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Antidiabetic effect of *Prosopis farcta* extract on human adipose-derived mesenchymal stem cells in 3D culture electrospun with PLA

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Abstract

Background Diabetes mellitus is a disordered syndrome which has destructive effects on cells. The aim of this study is to reduce the side effects caused by glucose using treating cells with hydroalcoholic extract of *Prosopis farcta* in electrospun 3D culture with poly lactic acid (PLA).

Materials and methods The extract was prepared by maceration method, and the plant compounds were identified by (GC–MS). Human adipose-derived mesenchymal stem cells **(**hADMSCs) was treated with different concentrations of extract, and the level of cytotoxicity was evaluated by MTT test. Then, the cells were cultured in hyperglycemic medium with concentrations of 25, 50 and 75 mM and hyperglycemic medium treated with optimal concentration of extract (200 µg/ml) for 24, 48 and 72 h. The expression of antioxidant genes SOD1 and HO1 was investigated by RT-PCR method. The scratch test was performed to investigate cell migration and JC-1 to measure the mitochondrial membrane potential, and the TUNEL test was performed to investigate DNA fragmentation.

Results The most compounds of the extract were (9-Octadecenoic acid (E)/heptadecene-(8)/carbonic acid-(1), oleic acid, and Z, E-2,13-Octadecadien-1-ol). The extract was able to increase the survival and proliferation of cells that were reduced in the hyperglycemic environment. It also decreased the expression levels of *SOD1* and *HO1* genes. In the scratch test, the rate of proliferation and migration of cells increased significantly in 48 and 72 h. DNA fragmentation decreased in the cells treated with the extract, but the mitochondrial membrane potential increased significantly.

Conclusion Due to its abundant antioxidant compounds, *Prosopis farcta* was able to reduce the harmful effects of glucose by modulating the expression of antioxidant genes, reducing apoptosis, increasing the rate of cell growth and migration, and mitochondrial membrane potential.

Keywords *Prosopis farcta*, Mesenchymal stem cells, High glucose, Mitochondrial membrane potential, DNA fragmentation

Background

Diabetes mellitus is a multifactorial metabolic disorder characterized by chronic hyperglycemia with impaired carbohydrate, fat, and protein metabolism due to defects in insulin secretion and insulin action. The consequences of diabetes include long-term damage, dysfunction and failure of various organs [1]. Oxidative stress can be mentioned among the complications of diabetes that occurs in a cellular system when the rate of formation of free radicals exceeds its antioxidant capacity. Free radicals

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have destructive effects on DNA and protein in cells and can activate cell death mechanisms including apoptosis. Apoptosis plays an essential role in the normal development and homeostasis of tissues, and if there is a disturbance in its regulation, it can play a role in the development of various diseases such as cancer, autoimmunity, and neurodegenerative disorders. This active form of cell death is characterized by morphological changes such as chromatin condensation and DNA fragmentation [2].

One of the most important problems faced by diabetic patients is diabetic foot ulcer [3]. Diabetic foot complication, which is the source of many sufferings and social costs for patients, makes diabetes more complicated [4]. When the skin on the foot becomes ulcerated, the area is prone to infection, which is considered a medical emergency. Only two-thirds of cases of leg ulcers eventually heal; and up to 82% of leg ulcers may lead to lower limb amputation. Every year, more than 1 million patients with diabetes lose at least part of their leg as a result of diabetes complications [4]. Because blood supply to the wound area is not suitable in diabetic people, the wound healing process is slow. Drug delivery in the wound environment is the newest method of wound healing [3]. Wound healing is a complex process that involves various chemical and biological interactions between cells, growth and inflammatory factors, and extracellular matrix compounds [5].

Various methods have been proposed to treat diabetes. Medicinal plants have been used since ancient times to control blood sugar, reduce complications, and increase the quality and length of life in diabetics. Many medicinal plants have antidiabetic and antioxidant properties [6, 7]. Because of the presence of alkaloids with antioxidant effects, flavonoids with anti-inflammatory effects and tannins with cell proliferation and angiogenesis effects, the *Prosopis farcta* is effective in the process of healing and regenerating of diabetic wounds, which do this by accelerating the reduction of inflammation, cell proliferation and hypoglycemia [8].

Prosopis farcta (Mimosaceae family) is one of the most important plants of Sistan and Baluchistan region, which was native to dry and semi-arid areas of America, Africa and Asia. Its effective compounds are: 5-hydroxyl tryptamine (alkaloid), l-arabinose, lectin, quercetin, tryptamine and apigenin. The plant has antioxidant, anti-inflammatory and anti-apoptotic properties [9].

Oxygen free radicals caused by oxidative stress can bind to various molecules such as nucleic acid, proteins and phospholipids and cause damage to them. Enzymes such as superoxide dismutase (SOD) and heme oxygenase 1 (HO1) reduce superoxide anions [10].

HADMSCs are a source of mesenchymal stem cells with multipotent differentiation and self-renewal properties [11].

Scaffolds play a decisive role in tissue regeneration and repair [12]. Polylactic acid is a type of synthetic polymer that is used in bone repair, drug release system and wound dressings due to its ideal mechanical properties and elasticity [13]. The reason for the use of this biopolymer in biomedical industries, especially tissue engineering, is the accepted mechanical properties (tensile strength of electrospun PLA nanofibers) along with desirable biological properties (biodegradability and biocompatibility). PLA scaffolds are a suitable option for the repair of all kinds of tissues and have a significant effect on the process and speed of repair and improvement of the considered tissue [14].

In the current study, the effects of the hydroalcoholic extract of the *Prosopis farcta* on the hADMSCs cultured on the PLA scaffold were investigated in order to reduce the harmful effects of high-glucose environment on the cells.

Methods

Preparation of hydroalcoholic extract

The maceration method was used to prepare hydroalcoholic extract of *Prosopis farcta*. The seedless fruit pods of the plant were collected from the desert area of Zabul city in July 2022 and then grounded (Voucher No. 1952).

Isolation and identification of plant compounds

In order to identify the compounds in the hydroalcoholic extract, a gas chromatography device connected to a mass spectrometer (GC-MS) was used. The length of column was 30 m, the inner diameter was 0.25 mm, and the thickness of the inner layer was 0.25µ m. The temperature program of the column was set from 50 to 250 °C with a temperature increase of 3 °C per minute. The extract was injected into the hot chamber with a microsyringe. Helium gas was used as a carrier gas with a flow rate of 15 ml/min. Extract components were identified by comparing their mass spectrometer with the spectrum bank and comparing their quats indices with the standard quats indices of the compounds.

Electrospinning of PLA scaffold

An electrospinning machine was used to prepare the PLA scaffold with 15% by weight. First, the granular PLA polymer with a weight of 0.75 g was dissolved in 3.5 cc of chloroform, and after complete dissolution, 1.5 cc of DMF (dimethylformamide) was added to the solution. These steps were performed on a heater stirrer (Heidolph) for 2 h. The obtained solution was transferred to a 5 cc syringe. The electrospinning process was carried

out with a voltage of 16 kV for 4 h and a distance of 15 cm from the syringe to the aluminum collecting plate. SEM microscope was used to examine the morphological characteristics. In order to check the biocompatibility of polylactic acid scaffold with cells, hADMSC cell line was cultured on it. The samples were dehydrated and fixed with alcohol. Then, photography was done with a microscope.

Cell culture

The hADMSC cell line was obtained from the stem cell center (Bone Yakhte, Tehran). The cells were cultured in DMEM/F12 medium with 10% FBS and incubated at 37 °C, 95% humidified atmosphere and 5% CO2. Cells from passage 4 to 6 were used for the experiment. Cells were cultured in three groups with for 72 h; a control group with culture medium containing plant extract with concentrations (25, 50, 100, 200 and 400 µg/ml), hyperglycemic group containing different concentrations of high glucose (20, 50 and 75 mM) and hyperglycemia group treated with different concentrations of the extract.

MTT assay

MTT assay was used to investigate the effect of hydroalcoholic extract of *Prosopis farcta* on cell viability in control and hyperglycemic environment. Different concentrations of the extract were added to the cells in the mentioned groups that were cultured in 96-well plates, and after 24, 48, and 72 h, the supernatant of the cells was removed, and 200 µl of MTT solution (Sigma) was added to the cells and incubated for 1 h at room temperature. Then, the supernatant was discarded, and DMSO (Merck, Germany) was used to dissolve the formazan crystals in each well. Finally, the OD of the solution was read with a spectrophotometer (EPOCH Bio Tek, USA) at a wavelength of 570 nm.

Scratch test

Wound healing process in hADMSC cell line cultured in hyperglycemic medium treated with extract was determined through a scratch method in vitro. The cells were placed on 6-well culture dishes with a density of 3×10^4 cells per well. A manual scratch was made across the cell monolayer with a 200 p pipette tip to remove cells from an area 1.5 ±0.5 mm wide. Cell debris was removed by washing with phosphate-buffered saline (PBS), and the medium was replaced. Cells were observed under a phase contrast microscope for 72 h, and digital photographs were taken. Area under the curve (AUC) or scratch area was calculated using AutoCAD software and reported.

Investigation of apoptosis by flow cytometry

DNA fragmentation is a hallmark of apoptosis. To measure apoptosis, TUNEL kit was used according to the instructions. The cells treated with plant extract were fixed with paraformaldehyde for 15 min. Cells were washed with PBS and centrifuged. Then, the cells were incubated in proteinase K and washed again with PBS and centrifuged. TUNEL enzyme solution and label TUNEL solution were used for staining from the TUNEL kit in a ratio of 1 to 9. After washing with PBS, the cells were centrifuged for 5 min at 1200 rpm at 4 °C. A flow cytometry device was used to read the wavelength.

Mitochondrial membrane potential measurement

To measure the mitochondrial membrane potential, the JC-1 (Cat number: T4069) staining was used. JC-1 is a lipophilic cationic and fluorescent dye with green fluorescent production property that easily enters normal and apoptotic cells. Under normal conditions, JC-1 enters the mitochondria and forms aggregate with red fluorescence. While in apoptotic cells due to the loss of electrochemical potential, it remains as a monomer and produces green fluorescent. Therefore when mitochondrial potential collapses, the JC-1 dye remains in cytoplasm with green fluorescence. The hADMSC cell line was treated with extract and then incubated with 2 µm of JC-1 dye for 30 to 60 min. After washing with PBS (three times), glycerol and PBS solution was poured on the sample and then the resulting fluorescence (green and red emissions) was read using a fluorescence reading device.

Investigating gene expression using real-time RT-PCR method

To investigate HO1 and SOD1 genes expression, cell RNA was determined according to the extraction kit protocol (RNX PLUS, Sinaclon), and its purity was determined with a nanodrop device (EPOCH Bio Tek) at wavelengths of 260–280 nm. CDNA was synthesized from the extracted RNAs by cDNA synthesis kit (Pars Tos, Iran). To perform the RT-PCR reaction, Master Mix kit and primers which designed by Oligo software (Oligo 7.60) and synthesized by Synaclon Company (Table 1) were used. *B-actin* gene was used as housekeeping gene. RT-PCR temperature program included 10 min at 95 °C,

Table 1 *HO1 and SOD1* primer sequences

Gene	Primer sequence	Length
Forward SOD1	CTCACTCTCAGGAGACCATTGC	22
Reverse SOD1	CCACAAGCCAAACGACTTCCAG	22
Forward HO1	ATGACACCAAGGACCAGAGC	20
Reverse HO1	GTGTAAGGACCCATCGGAGA	20

15 s at 95 °C, 15 s at 75 °C and finally 30 s at 72 °C. The results of the RT-PCR machine (Roche Light Cycler 96) were analyzed by using the $2^{-\Delta\Delta ct}$ formula.

Statistical analysis

The results were analyzed with SPSS software version 23 using one-way ANOVA and Tukey's post hoc test. *P* value < 0.05 was considered significant.

Results

Plant chemical compounds

Among the compounds identified by GC-MSS device (Fig. 1), the most constituent compounds of the hydroalcoholic extract are shown in Table 2.

PLA scaffold synthesis

The images obtained from SEM were indicated the cell adhesion on the scaffold (Fig. 2). According to the obtained figures, the synthesized PLA scaffold can be used as an in vitro environment in the laboratory for 3D cell culture. The results obtained from the first, third and seventh days of cell culture on the scaffold indicated the growth and orientation of the cells toward the scaffold. Therefore, it can be concluded that PLA scaffold has suitable biocompatibility and non-toxicity for this cell line.

Results of MTT assay

The results showed that with the increase in glucose concentration and time, the percentage of cell viability decreased ($P < 0.001$) (Fig. 3A). But after treating the cells with a concentration of 200 µg/ml extract, cell viability increased ($P < 0.05$) (Fig. 3B).

Cell migration and proliferation

Cell migration was disrupted in hyperglycemic medium with concentrations (25, 50 and 75 mM) (Fig. 4A). This disorder was observed under the microscope within 48 to 72 h. Cell migration increased in the groups treated with *Prosopis farcta* hydroalcoholic extract (200 µg/ml) and was slightly different from the control group. The results of the scratch test showed that the extract increased the migration performance of the cells (Fig. 4B). The AUC of each group was calculated and compared with the control group (Fig. 4C–F).

Examining the rate of apoptosis by tunnel method

The amount of DNA fragmentation (apoptosis) in the hADMSCs after 24 h of culture in the normal medium treated with the extract decreased and the cell viability increased (figure A and D). An increase in apoptosis and a decrease in the viability of cells exposed to a hyperglycemic environment with a concentration of 25 mM are shown in figure B (Fig. 5). A decrease in the percentage of apoptosis was observed in cells exposed to hyperglycemic environment treated with the extract (figure C and E).

Mitochondrial membrane potential assessment using JC-1 dye

According to the results obtained in the JC-1 test, when the cells are in a normal environment (control), the mitochondrial membrane potential is normal (red color). Also after the treatment with the extract, the potential of the mitochondrial membrane is normal (Fig. 6A). In the hyperglycemic group (glucose treatment), the potential of the mitochondrial membrane decreased (green color). With the effect of the hydroalcoholic extract on the cells in the hyperglycemic environment, the potential of the mitochondrial membrane increased (increase in the percentage of red to green color) (Fig. 6B), which indicates the positive effect of the extract in the direction of increasing the mitochondrial membrane potential (Fig. 6c).

Results of real-time q-PCR

The expression of *SOD1* and *HO1* genes was significantly increased in the hyperglycemic group (50, 25 and 75 mM) compared to the control group (Fig. 7A and B). In the 25 mM glucose group, the greatest effect on *SOD1* and *HO1* gene expression was observed compared to the control group. A significant difference was observed in the expression of *SOD1* gene among the groups treated with glucose 50 compared to the concentration of 25 and 75 mM ($P < 0.05$). A significant difference was observed in the expression of *HO1* gene among the groups treated with glucose 75 compared to the concentration of 25 and 50 mM ($P < 0.05$).

In diabetic groups treated with extract (200 µg/ml), the expression of genes decreased significantly compared to the control group ($P < 0.05$). The decrease in *HO1* and *SOD1* gene expression in these groups indicated the positive effect of the extract to compensate for the harmful effects of glucose.

Discussion

Skin wound healing is a process that involves the cooperation of different cells (including fibroblasts, monocytes, macrophages, endothelial cells, and epidermal cells), growth factors, and cytokines [15]. After injury, healing and repair of skin damage in diabetic patients shows a significant decrease. Many evidences show that high concentrations of glucose have destructive effects on the function of different cells in different tissues and organs [10]. Our results showed that the healing process leading to wound closure is faster in the hyperglycemic groups treated with the extract compared to the control group. Meanwhile, in the group of hyperglycemia

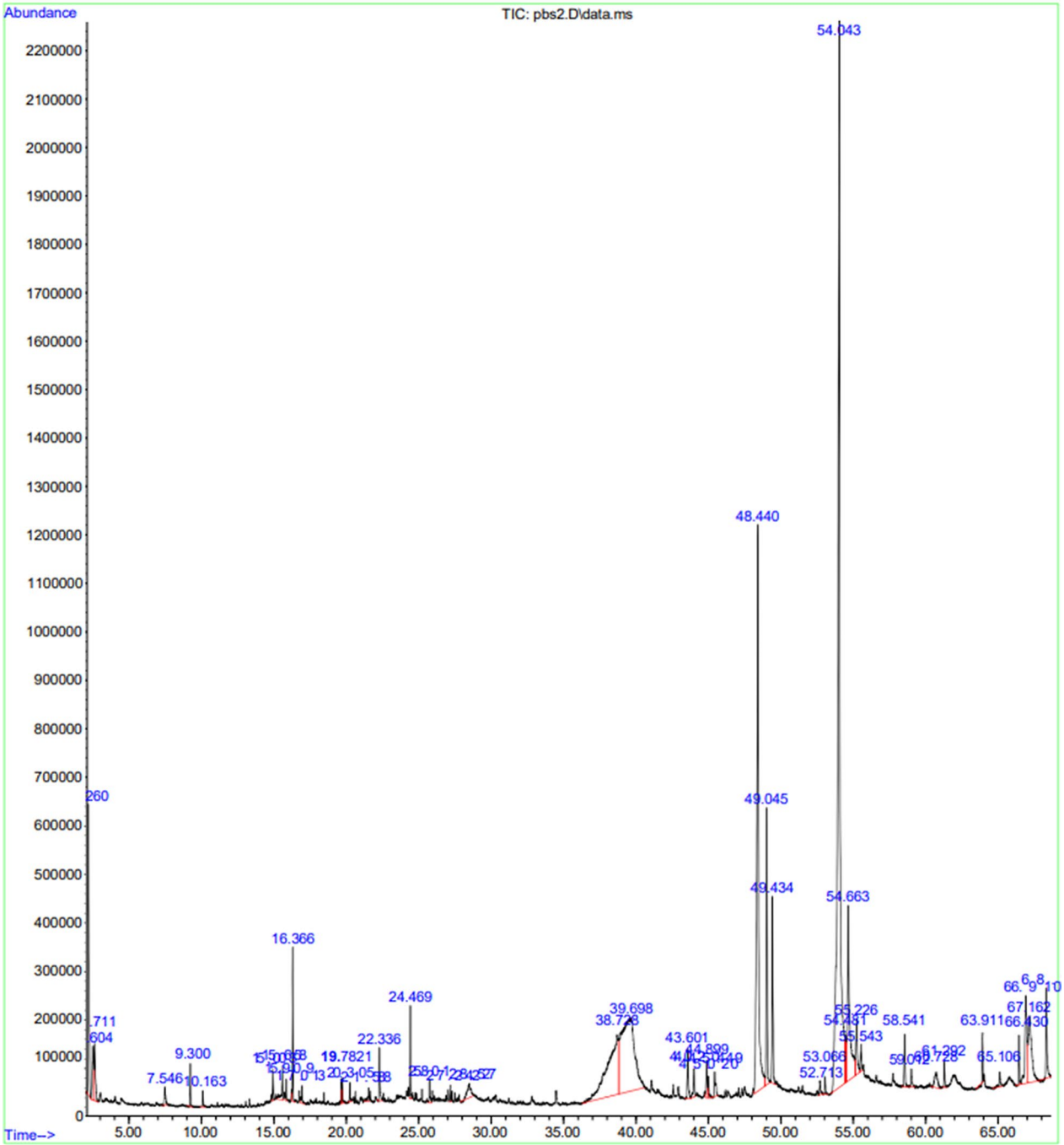


Fig. 1 GC/MSS chromatogram of *Prosopis farcta* hydroalcoholic extract. The X-axis represents the retention time and Y-axis represents the intensity of each peak

without treatment, the mentioned events are delayed. The results of this study are consistent with the results of other studies which showed that high glucose disrupts the migration and proliferation of cells and causes damage to skin fibroblasts [10, 16]. A study showed that the hydroalcoholic extract of *Prosopis farcta* leaves is effective in reducing blood glucose [9]. In a research, it was shown that Quercetin, which is a type of flavonoid, inhibits the expression of inflammatory cytokines. Therefore, it seems that due to the presence of flavonoids in the *P. farcta*, which is probably related to anti-inflammatory effects through the inhibition of inflammatory

Table 2 Chemical compounds *of Prosopis farcta* hydroalcoholic extract

%	Peak	Time (min)	Compounds
93	33	54.043	Z,E-2,13-Octadecadien-1-ol
96	33	54.043	Oleic acid
99	33	54.043	9-Octadecenoic acid(E)/HEPTADECENE-(8)/CARBONIC ACID-(1)

mediators, a reduction in the period of inflammation and acceleration of the healing of diabetic wounds have been observed [15]. The results of the study showed that flavonoids and triterpenoids increase wound contraction and epithelization [15]. The use of medicinal plants is especially significant when common treatments are not able to control the disease, and the patient needs insulin administration [6].

MTT results indicated that the best concentration of the extract for this study was 200 µg/ml (optimal concentration) and the plant extract increased cell survival, growth and proliferation. Concentrations higher or lower than these cause little damage to the cell.

The SEM results showed that the PLA scaffold in the in vitro environment is suitable for 3D cell culture and the cells grow well on this scaffold. The production of three-dimensional polylactic acid scaffolds made by wet electrospinning method has a porosity of over 80% and low density [13]. The results of a research showed that the PLA/CS (polylactic acid/chitosan) scaffold prepared by electrospinning method is a scaffold with biocompatibility and surface topography suitable for adhesion, survival and cell culture and does not cause any negative side effects due to grafting. This scaffold along with the simultaneous cultivation of fibroblasts and its placement in the wound improves the wound healing process. It seems that this scaffold has caused an increase in the number of fibroblasts at the wound site and an increase in the amount of collagen and possibly also an increase in the amount of angiogenesis, thus accelerating and shortening the healing time of the wound [17].

The results of our study showed that the expression level of *HO1* and *SOD1* genes in hADMSC in hyperglycemic environment (50, 25 and 75 mM) under treatment with plant extract (200 µg/ml) decreased compared to the control group but in the hyperglycemic group, the expressions increased with the mentioned concentrations and without extract treatment. These genes have an antioxidant and cell protective effect. When the cell is cultured in high glucose environment, due to the destructive effect of glucose, cell growth decreases, but by adding the extract, the antioxidant effect increases which causes the conditions to approach the normal condition and the expression of the mentioned genes decreases. *Prosopis farcta* with the antioxidant compounds such as apigenin and vicenin-2 [18] is effective in the treatment of diabetes and its complications. Increased levels of *HO1* result in increased levels of the antioxidant, bilirubin and anti-apoptotic, carbon monoxide, which are responsible for neutralizing free radicals, ICAM-1, VCAM-1, TNF and

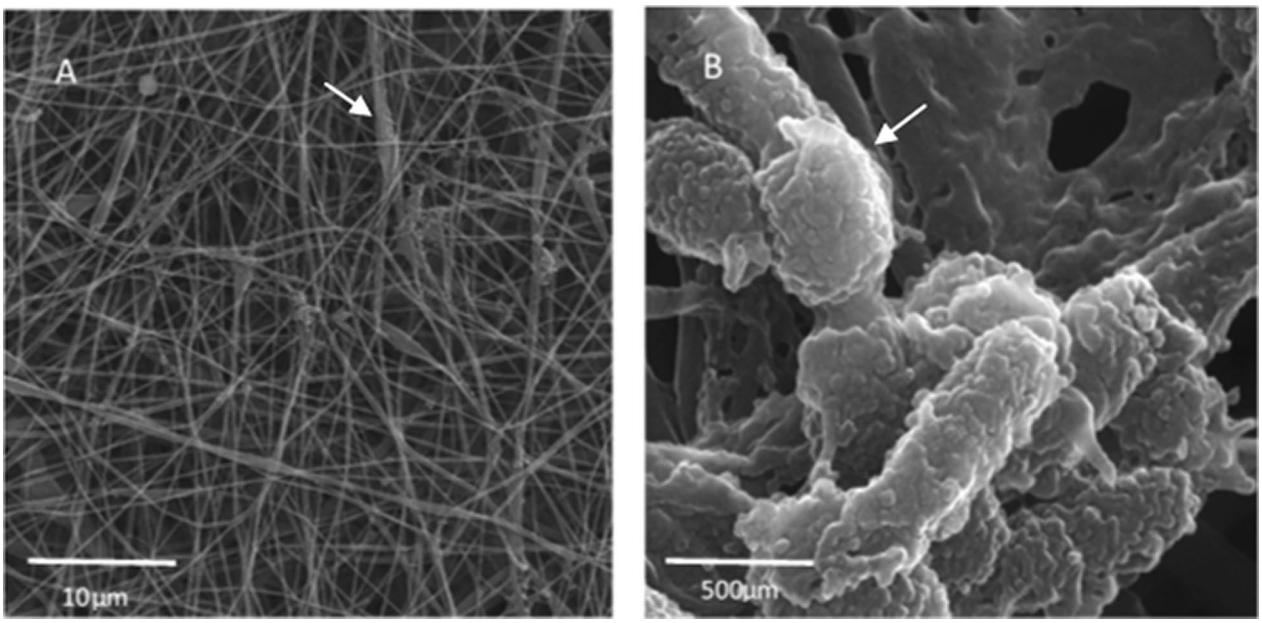


Fig. 2 SEM images of *hADMSc* cells on electrospun polylactic acid (PLA) scaffold. Arrows indicate human mesenchymal stem cells

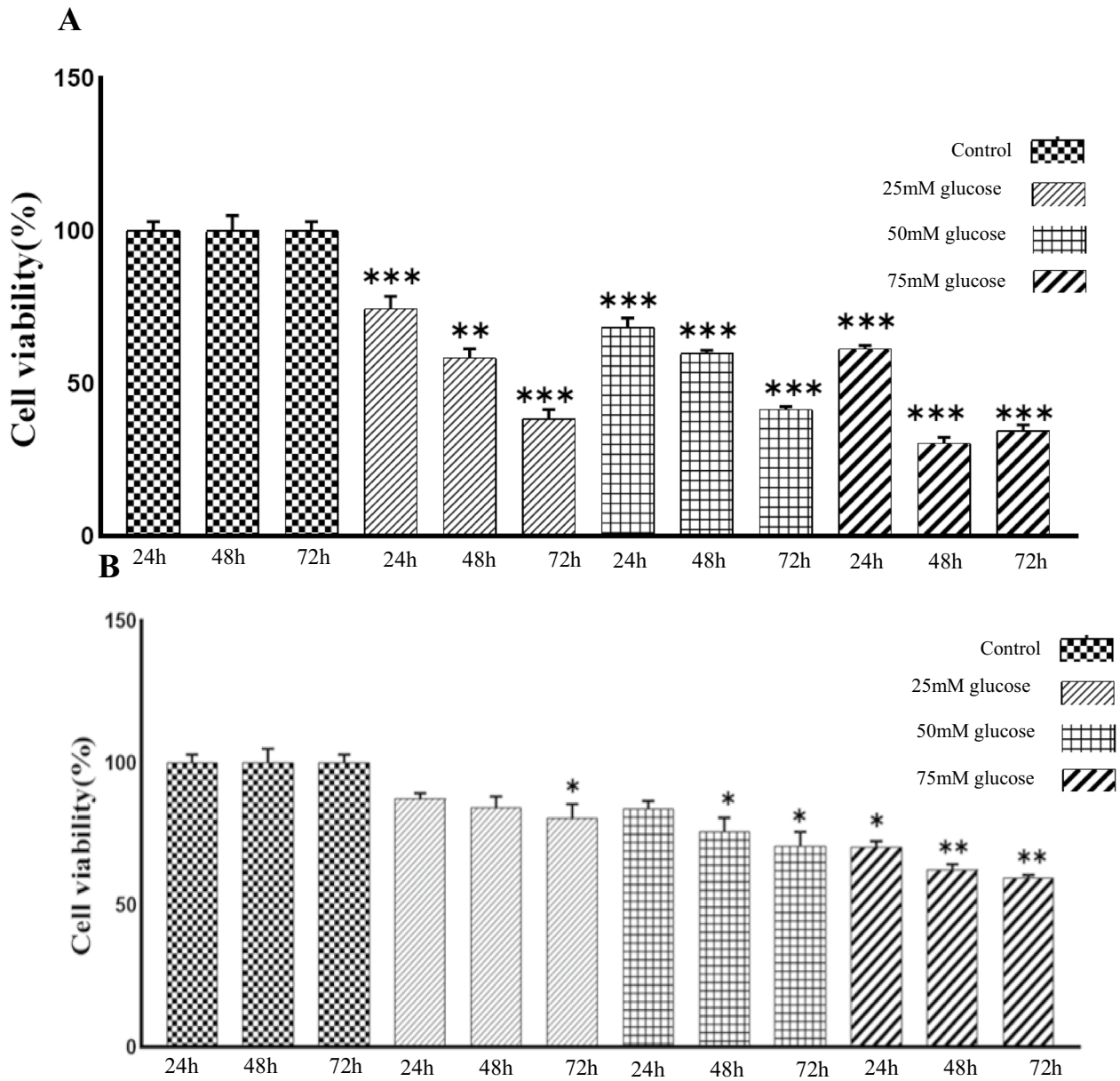


Fig. 3 Percent viability of *hADMSC*. **A** Cells cultured in hyperglycemic medium with concentrations (25, 50 and 75 mM) at 24, 48 and 72 h. **B** Treatment of cultured cells in hyperglycemic environment at 48, 24 and 72 with hydroalcoholic extract. Data are shown in mean ± SD. * Comparison of different groups with control group. * $p < 0.05$, ** $p < 0.01$, and *** $p < 0.001$

IL-18 [18]. Our study showed the antioxidant genes *HO1* and *SOD1* increase significantly in the condition that the cell is in a hyperglycemic environment to trap the ROS produced in the cell.

In eukaryotic cells, mitochondria are double-membrane organelles that provide the main energy for intracellular events through aerobic respiration and oxidation. During this process, the concentration of protons and other ions is asymmetrically distributed on both sides of the inner membrane, and the mitochondria show a negative potential difference, that is, the mitochondrial membrane potential (MMP) [19–21]. As a measure of mitochondrial function, MMP can indicate the healthy state of cells [22, 23]. Normal MMP usually fluctuates from − 150 to − 180 mV and can maintain stable biological activities, including ATP synthesis [24], signal transduction, ion exchange [25]. However, any abnormality in the level of MMP, even a small change, will have a great

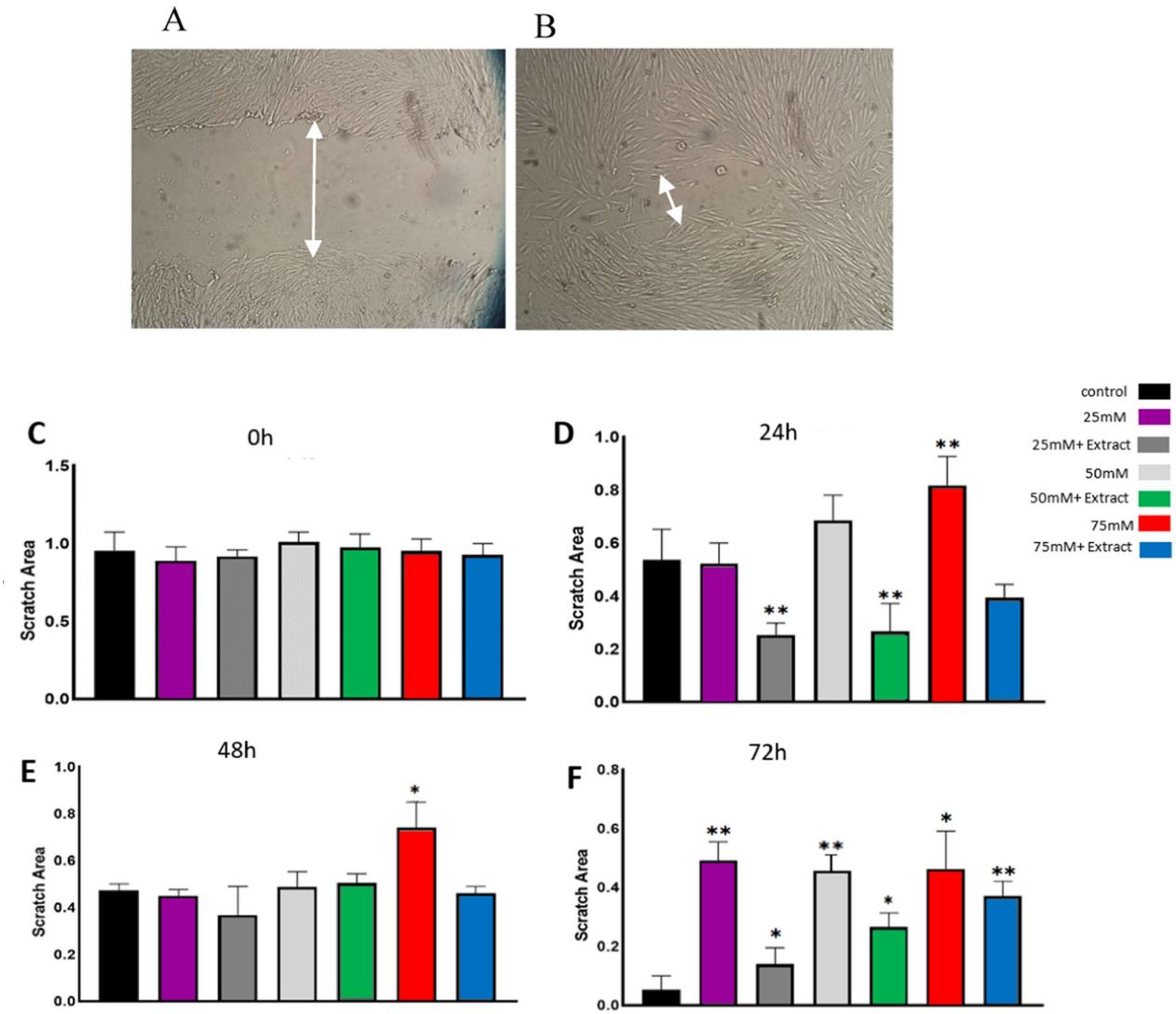


Fig. 4 Images show the scratch test in laboratory conditions at different glucose concentrations after 48 h. **A** Scratch in *hADMSCs* in hyperglycemic medium with a concentration of 25 mM. **B** Scratching in *hADMSCs* in hyperglycemic medium with a concentration of 25 mM and treatment with hydroalcoholic extract of *Prosopis farcta*. Magnification, 40X, **C**–**F** Comparison of AUC in different groups after 0, 24, 48 and 72 h, respectively. Data are mean ± SD, * $p < 0.05$, ** $p < 0.01$ and *** $p < 0.001$

impact on mitochondrial function, which causes mitochondrial dysfunction [26]. For example, loss of MMP level is a signal of some cellular disorder events that lead to autophagy, apoptosis and necrosis [27]. Recent reports showed that many diseases, such as diabetes, are associated with the loss of MMP [28].

In a study conducted on the relationship between mitochondrial membrane potential and sperm quality and fertility, it was shown that high mitochondrial membrane potential indicates normal and more mobile sperm. Considering that sperm mitochondrial membrane contains cytochrome C, in case of destruction of mitochondrial membrane by ROS, this cytochrome is released from mitochondria and activates caspases and induces apoptosis phenomenon. Apoptosis can aggravate DNA damage caused by ROS [29]. Oxidative stress causes damage to the nuclear and mitochondrial DNA in sperm cells and a decrease in glutathione and an increase in markers of oxidative stress and lipid peroxidation in the testicles of diabetics, all of which occur as a result of chronic hyperglycemia and an increase in free radicals that cause the inactivation of antioxidant factors [30]. In the present study, the increase in glucose concentration caused a decrease in mitochondrial membrane potential and apoptosis.

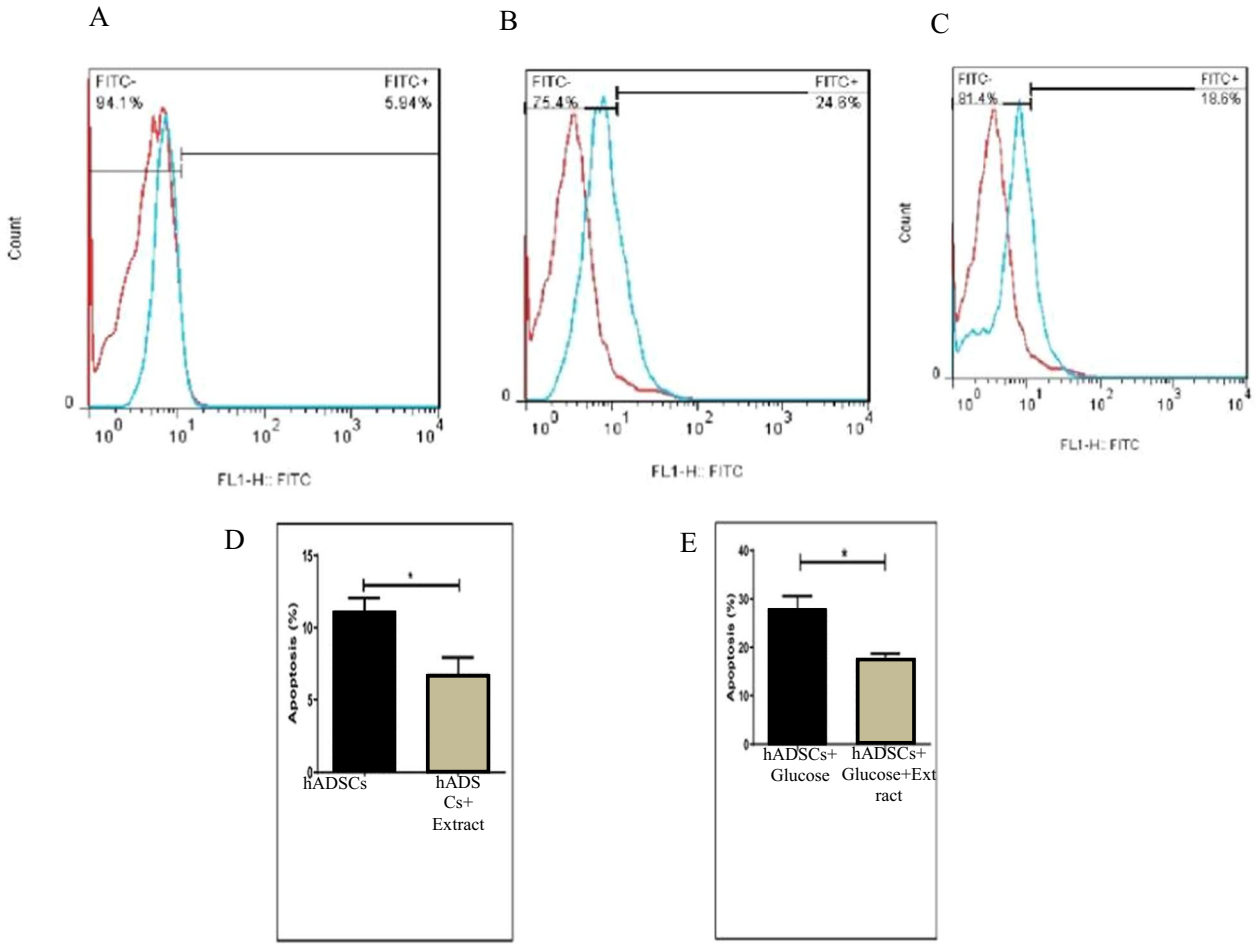


Fig. 5 TUNEL test in the *hADMSC* cell culture. **A** Extract group, **B** Hyperglycemic group, **C** hyperglycemic group treated with the extract, **D** Comparison of the percentage of apoptosis in the *hADMSC* in the control group and extract group, **E** Comparison of the percentage of apoptosis in the *hADMSC* in hyperglycemic and hyperglycemic treated with extract. *$P < 0.05$

Induction of type II diabetes in mice caused a significant decrease in BCL2 protein expression, an increase in Bax protein expression, and as a result, an increase in the ratio of Bax/Bcl-2 and a significant increase in apoptosis [31]. Increased oxidative stress and defects in mitochondrial function play a pivotal role in inducing apoptotic cell death. The opening of the mitochondrial permeability transition pore causes the depolarization of the transmembrane potential, the release of calcium (Ca2+) and cytochrome C, and the loss of oxidative phosphorylation, which leads to the loss of cell viability [32].

It has been shown that high concentration of glucose can cause the death of PC12 cells (similar to fetal neurons) through the internal pathway of apoptosis with the activation of caspases 9 and 3 [33]. DNA fragmentation is a biochemical hallmark of apoptosis. The enzyme responsible for DNA fragmentation/apoptosis is caspase-activated DNase.

Therefore, according to the results of our study, the harmful effects caused by glucose induce a decrease in the mitochondrial membrane potential. However, by treating the cells with hydroalcoholic extract of *Prosopis farcta*, we saw a decrease in DNA fragmentation and an increase in mitochondrial membrane potential.

Conclusion

Totally, high glucose disrupts cell proliferation and migration and damages cells; it also causes damage to mitochondrial membrane potential and therefore increases DNA fragmentation and apoptosis. The antioxidant effects of the hydroalcoholic extract of *Prosopis farcta* counteracted the destructive effects of glucose and increased the rate of cell proliferation, survival and the wound healing process. It also caused a decrease in the expression of *SOD1* and *HO1* genes (their expression were increased with an increase in glucose concentration) and apoptosis. The in vitro conditions created

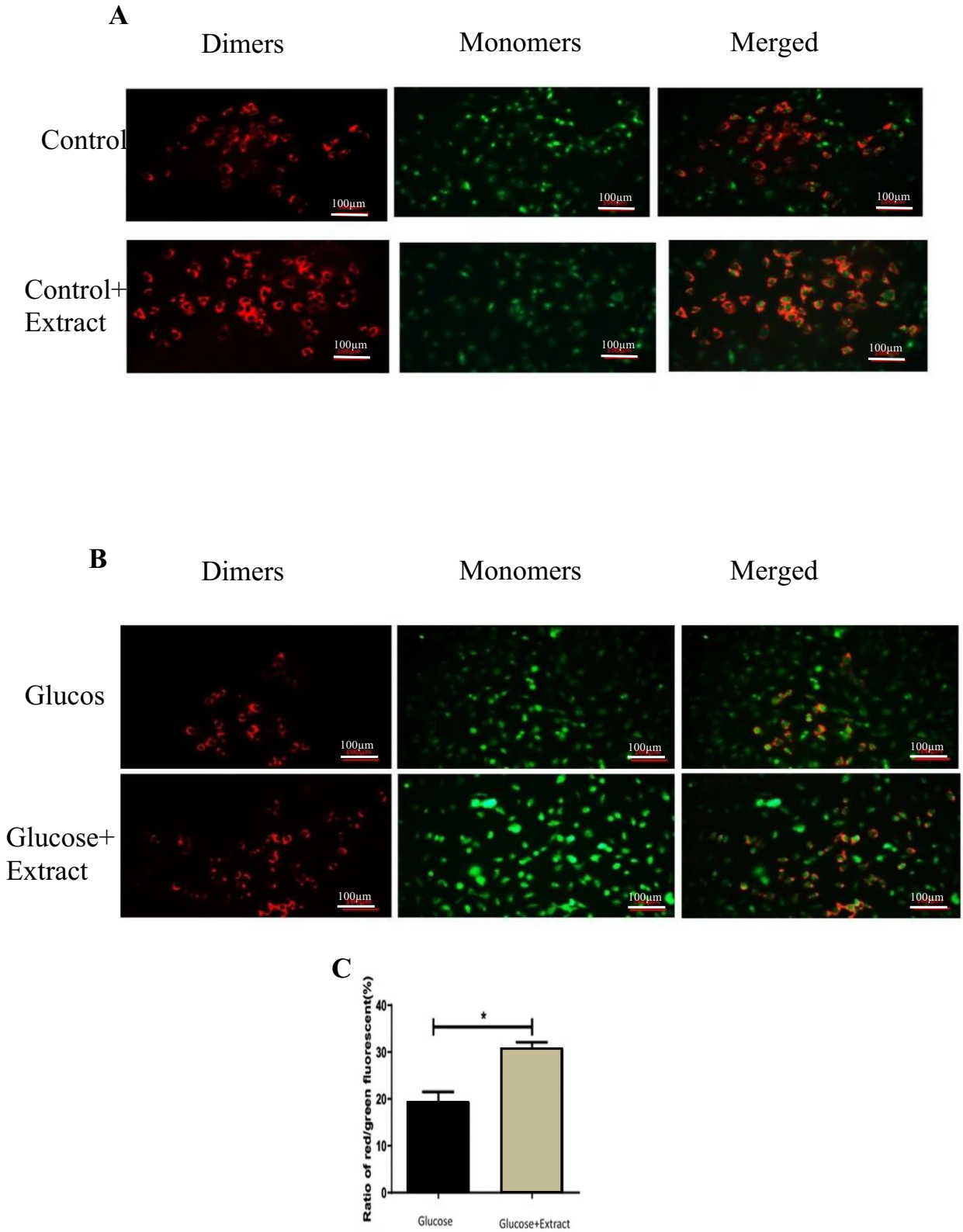


Fig. 6 Fluorescent microscope images of JC-1 assay. **A** Control group (up), extract group (down), **B** Hyperglycemic group (up), hyperglycemic group treated with the extract (down), **C** Comparison of hyperglycemic group with hyperglycemic group treated with the extract, $*p < 0.05$

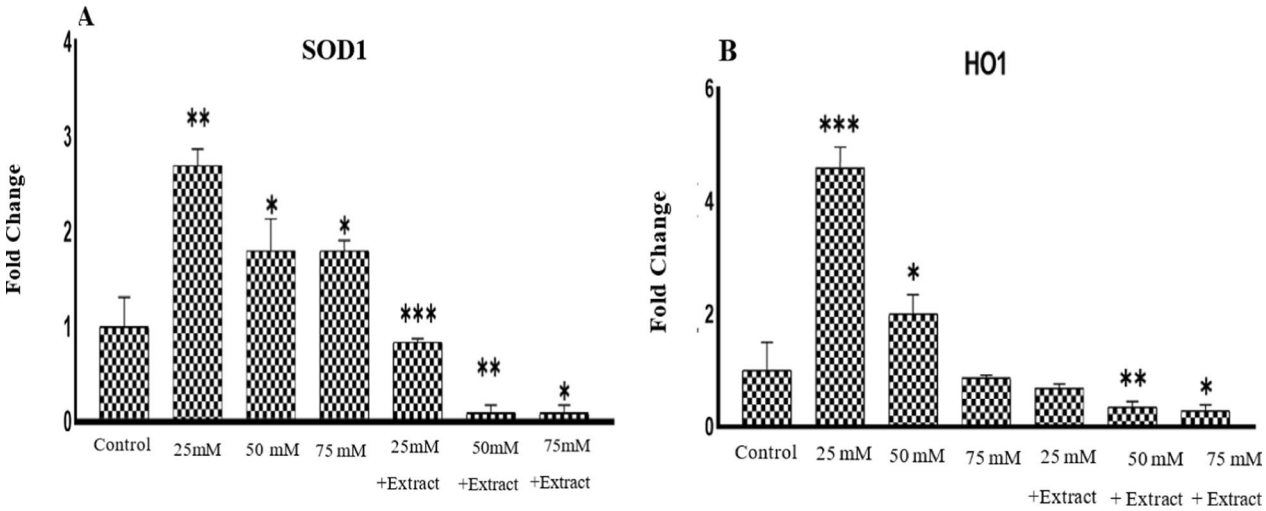


Fig. 7 Comparison of the expression of *HO1* and *SOD1* genes in control, hyperglycemic, and hyperglycemic treated with extract group. * Comparison of different groups with control group. * $p < 0.05$, ** $p < 0.01$, and *** $p < 0.001$

in this experiment mimic the diabetic conditions in the body. Therefore, this study can be a basis for further studies in the field of problems caused by high glucose, such as impaired wound healing and apoptosis in diabetic people. In this regard, more research is needed for the therapeutic applications of the *Prosopis farcta* for diabetic patients.

Abbreviations

PLA	Polylactic acid
GC–MS	Gas chromatography–mass spectrometer
hADMSCs	Human adipose-derived mesenchymal stem cells
MTT	(3-[4,5-dimethylthiazol-2-yl]-2,5 diphenyl tetrazolium bromide)
SOD	Superoxide dismutase
HO1	Heme oxygenase 1

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Author contributions
M.R. and Z.P. contributed to the study conception and design. Material preparation, data collection and analysis were performed by all authors. The first draft of the manuscript was written by N.H. and F.N., and all the authors commented on previous versions of the manuscript. All the authors read and approved the final manuscript.

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Availability of data and materials
The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Declarations

Ethics approval and consent to participate
Not applicable.

Consent for publication
Not applicable.

Competing interests
The authors have no relevant financial or non-financial interests to disclose.

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