## ORIGINAL ARTICLE

# Effect of niosomes containing saponin on hippocampus tissue damage in animal model of stroke

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**Abstract**
Cerebral ischemia is one reason for death and loss of movement ability of people, which imposes a large and significant cost on the global health system. Niosomes, as useful tools, can increase drug delivery to the brain. The purpose of this research is to investigate the effect of niosomes containing saponin (NS) on stroke- induced damage in the hippocampus of an animal model. The physicochemical characteristics of nanocarriers, such as zeta potential, size, and release test were investigated after the fabrication of thin film method. In this study, Wistar rats were divided into five experimental groups including sham group, stroke group, stroke group with empty niosome injection, stroke group with saponin injection, and stroke group with niosome saponin injection. The study examined various aspects of ischemia including stroke volume, blood-brain barrier (BBB) damage, neurological defects, levels of inflammatory cytokines, and cellular damage in the hippocampus. The findings indicate that NS, with a size of 85.92nm, zeta potential of -34.7 mv, and an entrapment efficiency (EE%) of 85.70% effectively reduced stroke volume, cerebral edema, BBB damage, expression level of TNF-α, and NF-kB genes and inflammation in hippocampal cells. Additionally, NS improved sensory and motor performance in rats. These results demonstrate that NS can mitigate stroke-induced damage in the hippocampus of the rat model by effectively crossing the BBB.

**Keywords:** *Hippocampus; Niosome; Neurological Deficits; Saponin; Stroke.*

## INTRODUCTION

Stroke with the rank of five is the leading cause of death among teens to middle-aged individuals globally [1]. It occurs due to a long-term decrease in blood flow or a defect in blood transfusion, resulting in reduced delivery of glucose and oxygen [2]. Reactive oxygen species (ROS) and oxidative stress are important factors involved in cerebral ischemia [3]. Apoptosis is the critical causes of nerve cell death during ischemia [4]. Therefore, there is a need for neuroprotective agents with fewer complications. Saponins, which consists of a hydrophobic core (Sapogenin) surrounded by a hydrophilic sugar chain attached to a triterpene or steroid aglycone, has antioxidant properties and affects the signaling pathway of inflammation. They can prevent neurodegenerative diseases caused by free radicals [5]. The main challenge in treating brain diseases is delivering the therapeutic substance across the blood-brain barrier(BBB) to the brain cells, which can be achieved using nanocarriers [6, 7]. Niosomes, a type of nanocarriers, offer advantages such as chemical stability, efficient drug storage, and cost-

effectiveness [8]. Niosomes can traverse cerebral vessels by inhibiting diffusion function [9, 10]. Furthermore, nanocarriers have several benefits, including enhancing effectiveness in target areas reducing the drug dosage and side effects [11]. As nanoparticles accumulate in organs like the spleen, kidney, and liver their ability to across the BBB and penetrate the central nervous system (CNS) increases [12]. Hence, this study aimed to investigate the protective effect of niosomes containing saponin (NS) on hippocampal tissue cells in an animal model of stroke. Parameters such as stroke volume, cerebral edema, and expression levels of inflammatory cytokines were investigated examined for this purpose.

## MATERIALS AND METHODS
### Chemicals

Evans Blue (EB), 2, 3, 5-triphenyl tetrazolium chloride (TTC), Chloral hydrate, and Trichloroacetic Acid (TCA) were purchased from Merck Company in Germany. Saponin, non-ionic surfactant (Tween 40), Cholesterol, Dialysis Bag (Cut-Off 12 kDa), Chloroform and Phosphate Buffer (PBS) were prepared from Sigma Aldrich Company in the USA.

### Preparation of nanocarriers

Nanocarriers were prepared using thin layer hydration with the same composition and molar ratio of Tween 40 and cholesterol. Initially, precise amounts of Tween 40 and cholesterol were dissolved in 8 mL of chloroform. . The resulting solution was then poured into a round bottom flask. Chloroform was evaporated under vacuum in a rotary device with a temperature of 60°C in 60 minutes. After the solvent evaporation, a thin layer of compounds remained on the wall of the round bottom flask. Subsequently, 10 ml of PBS was added to it  the flask and sonicated for 30 minutes at a temperature of 40°C by a sonicator (Faraz Teb Tajhiz) (4 cycles, 100) [13].

### Characteristics of nanocarriers
**The diameter size of nanoparticles and their surface charge**

The zeta potential and hydrodynamic diameter of nanocarriers were measured using Zetasizer Nano ZS (Malvern Company, England) with three repetitions. Dynamic light scattering (DLS) was employed for this analysis. Only data that met the qualitative criteria were reported [14].

### Entrapment Efficiency (EE%)

To determine the amount of saponin enclosed in nanoparticles, the niosomes were subjected to centrifugation (Pars Azma company, made in Iran) with PBS solvent for 1 hour, at a temperature of 4 degrees Celsius and a centrifuge speed of 15,000 rpm. The supernatant solution was then separated, and the light absorption was measured by a nanodrop device (2000 Thermo Fisher) with three repetitions (Only one of the data with the desired qualitative criterion is given here). Empty niosomes were used as a blank control.

The entrapment efficiency was calculated using the formula: $EE\% = (Cp \ / \ CT \times 100$ ($Cp$: concentration of saponin enclosed in niosomes, $CT$: initial concentration of saponin added to the formulation) [15].

### Release test

To evaluate the release of saponin from niosomes under these conditions, 1 ml of niosome solution containing saponin and free saponin was poured separately placed into a dialysis bag. To simulate the environment inside the body, PBS was placed at the temperature of 37°C, and the dialysis bags containing the samples were placed in a container containing PBS in a shaker incubator (Pars Azma Company, Iran). At specific times, the environment around the dialysis bag was done sampled, and the same amount of PBS was immediately added to maintain the volume. The optical absorption of the prepared samples was measured by a nanodrop device (2000 Thermo Fisher) [16, 17].

### Imaging of nanoparticles

To investigate the appearance and structure of NS, images were taken using an electronic microscope (FE-SEM TESCAN MIRA3, Czech Republic). A drop of the sample was poured on a slide dried, and then coated with gold before imaging. Finally, the SEM image was prepared.

### Animals and groups

This article is the result of the thesis of Ms. Elmira Shiri, a PhD student in Animal Physiology, and received the Code of Ethics IR.IAU.SRB. REC1398.197 was conducted by the Ethics Committee of Islamic Azad University, Tehran Science and Research Unit. The regulations and principles of using laboratory animals were fully and strictly followed.

The study used 100 mature male Wistar rats, which were kept separately in five pairs in cages. The ambient temperature of the cages was maintained at 21-25 degrees Celsius. The relative humidity (RH) of the animal-keeping environment was about 50, and the illumination level was 12 hours of light and 12 hours of darkness. After a week of adaptation to the maintenance conditions (when the body weight was about 250 to 350 grams), the rats were randomly grouped, including the sham group, stroke group middle cerebral artery occlusion (MCAO), stroke group + empty niosome (MCAO+N), stroke + saponin group (MCAO+S), and stroke + NS group (MCAO+NS). All five groups were divided into three subgroups of 5 such as stroke volume, brain edema, BBB (blood-brain barrier), and two groups of 3 (histology, gene expression examination). In this study, in the MCAO+S group saponin, rats received saponin (3 mg/kg), 30 minutes before ischemia. In the MCAO+NS group, rats received niosome saponin (1mg/kg) before ischemia. The MCAO+N group received empty niosome and the MCAO group received saline intravenously [18]. The sham group did not have a stroke volume subgroup. The study followed ethical regulations and principles for using laboratory animals.

*Induction of cerebral ischemia*

To induce cerebral ischemia, we followed the method described by Lunga *et al.* Briefly, the rat was anesthetized and a 0-3 nylon thread, approximately 20 mm in length, was inserted into the external carotid artery (ECA). The thread was then advanced through the internal carotid artery (ICA) until it reached the anterior cerebral artery (ACA) while ensuring that the pterygopalatine vein was closed. The blood flow in the middle cerebral artery (MCA) of the right hemisphere was completely occluded using a suture for 60 minutes. The occlusion was confirmed by the resistance felt when moving the suture thread to the ECA [19].

*Evaluation of neurological deficits score (NDS)*

To evaluate the NDS, an investigator blinded to grouping conducted a thorough assessment of neurological damage using the modified neurologic severity scores (mNSS) after 24 hours of ischemia induction. The assessment covered four aspects: motor function, sensory function, balance, and reflexes. The scoring system used

was as follows: 0 indicated no deficiency, 1 to 6 indicated slight deficiency, 7 to 12 indicated intermediate deficiency, and 13 to 18 indicated intense deficiency [20].

*Examining the amount of damage to the BBB*

The extent of BBB damage was assessed by measuring the release of Evans blue (EB), a dye that binds to albumin and is normally excluded from crossing the intact BBB. During surgery and after 30 minutes of ischemia induction, the animal received a 2% EB solution at a dose of 4mg/kg through the ischemic tail vein. After 24 hours, the rats' thoraxes were opened after anesthesia, and the injected EB was removed from the veins using 250 ml of saline until colorless saline exited from the right atrium. The hippocampus of each brain hemisphere was then separated and homogenized in phosphate buffer. Protein isolation was performed by adding 60% TCA (Trichloroacetic acid) to precipitate proteins. After vortexing for 3 minutes and storing in the refrigerator for 30 minutes, the homogenate was centrifuged at 1000 rpm for half an hour to separate the precipitated proteins. The light absorption of the supernatant solution was measured using an ELISA microplate reader (BIOTEK ELX-800TS, USA) at a wavelength of 610 nm. Finally, the concentration of EB in the solution was determined by comparing the absorbance values to a standard curve, allowing for quantification of BBB damage based on the amount of EB released into the brain tissue.

*Infarct volume measurement*

To assess the amount of brain tissue damage, their brains were quickly removed from the skull after 24 hours of ischemia induction and kept in cold normal saline at 4°C for 5 minutes. Coronal slices of the brain with a thickness of 2 mm were then cut using a matrix. To determine the volume of tissue damage, the obtained coronal brain sections were placed in a solution of TTC (2%) in a Bain Marie (Memmert, Germany) at a temperature of 37 °C for 15 minutes. Photos were taken from the slices using a digital camera equipped with a computer connection [21]. The area of the damaged parts (colorless areas) was measured using image tools software, and the volume of the tissue damage area in the hippocampus section was calculated by multiplying it by the thickness of 2 mm and summing the obtained numbers.

*Cerebral edema rate*

After 24 hours of ischemia induction, the rats were euthanized and the brain was carefully removed. The hippocampus was separated and the wet weight (WW) of each hemisphere was measured separately using a scale. The dry weight (DW) of each part was measured after placing it in an autoclave or dry oven at 120 °C for 24 hours. The formula for calculating brain interstitial water is ((WW-DW))/WW×100) [22].

*Preparation of histology samples*

Following euthanasia, the brains of the rats were promptly and carefully extracted from the skull and placed in containers filled with formalin. Subsequently, paraffin blocks were prepared using standard histological methods. The process involved alcohol dehydration, clarification, paraffin embedding, molding, sectioning, and staining with hematoxylin and eosin (H&E). Histological observations were evaluated by the pathological scoring scale developed by Gilat *et al.* The scale consisted of the following categories to assess the extent of brain damage: Normal morphology (0), minor damage including edema and a few pyknotic cells (1), moderate damage including structure disorganization, edema, intermediate pyknotic cells, vacuolization, and inflammatory cells (2), and intense damage including structured disorganization, edema, intense pyknotic cells, vacuolization, and inflammatory cells (3) [23].

*Gene analysis*

In this study, the relevant region of the rat brain was isolated immediately after 24 hours of ischemia and transferred to a refrigerator set at -80 °C.

**RNA isolation and cDNA (complementary deoxyribonucleic acid) synthesis**

RNA extraction from tissue samples was performed using Tripure isolation reagent solution manufactured by Roche (Cat No. 11667165001), following the provided manual. The quality and quantity of the extracted RNAs were assessed by measuring the optical density (OD) using a nanodrop device (Thermo). Subsequently, cDNA synthesis was carried out according to the protocol of the cDNA synthesis kit (TAKARA Cat No. 6130).

**Real-time PCR**

Quantitative analysis was conducted using the Step One Plus model of a real-time PCR machine (Applied Biosystems, Foster City, CA, USA). Specific gene primers and SYBR Green master mix kits (TAKARA Cat No. RR 820W) were utilized to determine the relative quantification of the expression levels of nuclear factor kappa (NF-kB), Bcl-2-associated X protein (Bax), and tumor necrosis factor-alpha (TNF-α) genes in the hippocampal tissue samples. The GAPDH gene was used as a reference gene and internal control to monitor changes in the expression levels of NF-kB, Bax, and TNF-α gene. Primer sequences were designed using Oligo 7 software, and their accuracy was confirmed by the National Center for Biotechnology Information (NCBI) website [24]. The sequence of primers was as follows: Bax (Forward (F): 5'TGAAGACAGGGGCCTTTTTG 3', Reverse (R): 5'AATTCGCCGGAGACACTCG 3'), NF-kB (F: 5'TGCATCCACAGTTCCCAT 3', R: 5'AAATCGGATGCGAGAGGA 3'), TNF-α (F: 5' CTCTTCAAGGGACAAGGCT 3', R: 5' CTTGATGGCAGAGAGGAGG 3'), GAPDH (F: 5'CAAGATCATCAGCAATGCCTCC 3', R: 5'GCCATCACGCCAGTTTCC 3 ').

*Statistical analysis*

The mean values and standard deviation (mean ± SEM) were analyzed using SPSS 11 statistical analysis software. A one-way ANOVA test was used to assess cerebral edema, stroke volume, BBB damage, and expression levels of TNF-α, Bax, and NF-kB genes. The Kruskal-Wallis test was utilized to evaluate NDS and histological scores. Significant differences in the values were indicated by a P-value of less than 0.05.

**RESULTS AND DISCUSSION**

*Size and surface charge of nanoparticles*

The diameter of the niosome control particles was measured to be 83.30 nm with a PdI of 0.149 (Fig. 1a). The diameter and zeta potential of NS were determined to be 85.92 nm and PdI of 0.240 (Fig. 1b), respectively, with a zeta potential of -34.7 mV (Fig. 1c).

*Electron microscopy imaging*

According to the electron microscope image (Fig. 1d), it was found that the synthesized nanoparticles have a uniform and globular structure and an approximate size of 17.71 nm. Based on the electron microscope image (Fig. 1d), it was observed that the synthesized nanoparticles
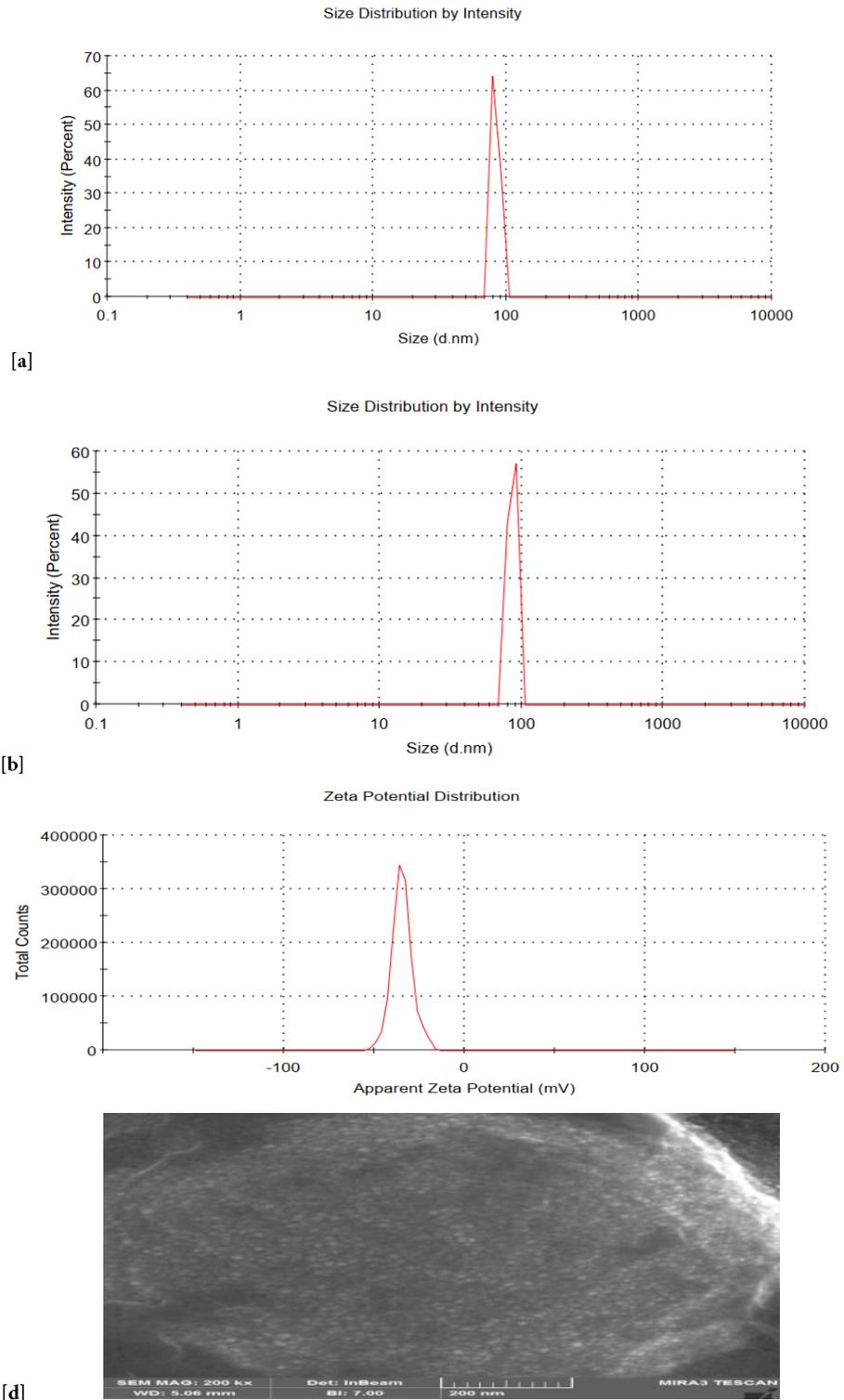
Fig. 1. Examining the Size, surface charge, and SEM image of niosomes. [a] Size of control niosomes, [b] Size of NS, [c] Surface load size of NS, [d] SEM image of NS, which shows the appropriate morphology of nanoparticles.

have a uniform and globular structure with an approximate size of 17.71 nm.

**EE%**

The saponin-loaded nanoparticles synthesized in this study exhibited an EE% of 85.70%, indicating successful loading of saponin. The nanoparticles had a small size of 85.92 nm and a suitable EE%, which could potentially facilitate their passage through the blood-brain barrier (BBB) and enhance the therapeutic effects of saponin. Previous studies have also supported the use of similar nanocarriers for the effective delivery of bioactive compounds to the brain. For example, niosomes containing Ginkgolide B (GB) to improve Parkinson's disease were shown to have a size of 187.3 nm and an EE% of 68.2%, resulting in improved stability, pharmacokinetics, and brain permeability of GB [10]. The presence of cholesterol in the niosome structure was found to enhance vesicle strength and prevent leakage of the active substance, leading to increased EE% [11]. In a similar study, Varshosaz *et al.* prepared bilayer nanocarriers containing vitamins using the thin film method, with a size of 5.06 ± 0.13 micrometers and an EE% of 55.24 ± 0.17% and demonstrated positive effects in ischemic rats [12]. Detoni *et al.* prepared nanoliposomes containing *Zanthoxylum tingoassuiba* were also synthesized using the thin film method, with a size of 937 nm and an EE% of 43.7%, resulting in good drug penetration [25].

On the other hand, the chemical structure of the lipids comprising the niosome layer was found to significantly influence drug release [12]. Additionally, the type of trapped molecule and the presence of electrically charged agents on the niosome surface should be investigated in drug penetration studies using carrier nanosystems. In this study, nanocarriers were synthesized using a combination of Tween 40 and cholesterol, and their zeta potential and release rate were investigated, yielding favorable results such as a zeta potential of -34.7 mV.

Fang *et al.* by examining niosomes and liposomes observed that the inclusion of cholesterol in nanocarriers has been shown to improve drug stability in liposome and niosome nanoparticles [26]. In another study Markova *et al.*, synthesized niosomes containing sage extract to cross the BBB, and according to their results, the successful use of niosomes for crossing the

BBB, with optimized formulations exhibiting zeta potentials of -25.6 ± 0.404 mV and 6.74 ± 0.609 mV[27], for regular and poloxamer-coated niosome have been also reported [28] .The type of surfactant used can influence the zeta potential. Furthermore, in a study utilizing the optimal formulation for drug delivery, the synthesized nanocarriers exhibited a surface charge of -14.2 ± 1.8 mV. This negative surface charge suggests that it can facilitate the attraction of hydroxyl ions on the surface of niosomes, providing an advantage for surface interaction [11]. These findings are consistent with results obtained by other researchers, further validating the synthesis of the niosome structure in this study.

*Release test results*

The release rate of saponin from the nanocarriers in NS was found to be gradual and slow, as shown in Table 1. Initially, there was an upward trend in the release of saponin, with 32.85% being released in the first hour. Subsequently, the release continued gradually over time. In contrast, the saponin-free formulation exhibited a continuous ascending release pattern, with 55.28% being released in the first hour.

The results of this release test confirm the slow-release capability of niosomes, which is consistent with findings from other studies involving niosomes containing different drugs such as Topotecan [29] Quercetin [30] and Acyclovir [31].

By synthesizing nanocarriers containing saponin, we were able to enhance its availability to brain cells and amplify its positive effects against ischemia. This research aligns with previous studies conducted by other researchers, such as Difuse *et al.*, who observed that niosomes facilitate drug entry into the rat brain [9]. Moreover, our nanocarrier NS demonstrated significant reductions in cell damage, stroke volume, and brain edema caused by ischemia. This can be attributed to its ability to cross the blood-brain barrier and gradually release saponin in the brain tissue of the animal model used in this study.

*Effect of NS on NDS*

The scores for total neurological defects, raising rat by the tail (RT), motor function (MF), sensory function (SF), beam test (BT), and reflex activity (RA) are presented in Figs. 2a, 2b, 2c, 2d, 2e, and 2f. Rats in the MCAO+S and MCAO+NS

Table. 1. Saponin release rate from niosomes at 37 °C.

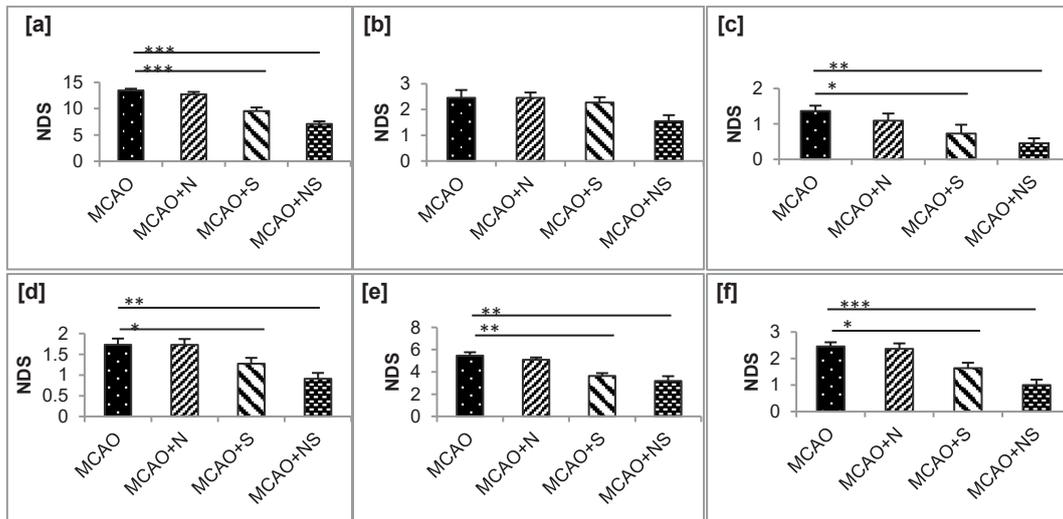| Time→ | 1/4 hour | 1/2 hour | 1 hour | 2 hour | 4 hour | 6 hour | 12 hour | 24 hour |
|---|---|---|---|---|---|---|---|---|
| NS | 18.85% | 25.57% | 32.85% | 35.42% | 39.85% | 41.85% | 43.71% | 44.71% |
| saponin | 31.85% | 43.28% | 55.28% | 64.42% | 69.42% | 81.71% | 86.71% | 91.07% |



Fig. 2. The effect of saponin and NS on the total scores of neurological deficits, expressed as mean ± SEM. The results were obtained by SPSS software, and the Kruskal-Wallis test. (Statistically, $P<0.05$ is significant and marked with an abbreviated sign (*), $P<0.01$ is significant and marked with an abbreviated symbol (**), and $P< 0.001$ is significant and indicated by an abbreviation (***)). (The experimental groups have been compared to the MCAO group.) ([a]: total, [b]: raising rat by the tail, [c]: motor function, [d]: sensory function, [e]: beam test, [f]: reflex activity).

groups exhibited significantly fewer defects in all tests except the RT test compared to rats that did not receive any drugs ($P<0.05$).

*Degree of damage to the BBB*

The results of examining the concentration of EB in the hippocampus of the damaged hemisphere (right) showed a lower level in the pretreatment group with niosome saponin. In contrast, the concentration of EB was high in the groups that did not receive drugs and suffered ischemia. Its details are shown in Fig. 3.

*Stroke volume*

Regarding the pretreatment with saponin, a dose of 3 mg/kg administered half an hour before ischemia resulted in a reduction in stroke volume in the hippocampus of the right hemisphere compared to the MCAO group and MCAO+N group. However, this decrease was even more significant when pretreated with niosome saponin at a dose of 1mg/kg (Fig. 4). Additionally, as shown in Fig. 5, the colorless areas in the MCAO and MCAO+N

groups are clearly defined. In the pretreatment group with NS, the extent of the colorless regions has been significantly reduced.

*Degree of cerebral edema*

The rats in the pretreatment group with NS showed a significant reduction in cerebral water content in the brain hippocampus compared to the rats that did not receive any drug (Fig. 6). These results indicate that the synthesized niosomes containing saponin with antioxidant properties can effectively reduce stroke-induced damage. Previous studies have also demonstrated the ability of saponin to reduce oxidative stress caused by ischemia. For example, studies on the effect of saponin extracted from ginseng on ischemic rats have shown a reduction in stroke volume and neurological deficits [32, 33]. Similarly, the prescription of total triterpenoid saponin extracted from A taibaiensis (SAT) for seven consecutive days before ischemia in rats has been shown to reduce stroke volume and neurological dysfunction by reducing oxidative stress [34].
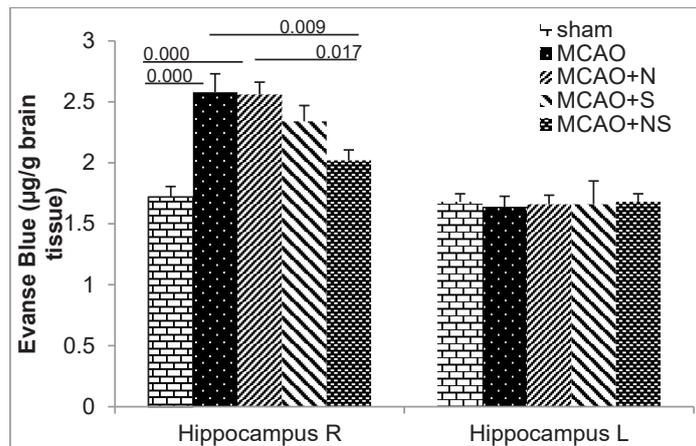
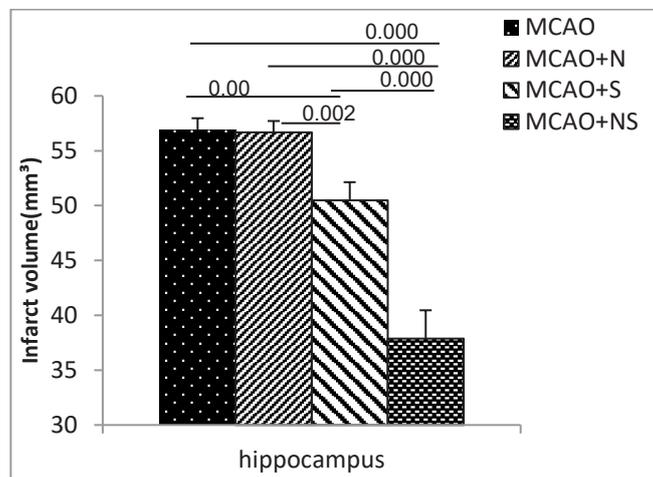Fig. 3. EB concentration in the hippocampus section of the rat brain in the studied groups n=5 (P<0.05).



Fig. 4. Effect of saponin and NS on tissue damage volume in the hippocampus section of the brain. The data analysis was in the form of mean ± standard deviation (n=5).
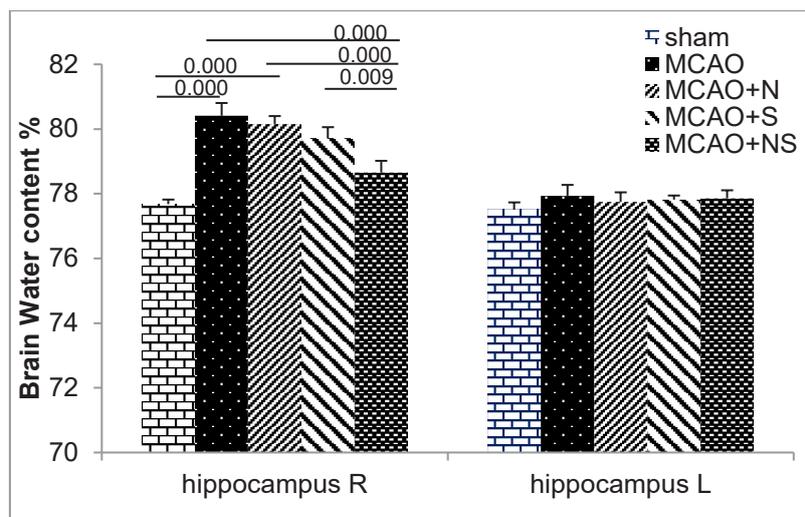


Fig. 5, Sections of the brains of rats of the studied groups stained with TTC.

Sham     MCAO     MCAO+N     MCAO+S     MCAO+NS

Fig. 6. The amount of cerebral edema changes in the brain's hippocampus in experimental groups. The data analysis was in the form of mean ± standard deviation (n=5).

*Expressing Bax, NF-kB, TNF-α genes*

Fig. 7a demonstrates a decrease in the expression level of the Bax gene in the brain's hippocampus with NS pretreatment, although this decrease was not statistically significant. However, the expression levels of NF-kB and TNF-α genes in this brain region were significantly lower in rats that received NS pretreatment compared to those that did not receive NS. Further details can be seen in figs.7b and 7c.

During the cerebral ischemia process, microglia become activated and release inflammatory cytokines, such as TNF-α [35]. Also, the adhesion of leukocytes to cerebral vessels is increased by TNF-α and directly affects cerebral capillaries [36]. The findings show that inhibiting TNF-α can reduce the volume of cortical lesions in Syrian rats with permanent cerebral ischemia [37]. Additionally, stroke as an external stimulus to the cell activates the NF-kB signaling pathway during a process, leading to cell damage [38].

In this study, gene expression analysis revealed a significant increase in the expression of NF-kB and TNF-α genes in the right hemisphere of the brain in rats with induced ischemia compared to rats without ischemia. However, pretreatment with NS reduced this increase, suggesting that inhibiting the expression of TNF-α and NF-kB genes may be another neuroprotective mechanism of NS.

According to other researchers, saponin DT-13 has been found to have a preventive effect on TNF-α-induced adhesion in endothelial cells. In these studies, a dose of 4 milligrams per kilogram of saponin DT-13 was prescribed for a week, resulting in a decrease in the expression of molecules such as intercellular adhesion molecule 1 (ICAM-1) [39]. Furthermore, Zhang *et al.*, conducted a study

on saponin in *Platycodon Grandiflorus* (PG) and its effects on inflammation. They discovered that PG saponin suppresses NF-kB transmission and inhibits LPS-induced MAPKs and AKT phosphorylation. The results of their study indicated that PG saponin can inhibit the inflammatory response in microglial cells, potentially by preventing the activation of processes like NF-kB. These findings suggest that the saponins in PG may have beneficial effects on the health and healing of irritated brain and nerve cells by reducing inflammation [40].

The Bax gene plays a crucial role in controlling programmed cell death. When the cells are exposed to oxidative stress, the mitochondrial pathway-dependent apoptosis pathway is triggered through the activation of Bax. However, the details of its activation mechanism have not been identified [41]. In a related study investigating saponin, Zhong *et al.* discovered that saponin has the ability to decrease the expression of Bax, beta-amyloid (Abeta), and beta-amyloid precursor protein (betaAPP) in the hippocampus and cortex of the brain. As a result, the occurrence of cell apoptosis is reduced [42].

*Examining histology samples*

The results of microscopic observation of the tissues prepared by H & E staining from the brain tissue of rats in the experimental groups are presented in Table 2. Representative microscopic images of histology slides can be seen in Fig. 8. The details of the images are as follows: Fig. 8a shows the hippocampus section of the brain in the sham group, displaying the natural structure. Fig. 8b shows the hippocampus section of the brain in the MCAO group, exhibiting severe necrosis of neurons (indicated by an arrow). Fig. 8c shows the

Table. 2. The results of examining histology samples.

| Groups→ | sham | MCAO | MCAO+N | MCAO+S | MCAO+NS |
|---|---|---|---|---|---|
| Hippocampus | 0* | 2 | 2 | 1* | 1* |

The results were obtained by SPSS software, and the Kruskal-Wallis test. (statistically, P<0.05 is significant and marked with an abbreviation (*), P<0.01 is significant and marked with an abbreviation (**), and P<0.001 is significant and marked with an abbreviation (***). The experimental groups were compared to the MCAO and MCAO+N groups.)
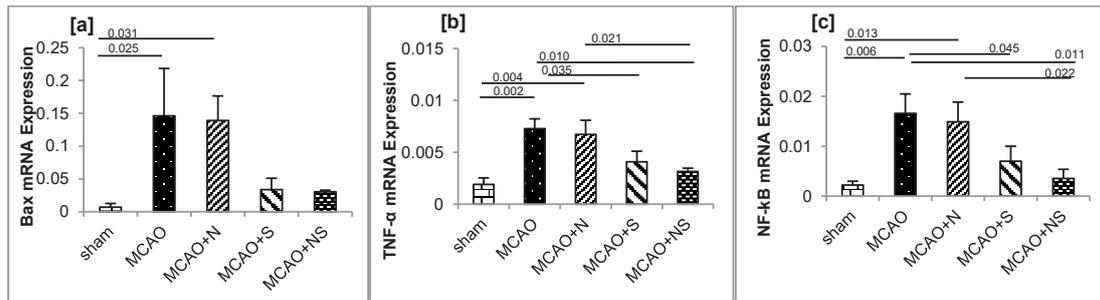


Fig. 7. The effect of saponin and NS on expressing Bax, NF-kB, and TNF-α genes in the hippocampus section of the brain. The results were obtained by SPSS software, a one-way ANOVA test (n=3). (Expressing Bax gene in hypocampus [a], Expressing TNF-α gene in hypocampus[b], Expressing NF-kB gene in hypocampus [c]).
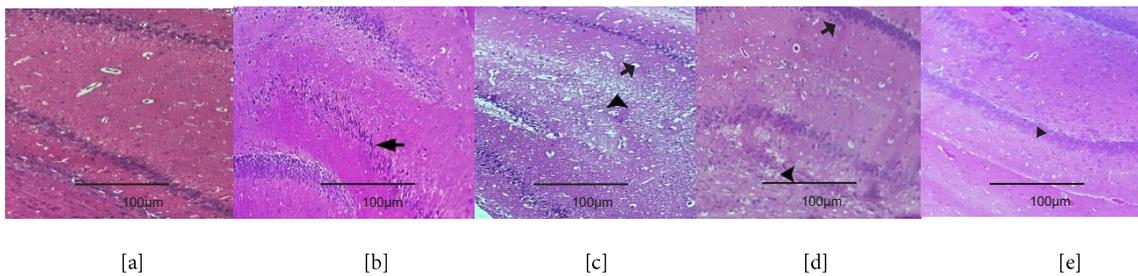


[a]    [b]    [c]    [d]    [e]

Fig. 8 (a, b, c, d, e). Microscopic photographs of the brain tissue of experimental groups of rats (H&E, 10X).

hippocampus section of the brain in the MCAO+N group, demonstrating severe necrosis (arrow) and severe edema and necrosis (arrowhead). Fig. 8d shows the hippocampus section of the brain in the MCAO+NS group, displaying mild necrosis (arrow) and mild edema and necrosis (arrowhead). Fig. 8e shows the hippocampus section of the brain in the MCAO+S group, showing mild necrosis (arrow).

The results obtained from the histological changes of the neurons in the hippocampus of the brain of MCAO and MCAO+N groups by light microscopy and hematoxylin and eosin staining show that the neurons suffered severe inflammation, pyknosis, edema, and necrosis, that the extent of this damage and cell inflammation has been significantly reduced by pretreatment of NS. The above analysis results, including the examination of TNF-α, an inflammatory cytokine, can confirm the histological findings.

## CONCLUSION

This research has shown that nanocarrier niosomes can deliver saponins to a significant extent to hippocampal cells in the ischemic brain and have excellent and acceptable effects without reducing the benefits of saponins. It can also prevent damage and injuries caused by ischemia, including brain edema, stroke volume, BBB damage, and expression of inflammatory cytokines. According to the desirable and evident results, as well as the high efficacy of these nanoparticles in delivering antioxidants to brain tissue, there is great potential in this pathway. By further investigating in other animal models of stroke and aligning with it for the prevention of stroke in individuals, especially those at high risk of stroke during certain surgeries, clinical use of nanoparticle carriers containing saponin can be employed.

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**Access to research data**

The results and information obtained from the steps of this study will be made available by the responsible author upon request.

**Declarations**

All authors of this study have clearly declared that they do not have any conflict of interest.

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