ameliorating oxidative stress and neuroinflammation two key contributors to Alzheimer's pathogenesis but also in enhancing bioavailability and sustained release. Mowali et al. conducted a study about the synergistic effect of quercetin and exercise against Alzheimer's disease-induced complications in mice. For this purpose, quercetin was administered by gavage for 60 days to mice with Alzheimer's disease. The brain histopathology data indicated quercetin's beneficial effects in mice (18). Takashi Mori et al. show, that gallic acid administration by gavage for 6 months daily to mice suffering from Alzheimer's disease, illustrates a good effect on behavioral and histopathological results of the brain (19). In this study, after the administration of aluminum chloride, a significant increase in malondialdehyde was observed compared to the control group, which indicated an increase in tissue damage. Furthermore, co-administration of quercetin and gallic acid to other groups reduced the amount of this marker, demonstrating the drug's effect both in its free form and in its nanocapsules form, likely due to their antioxidant properties. The increase in the

amount of TAC in the treatment and control groups with drugs indicates the same issue that the combination of both gallic acid and quercetin can benefit the body's oxidant balance to reduce radicals or neutralize them, the group treated with nanocapsules medicine also had a significant difference compared to the conventional treatment group (20-22). Also, in the following, we saw a decrease in SOD enzyme in the group with aluminum chloride, which indicated an increase in the amount of superoxides in the desired tissue, which itself indicates an increase in free radicals and tissue oxidation and oxidative stress, which fortunately in the treatment groups show an increase in the amount of SOD, which can indicate both the reduction of superoxides and the increase in the production of SOD, both of which testify to the reduction of oxidative stress and the overcoming of the body's oxidant balance (23). Following the administration of aluminum chloride, which destroys mainly neutrophils, we saw a significant increase in the amount of myeloperoxidase in the group treated with aluminum chloride compared to the control group. In the treatment groups, we saw a significant decrease, which is caused by the decrease in the death rate of neutrophils, which is a sign of the decrease in tissue inflammation. The reduction of inflammation, in turn, was probably due to the reduction of tissue stress and the return of inflammatory agents from the tissue to the blood (5). Regarding the catalase enzyme, we saw a significant decrease in the aluminum chloride group, which was caused by the increase in the consumption of this enzyme to eliminate oxidative stress in the tissue. No significant difference, regarding the catalase enzyme was observed between treatment groups (24). In the case of glutathione, which is a strong antioxidant and antiradical in the body and detoxifies in combination with free radicals, a significant decrease was observed in the aluminum chloride group, which significantly increased to the normal level in the treatment groups. This rate was higher in the group treated with nanocapsule by gavage form than conventional medicine, but no significant difference was observed between these two treatments (25). Regarding the level of the two enzymes glutathione reductase and glutathione peroxidase, it should also be said that despite the reduction property of aluminum chloride and the significant reduction caused by this substance in the control group, and on the other hand, the antioxidant property and reduction of oxidative stress of these two enzymes, there was a significant reduction. Both enzymes are in the group treated by aluminum chloride, which are consistent with the rest of the investigated oxidative indices. In both treatment groups, we see a significant increase in the amount of these two enzymes compared to the control group with aluminum chloride, which indicates the improvement of the condition and reduction of the oxidative imbalance in the desired tissue, although, of course, no significant difference was observed between the two treatment groups (26). It is also important that the administration of drugs in the control groups alone did not cause any side effects and no oxidative imbalance was observed in any of the stress indicators, and in the dose used, there was no effect of cell poisoning or There was no texture. In general, it should be said that due to the oxidative properties of aluminum chloride, we are witnessing oxidative stress in the tissue, and all the tissue stress indicators confirm the existence of this phenomenon. On the other hand, the administration of the drug in both the normal form and the nanocapsules has improved the condition and returned the stress indicators to the normal state. It is to be noted that not only nanocapsule drugs show an increased protective effect compared to the free form of the drug, but these effects are seen in lower doses of nanocapsule drugs (10 mg/kg) compared to the free form of the drug (50 and 20 mg/kg). On the other hand, it should be stated that the nanocapsules drug was able to provide the same protective effects as the normal drug and even better than that in a lower dose than the normal way of administering the drug and show that the use of nanocapsules for oral administration of the drug can improve the effectiveness and reach of the drug. accelerate the target cells and increase the efficiency of the treatment so that a similar result can be achieved with a lower dose of the effective substance. While our findings highlight the potential neuroprotective effects of encapsulated quercetin and gallic acid against Alzheimer's rat model, we propose to evaluate the therapeutic efficacy of encapsulated quercetin and gallic acid in transgenic Alzheimer's animal models and long term evaluation of in vivo toxicity and pharmacokinetic studies. In summary, the combination of 20 mg/kg quercetin and 50 mg/kg gallic acid can reduce the oxidative imbalance of the tissue, improve the oxidative stress indicators, and reduce the

histopathological lesions caused by Alzheimer's disease. Also, the nanocapsules formed at a dose of 10 mg/kg of this drug combination can lead to better results.

Conclusion

In this study, the protective effects of both quercetin and gallic acid by gavage on aluminum-induced brain damage were investigated. As the results show, the nanocapsule form of both quercetin and gallic acid by gavage at a lower dose can protect against aluminum-induced damage by generating a protective effect, and the nanocapsule form does not have the adverse effects that the free form of the drug causes. Therefore, the nanocapsule form of quercetin and gallic acid may offer a promising direction for future preclinical studies to protect against the adverse effects of aluminum.

Author contribution

RTT, performed the experiments, collected and analyzed the data, interpreted the findings, and wrote the initial draft of the manuscript. **AM**, helped with data analysis and laboratory work and helped create figures and tables. **MG**, participated in the interpretation of the results and contributed to the biochemical and histological assessments. **FZGh**, controlled the data analysis procedure, offered scientific advice and assistance with the study design, and made significant revisions to the manuscript. **AF**, aided with the validation of the results and helped prepare and characterize the nanocapsule formulations. Technical support, statistical analysis, and manuscript revision were all contributed by **AHE**. **AT**, supervised the entire investigation, created the experimental setup, provided direction for interpreting the findings, and edited the manuscript for important intellectual content.

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Conflicts of interest

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