



Microbial Contamination of Handmade Ice Creams Sold in Zanjan, Iran



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ABSTRACT

Background: Ice cream is a widely consumed dairy product that provides a favorable environment for microbial growth. This study aimed to assess the microbiological quality and contamination levels of handmade and industrial ice creams sold in Zanjan, Iran.

Methods: A total of 70 ice cream samples, including traditional, industrial, chocolate, and fruit-flavored types, were randomly collected from different regions of Zanjan during the summer of 2024. The samples were analyzed for *Staphylococcus aureus*, *Escherichia coli*, *Salmonella* spp., *Bacillus cereus*, lactic acid bacteria, and coliforms using standard microbiological methods according to Iranian National Standards.

Results: The results showed that 85% of the ice cream samples exceeded permissible microbial limits. Contamination rates were as follows: *Bacillus cereus* (87%), *Escherichia coli* (84%), *Staphylococcus aureus* (68%), *Salmonella* spp. (80%), coliforms (81%), and lactic acid bacteria (84%). The highest total bacterial counts were observed in machine-made and traditional ice creams, suggesting poor hygienic conditions during processing and handling.

Conclusion: The presence of multiple pathogenic bacteria highlights the urgent need for strict hygienic control during ice cream production, the use of pasteurized milk, regular monitoring of production environments, and training of food handlers to reduce microbial hazards and protect public health.

1. Introduction

Ice cream, as one of the most popular frozen dairy products, has a special place in the dietary habits of many communities, especially during the warm seasons. This product is widely consumed among various age groups due to its characteristics, such as a soft texture, desirable flavor, and high nutritional properties. It is made by freezing a mixture of raw materials such as milk, sugar, cream, and eggs. Additionally, colors, flavorings, fruits, and edible nuts can be added. It typically contains 12% fat, 11% non-fat milk, and 15% sugar or other sweeteners, which can simultaneously act as an emulsifier, a dispersant, and a foam (Cook & Hartel, 2010; Salehian et al., 2013). Due to its

nutrient-rich environment, near-neutral pH (6-7), and long shelf life, ice cream can provide a suitable medium for the growth of pathogenic and spoilage microorganisms. The ice cream production process involves several stages that can introduce various microbiological hazards. However, pasteurization, freezing, and hardening can significantly reduce most of these risks (Khalkhali et al., 2021). Pasteurization of milk can eliminate many pathogens that harmful to public health. However, potential microbiological hazards can still persist in the final products, especially due to the addition of contaminated ingredients or the failure to observe hygiene practices after pasteurization. This issue is particularly significant in the production of handmade ice cream, as the final stage of production occurs at the point of



sale and distribution. Traditional production and storage methods expose the product to various sources of microbial contamination that can threaten consumer health. Microbial contamination is considered one of the most important challenges in the production, supply, and consumption of ice cream, especially its traditional type (Ghorbani Ranjabari et al., 2016; Kanbakan et al., 2004). Unsanitary conditions in the production environment, low-quality raw materials, failure to adhere to personal hygiene principles by staff, improper storage conditions, and inadequate quality control can lead to the growth and proliferation of pathogenic microorganisms in this product (Yaman et al., 2006). Some of these pathogens, such as *Staphylococcus aureus*, *Escherichia coli*, *coliforms*, *Salmonella*, *Bacillus cereus*, and other opportunistic bacteria, can cause food poisoning, gastroenteritis, fever, diarrhea, and other gastrointestinal issues. These infections are particularly dangerous for children, the elderly, and individuals with weakened immune systems (Shahyari et al., 2010). According to a study by the World Health Organization (WHO), approximately one and a half billion children under the age of five suffer from diarrhea due to the consumption of contaminated foods such as milk and dairy products, with five million deaths occurring as a result of this illness (Anvarinejad & Mirzaei, 2013). The WHO also estimates that the actual number of foodborne illness cases is 30 to 35 times higher than officially recorded statistics (Ranjbar et al., 2016). Therefore, numerous studies in various parts of the world, including Iran, have investigated the microbiological status of different dairy products, including traditional ice cream. These studies have consistently emphasized the need for greater attention to health supervision, training of producers, and the enhancement of production standards (Bagheri Kakesh & Saeebi, 2021). Hassanzadazar et al. (2012), in a study conducted in Iran, reported that traditional ice creams in Urmia County had a high level of contamination, with 78% of samples containing pathogenic microorganisms (Hasanzadeh Azar, 2012). Similarly, Shadan et al. (2002) investigated the level of contamination of ice creams in Zahedan across different seasons. They reported that contamination by mesophilic aerobic microorganisms was 56% in spring and 53.3% in summer. Contamination by *coliforms* was 72% in spring and 64% in summer, while *E. coli* contamination was 2% in spring and 5.3% in summer. None of the samples were contaminated with *Salmonella* (Shadan et al., 2002). Therefore, a thorough examination of contamination levels in traditional ice cream can serve as an indicator for assessing food safety status and the effectiveness of health supervision within the community. Given the high consumption rate of traditional ice cream in Zanjan City and the lack of comprehensive data regarding the microbial quality of this product in the region, conducting a study to assess the level of microbial contamination in traditional ice creams available in the city is essential. This study aims to evaluate the microbial load of traditional ice cream samples collected from Zanjan City to clarify the health status of this product and highlight the need for improved hygiene standards in the production and

distribution processes. The results of this research can help health authorities, traditional ice cream producers, and consumers enhance food safety and mitigate microbiological risks.

2. Materials and Methods

2.1 Sample collection

This descriptive-cross-sectional study was conducted to investigate the status of microbial contamination in various types of traditional, industrial, fruit-flavored, and chocolate ice creams available in Zanjan city during the summer of 2024. Initially, Zanjan city was divided into four clusters based on the density of ice cream vendors, in proportion to consumption and demand. A total of 70 ice cream samples were randomly collected from four urban areas of Zanjan. The sample size was chosen based on previous similar studies (Hassanzadazar et al., 2012; Khalkhali et al., 2021). All samples were collected aseptically, transported to the laboratory, and stored at -20 °C before examination. The samples included 29 industrial ice creams, 17 chocolate ice creams, 12 traditional ice creams, and 12 fruit-flavored ice creams.

2.2 Preparation of samples

All preparation steps were carried out according to the method described by Eghbali and Jafarpour (2021). The samples were held at room temperature (25 ± 5 °C) for approximately 10 min to thaw slightly before detection. A 25 g of ice cream sample was added to 225 mL peptone water (0.1%) solution and homogenized for 2 min to make a 10^{-1} dilution. Serial dilutions were prepared with peptone water (0.1%) solution. Three suitable serial dilutions were chosen according to the contamination status of the samples.

2.3 Detection and evaluation of microbial contamination

Microbiological tests included enumeration of total bacterial count, *S. aureus*, *E. coli*, *coliforms*, *Salmonella*, *B.cereus*, and *lactic acid bacteria*, performed according to Iranian National Standard protocols numbered 2946, 2324, 9263, 5272, 6806-3, 1810, and 4721, respectively, using the drop plate method. After incubation at appropriate temperatures (depending on the type of test), colonies were counted.

For total bacterial counting, Plate Count Agar (PCA) was used and incubated at 37 °C for 48 h (ISIRI, 2005). To enumerate *S. aureus*, prepared diluted solutions were cultured on Baird-Parker Agar and incubated at 37 °C for 24 h (ISIRI, 2004). *Coliforms* were identified using MacConkey Agar (MAC), and *E. coli* was identified using Eosin Methylene Blue Agar (EMB); the plates were incubated at 37 °C for 48 h (ISIRI, 2015; ISIRI, 2008). Additionally, *Salmonella* was cultured on *Salmonella-Shigella* Agar (SSA), and colonies with black centers were identified after incubation at 37 °C for 24-48 h (ISIRI, 2007). Lactic acid bacteria were counted by culturing on MRS Agar and incubating at 30 °C for 72 h (ISIRI,

1998). *B. cereus* was identified by culturing on Mannitol Egg Yolk Polymyxin Agar (MYP) and observing large pink colonies with a precipitate halo after 24-48 h at 30 °C (ISIRI, 1998).

2.4 Quality assurance and quality control procedures

To ensure the accuracy and reliability of microbiological analyses, standard quality assurance and quality control (QA/QC) procedures were applied throughout the testing process. All microbiological media were prepared according to the manufacturer's instructions and Iranian National Standard protocols and were checked for sterility before use. Positive control strains were used to confirm the performance of selective and differential media, including *Escherichia coli* ATCC 25922, *Staphylococcus aureus* ATCC 25923, *Salmonella enterica* ATCC 14028, and *Bacillus cereus* ATCC 11778. Sterile peptone water was used as a negative control to detect any potential laboratory contamination. Colony counts were performed only on plates within the acceptable counting range. Equipment such as incubators, autoclaves, and refrigerators was regularly calibrated, and aseptic techniques were strictly followed during sample handling, dilution, and plating. These measures were implemented to minimize analytical errors and ensure the validity and reproducibility of the results (ISIRI, 2012).

2.5 Statistical analysis

All analyses were conducted in triplicate, and the results were expressed as Mean \pm SD (Log CFU/g) calculated using Excel software.

3. Results and Discussion

The results of the microbiological tests on 70 ice cream samples collected from ice cream vendors in Zanjan city

revealed that a significant portion of the samples had microbial contamination levels exceeding the permissible limits set by the Iranian National Standard. According to the data presented in Table 1, 60 samples (85%) of the ice creams were found to have higher total bacterial counts above the allowable limits. Additionally, contamination with *E. coli* was detected in 59 samples (84%), *S. aureus* in 48 samples (68%), *Salmonella spp.* in 56 samples (80%), *coliforms* in 57 samples (81%), *B. cereus* in 61 samples (87%), and *lactic acid bacteria* in 59 samples (84%), all of which exceeded the permissible values declared by the National Standard Organization of Iran (ISIRI, 2020).

One of the primary causes of illness and death worldwide is bacterial gastrointestinal infections induced by contaminated food consumption. Despite the presence of successful control programs in some developed countries, these infections continue to have a significant impact on public health and the economy. Milk and dairy products provide an ideal environment for the growth and proliferation of various microorganisms (ISIRI, 2015).

Ice cream, as a popular frozen dairy product, is enjoyed by individuals of all ages due to its delicious taste and nutritional value. With the increased consumption of ice cream during the summer season and the heightened risk of food contamination during this time, the importance of monitoring ice cream contamination becomes even more critical, especially for children and the various ingredients used in its preparation that may serve as sources of contamination and illness. However, this product can pose public health risks due to contamination by pathogenic microorganisms resulting from the use of substandard raw materials, inadequate processing of milk and equipment, improper storage of stabilizers, sugar, and vanilla in open containers, and the use of unclean hands instead of sterilized scoops (Ghadiri Hakim et al., 2021; Bagheri Kakesh & Saeebi, 2021; Ghorbani Ranjabari et al., 2016).

Table 1. Total bacterial count in different types of ice cream (numbers are expressed as Mean \pm SD (Log CFU/g))

Ice Cream Type	<i>Lactic Acid Bacteria</i>	<i>Salmonella</i>	<i>Staphylococcus Aureus</i>	<i>Bacillus Cereus</i>	Total Count	<i>Coliform</i>	<i>E. Coli</i>
Industrial	4.87 \pm 0.59	2.37 \pm 1.18	3.45 \pm 0.78	3.99 \pm 0.48	4.99 \pm 0.62	4.07 \pm 1.35	4.28 \pm 0.97
Traditional	3.24 \pm 0.78	2.55 \pm 1.17	3.12 \pm 0.97	4.14 \pm 1.15	4.25 \pm 0.41	3.12 \pm 1.08	3.53 \pm 1.51
Chocolate	4.34 \pm 0.39	1.66 \pm 1.32	1.48 \pm 1.09	3.26 \pm 0.90	3.81 \pm 1.91	2.28 \pm 1.98	2.29 \pm 1.47
Fruity	3.43 \pm 0.66	1.71 \pm 1.84	2.08 \pm 0.67	3.65 \pm 0.55	4.60 \pm 0.40	3.41 \pm 1.14	2.49 \pm 1.28
Total Count	4.22 \pm 0.59	2.11 \pm 1.18	2.68 \pm 0.87	3.78 \pm 0.69	4.53 \pm 0.60	3.36 \pm 1.42	3.36 \pm 1.24

The results of this study indicated that the contamination levels of handmade ice creams with pathogenic bacteria such as *S. aureus*, *E. coli*, *Salmonella*, and *B. cereus* exceeded permissible limits, with 80% of the handmade ice creams being contaminated with these pathogens. Additionally, contamination of these samples with coliform bacteria, which serves as an indicator for fecal contamination, was found to be significantly higher than the national standard, which requires this parameter to be negative in each gram of ice cream according to Iranian National Standards (ISIRI, 2020). Therefore, the findings of this research highlight a high likelihood of microbial contamination in handmade ice cream and a potential risk of transmission to consumers.

Based on the results of this study, 85% of the samples were deemed unsuitable for human consumption according to the Iranian National Standards presented in Table 2, with the percentage of contamination by various microorganisms illustrated in Table 3 (ISIRI, 2020).

According to Figure 1, the contamination levels in both machine-made and traditional ice cream samples were significantly high for various pathogens. One reason for this contamination is the use of unsuitable raw materials, including unpasteurized and low-quality milk, as well as improper storage of milk in contaminated containers or environments before processing. Additionally, failure to adhere to environmental hygiene and sanitary principles in

washing and disinfecting containers, working tools, ice cream machine reservoirs, freezers, and other equipment in these units also contributes to increased contamination in these production units.

Table 2. Permissible levels of each bacterium in ice cream based on (CFU/g)

Feature/ Product	Ice Cream	Ice Cream with Edible and Fruity Fillings
Total bacterial count	5×10 ⁴	5×10 ⁴
<i>Enterobacteriaceae</i>	10	10
<i>Escherichia coli</i>	Negative	Negative
<i>Staphylococcus aureus</i>	Negative	Negative
<i>Salmonella</i> (CFU/25 Grams)	Negative	Negative
<i>Bacillus cereus</i>	Negative	Negative
<i>Lactic Acid Bacteria</i>	Negative	Negative

Furthermore, as shown in Table 1, machine-made ice cream had the highest total bacterial counts and contamination with *E. coli*, indicating poor hygienic conditions during its production. In comparison, traditional ice cream exhibited lower contamination levels, although it still showed significant contamination in certain cases, such as with *B. cereus* and total bacterial counts. Chocolate ice

cream generally performed better, with significantly lower contamination levels of *Salmonella* and *S. aureus*. This improved condition in chocolate and fruit ice creams may be due to different ingredients and preparation methods, as well as reduced direct contact with producers. However, chocolate and machine-made ice creams showed high contamination levels with spoilage bacteria, such as lactic acid bacteria, which may indicate prolonged storage in stores. These bacteria often increase under poor storage conditions or due to unwanted fermentation processes, potentially leading to off-flavors or quality degradation of the product. Overall, there is a need for improved adherence to hygiene principles in the production of machine-made ice cream.

Based on the results of this study, regarding total bacterial counts, the contamination percentage in 85% of traditional ice creams was above permissible limits. In similar studies, such as that by Ranjbari et al. (2016), 37.08% of the tested ice creams were deemed unsuitable based on total bacterial counts, and in the study by Anvarinejad & Mirzaei (2013) conducted in Maragheh, 69.4% of the samples were found to be contaminated based on total bacterial counts.

Table 3. Percentage of sample contamination with pathogenic bacteria

Feature	Total Count	<i>Coliform</i>	<i>Escherichia Coli</i>	<i>Bacillus Cereus</i>	<i>Staphylococcus Aureus</i>	<i>Salmonella</i>
Contaminated Samples	60	57	59	61	48	56
Healthy Samples	10	13	11	9	22	14
Total Samples	70	70	70	70	70	70
Contamination Percentage	85%	81%	84%	87%	68%	80%

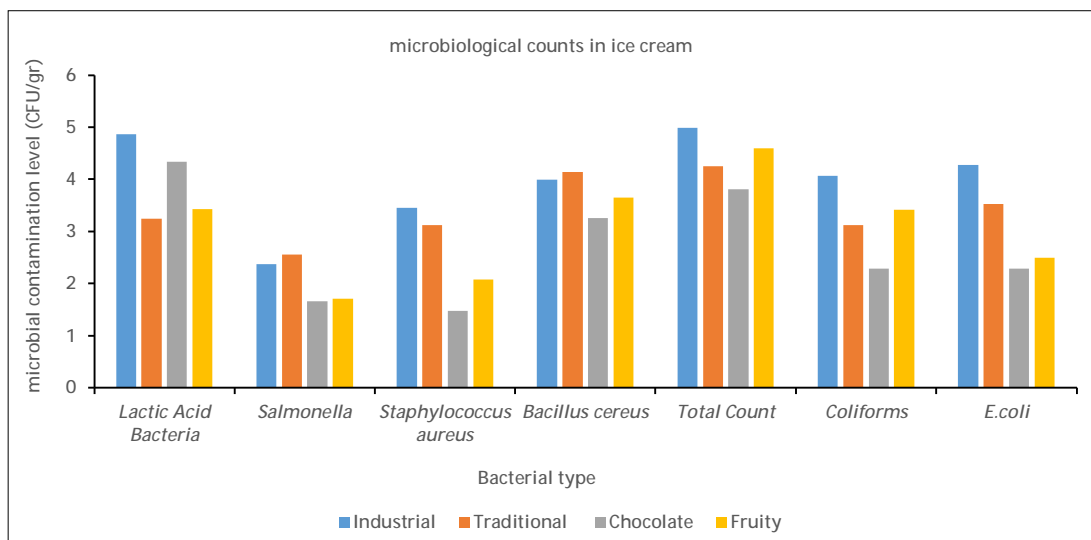


Figure 1. Contamination Levels of Different Types of Ice Cream Based on Various Bacteria

Another parameter obtained was that contamination with *coliforms* and *E. coli* was significantly higher than permissible limits. The results of other similar studies, such as the study conducted by Hassanzadazar et al. (2012), also indicated high levels of coliform contamination and unsatisfactory hygienic conditions in ice cream samples. The high prevalence of coliforms in traditional ice cream may be attributed to environmental contamination or unhygienic production and storage methods (El-Mossalami & Abd-El-

Rahman, 2008). The counts of *Enterobacteriaceae* and *coliforms* in food are considered significant indicators of food hygiene. In this study, the contamination levels of *Salmonella* bacteria were also found to be above permissible limits. This finding is inconsistent with the results of studies conducted by Shadan et al. (2002) in Zahedan and Anvarinejad & Mirzaei (2013) in Maragheh, in which the tested ice cream samples were reported to be free of from *Salmonella* contamination. The consumption of food contaminated with

Salmonella can lead to enterocolitis, and the feces of infected individuals and carriers serve as sources of infection (Salehian et al., 2013).

Regarding another parameter, 68% of the ice cream samples were contaminated with *S. aureus*. In similar studies, Khalkhali et al. (2021) found that 18% of samples were contaminated with *S. aureus* in East Azerbaijan. In another study conducted by Abolhasan Nejad (2014) in Birjand, ice cream samples exhibited the highest levels of contamination. This bacterium is commonly found in the nasal passages of many individuals and can be transmitted through infected skin lesions or via respiratory droplets from sneezing and coughing. Poor personal hygiene and the lack of gloves and masks during production and sales increase the likelihood of this bacterium being transferred to the products. Furthermore, this bacterium can spread through water, soil, dust, and air. Milk and dairy products, such as ice cream, provide a suitable environment for the growth of pathogenic bacteria; therefore, the risk of contamination of milk from cows affected by mastitis and its subsequent transfer to dairy products is quite high (ISIRI, 2015).

Another important parameter in this study is the contamination of ice cream samples with *B. cereus*. In the present study, the contamination levels of ice cream samples with *B. cereus* were found to be above permissible limits, consistent with the results of the study by Shakarian et al. (2005) in Shahrekord, which showed that 12% of ice cream samples were contaminated with *Bacillus cereus* and exceeded the national standard limits of Iran. In another study conducted by Pourmahmoodi et al. (2003) in Yasuj, 10% of the samples were also found to be contaminated with *B. cereus*. This bacterium primarily contaminates materials such as sugarcane, gelatin, and starch through its spores, which are dispersed in the soil, ultimately leading to contamination of ice cream (Khorramruz et al., 2015).

In the present study, although direct observational or survey-based data regarding handling practices, storage conditions, and environmental hygiene of ice cream production and sales units were not collected, the high prevalence of microbial contamination strongly indicates the presence of multiple contamination pathways. According to previous studies, the most important sources of contamination in traditional and machine-made ice creams include raw materials, processing equipment, storage environments, and food handlers. The use of unpasteurized or inadequately pasteurized milk is considered one of the primary risk factors, as milk can serve as a vehicle for pathogens such as *E. coli*, *Salmonella* spp., and *S. aureus*. In addition, contaminated additives such as sugar, flavorings, fruits, nuts, and stabilizers may introduce spores of *B. cereus* and other microorganisms into the final product. Equipment and utensils used during ice cream production and serving, including mixers, ice cream machines, storage containers, scoops, and freezers, represent another critical route of post-pasteurization contamination. Inadequate cleaning and disinfection of these tools have been repeatedly reported as major contributors to microbial contamination in ice cream, particularly in small-scale and traditional production units.

Furthermore, improper storage conditions, such as temperature abuse during freezing, prolonged storage, and repeated thawing and refreezing cycles, can facilitate the survival and growth of psychrotrophic and spore-forming bacteria. Personnel-related factors are also highly influential. Previous research has shown that poor personal hygiene practices, including insufficient hand washing, lack of protective clothing (gloves, masks, and hair covers), and direct hand contact during serving, significantly increase the risk of contamination with *S. aureus* and coliform bacteria. Environmental contamination from air, dust, water, and surrounding surfaces further exacerbates the problem, especially in open or poorly controlled retail environments. Similar contamination pathways have been documented in studies conducted in different regions of Iran and other countries, suggesting that the observed contamination patterns in the present study are consistent with broader findings in the literature.

From an applicability perspective, these findings emphasize the urgent need for practical and enforceable interventions. Ice cream vendors should be encouraged to use only pasteurized milk and approved raw materials, ensure regular and effective cleaning and disinfection of all equipment and utensils, and strictly maintain the cold chain during storage, transportation, and sale. Adherence to personal hygiene principles, including mandatory hand washing, use of gloves and masks, and avoidance of direct hand contact with products, is essential. Regular participation in food safety and hygiene training programs should be considered mandatory for all personnel involved in ice cream production and sale.

Regulatory and health authorities play a key role in reducing microbiological risks. Strengthening routine inspections, especially during peak consumption seasons, implementing standardized hygiene assessment checklists, and enforcing compliance with national microbiological standards can significantly improve product safety. Periodic microbiological surveillance of ice cream samples, combined with corrective actions and penalties for non-compliant units, is strongly recommended. In addition, public education campaigns can raise consumer awareness regarding the risks associated with improperly handled ice cream and promote safer purchasing behaviors. Future studies should focus on molecular identification of pathogens and assessment of antibiotic resistance patterns to better understand and control foodborne hazards in dairy-based products.

4. Conclusion

The present study revealed a high prevalence of microbial contamination in handmade ice creams sold in Zanjan city, with 85% of the analyzed samples exceeding the permissible microbial limits established by the Iranian National Standards. The dominant contaminants included *Bacillus cereus* (87%), *Escherichia coli* (84%), *Staphylococcus aureus* (68%), *Salmonella* spp. (80%), and coliforms (81%), indicating serious lapses in hygiene during production, handling, and

storage. These findings demonstrate that both traditional and machine-made ice creams are potential vectors for foodborne pathogens and may pose significant public health risks, particularly to children, the elderly, and individuals with compromised immune systems.

Although this study did not include direct observational or survey-based assessments of handling practices, storage conditions, or environmental hygiene, the findings, supported by extensive literature evidence, clearly indicate that deficiencies in these areas are likely responsible for the high levels of microbial contamination observed. Therefore, improving hygiene practices across the entire production-to-consumption chain, through vendor training, strict regulatory oversight, and continuous monitoring, remains essential for reducing foodborne risks and protecting public health.

Authors' Contributions

Sara Balali: Project administration; Writing-original draft; Software; Methodology; Visualization; Investigation. **Hassan Hassanzadazar:** Resources; Methodology; Writing-review & editing; Supervision; Funding acquisition; Conceptualization; Data curation. **Majid Aminzare:** Methodology; Writing-review & editing. **Adel Mirza Alizadeh:** Validation; Resources; Methodology. **Sara Moradpey:** Project administration; Resources; Investigation. **Nessa Azimzadeh:** Project administration; Resources; Investigation. **Pegah Homauoni:** Project administration; Resources; Investigation.

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Conflicts of Interest

The authors declare that there are no conflicts of interest regarding the publication of this article.

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Ethical Considerations

All Ethical issues including have been confirmed by Iranian National committee for Ethics in Biomedical Research (IR.ZUMS.BLC.1403.020).

Using Artificial Intelligence

We hereby declare that artificial intelligence was not used in the current manuscript.

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