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Niosomal hesperidin ameliorates depression-induced behavioral, histological, and molecular alterations in the rat hippocampus

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ABSTRACT

Aim: Niosomal carriers offer a promising drug delivery in neurological disorders. This study evaluated the therapeutic effects of niosome-encapsulated hesperidin on reserpine-induced depression in rats, focusing on oxidative stress, transient receptor potential melastatin2 (TRPM2) and transient receptor potential canonical 1 (TRPC1) gene expression levels, and histo-striology changes in hippocampal tissue.
Methods: 36 rats were randomly divided into six groups, including Control, Depression, Hes (Hesperidin), nHes (Niosomes of Hesperidin), Dep+Hes, and Dep+nHes. Reserpine 0.5 mg/kg was administered intraperitoneally to induce the Dep Model for 14 days. Then, with intraperitoneal injection, rats received Hes (20 mg/kg for 14 days) and nHes (20 mg/kg for 7 days). Next, the Forced Swimming Test (FST) and the Sucrose Preference Test (SPT) were applied to assess Behavioral disorders. Finally, Glutathione peroxidase (GPX) levels, malondialdehyde (MDA), TRPM2, and TRPC1 gene expression, as well as histo-stereological studies, were measured in hippocampal tissue.
Results: The nHSP treatment reduced immobility duration and increased sucrose solution consumption compared to the depression group in behavioral tests. The expression of genes TRPM2 decreased in the Dep+nHSP group compared with the depression group, and TRPC1 decreased in the HSP group compared with the depression group. Histological and stereological studies revealed improved apoptosis and cell death in nHSP-treated rats, with an increase in live cells compared to the depressed group.
Conclusion: Our findings suggest that nHes improves depression phenotypes induced by reserpine by reducing hippocampal oxidative stress damage and histopathological alterations. This may be related to enhanced therapeutic efficacy and bioavailability due to liposomal carriers.

1. Introduction

The word Major Depressive Disorder means stagnation, decline, and distress [1]. Depression is a multifactorial disease and can be caused by various factors such as genetics, environment, biological factors, psychological factors, pathophysiology, and social and family factors [2]. Several theories have been proposed to explain the pathophysiology of depression. Other significant hypotheses include the loss of brain monoamines and dysregulation of the hypothalamus-pituitary-adrenal (HPA) axis, with the latter considered a more impactful cause. The regulation of the HPA axis by hippocampal glutamate receptors and disruptions in nervous system signaling pathways, such as ion channel abnormalities, are also linked to depression. One of the main causes of depression that can lead to changes in brain structure and function is oxidative stress [3,4]. A key theory posits an imbalance between reactive oxygen species (ROS) and antioxidants, which can lead to

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inflammation, oxidative stress, and depression. Increased oxygen consumption can produce excess ROS [5]. As a result, due to an imbalance between antioxidant defenses and ROS, brain function is impaired, and neuronal abnormalities occur [6,7]. TRPM2 represents a unique channel that activates in response to nitrogen species (ROS/RNS) and reactive oxygen [8]. It is generally accepted that exposure to ROS/RNS activates these channels by producing ADPR, an agonist for TRPM2 [9]. TRPC channels play multiple crucial roles in the brain. These functions include axon guidance, neurite outgrowth, neuronal survival or apoptosis, and neural progenitor cell proliferation and differentiation [10,11].

Additionally, TRPC channels contribute to modulating neuronal excitability and enhancing [12,13]. TRPC channels are another vital subfamily of TRP ion channels in depression. This gene, which is expressed in the hippocampus, acts as an environmental sensor and regulates cation currents, particularly within cells [14].

The TRPC channel plays a role in brain function, neurological development, and neurological diseases. The TRPC-1 develops and differentiates neurons by regulating calcium homeostasis[15]. The MRI images taken from depressed people show a reduction in the density and size of neurons and glial cells. Besides, a decrease in the volume of specific limbic system structures such as the amygdala, prefrontal, and hippocampus has been observed. The hippocampus is a part of the limbic system and includes the subiculum, dentate gyrus, and three other areas named C1, C2, and C3. The death of nerve cells causes a decrease in the volume of the hippocampus, changes in neurons and glial cells, and a reduction in neurogenesis. Finally, these neuronal changes cause neurocognitive disorders in depressed people[16,17].

Since one of the significant causes of depression is ROS, antioxidants are considered potential drugs to balance the ROS level. Hesperidin is a well-known member of flavonoids that contains antioxidant properties and is extracted from the peel of citrus fruits such as bitter orange and sweet orange [18,19]. Despite the benefits of flavonoids, they face limitations due to their low solubility and stability in water, which makes studies to improve their bioavailability [20]. Therefore, nano drug carriers are being investigated for their ability to combat oxidative stress and disturbances in signaling pathways during disease progression [21]. Niosomes are a novel drug delivery system that traps hydrophilic drugs in the core cavity and hydrophobic drugs in the nonpolar region of the bilayer, thus allowing the incorporation of both hydrophilic and hydrophobic drugs into niosomes [22]. Niosomes are amphiphilic, in which the drug is encapsulated in a vesicle formed by a nonionic surfactant; hence the name niosomes. Niosomes are microscopic [23]. This study aims to examine the effects of niosome Hesperidin on the level of oxidative stress parameters, expression of TRPM2 and TRPC1 genes, and changes in histological and striological features of the hippocampus in depressed rats over 7 days.

2. Materials and methods

2.1. Drug administration

In this study, chronic low doses of reserpine (0.5 mg/kg, i.p.; Sigma-Aldrich, USA) were used to induce depressive illness [24]. Reserpine was dissolved in saline (0.3 ml) and injected intraperitoneally once a day for two weeks. In the treatment groups, nHSP and Hesperidin(Formulation of Hesperidin C28H34O15, Sigma Aldrich CAS Number:520–26–3) with a 20 mg/kg dose were injected intraperitoneally for 7 and 14 days, respectively.

2.2. Synthesis of nHSP

A thin film hydration method was used to prepare niosomes. The thin film was prepared by dissolving a 1:1 molar ratio of cholesterol as a Nisome membrane stabilizer and non-ionic surfactant (Tween 60) in 10 ml of chloroform in a round-bottomed flask and then evaporating chloroform at a temperature of 60°C by rotary evaporation under vacuum conditions. The synthesized thin film on the inner wall of the round-bottom balloon was hydrated with 7 ml of PBS solution at 45°C. Finally, the synthesized niosomes were subjected to ultrasonic waves with an intensity of 100 degrees for 30 min at room temperature to reduce their size [25]. To load the drug into the nisome, we first dissolved the drug in 1 ml of DMSO solvent and then added the drug to the formulation drop by drop at the same time while sonication. To evaluate the physicochemical properties and biological performance of niosomal Hesperidin formulation, determination of nanoparticle size, dispersion coefficient, and zeta potential, morphology, determination of the percentage of drug entrapped in niosomes, study of Hesperidin release from niosomal nanoparticles, and in vitro cytotoxicity assay (MTT) were performed.

2.3. Animals

Thirty-six male Wistar rats (Pasteur Institute, Orumieh, Iran; weighing 250 ± 20 g) were used for this study. There were six cages with 24-hour light/dark cycles (6:00 a.m. to 6:00 p.m. with lights) in the colony room at 220 C. Food and tap water were freely available except during experiments [26]. Animal care, treatment, and surgical procedures were approved by the scientific and ethics committees of Tabriz University (IR.TABRIZU.REC.1402.055) and performed based on the Guide for Care and Use of Laboratory Animals published by the National Institute of Health, United States (NIH Publication No. 85–23, revised 1985). All efforts have been made to minimize suffering. Operations that could create pain and distress were performed on other animals.

2.4. Experimental design

Animals were randomly divided into six groups (n = 6), including 1) Control, 2) Depression (received reserpine 0.5 mg/kg for 14 days) [24, 27], 3) Depression + the treatment with Hesperidin (first received reserpine 0.5 mg/kg for 14 days and then received Hesperidin 20 mg/kg for 14 days, Dep+hes) [26], 4) Depression+nHSP treatment (first received reserpine 0.5 mg/kg for 14 days and then received nHSP 20 mg/kg for seven days), 5) Hesperidin group (received Hesperidin 20 mg/kg for 14 days), and 6) nHSP group (20 mg/kg for seven days).

2.5. Behavioral assessment

2.5.1. Force swimming test

The forced swim test examined depressive behavior using the following procedure. The test was performed over two days. On the first day, each rat was placed in a light-blocking cylindrical container (30 cm diameter, 60 cm height) containing 25 cm of water at 25–26°C for 15 min to acclimatize. On the second day (the day of testing), each rat was placed in the water-containing cylinder for 6 min. The duration of immobility of the animal during the test day (the period of minimal locomotor activity sufficient to keep the head afloat) is a parameter that determines the degree of depression[27].

2.5.2. Sucrose preference test

The sucrose preference test was performed based on sucrose solution consumption to investigate the lack of pleasure as a pseudo-depressive behavior. To accomplish this test according to the protocol, 72 h before the test, the animals were fed for 24 h using two bottles containing 1 % sucrose. Then one of these bottles was replaced with pure water after 24 h. For the next 24 h, the animals will have food and water. On the day of the test, each animal was placed in a special cage, and a bottle containing 200 ml of sucrose solution and pure water was provided to them for 24 h [26].

Sucrose preference % = Sucrose consumption/ Sucrose + Water consumption × 100

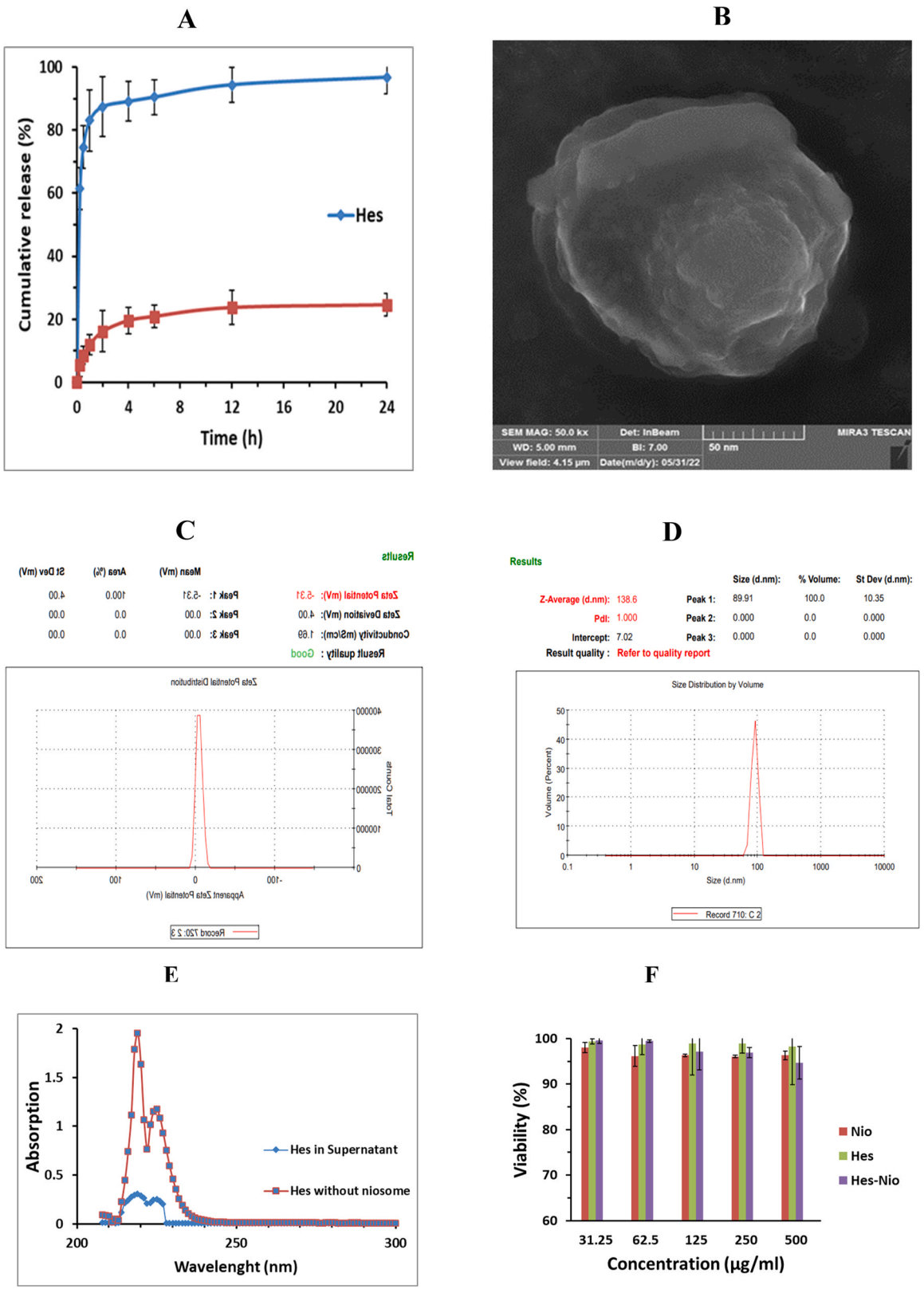


Fig. 1. A shows the amount of drug released at different times. B shows niosome FESEM image at a 50 nm scale. C showed a nanoparticle zeta potential diagram. D showed a nanoparticle size chart. E showed determining the amount of hesperidin inclusion in niosomal nanoparticles (%EE). F showed a diagram of cell viability in increasing concentrations of nisome, hesperidin, and nisome-hesperidin.

2.6. Brain homogenate preparation and the assessment of Trpm2 or TRPC-1 levels in the hippocampus and qRT-PCR Analysis

Twenty-four hours after completing all the tests, the animals were anesthetized with 40 mg/kg chloral hydrate, and then the brain tissue was removed. The hippocampus tissue was transferred to the 80 °C freezer for molecular tests.

Initially, 800 microliters of Trizol solution were used to extract RNA from the hippocampus tissue [22], followed by homogenization at a speed of 2000 rpm for 5 s. Subsequently, the mixture was centrifuged at 13,000 rpm at 4 °C for 15 min. The upper phase was removed, and 1 ml of cold isopropanol was added and then placed in a −20°C freezer for 45 min to 24 h [28]. Afterward, it was centrifuged at 13,000 rpm for 15 min at 4°C. The supernatant was removed, washed with 1 ml of 75 % alcohol, and centrifuged at 8000 rpm at four °C for 10 min. Finally, it was dried in a dry bath at 56–58 °C to eliminate excess alcohol. The concentration of the samples using a nanodrop device was measured; all RNA samples had 260/280 nm ratios≥ 1.8. The qPCR was performed using one µl of cDNA, 0.5 µl master mix (Smobio), five µl SYBR Green and 3.5 µl DEPC-treated water. Specific oligonucleotide primers were used to analyze Trpm-2 or TRPC-1 mRNA expression. The qPCR was performed according to the manufacturer's instructions. The primers used were tested for specificity by melting curve analysis, agarose gel electrophoresis, and using a negative control (TRPM2: F: AGAGCCCC-CAACCTCACAAA, R: ATTGCTTCGTCGGAGATTGGG), (TRPC1: F: AGGACAGCCTCAGACATTCCA, R: ACATTTTGCACTGAGG). The relative expression values using the method were calculated. Samples were reverse-transcribed into cDNA (37 °C for two hours) using the parstouse kit (Iran) [29].

2.7. Oxidative stress parameters change.GPx)

The activity of this enzyme was calculated using the RANSEL kit (Randox Laboratories Ltd, Crumlin, UK) [30]. The basis of the reaction is that initially reduced glutathione is oxidized by glutathione peroxidase. Then, in the next step, the oxidized glutathione is converted to reduced glutathione in the presence of glutathione reductase and NADPH. The decrease in absorbance due to the conversion of NADPH to NADP+ is proportional to the GPx concentration. The absorbance of the cuvette samples was measured at a wavelength of 340 nm and a temperature of 37 °C against distilled water.

The number obtained from the optical absorption is inserted into the following formula:

$$\text{U/l of hemogenate} = 8412 \times \Delta A\ 340\ \text{nm/minute}$$

An autoanalyzer performed all the above steps. The values obtained were expressed as units per milligram of protein.

$$GPx(U/l)/protein(mg/dl) \times 10 = GPX\ (u/mg\ protein)$$

2.8. Oxidative stress parameters change(MDA)

Malondialdehyde was measured using the thiobarbituric acid reactive substances (TBARS) method [31]. In this method, to measure MDA concentration, first, the supernatant was diluted 1:10, and 500 µL of the diluted solution was mixed with 3 ml of 1 % phosphoric acid. After vortexing, 1 ml of 0.67 % TBA solution was added, and the samples were placed in a boiling water bath for 45 min. Then, after cooling, 3 ml of n-butanol was added, the sample was vortexed for 1–2 min, and then centrifuged at 3000 rpm for 10 min. The organic phase was separated, and the optical absorption was measured at a wavelength of 532 nm against a blank (n-butanol). The results were expressed as nanomoles of MDA per mg of protein using a standard curve. An autoanalyzer performed all the above steps. The values obtained were expressed as units per milligram of protein.

$$\text{MDA (nmol/ml)/protein(mg/dl)} \times 1000 = \text{MDA (nmol/mg protein)}$$

2.9. Histological assessment

After removing the brains, a paraformaldehyde solution was used to fix the samples. Then, the steps of dehydrating the samples, passing them through different dilutions of alcohol, *xylol,* and soaking in paraffin were done, and Lockhart molds were prepared from paraffin blocks. After that, sections with a thickness of 5 µm were prepared using a microtome. The samples were then stained with H&E [32].

2.10. Striological assessment

First, the hippocampal tissue was completely lysed with an ultrasonic homogenizer under an ice bath. The nuclei in it were stained with specific nucleus-like staining with DAPI-(4′6-diamidino-2phenylindole) DAPI, and the neurons were stained with a particular antibody to neuronuclear antigen (NeuN), which is expressed in all the nuclei of the majority of nerve cells (notable exceptions are Purkinje cells, inferior olivary neurons, mitral cells, and photoreceptors). The total number of "non-neuronal nuclei" is calculated by subtracting the number of NeuN-containing nuclei from the total number [33].

2.11. Statistical analysis

Statistical analysis was performed using one-way repeated-measures ANOVA with Tukey's post hoc tests in GraphPad Prism 8 software to compare values at each point with those of the control. It expressed all values as mean ± S.E.M. The $P < 0.05$ was considered significant.

3. Result

3.1. Nano Hesperidin characterization results

3.1.1. Determination of Hesperidin release rate

The results of the release rate of free Hesperidin and Hesperidin enclosed in niosomes from the dialysis bag indicate the controlled release of Hesperidin after encapsulating it inside the niosome nanoparticles. The results showed that during the first 30 min of the experiment, more than 50 % of free Hesperidin was released from the dialysis bag, while the encapsulation of Hesperidin inside the niosomal nanoparticle significantly reduced its release rate. As a result, controlling the release rate improves the bioavailability and increases the shelf life of free Hesperidin in the body. The release test showed that the release rate of nano hesperidin was prolonged from 25 min to 24 h. In comparison, free Hesperidin was discharged during the first hours (Fig. 1 A).

3.1.2. Niosome surface morphology

FESEM (field emission scanning electron microscope) confirmed the uniformity, order, and spherical shape of nanoparticles and did not show any aggregation. The results also showed that the diameter of the synthesized nanoparticles is between 50 and 90 nanometers (Fig. 1 B).

3.1.3. Determination of size, zeta potential, and dispersion index of synthesized niosomes

In the present study, niosomes were synthesized by thin-film hydration and the Sonicishin method. Combining the 1:1 molar ratio of cholesterol and Tween 60, niosomes with an appropriate and optimal size of 89.91 nm and a dispersion index of 0.2 and 0.392 mV were synthesized for the empty niosome and the Hesperidin niosome,

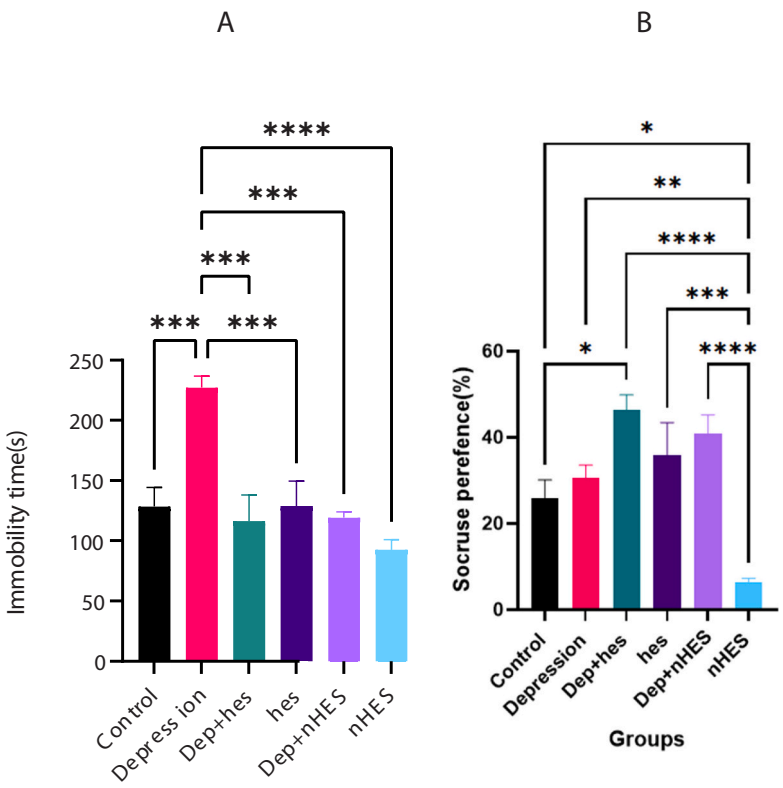


Fig. 2. A and B: the immobility time and the preferred intake of sucrose. The bars indicate the mean ± S.E.M.

respectively. The zeta potential of the synthesized niosomes is in the range of −5.31 mV (Fig. 1, C, D).

3.1.4. Determining the amount of Hesperidin inclusion in niosomal nanoparticles (%EE)

The results of the encapsulation efficiency study show a high rate of Hesperidin incorporation into niosomes (84 %), which can be attributed to the lipophilic nature of Hesperidin. These findings confirm the importance of Hesperidin encapsulation in niosomal particles for proper and controlled release of hesperidin during intra-body injection without increasing the injection dose (Fig. 1E).

3.1.5. Assessment of cytotoxicity of nanoparticles in vitro using MTT test

This study evaluated the cytotoxicity of Hesperidin, niosome, and niosome Hesperidin on a mouse fibroblast cell line (NIH-3T3) using the MTT test. The results showed that hesperidin, niosome, and niosome Hesperidin do not have any toxic effects on normal, healthy cells. Additionally, by increasing the concentration of Hesperidin, niosome, and niosome Hesperidin up to 500 μg/ml, no harmful effects were observed on the cells (Fig. 1 F).

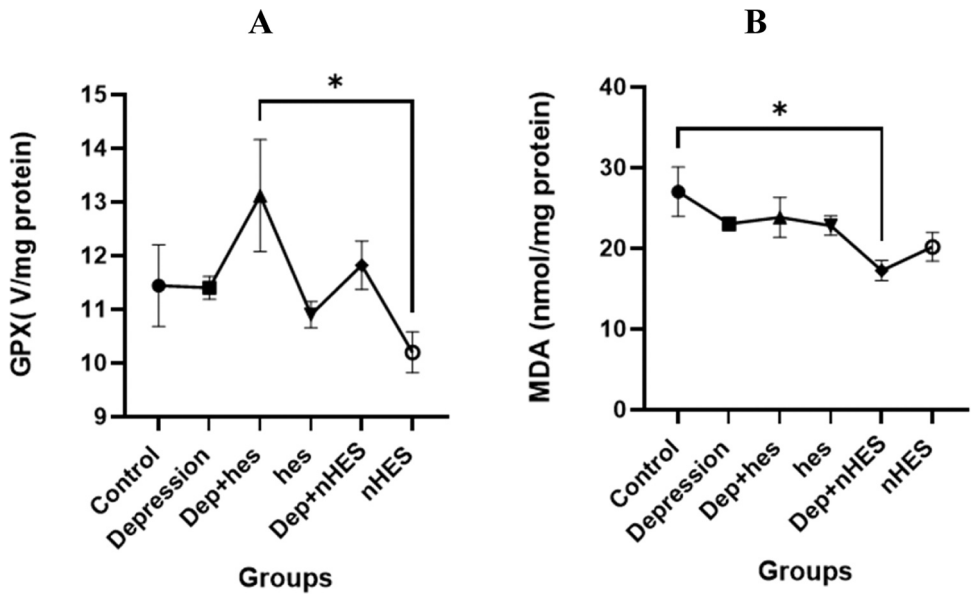


Fig. 3. A and B: Mean changes in the levels of MDA and GPX compared to the control group, and shows the intragroup comparison of MDA and GPX. The bars show the mean ± S.E.M.

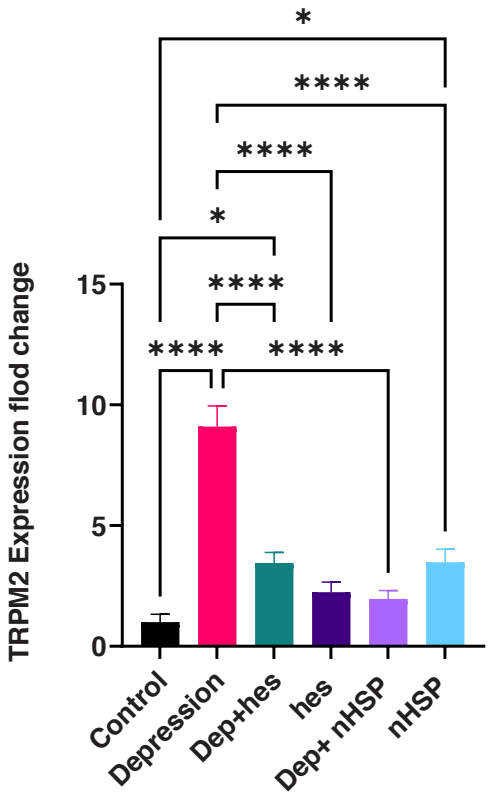


Fig. 4. Mean changes in mRNA levels of TRPM2 compared to the control group, and shows the intragroup comparison of TRPM2. The bars show the mean ± S.E.M.

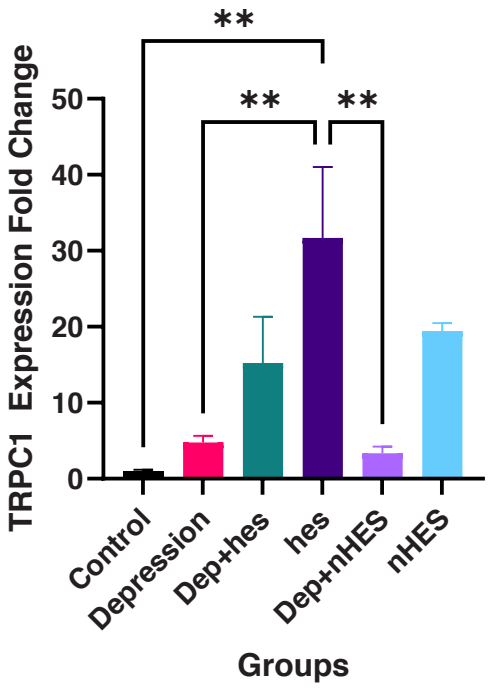


Fig. 5. Mean changes in mRNA levels of TRPC1 compared to the control group. The chart shows the intragroup comparison of TRPC1. The bars show the mean ± S.E.M.

3.2. Comparison of immobility time in the forced swimming test between groups

The results showed a significant decrease in immobility time in the control group compared to the depressed group (95.00 % CI of diff: −163.3 to −34.40, P < 0.001). Also, the duration of immobility in the depressed group (0.5 mg/kg) has increased significantly compared to the depression+Niosome Hesperidin(95.00 % CI of diff: 70.06–198.9, P < 0.0001) group, the Hesperidin group(95.00 % CI of diff:33.90–162.8), and the depression + Hesperidin group (95.00 % CI of diff: 46.40–175.3, P < 0.001). Additionally duration of immobility in the depressed group (0.5 mg/kg) has increased significantly compared Niosome hesperidin group (95.00 % CI of diff: 43.56–172.4, P < 0.001, Fig. 2.A).

3.3. Comparison of sucrose solution consumption in the sucrose preference test between groups

Statistical analysis of the data from the sucrose preference test indicated significant changes. Tukey's test showed that sucrose consumption decreased significantly in the control group compared to the treatment with the Hesperidin group (95.00 % CI of diff: −39.56 to −1.438, p < 0.05). On the other hand, the control group's sucrose consumption significantly increased compared to the Niosome Hesperidin group (95.00 % CI of diff: 0.5049–38.63, p < 0.05). Additionally, sucrose consumption in the depressed group was significantly lower compared to the Niosome Hesperidin group (95.00 % CI of diff: 5.172–43.30,p < 0.01). In the intra-group comparison, sucrose consumption in the group was significantly increased in the treatment with Hesperidin compared to the Niosome Hesperidin (95.00 % CI of diff: 21.00–59.13,p < 0.0001). Furthermore, the Hesperidin group showed a significantly increased value compared to the Niosome Hesperidin group (95.00 % CI of diff: 10.50–48.63, p < 0.001). Also, A significant increase in sucrose consumption has been observed in the treatment with the Niosome Hesperidin group compared to the Niosome Hesperidin group (95.00 % CI of diff: 15.50–53.63, p < 0.0001; (Fig. 2.B).

3.4. Comparison of the MDA and GPX levels among different groups

Observations revealed that administration of the level od MDA in the control group significantly increased the level of MDA compared to the Dep+nHES group (95.00 % CI of diff: 1.320–18.20, p < 0.05, Fig. 3 B). The level of GPX in the Dep+hes group significantly increased compared to the nHES group (95.00 % CI of diff:0.3243–5.516, p < 0.05, Fig. 3 A).

3.5. Comparison of TRPM2 gene expression levels among different groups

The results of real-time PCR showed that in the level of TRPM2 gene expression, the control group had a significant decrease compared to the depressed group (95.00 % CI of diff: −10.38 to −5.814, P < 0.0001). Also, the control group showed a significant decrease in the level of TRPM2 gene expression compared to the treatment group with Hesperidin(95.00 % CI of diff: −4.728 to −0.1589) and the nHSP group (95.00 % CI of diff: −4.759 to −0.1904, P < 0.05). In the intragroup comparison, the results showed that the depressed group had a significant increase compared to the Hesperidin treatment group(95.00 % CI of diff: 3.370–7.939), the treatment with Niosome Hesperidin group (95.00 % CI of diff: 4.854–9.423), Hesperidin(95.00 % CI of diff: 4.577–9.146), and Niosome Hesperidin groups (95.00 % CI of diff: 3.339–7.908, P < 0.0001; Fig. 4).

3.6. Comparison of TRPC1 gene expression levels among different groups

Real-time PCR results showed that the TRPC1 gene in the control group was significantly lower compared to the Hesperidin group (95.00 % CI of diff: −50.75 to −10.67, P < 0.01). Also, the results of the intra-group comparison show that the TRPC1 gene was significantly increased in the Hesperidin group compared to the depression(95.00 %

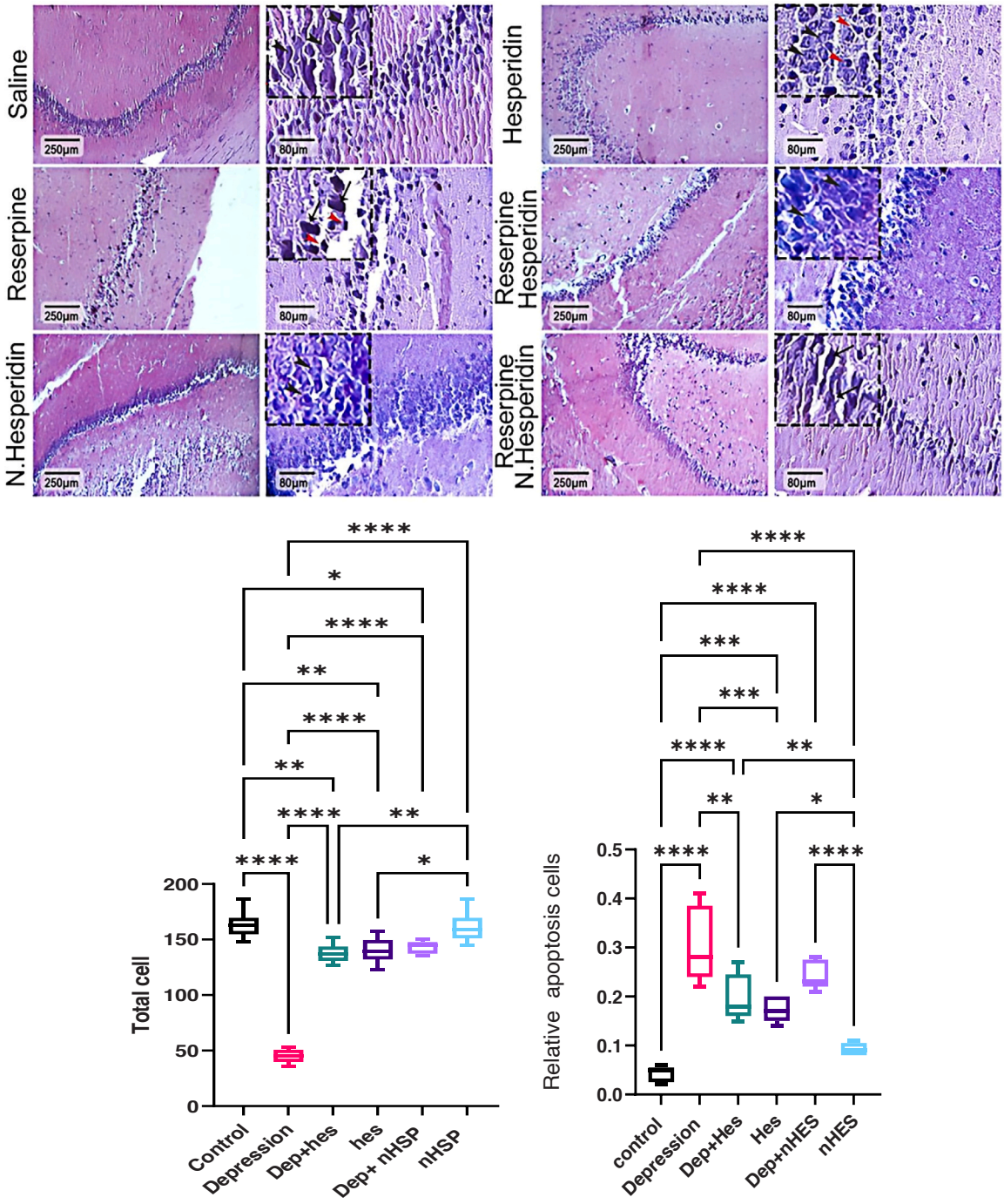


Fig. 6. The photomicrograph of the CA1 hippocampus from different groups; the histologically intact cells in the section from the saline group (blackhead arrows) are detectable in a photomicrograph with higher magnification. The sections from reserpine represent massive cell death with eosinophilic cytoplasm (arrows) and apoptotic cells (redhead arrows) in the CA1 zone, H&E staining. A shows the total number of cells, and B shows the results of counting the number of apoptotic cells. The bars show the mean ± S.E.M.

CI of diff: 6.899–46.98) and treatment with the Niosome Hesperidin group (95.00 % CI of diff: 8.322–48.40, $P < 0.01$; Fig. 5).

3.7. Comparison of histological changes of hippocampal tissue among different groups

The results of pathohistological investigations showed that cellular popularity is normal in the control group with typical histological nuclei and cytoplasmic characteristics. The Depression group exhibits massive cell loss in one mm2 of tissue, the distribution of apoptotic cells (cells with shrunken nuclei), and damaged cells with acidophilic cytoplasm. Moreover, severe edema is revealed in the intercellular space. The general histological characteristics are shown in the Niosome Hesperidin and Hesperidin groups. The Niosome Hesperidin and Hesperidin could significantly decrease cellular damage, apoptotic cell distribution, and edema in the intercellular area (Fig. 6).

3.7.1. Comparison of total cell counts among different groups

The histological studies and the counting of neuroglia cells show a significant increase between the control groups and the depressed group

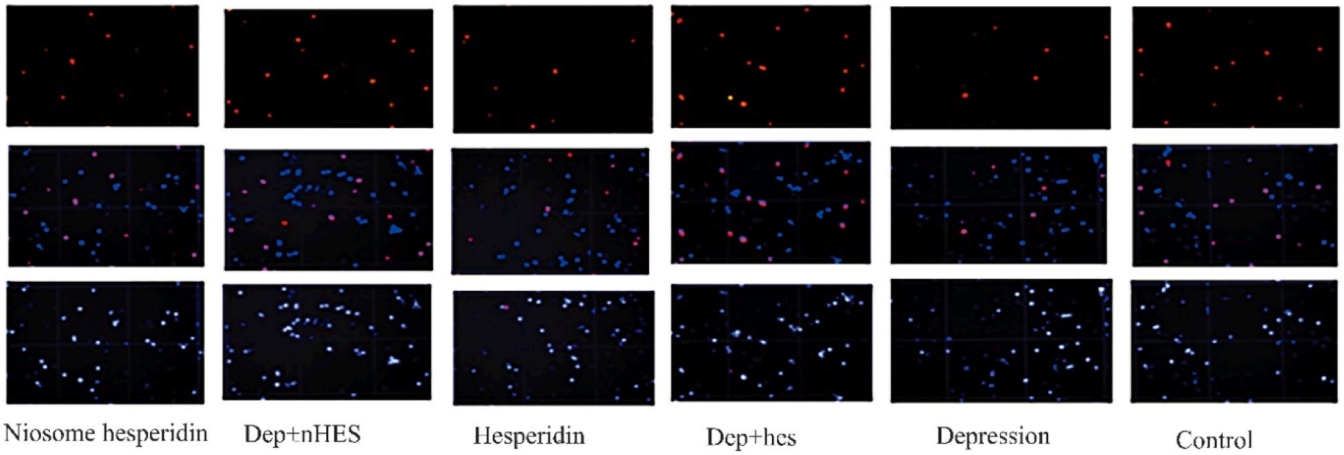


Fig. 7. Checking the number of cells using a Neobar slide. The resulting images are from the immunofluorescent method. For each group, a picture of neu N-positive red cells, an image with DAPI-positive blue cells, and a MERGED image are placed.

(95.00 % CI of diff: 100.2–136.1, $p < 0.0001$). Also, a significant increase was observed between the control group and treatment group with Hesperidin (95.00 % CI of diff: 7.729–43.60), and between the control group and the Hesperidin group(95.00 % CI of diff: 5.395–41.27, $p < 0.01$). Also, a significant increase was observed between the control group and the treatment group with Niosome Hesperidin (95.00 % CI of diff: 2.229–38.10, $P < 0.05$). Also, in the intra-group Comparison, a significant decrease was observed between the depressed group and the treatment groups with Hesperidin(95.00 % CI of diff: −110.4 to −74.56), the group of hesperidin(95.00 % CI of diff:-112.8 to −76.90), the treatment group with Niosome Hesperidin(95.00 % CI of diff:-115.9 to −80.06), and the Niosome Hesperidin group (95.00 % CI of diff:-133.8 to −97.90, $p < 0.0001$). There was a significant decrease between the treatment group with Hesperidin and the Niosome Hesperidin group (95.00 % CI of diff: −41.27 to −5.395, $p < 0.01$). The number of total cells was significantly decreased between the Hesperidin groups and the Niosome Hesperidin (95.00 % CI of diff: −38.94 to −3.062, $p < 0.05$; Fig. 6.A).

3.7.2. Comparison of counting the number of relative apoptotic cells among different groups

In histological studies and by counting the ratio of relative apoptotic cells, a significant decrease is observed among the control group and depression(95.00 % CI of diff: −0.3448 to −0.1832, $p < 0.0001$), treatment with Niosome Hesperidin(95.00 % CI of diff:-0.2828 to −0.1212, $p < 0.0001$), the treatment with Hesperidin (95.00 % CI of diff:-0.2368 to −0.07517, $p < 0.0001$), and the Hesperidin group (95.00 % CI of diff: −0.2128 to −0.05117, $p < 0.001$). Also, a significant increase was observed in the depressed group compared to the Hesperidin treatment group (95.00 % CI of diff: 0.02717–0.1888, $p < 0.01$). The number of relative apoptotic cells in the depressed group has increased significantly compared to the Hesperidin(95.00 % CI of diff:0.05117–0.2128,$p < 0.001$) and the Niosome Hesperidin groups (95.00 % CI of diff: 0.1332–0.2948, $p < 0.0001$). Also, the number of relative apoptotic cells in the treatment with the Niosome Hesperidin group has increased significantly compared to the Niosome Hesperidin group (95.00 % CI of diff: 0.07117–0.2328, $p < 0.0001$). Additionally, the treatment with Hesperidin showed a significant increase compared to the Hesperidin group(95.00 % CI of diff: 0.02517–0.1868, $P < 0.01$). On the other hand, a significant increase was observed in the Hesperidin groups and the Niosome Hesperidin group (95.00 % CI of diff: 0.001175–0.1628, $p < 0.05$; Fig. 6. B).

3.8. Comparison of stereological changes among different groups

Stereological studies and counting of neuroglia cells showed a decrease in the number of cells in the depressed group, which showed apoptosis and cell damage (Fig. 7). The results of counting the cells in the Neobar slide, calculating the percentage of nerve cells, and calculating the rate of cells in the hippocampus tissue also confirm the histological results and show that the number of nerve cells in the depressed group has decreased. In contrast, the percentage of nerve cells in the group treated with Hesperidin nanoparticles increases. Also, the weight of the hippocampus tissue decreased in the depressed group, while the importance of the hippocampus tissue increased in the group treated with Hesperidin nanoparticles. The percentage of total brain cells also confirms the same results (Table 1).

4. Discussion

In the current century, MDD has been counted as one of the leading causes of disability that can contribute to mortality. Regardless of social context, this mood disorder occurs in all populations [34]. Several neurotransmitters or metabolic systems are implicated in the pathogenesis of MDD. Based on previous findings in humans and animals, the serotonergic (serotonin (5-HT)) and norepinephrine/noradrenergic systems are implicated in depression [35,36]. We found that the mobility time in the depression group significantly increased compared to the treatment group with the nHES group. While the Sucrose consumption was reduced in the depressed group compared to the Hesperidin neosome group. It has been shown that in depressed patients, monoamines, which are excellent scavengers of hydroxyl and oxygen radicals, are deficient due to increased monoamine catabolism [37].

Furthermore, depression is associated with changes in brain neuroplasticity and function, besides a reduction in frontal cortex and hippocampus volume. During depression, these abnormalities in the function and structure of the brain are linked to oxidative stress. Nevertheless, the oxidative stress and a combination of factors may contribute to reduced hippocampal volume during depression, including dysregulation of the HPA axis, inflammation, and altered neurotrophic signaling [3,4,38]. Furthermore, it has been proven that stress can also reduce dendritic spine density and impair synaptic plasticity [39].

Regarding oxidative stress, antioxidants play a defensive role by neutralizing free radicals. This happens through nonenzymatic and enzymatic physiological components, by antioxidants donating their electrons to ROS and undergoing oxidation. Due to the key role of antioxidants, a reduction in their capacity leads to subsequent damage to DNA, protein, and fatty acids by ROS and nitrosative stress [40]. Our

Table 1
The results of counting cells and calculating the weight of hippocampal tissue and the percentage of cells.

Brain	Weight(mg)	nbrainCell(x106)	Cells(x103)/mg	nbrainNeuN + nuclei(x106)	nnon − neuronalcells(x106)	HIPPOCAMPUSWeight(mg))	ncells(x106)	nneurons(x106)	HIPPOCAMPUSWeight(mg))	ncells(x106)	nneurons(x106)	%ofbrainweight	%ofallcellsinbrain	%ofallneuronsinbrain	Cells(x103)/mg	%ofNeuN + nuclei	Neurons(x103)/mg	nnon − neuronalcells(x106)
Control	1778 ± 213.36	312 ± 12.48	175.4781 ± 17.54	182 ± 18.2	130 ± 10.4	86 ± 6.88	8.2 ± 5.74	0.9 ± 0.06	86 ± 6.88	8.2 ± 5.74	0.9 ± 0.06	4.8	2.6	0.5	95.3	11.0	10.5	7.3
Dep+nHES	1829 ± 219.48	338 ± 13.52	184.8004 ± 18.48	176 ± 17.6	162 ± 12.96	82 ± 6.56	7.7 ± 5.39	1.0 ± 0.07	82 ± 6.56	7.7 ± 5.39	1.0 ± 0.07	4.5	2.3	0.6	93.8	13.0	12.2	6.7
Dep+hes	1731 ± 207.72	302 ± 12.08	174.4656 ± 17.44	166 ± 16.6	136 ± 10.88	81 ± 6.48	7.9 ± 5.53	1.1 ± 0.07	81 ± 6.48	7.9 ± 5.53	1.1 ± 0.07	4.7	2.6	0.7	97.5	13.9	13.6	6.8
Niosome hesperidin	1982 ± 237.84	319 ± 12.76	160.9485 ± 16.09	185 ± 18.5	134 ± 10.72	84 ± 6.72	8.4 ± 5.88	1.2 ± 0.08	84 ± 6.72	8.4 ± 5.88	1.2 ± 0.08	4.2	2.6	0.6	100.0	14.3	14.3	7.2
Depression	1609 ± 193.08	337 ± 13.48	209.4469 ± 20.94	151 ± 15.1	186 ± 14.88	66 ± 5.28	5.9 ± 4.13	0.5 ± 0.03	66 ± 5.28	5.9 ± 4.13	0.5 ± 0.03	4.1	1.8	0.3	89.4	8.5	7.6	5.4
DEP-HES	1892 ± 227.04	328 ± 13.12	173.3615 ± 17.33	159 ± 15.9	16913.52	73 ± 5.84	7.2 ± 5.04	0.8 ± 0.05	73 ± 5.84	7.2 ± 5.04	0.8 ± 0.05	3.9	2.2	0.5	98.6	10.8	10.7	6.4

research demonstrated that Hesperidin-niosome reduced malondialdehyde levels, while Hesperidin as an antioxidant increased glutathione peroxidase levels. This may be because nanoparticles can increase the solubility and stability of polyphenols, enhance their absorption, protect them from premature degradation in the body, and prolong their circulation time [41,42].

Our findings also showed that the expression of the TRPM2 gene was decreased during treatment with Hesperidin-niosome, which could explain how oxidative stress may induce ADPR. Initially, ROS/RNS induce DNA damage, triggering the activation of poly-ADP-ribose polymerase (PARP) and poly-ADP-ribose glycohydrolase (PARG). These enzymes combine to convert nicotinamide adenine dinucleotide (NAD) into ADPR polymers, which are broken down into ADPR monomers. The resulting ADPR monomer is expected to serve as an intracellular ligand that activates TRPM2 channels [43,44]. This suggests that Hesperidin-niosome, by reducing free radicals, reduced the expression of this gene in a short period. The result indicated that noisome-Hesperidin treatment led to lower expression of the TRPC1 gene. This can be due to heterotetramers composed of TRPC1, TRPC4, and TRPC5 members of the canonical TRPC subfamily of TRP ion channels, which regulate hippocampal synaptic transmission. These channels are implicated in MDD, as M084, a TRPC4/5 inhibitor, has demonstrated antidepressant and anxiolytic properties in mice as preclinical models. Elevated intracellular Ca2 + signaling with increased TRPC1 and TRPM4 expression could promote oxidative stress and apoptosis, potentially driving neuronal loss in recurrent episodes of mania and depression. Thus, the blockade of TRPC1 and TRPM4 may confer therapeutic benefits by normalizing dysregulated neurotransmission, lowering intracellular calcium influx, and mitigating oxidative stress and apoptosis, simultaneously [45]. Also, histological analysis revealed extensive cell death and severe cellular damage in the depressed group.

Furthermore, stereological analysis showed decreased neuroglial cell density in the depressed group. Moreover, the number of apoptotic cells was increased in the depressed group. The reduced hippocampal volume is mainly due to neuronal cell death, neuronal and glial cell population changes, and reduced neurogenesis. Ultimately, these neuronal changes contribute to cognitive impairments observed in the depressed group [16]. For therapeutic purposes, numerous studies show that niosome-based drug delivery increases therapeutic efficacy compared to naturally derived drugs[46,47]. The most significant advantage of noisome-based drug delivery is that it enhances the bioavailability of poorly water-soluble drugs while reducing potential adverse effects. Additionally, niosomes are osmotically active and stable. Moreover, niosomal formulations of natural-based drugs can improve therapeutic outcomes by prolonging blood circulation time, protecting the drug from biological interactions, and ensuring targeted delivery to specific cells [48]. According to this study, Hesperidin-loaded niosomes reduced the duration of immobility, increased pleasure, decreased glutathione peroxidase levels, and increased malondialdehyde levels in depressed rats over 7 days. Hesperidin niosomes also reduced the expression of the TRPM2 and TRPC1 genes and reduced apoptosis and cell damage in the hippocampal tissue of depressed rats. This study showed that Hesperidin nanoniosomes were more effective in a short period than the antioxidant hesperidin over the same period.

Author Agreement Statement

We, the undersigned, declare that this manuscript is original, has not been published before, and is not currently being considered for publication elsewhere.

We confirm that the manuscript has been read and approved by all named authors and that there are no other persons who satisfied the criteria for authorship but are not listed. We further confirm that the order of authors listed in the manuscript has been approved by all of us.

We understand that the Corresponding Author is the sole contact for

the Editorial process. He/she is responsible for communicating with the other authors about progress, submissions of revisions, and final approval of proofs

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Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

No data was used for the research described in the article.

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Corrigendum to "Niosomal hesperidin ameliorates depression-induced behavioral, histological, and molecular alterations in the rat hippocampus" [Biomed. Pharmacother. 191 (2025) 118473]

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The authors regret that the figure legends in the published version were not updated according to the revised submission. I've shared the corrected figure legends below.

Fig. 2 A and B: the immobility time and the preferred intake of sucrose in the control group and other groups, respectively, as well as the comparison within the group. The bars indicate the mean ± S.E.M. In A, Control as compared to Depression *** $P < 0.001$, Depression as compared to Dep + nHES; the treatment group with Niosome loaded with Hesperidin, *** $P < 0.001$, nHES; Niosome loaded with Hesperidin **** $P < 0.0001$, Dep + hes; the treatment group with Hesperidin *** $P < 0.001$, hes; hesperidin *** $P < 0.001$. In B Control as compared to Dep + hes, the treatment group with Hesperidin * $P < 0.05$, nHES Niosome loaded with Hesperidin * $P < 0.05$, Depression as compared to nHES Niosome loaded with Hesperidin ** $P < 0.01$, Dep + nHES; the treatment group with Niosome loaded with Hesperidin as compared to nHES Niosome loaded with Hesperidin **** $P < 0.0001$, Dep + hes; the treatment group with Hesperidin as compared to nHES; Niosome loaded with Hesperidin **** $P < 0.0001$, hes; Hesperidin as compared to nHES Niosome loaded with Hesperidin *** $P < 0.001$.

Fig. 3 A and B: Mean changes in the levels of MDA and GPX compared to the control group, and show the intragroup comparison of MDA and GPX. The bars show the mean ± S.E.M. In A Dep + hes; the treatment group with Hesperidin as compared to nHES; Niosome loaded with Hesperidin * $P < 0.05$. In B Control as compared to Dep + nHES, the treatment group with Niosome loaded with Hesperidin* $P < 0.05$.

Fig. 4. Mean changes in the expression of the TRPM2 gene compared to the control group, and show the intragroup comparison of TRPM2. The bars show the mean ± S.E.M. Control as compared to Depression (****P < 0.0001), nHES; Niosome loaded with Hesperidin * P < 0.05, Dep + hes; the treatment group with Hesperidin * P < 0.05. the depression as compared to Dep + nHES, the treatment group with Niosome loaded with Hesperidin **** P < 0.0001, Dep + hes; the treatment group with Hesperidin **** P < 0.01,nHES; Niosome loaded with Hesperidin **** P < 0.0001, hes; Hesperidin **** P < 0.0001.

Fig. 5. Mean changes in the expression of the TRPC1 gene compared to the control group, and show the intragroup comparison of TRPC1. Control as compared to hes; Hesperidin ** P < 0.01. Depression as compared to hes; Hesperidin ** P < 0.01, hes; Hesperidin as compared to Dep + nHES, the treatment group with Niosome loaded with Hesperidin** P < 0.01.

Fig. 6. The photomicrograph of the CA1 hippocampus from different groups; the histologically intact cells in the section from the saline group (blackhead arrowheads) are detectable in a photomicrograph with higher magnification. The sections from reserpine represent massive cell death with eosinophilic cytoplasm (arrows) and apoptotic cells (redhead arrows) in the CA1 zone, H&E staining. A shows the total number of cells, and B shows the results of counting the number of apoptotic cells. The bars show the mean ± S.E.M. In A, the control group as compared to the Depression(****P < 0.0001), Dep + hes; the treatment group with Hesperidin, Hes; hesperidin(**P < 0.01), and Dep + nHES; the treatment group with Niosome loaded with Hesperidin (*P < 0.05). In the intragroup depressed group as compaired to the Dep + hes; the treatment group with Hesperidin (****P < 0.0001), hes; Hesperidin group (****P < 0.0001), Dep + nHES; the treatment group with Niosome loaded with Hesperidin (****P < 0.0001), and nHES; the Niosome Hesperidin group (**p < 0.01). Dep + hes; the Niosome Hesperidin group as compared to the nHES; the Niosome Hesperidin group (**p < 0.01).hes; Hesperidin group as compaired to nHES; the Niosome

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Hesperidin groups ($p < 0.05$). In B, the control group compared to the depression group(****$P < 0.0001$), Dep + nHES, the treatment group with Niosome loaded with Hesperidin (****$P < 0.0001$), Dep + hes; the treatment group with Hesperidin (****$P < 0.0001$), and Hes; Hesperidin group (***$P < 0.001$). In the intragroup depressed group as compared to Dep + hes; treatment groups with Hesperidin group(**$P < 0.01$), Hes; hesperidin group (***$P < 0.001$), and nHES; the Niosome Hesperidin group (****$P < 0.0001$). Dep + hes; treatment group with Hesperidin as compared to the nHES; Niosome Hesperidin group (**$p < 0.01$). Hes; Hesperidin groups and nHES; the Niosome Hesperidin(*$P < 0.05$).

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