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Experimental Research Article

High concentration of nitric oxide impaired proliferation of bone marrow mesenchymal stem cells due to cell cycle arrest at G1 stage

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ABSTRACT

Introduction: Although exogenous nitric oxide (NO) is used as medicine, in the previous we showed its inhibitory effect on the proliferation ability of rat bone marrow mesenchymal stem cells (BMSCs). In the present investigation, the inhibitory role of exogenous NO on BMSCs cell cycle was studied.

Methods: BMSCs after the third passage were treated for one hour every 48 hours with 100µM of sodium nitroprusside as an NO donor. Then, after 5,10,15, and 20 days of treatment, the viability, proliferation, and cell cycle of the BMSCs was investigated. In addition, the expression of the *Raf1*, *CDK2*, *CDK4*, *P53*, and *GAPDH* genes was studied.

Results: Cell treatment caused a significant reduction in viability and proliferation at 5,10,15, and 20 days. Also, the treatment caused cell cycle arrest at G1 after 20 days. In addition, it was found that the *CDK2* and *CDK4* expression were down-regulated whereas the *P53* expression was up-regulated, but the expression of *Raf1* as well as GAPDH remained the same.

Conclusion: This study showed that prolonged treatment with a NO donor arrest the BMSCs cell cycle due to overexpression of *P53*, which inhibits the expression of *Cdk2* and *Cdk4*.

Keywords:
Cell cycle
Nitric oxide
Mesenchymal stem cells
Cyclin-dependent kinases
Cell proliferation

Introduction

Nitric oxide (NO) is a free radicals that functions as a signaling molecule in biological systems. In cells, NO is produced following L-arginine conversion to L-citrulline by a family of enzymes called nitric oxide synthases (NOSs) (Hill et al., 2010). In a variety of cells, NO is produced by three isoenzymes of NOSs, namely neuronal (nNOS), inducible (iNOS), and endothelial (eNOS) (Wimalawansa 2008). The level of NO production by these three enzymes was defined to be low in eNOS and nNOS, whereas iNOS produces a high level of NO when activated (Wimalawansa 2010). In physiologic conditions, NO exists with a half-life of 2-3 seconds and due to its hydrophobic properties, moves freely across cell membranes (Wood and Garthwaite 1994). It is shown that NO has a dual effect on the activity of

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osteoblasts. *In vitro* investigations revealed that a small amount of NO produced by osteoblasts or released by NO donors acts as an osteoblast growth stimulator and promotes its differentiation (Turner et al., 1997), whereas, a high concentration of NO showed an inhibitory effect on osteoblastic growth and differentiation (Mancini et al., 2000). In our recent publication, it was shown that the 100 μM of exogenous NO, when applied for 1 hour every 48 hours, was able to improve osteogenic differentiation of rat bone marrow mesenchymal stem cells (BMSCs) via up-regulation of RUNX2, which is an important gene in osteogenic differentiation, whereas 1000 μM reduced the differentiation significantly (Abnosi et al., 2022). Regulation of cellular responses depends on NO concentration, which at low concentrations (1 and 10 μM) promotes cell survival, proliferation, and prevents apoptotic responses (Mohammadi et al., 2017), whereas at high concentrations (100 μM and more), NO causes a reduction in proliferation, especially when the treatment time was increased (Pari et al., 2017; Mohammadi et al., 2017), apoptosis, and senescence (Thomas et al., 2008). In 1974, Sodium nitroprusside (SNP) was approved by the FDA as a vasodilator for the treatment of hypertension. SNP is water-soluble and is used asmedication to decrease blood pressure (Hottinger et al., 2014), which reacts with sulfhydryl groups of proteins to produce NO. The released NO immediately binds to vascular smooth muscle to activate protein kinase G, producing cyclic guanosine monophosphate (cGMP) which mediates myosin light chains inactivation that results in the reduction of blood pressure by relaxation of vascular smooth muscle (Fok et al., 2012).

As it was mentioned before, in our *in vitro* investigation, we found that NO has a dual action on BMSCs, where 100 μM of SNP as an exogenous NO-donor has no significant effect on cell viability in the short term whereas prolonged exposure reduces the viability and proliferation property of the BMSCs (Pari et al., 2017; Mohammadi et al., 2017). Our results were confirmed by Oleson and Corbett (2018), whohave discussed the dual function of NO on regulation of β-cells of the pancreas (Oleson and Corbett 2018).

The fundamental requirement for organismal development is cell proliferation through cell division, which ensures complete and precise genome duplication and segregation. The cell cycle involves two consecutive processes, called interphase and mitotic phase, which are characterized by the replication of DNA and chromosomal segregation into daughter cells (Vermeulen et al., 2003). The mitotic phase consists of different phases called prophase, metaphase, anaphase, and telophase, during which the chromosomes are separated, and the cell divides to produce two equal daughter cells (Vermeulen et al., 2003). Interphase, which comprises G1, S, and G2 phases, is characterized by a lack of cell division, but synthesis of necessary material takes place (Coller et al, 2006). The cell cycle is controlled by proteins which are heterodimeric kinases that consist of a cyclin subunit (regulatory subunit) and a cyclin-dependent kinase subunit (catalytic subunit). In the phase of the cell cycle, different heterodimers such as (CyclinD-CDK4/CDK6 and CyclinE-CDK2), S (CyclinA/E-CDK2), G2/M (CyclinA/B-CDK1) are functioning (Vermeulen et al., 2003).

BMSCs, which differentiate into osteoblasts, the matrix-producing cells (Zhou et al., 2021), are widely used in regenerative cell-based therapy due to their low immunogenicity and T-cell suppression, which is said to be mediated by NO production via the activity of iNOSs (Ren et al., 2008). Since high concentrations of NO decreased BMSCs viability and proliferation, therefore, in this study, we investigated the effect of NO on the cell cycle of the BMSCs during long-term *in vitro* incubation.

Material and Methods

Cell extraction and isolation

In the present experimental study, we purchased 6 to 8-week-old Wistar rats from Pasteur Institute (Tehran-Iran), and housed them in an animal house with access to food and water at standard temperature. After one week, the rats were anesthetized using chloroform inhalation and sacrificed by cervical dislocation. Subsequently, femurs and tibias were removed. under sterile conditions, connective tissues were dissected from the bones, and transferred to a clean room. Both ends of the bone were cut, and the marrow was extracted using a 2 ml syringe filled with culture media. We used complete DMEM media (Gibco-Germany) containing 15% FBS (Gibco-Germany) and 1% penicillin-streptomycin (PEN/STREP) (Gibco-Germany). Extracted bone marrow was centrifuged at 250g for 5 min. The resulting cell pellet was then resuspended in fresh media and transferred to T25 culture flasks. The flasks were kept

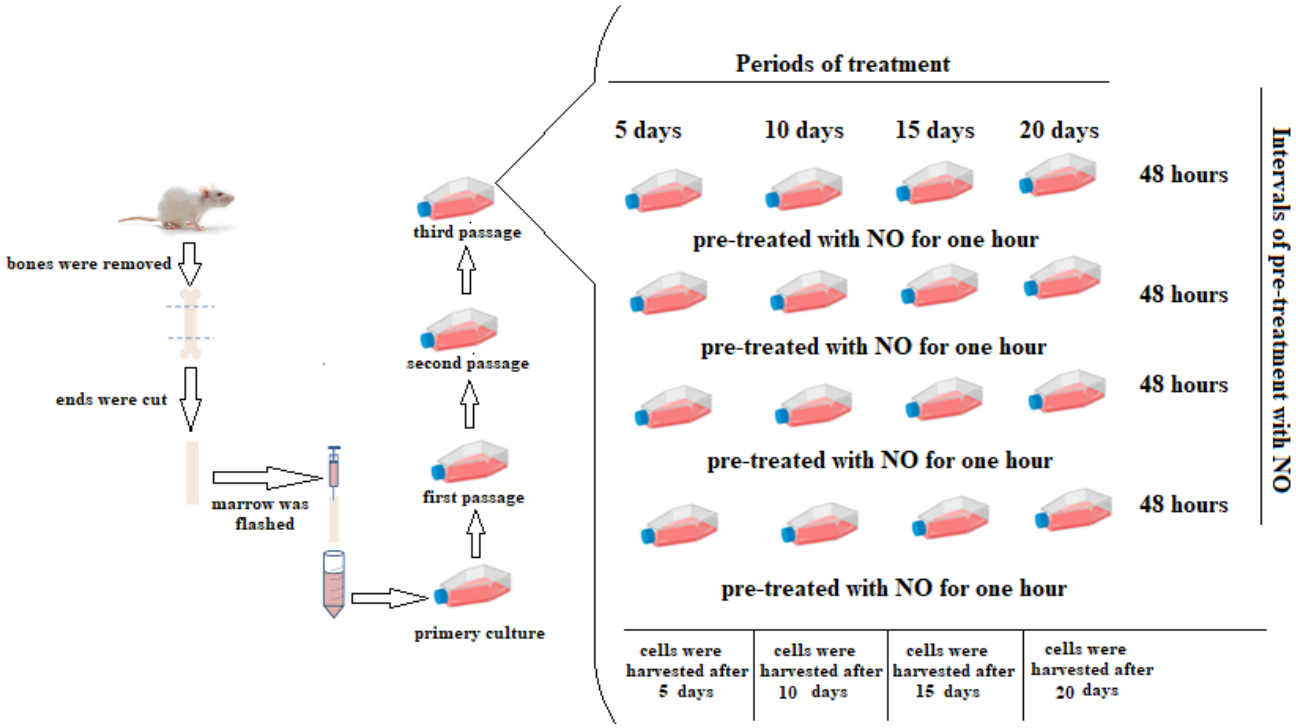

FIGURE 1. Schematic overview of experimental design

in an incubator (37 °C, 5% CO_2) in a humid condition. The media were replaced every 3 days with fresh ones. Once the cells reached confluence in the flask, they were detached using trypsin-EDTA (Gibco-Germany), centrifuged, and washed with PBS (20mM, pH 7.2). The cells were then seeded in new T25 culture flasks (Abnosi and Yari, 2018). This sub-culturing process was repeated twice more, and at the third passage, the purity of the cells was confirmed using a flow cytometer (Germany, PARTEC (PAS)) before proceeding with further analyses.

Exposure to sodium nitroprusside

A stock solution of SNP (pH 7.2) was prepared in deionized water, and then, based on previous studies, the cells were treated with 100 µM of SNP (Merck, Germat ny) for 1 hour every 48 hours in the presence of a control group (see Figure 1). This treatment was repeated for 5, 10, 15, and 21 days, as described in previous works (Pari et al., 2017; Abnosi and Pari, 2019). Each analysis was performed three times.

Viability assay

MTT Assay

The viability of the cells was assessed using the MTT assay. Briefly, BMSCs were cultured in a sterile plate and then treated with SNP as described previously. After washing the cells with PBS, 10 µL of MTT solution (5 mg/ml in PBS) per 100 µL of culture media (free from FBS) was added to each plate and incubated for 4 hours. Formazan crystals were then extracted by adding DMSO to the plate and incubating them at room temperature for 30 minutes. Subsequently, 100 µl of the extracted solution was transferred to a fresh ELISA plate, and the absorbance was measured at 505 nm using an ELISA reader (Medical SCO GmbH, Germany). To calculate the number of viable cells, a linear formula (y=0.006x+0.0171) with R2=0.999 was utilized, where y represents absorbance and x represents the number of viable cells (Pari et al., 2017).

Trypan Blue Assay

The viability of the cells was determined using the trypan blue (TB) exclusion assay. Briefly, BMSCs were cultured and after the same incubation period as previously described, the cells were detached from the plates using trypsin/EDTA, washed with PBS, and homogenized in culture media (free from FBS). Subsequently, 50 µl of the homogenized cell suspension was mixed with 50 µl of TB solution (Sigma Co., Germany), and

TABLE 1: Primers used

Genes	primers	Annealing temperature	Product length (bp)
CDK2	F: CATGTTTCCTGGGAGATGGTA	58	164
	R: GTGTTGTACGTCTTGGAGAGA	58	
CDK4	F: AACGGTGGTGCCATAGATGC	55	85
	R: AGGAGGCTCTCTCTGCTCAC	62	
Raf1	F: CCGCACGACAACCGCACCAT	54	139
	R: CGCTCCGGCCCACAAATCTC	58	
P53	F: AAACATGGCAAGGTGTGTGA	62	75
	R: AGGTGACCAGGACGTTTTTG	63	
GAPDH	F: TCGTCTCATAGACAAGATGG	56.4	136
	R: GTAGTTGAGGTCAATGAAGGG	59.4	
F: forward primer and R: reverse primer			

the total number of cells as well as the number of dead cells were counted using a hemocytometer chamber. The percentage of viable cells was then calculated and reported (Pari et al., 2017).

Quantification of Cell Proliferation

The investigation of cell proliferation was conducted using both colony forming assay (CFA) and population doubling number (PDN) as follows:

Colony Forming Assay

The colony forming ability of BMSCs treated with NO (following the same protocol as previously described) was studied after staining with crystal violet solution (0.5 g crystal violet in 100 mL methanol) for 15 minutes. Photographs of the colonies were captured using a light microscope equipped with a camera, and the number of colonies and their diameters (in micrometers) were determined using Motic Imaging software (Pari et al., 2017).

Population Doubling Number (PDN)

To calculate the PDN, a known number of BMSCs was plated and treated as previously described. Subsequently, after 5, 10, 15, and 20 days, the cells were detached using trypsin/EDTA. The PDN was calculated using the equation , where N0 represents the initial number of cells and N stands for the total cells harvested (Pari et al., 2017).

Cell Cycle

In T25 culture flasks, treatment of the cells (3rd passage) with SNP (100 µM) was performed as previously described (1 hour every 48 hours), alongside a control group. Then, after 5, 10, 15, and 20 days of treatment, the cells were detached, washed with PBS, and homogenized in 1 ml of PBS. To fix the cells, 3 ml of cold 95% ethanol was added dropwise. After 2 hours at 4°C, the cells were precipitated by centrifugation at 250 g for 5 min and resuspended in 1ml Diaminophenyl indole phosphate (10µg/ml), then kept at 4°C for 30 min (Darzynkiewicz and Juan, 1997; Abnosi and Massomi, 2019). The cell cycle analysis was carried out using a flow cytometer (Germany, PARTEC (PAS)).

Passage ability Test

Under treatment with 100 µM of SNP, to assess the tolerance of the cells across multiple passages, BMSCs were cultured and treated as described previously. Once the bottom of the culture flask was covered with a monolayer of cells, the BMSCs were detached using trypsin-EDTA and sub-cultured again. This process of repeated passages was carried out, and the results were compared with their respective control groups.

Gene expression analysis

Reverse transcription PCR (RT-PCR) was performed after total RNA extraction using the Super RNA Extraction Kit (YT9080) and cDNA synthesis with the BioFACT cDNA Synthesis Kit (BR631-096). Amplification of the genes Cyclin dependent kinase 2 (*CDK2*), Cyclin-dependent kinase 4 (*CDK4*), *RAF1, P53*, and glyceraldehyde dehydrogenase (*GAPDH*) was conducted three times using a PCR machine (Eppendorf Mastercycler Gradient, Eppendorf Co., Hamburg, Germany) with specific primers (see Table 1). The PCR conditions

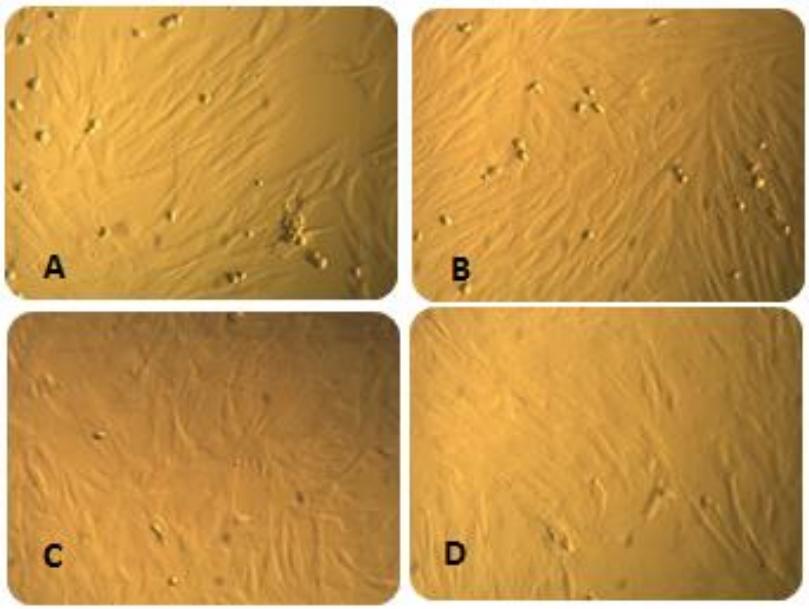

FIGURE 2. Photograph show cells at different stage of the culture A) primary culture B) 1st passage C) 2nd passage D) 3rd passage (magnification X 40).

were set as follows (Abnosi and Massomi, 2020):

Initiation temperature: 95 °C, 5 min

Denaturation temperature: 95° C, 1 min

Annealing of the specific primer: 1 min

Elongation temperature: 72° C, 1 min

Final elongation temperature: 72 °C, 7 min

Amplification was repeated for 35 cycles, and the products were run on a 1.5% agarose gel. The gel was photographed using Gel documentation (Gel flash, Syngene Bio imaging, England) and analyzed with Gel Quant software (Gel Quant: 1.8.2).

Statistical analysis

The data was analyzed using GraphPad Prism 8.4.3 with an unpaired t-test. Results were presented as mean values ± standard deviation, with a confidence level of 95%. Graphs were also generated using the same software.

Results

BMSCs purity

It was observed that at the 3rd passage, only thin elongated BMSCs with a fibroblast-like morphology were uniformly attached to the bottom of the culture flask (Figure 2-D). In contrast, in the 1st and 2nd passages (Figure 2-B & C), some round-shaped cells could also be observed. Additionally, at the 3rd passage, BMSCs exhibited a purity level of up to 93.32% (Figure 3-D), and this level of purity did not change significantly up to the 7th passage (Figure 3-H). Therefore, based on the flow cytometry analysis, cells from the 3rd passage were selected for further investigation.

Cell Viability

The results of the MTT assay (Figure 4-A) and trypan blue test (Figure 4-B) confirmed that treatment with 100 µM of SNP significantly reduced cell viability ($P<0.01$) after 5 days of treatment. It is noteworthy that the reduction in viability at days 10, 15, and 20 was also highly significant ($P<0.0001$).

Proliferation ability of the BMSCs

Data analysis revealed that treatment of cells with 100µM of SNP significantly reduced the number of colonies (Figure 5-A) at 5, 10, 15, and 20 days compared to the control group ($P<0.01$). However, there were no significant changes ($P>0.05$) observed in colony diameters (Figure 5-B). Photographs of the plates further confirmed the statistical data (Figure 6). Additionally, the population doubling number (PDN) was significantly reduced ($P<0.05$) at day 5, with a highly significant reduction ($P<0.0001$) observed at days 10, 15, and 20 of treatment (Figure 5-C).

Passage Test

The time required for sub-culturing BMSCs was

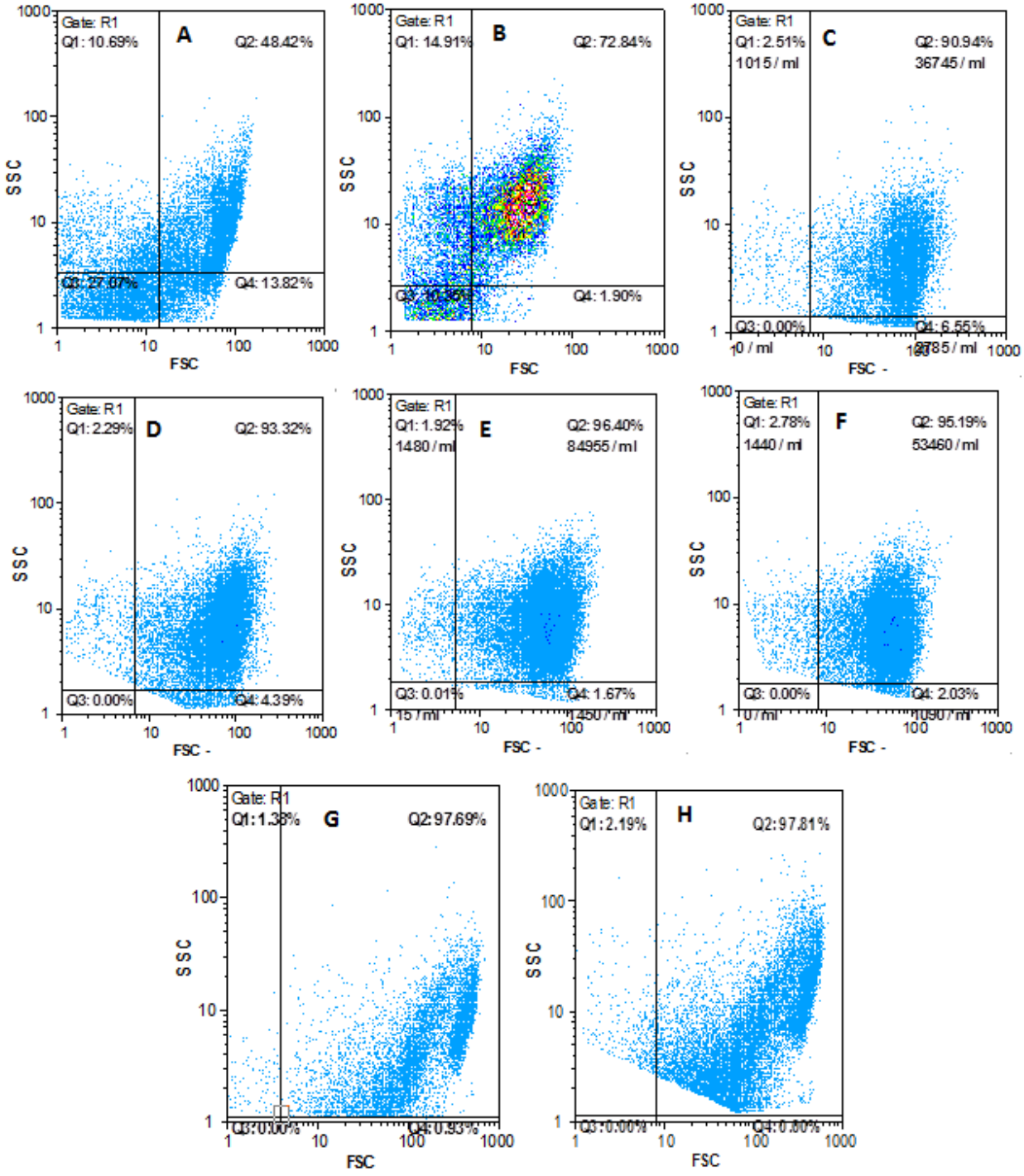

FIGURE 3. Side Scatter and forward Scatter plots analysis diagram of different passages of the BMSCs. A) primary culture B) 1st passage C) 2nd passage D) 3rd passage E) 4th passage F) 5th passage G) 6th passage H) 7th passage

found to be consistent between the treated and control groups up to the 10th passage. However, from the 11th passage onward, the time for sub-culturing in the treated group was significantly longer ($P < 0.01$) compared to the control group. Furthermore, at the 12th and 13th passages, the cells did not proliferate (Figure 7).

Regarding the cell number for each passage, it was observed that treatment with SNP resulted in a significant reduction ($P < 0.05$) in cell number from the 1st to 4th passages. Subsequently, the reduction in cell number became highly significant ($P < 0.01$) from the 5th to 11th passages, and no cell division was recorded at the 12th

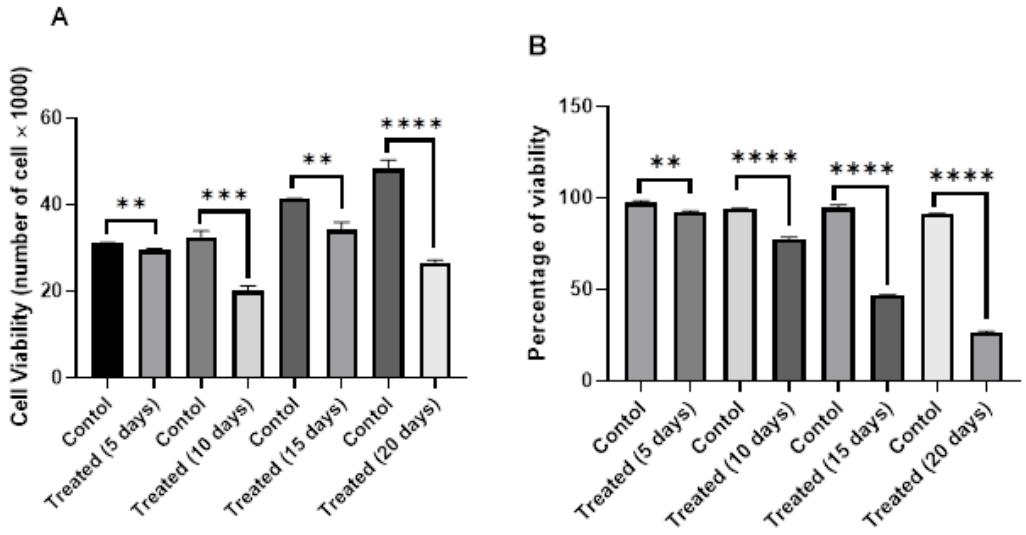

FIGURE 4. The Cell viability of BMSCs after 1 hour treatment with 100 µM of SNP for every 48 hours based on A) MTT assay and B) trypan blue. Values are presented as means ± standard deviation. Statistical significance is represented as follows: $^{**}P< 0.01$, $^{***}P< 0.001$, and $^{****}P< 0.0001$.

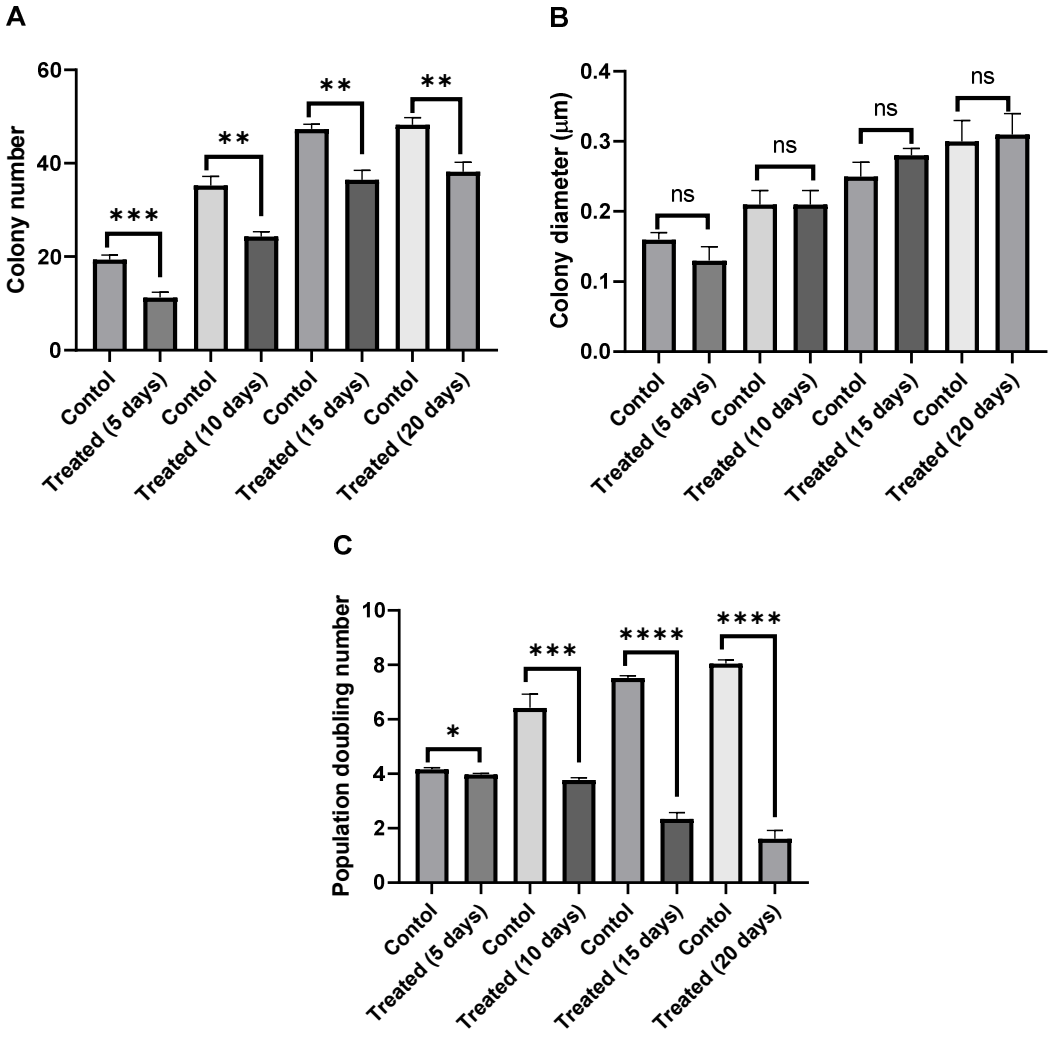

FIGURE 5. Proliferation Analysis: Comparing colony forming ability and population doubling number (PDN) of the BMSCs after treating for 1 hours with 100 µM of SNP in every 48 hours or in the period of 5,10,15 and 20 days. A) colony number, B) colony diameter, and C) PDN. Values are presented as means ± standard deviation. Statistical significance is represented as follows: ns (none significant), $^{*}P< 0.05$, $^{**}P< 0.01$, $^{***}P< 0.001$, $^{****}P< 0.0001$.

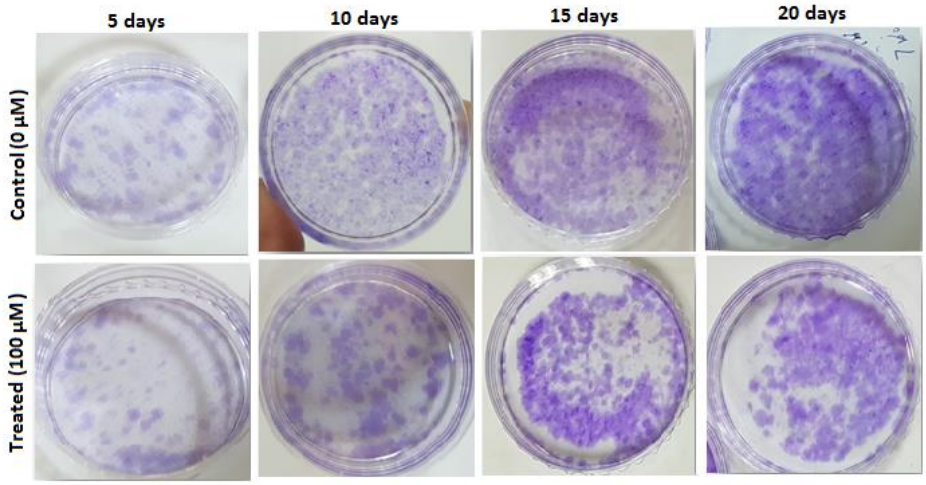

FIGURE 6. Colonies formed after 1-hour treatment with 100 µM of SNP administrated every 48 hours for 5, 10, 15, and 20 days of incubation. Digital photograph was taken by camera showed that treatment caused reduction of colonies when compared to the control group.

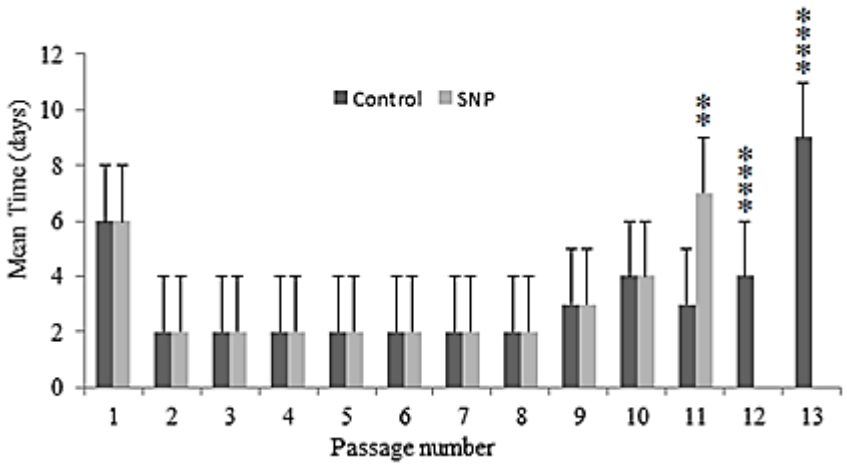

FIGURE 7. The mean time (days) required for BMSCs to be subculture (reach Passage). Values are presented as means ± standard deviation. Statistical significance is represented as follows: $^{**}P < 0.01$ and $^{****}P < 0.0001$.

and 13th passages (Figure 8).

Cell Cycle

Data analysis revealed that the treatment of cells for 1 hour every 48 hours caused cell cycle arrest at the G1 phase after 5, 10, 15, and 20 days compared to the control group (Figure 9-A and Figure 10). While there was a highly significant ($P < 0.0001$) and time-dependent increase in the total number of cells at the G1 phase, a highly significant and time-dependent reduction in the total number of cells at the S and G2/M phases was observed (Figure 9-B and C).

Gene expression analysis

Analysis of RT-PCR data revealed that the expression of *CDK2*, *CDK4*, and *P53* was significantly down-regulated ($P < 0.01$). However, there were no significant changes ($P > 0.05$) observed in *Raf1* expression (Figure 11-A). *GAPDH* was used as an internal control, and no change in expression was observed compared to the control group. Additionally, the photograph of the agarose gel (Figure 11-B) confirmed the statistical results.

Discussion

In our previous studies, while low concentrations of exogenous NO were found to enhance the osteogenic differentiation of BMSCs (Abnosi et al., 2022), it was demonstrated that prolonged treatment of BMSCs

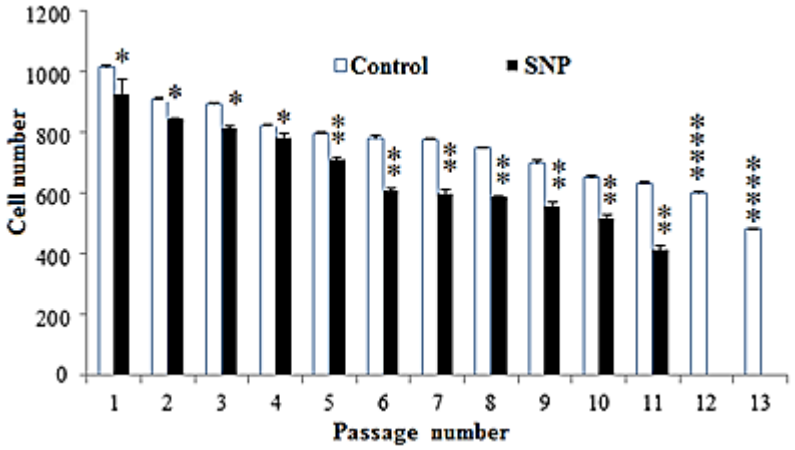

FIGURE 8. The mean number of BMSCs per passage. Values are presented as means ± standard deviation. Statistical significance is represented as follows: $^{*}P<0.05$, $^{**}P<0.01$, and $^{****}P<0.0001$.

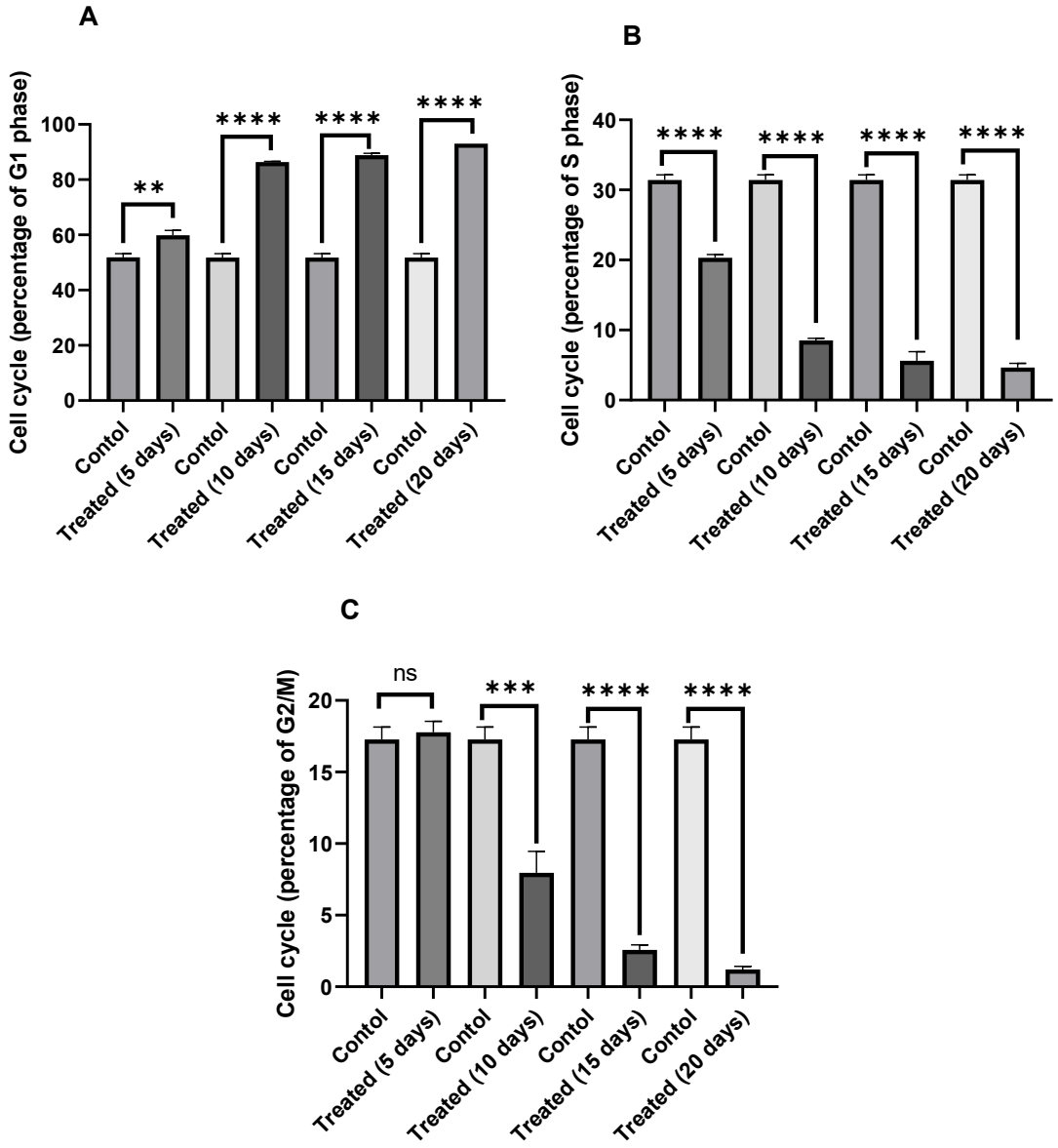

FIGURE 9. Cell cycle analysis based on DNA content of BMSCs after 1-hour treatment with SNP every 48 hours for 5,10,15, and 20 days of incubation. Values are presented as means ± standard deviation. Statistical significance is represented as follows: ns (none significant), $^{**}P<0.01$, $^{***}P<0.001$, and $^{****}P<0.0001$.

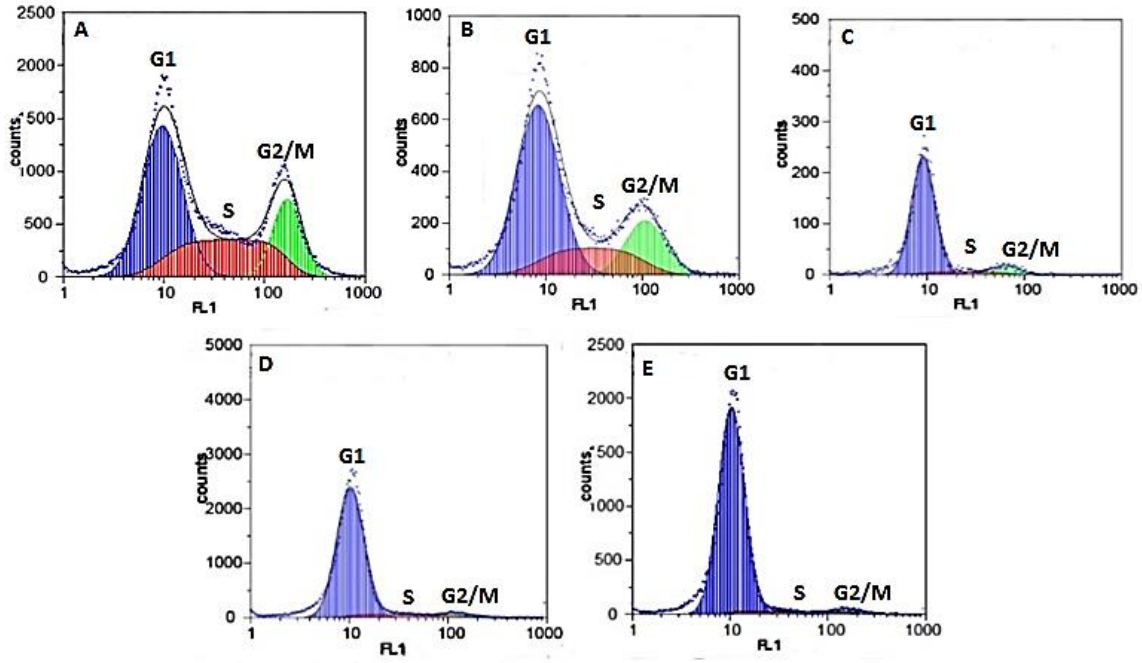

FIGURE 10. Cell cycle analysis of BMSCs after 1-hour treatment with SNP every 48 hours: A) control (0 µM) group incubated for 20 days. B) SNP-treated group of cells incubated for 5 days. C) SNP-treated group of cells incubated for 10 days. D) SNP-treated group of cells incubated for 15 days. E) SNP-treated group of cells incubated for 20 days.

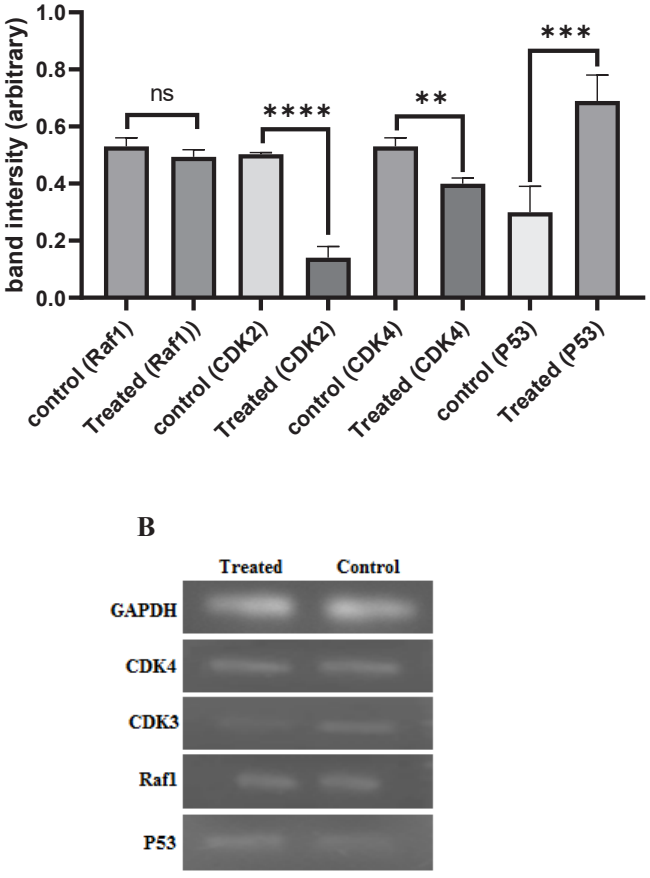

FIGURE 11. A) graph showing the effect of SNP on the relative expression of genes involved in BMSCs cell cycle after 1-hour treatment every 48 hours for 20 days of incubation. Values are presented as means ± standard deviation. Statistical significance is represented as follows: ns (none significant), $^{**}P < 0.01$, and $^{****}P < 0.0001$. B) the figure shows the expression of each gene on a 1.5 % agarose gel.

with SNP (100 μM) significantly reduced cell viabilie ty and proliferation (Pari et al., 2017; Mohammadi et al., 2017). Our findings, along with those of Chu et al. (2008) , indicated that a 48-hour treatment of bone marrow multipotent progenitor cells extracted from adult mice with SNP (100 μM) led to a dramatic reduction in cell proliferation (Chu et al., 2008). Furthermore, it has been observed that SNP, as an NO donor, inhibits cell proliferation in various cell types, including mouse macrophage-like Mm1 cells, mouse microglial MG5 cells, mouse neural progenitor cells, and rat cerebellar glial cells (Villalobo, 2006). In terms of proliferation ability, our study demonstrated that NO significantly reduced the PDN, indicating a decrease in the ability of individual cells to replicate over time. Additionally, our results showed that the number of colonies, but not their diameter, was affected by long-term NO treatment, which is consistent with our findings regarding PDN. This effect was also observed across different passages of the cells, indicating that the duration of treatment is a critical factor affecting cell proliferation ability. Therefore, prolonged and continues exposure of BMSCs to exogenous NO would certainly detrimental effects of NO.

It is known that inflammation and inflammatory factors can upregulate the expression of inducible nitric oxide synthase (iNOS), and this enzyme is known to be present in BMSCs (García-Aranda, 2020). Continuous and prolonged exposure of BMSCs to exogenous NO has been shown to inhibit their proliferation (Pari et al., 2017). We may postulate that BMSCs under prolonged stress could produce large amounts of NO, leading to a loss of their proliferation ability. Inflammatory factors such as INFγ, INFα, and IL-1 are known to play roles in the immunosuppression of BMSCs, possibly through overactivation of iNOS (Ren et al., 2008). Stressful conditions may also contribute to significant reduction in bone density. Therefore, exogenous NO could act as a stress factor, reducing the proliferation ability of BMSCs.

Proliferation of stem cells occurs through the process of cell division, which is tightly regulated by the cell cycle. During the cell cycle, DNA replication takes place, along with the synthesis of other biomolecules and organelles. As the cell cycle progresses, the cytoplasm undergoes partitioning, and ultimately, daughter cells are produced. Regulatory molecules called CDKs, control cell division as heterodimer complexes (Ding et al., 2020). Cyclins serve as regulatory subunits, while CDKs have catalytic activity but remain inactive in the absence of their cyclin partner (Dong et al., 2014). Our data analysis showed that exogenous NO arrests the BMSCs cell cycle at the G1 phase in a time-dependent manner. Heterodimer of CDKs and cyclins activate the target proteins required for cell to enter the next phase of the cell cycle (Ding et al., 2020). Cyclin D/CDK4 phosphorylates retinoblastoma during the early G1 phase, releasing E2F and allowing the expression of genes required for the G1 phase (Hinrichsen et al., 2008), whereas Cyclin E/CDK2 is necessary for the transition from G1 to S (Hume et al., 2020). Our results revealed that NO reduces the expression of the *cdk 2* and *cdk 4*, leading to cell cycle arrest at the G1 phase. In addition, several proteins such as P21, P27, and P57 act as inhibitory factors for cyclin/CDK complexes (Pack et al., 2021). P21 is activated by P53 (Engeland, 2022), and our results showed that continuous exposure of BMSCs to exogenous NO up-regulates the expression of the P53 gene and protein (photograph not shown). Consequently, P21 downregulates the expression of *Cdk2* and *Cdk4*, essential CDKs for the transition from G1 to S phase. Therefore, cell cycle arrest in BMSCs occurs due to reduced kinase activity resulting from inhibition of CDK production and heterodimer formation necessary for cell cycle progression. Takagi et al. (1994) demonstrated in an *in vitro* study that different concentrations of SNP (0.01, 0.1, and 1 mM) arrested the cell cycle of mouse macrophage-like cells at the G1 phase, which confirms our results (Takagi et al., 1994).

Conclusion

As mentioned previously, BMSCs serve as a cellular reserve that differentiates into osteoblasts, the cells responsible for bone matrix production. The reduction in viability and proliferation of BMSCs could potentially lead to a decrease in bone tissue's ability to maintain its matrix content and density. Our findings suggest that prolonged exposure of BMSCs to NO results in reduced viability and proliferation, primarily due to the down-regulation of *Cdk2/4* and inhibition of heterodimer complex formation, which is caused by indirect interference of P53. While further research is warranted to fully understand these mechanisms, it is plausible that cell cycle arrest of BMSCs due to overproduction of endogenous NO may contribute to reductions in bone

density observed during periods of stress and in bone inflammatory diseases.

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Conflicts of interest

There are no conflicts of interest.

Ethics approval and consent to participate

All procedures performed in studies on animals were in compliance with ethical standards of the Arak Medical University ethics committee, Iran, and the approval number is IR.ARAKMU.REC.1397.110.

References

Abnosi M H, Masoomi S. Para-nonylphenol toxicity induces oxidative stress and arrests the cell cycle in mesenchymal stem cells of bone marrow. Iranian Journal of Toxicology 2019;13 (3): 1-8. http://dx.doi.org/10.32598/IJT.13.3.496.2

Abnosi M H, Masoomi S. p-Nonylphenol impairment of osteogenic differentiation of mesenchymal stem cells was found to be due to oxidative stress and down-regulation of RUNX2 and BMP. Endocrine, Metabolic & Immune Disorders - Drug Targets. 2020; 20: 1336-1346. https://doi.org/10.2174/1871530320666200505114058

Abnosi M H, Pari S. Exogenous nitric oxide induced early mineralization in rat bone marrow mesenchymal stem cells via activation of alkaline phosphatase. Iranian Biomedical Journal. 2019; 23 (2): 142-152. https://doi.org/10.29252/.23.2.142

Abnosi M H, Sargolzaei I D J, Maleklou M. Exogenous nitric oxide up-regulates the runx2 via bmp7 overexpression to increase the osteoblast matrix production in vitro. Avicenna Journal of Medical Biochemistry 2022; 10(1): 58-64.

Abnosi M H, Yari S. The toxic effect of gallic acid on biochemical factors, viability and proliferation of rat bone marrow mesenchymal stem cells was compensated. by boric acid. Journal of Trace Elements in Medicine and Biology. 2018; 48: 246-253. https://doi.org/10.1016/j.jtemb.2018.04.016

Fok H, Jiang B, Clapp B, Chowienczyk P. Regulation of vascular tone and pulse wave velocity in human muscular conduit arteries: selective effects of nitric oxide donors to dilate muscular arteries relative to resistance vessels. Hypertension. 2012; 60(5): 1220-5. https://doi.org/10.1161/hypertensionaha.112.198788

Chu L, Jiang Y, Hao H, Xia Y, Xu J, Liu Z, et al. Nitric oxide enhances Oct-4 expression in bone marrow stem cells and promotes endothelial differentiation. European Journal of Pharmacology 2008; 591(1-3): 59-65. https://doi.org/10.1016/j.ejphar.2008.06.066

Coller H A, Sang L, Roberts J M. A new description of cellular quiescence. PLOS Biology 2006; 4: e83. https://doi.org/10.1371/journal.pbio.0040083

Darzynkiewicz Z, Juan G. DNA content measurement for DNA ploidy and cell cycle analysis. Current Protocols in Cytometry 1997; 00(1): 7.5.1–7.5.24. https://doi.org/10.1002/0471142956.cy0705s00

Ding L, Cao G, Lin W, Chen H, Xiong X, Ao H, Yu M, Lin J, Cui. The roles of cyclin-dependent kinases in cell-cycle progression and therapeutic strategies in human breast cancer. International Molecular Science. 2020; 21(6): 1960. https://doi.org/10.3390/ijms21061960

Dong P, Maddali M V, Srimani J K, Thelt F, Nevins J R, Mathey-Prevot B, You L. Division of labour between Myc and G1 cyclins in cell cycle commitment and pace control. Nature Communications 2014; 5:4750. https://doi.org/10.1038/ncomms5750

Engeland K. Cell cycle regulation: p53-p21-RB signaling. Cell Death & Differentiation 2022; 29: 946–960. https://doi.org/10.1038/s41418-022-00988-z

García-Aranda M I, Gonzalez-Padilla J E, Gómez-Castro C Z, Gómez-Gómez Y M, Rosales- Hernández M C, et al. Anti-inflammatory effect and inhibition of nitric oxide production by targeting COXs and iNOS enzymes with the 1,2-diphenylbenzimidazole pharmacophore. Bioorganic & Medicinal Chemistry. 2020; 28(9): 115427. https://doi.org/10.1016/j.bmc.2020.115427

Hill BG, Dranka BP, Bailey S M, Lancaster J R Jr, Darley-Usmar V M. What part of NO don't you understand? Some answers to the cardinal questions in nitric oxide biology. Journal of Biological Chemistry 2010; 285(26): 19699-19704. https://doi.org/10.1074/jbc.r110.101618

Hinrichsen R Hansen, A H, Haunsø S, Busk P K. Phosphorylation of pRb by cyclin D kinase is necessary for development of cardiac hypertrophy. Cell Proliferation 2008; 41(5): 813-829. https://doi.org/10.1111/j.1365-2184.2008.00549.x

Hottinger D G, Beebe D S, Kozhimannil T, Prielipp R C, Belani K G. Sodium nitroprusside in 2014: A clinical concepts review. Journal of Anaesthesiology Clinical Pharmacology 2014; 30(4): 462-71. https://doi.org/10.4103/0970-

9185.142799

Hume S, Dianov GL, Ramadan K. A unified model for the G1/S cell cycle transition. Nucleic Acids Research 2020; 48(22): 12483–12501. https://doi.org/10.1093/nar/gkaa1002

Mohammadi A, Abnosi M H, Pakyari R. Low concentration of sodium nitroprusside promotes mesenchymal stem cell viability and proliferation through elevation of metabolic activity. Avicenna Journal of Medical Biochemistry. 2017;5(1):9-16. http://dx.doi.org/10.15171/ajmb.2017.02

Oleson B J, Corbett J A. Dual role of nitric oxide in regulating the response of β cells to DNA damage. Antioxidants & Redox Signaling 2018; 29(14):1432-1445. https://doi.org/10.1089/ars.2017.7351

Pack L R, Daigh L H, Chung M, Meyer T. Clinical CDK4/6 inhibitors induce selective and immediate dissociation of p21 from cyclin D-CDK4 to inhibit CDK2. Nature Communications 2021; 12: 3356. https://doi.org/10.1038/s41467-021-23612-z

Pari S, Abnosi M H, Pakyari R. Sodium nitroprusside changed the metabolism of mesenchymal stem cells to an anaerobic state while viability and proliferation remained intact. Cell Journal (Yakhteh). 2017; 19(1): 146-158. https://doi.org/10.22074/cellj.2016.4875

Ren G, Zhang L, Zhao X, Xu G, Zhang Y, Roberts A I, Zhao R C, Shi Y. Mesenchymal stem cell-mediated immunosuppression occurs via concerted action of chemokines and nitric oxide. Cell Stem Cell 2008; 7;2(2):141-50. https://doi.org/10.1016/j.stem.2007.11.014.

Takagi K, Isobe Y, Yasukawa K, Okouchi E, Suketa Y. Nitric oxide blocks the cell cycle of mouse macrophage-like cells in the early G2+ M phase. FEBS Letters 1994; 340(3): 159-162. https://doi.org/10.1016/0014-5793(94)80128-2

Thomas D D, Ridnour L A, Isenberg J S, et al. The chemical biology of nitric oxide: implications in cellular signaling. Free Radical Biology and Medicine 2008; 45:18-31. https://doi.org/10.1016/j.freeradbiomed.2008.03.020

Vermeulen K, Van Bockstaele D R, Berneman ZN. The cell cycle: a review of regulation, deregulation and therapeutic targets in cancer. Cell Proliferation 2003; 36(3):131-149. https://doi.org/10.1046/j.1365-2184.2003.00266.x

Villalobo A. Nitric oxide and cell proliferation. The FEBS Journal 2006; 273(11): 2329-2344. https://doi.org/10.1111/j.1742-4658.2006.05250.x

Wimalawansa S J. Nitric oxide: novel therapy for osteoporosis. Expert Opinion on Pharmacotherapy 2008; 9(17): 3025-3044. https://doi.org/10.1517/14656560802197162

Wood J, Garthwaite J. Models of the diffusional spread of nitric oxide: implications for neural nitric oxide signaling and its pharmacological properties. Neuropharmacology 1994; 33(11): 1235-1244. https://doi.org/10.1016/0028-3908(94)90022-1

Zhou Z, Hossain M S, Liu D. Involvement of the long noncoding RNA H19 in osteogenic differentiation and bone regeneration. Stem Cell Research & Therapy 2021; 12(1):74. https://doi.org/10.1186/s13287-021-02149-4