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Original Article

Innovation in ovary decellularization methods: Chemical and herbal detergents

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ABSTRACT

The ovary provides an ideal environment for egg survival due to its distinct structure that directly contributes to the growth and maintenance of the follicle. The purpose of this study is to compare ovarian decellularization with herbal and chemical detergents. Sheep ovarian was used in this study. 1 % sodium dodecyl sulfate (SDS) as a chemical detergent and 1, 2.5, and 5 % Acanthophyllum (ACP) were used as herbal agents for decellularization. DNA content, histological characteristics, attenuated total reflection Fourier transform infrared spectroscopy (ATR-FTIR), biocompatibility, antibacterial test, hemocompatibility, and scanning electron microscope (SEM) were investigated. The results showed the DNA content in decellularization scaffolds with 1 % SDS and 5 % ACP was reduced suitably. Also, histological observations confirmed this finding, and the nuclei were completely removed in these two groups. Disorganization of collagen fibers and tissue architecture was observed more in the SDS group than in the ACP group. No group reported cytotoxicity and the best blood compatibility in decellularization with herbal agents was reported. Protein bands are largely conserved in all methods. Higher antibacterial properties were observed in the decellularization technique with ACP. Decellularization with 5%ACP, in addition to being able to completely remove cells in the tissue, can help preserve the ultrastructure of the ovary. Therefore, this plant agent can be introduced as a decellularization method for studies in this field.

1. Introduction

Because of its distinct structure, which directly aids in the follicle's growth and maintenance, the ovary is thought to provide an ideal milieu for the survival of the egg. Systemic metabolic diseases, chemotherapy, and ovarian pathologies including polycystic ovary syndrome (PCOS) can all contribute to ovarian tissue malfunction. By keeping germ cells in an environment that is conducive to their survival, tissue, and egg freezing procedures aim to maintain women's fertility. Despite their effectiveness, these treatments are constrained by methodological issues such as the high implementation costs, the risk of cancer cell proliferation, the use of cytotoxic cryopreservation, and the destruction of oocytes during the freeze/thaw or cryopreservation procedures [1].

Natural and manmade polymers are employed to create bioengineered ovaries. Studies have shown that extracellular matrix (ECM) derived from decellularized tissues is the preferred matrix for artificial ovaries, while synthetic polymeric materials lack some of the characteristics of decellularized tissue [2,3]. Decellularized biological scaffolds have garnered considerable attention due to their exceptional biocompatibility, bioactivity, and moderate mechanical capabilities for cell support. The current techniques for decellularization are designed to eliminate the cellular and nuclear material while preserving the remaining ECM's composition, biological activity, and mechanical integrity [4,5]. Growth factors, glycosaminoglycans, and collagenous proteins are all harmed by chemical detergents. Furthermore, the ECM that has been

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decellularized still contains residual cytotoxicity that is caused by specific chemicals and enzymes. Therefore, researchers have turned their attention to herbal detergents for tissue decellularization [6]. Since saponin is thought to be the primary component of Acanthophyllum (Caryophyllaceae) (ACP) its thick roots are a significant source of these compounds. The saponin found in ACP extract possesses antimicrobial and antioxidant properties [7]. Several studies have used soap nut pericarp extract as a herbal detergent due to its saponin compounds to decellularize different tissues, and interesting and effective results have been reported [8–10]. Therefore, in this study, our goal is to use ACP for the first time in tissue decellularization, we have chosen ovarian tissue according to the problems in infertility, which can provide a suitable three-dimensional platform in studies related to infertility.

2. Materials

Penicillin-streptomycin, trypsin- EDTA, and fetal bovine serum (FBS) were obtained from Bioidea, Iran. Xylene and paraffin were purchased from Asiapajohesh, Iran. Sodium dodecyl sulfate (SDS) was obtained from Merck, Germany. Phosphate-buffered saline (PBS), Dulbecco's Modified Eagle's medium (DMEM), 3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT), dimethyl sulfoxide (DMSO), glutaraldehyde, hematoxylin-eosin (H-E), Masson's trichrome (MT), Alcian blue (AB), and glutaraldehyde were purchased from Sigma Aldrich, Germany. Adipose mesenchymal stem cells (AMSCs) were obtained from Pasteur Institute, in Iran. Acanthophyllum (Caryophyllaceae) (ACP) was obtained from the Atrifal store, in Iran.

- **Decellularization definition:** ECM should contain <50 ng DNA per mg dry weight and there should be no visible nuclear component in the ECM [11].

3. Methods

3.1. Preparation of ovary tissues

One of the food sources in many countries is sheep meat. Therefore, after sacrificing these animals, their waste tissues can be used for research. The uterus of a female Sanjabi breed sheep was removed from the Kermanshah animal slaughterhouse, in Iran, after slaughtering these animals. The tissues were placed in phosphate-buffered saline (PBS) containing 2 % penicillin-streptomycin (P/S) and transferred to the laboratory, and then the ovaries were gently separated from the surface of the uterine tissue and made available for further processing.

3.2. Preparation of detergent

In this study, two chemical and herbal detergents were used. A 1 % solution of sodium dodecyl sulfate (SDS) (chemical) was prepared with distilled water. The root of Acanthophyllum (Caryophyllaceae) (ACP) (herbal), which grows in different regions of Iran [12], was used to prepare herbal detergent. The root of the plant was ground into powder and weighed. To prepare different percentages of herbal detergent according to the study, the solution (ACP powder in distilled water) was placed on a stirrer for 24 h and then passed through a filter paper and kept in the refrigerator until use.

3.3. Decellularization process

Each ovary was divided into two halves from the middle and washed in PBS solution to remove the remaining blood from the tissue. Then the tissues were divided into 5 groups for experimental studies and the decellularization process. Group 1 was native tissue (no treatment was performed on this tissue), and the other 4 groups were respectively in detergents of 1%SDS, 1%ACP, and 2.5% ACP, and 5 % ACP were immersed and shaken at room temperature for 48 h at a speed of 70 revolutions per minute (rpm). The solution was changed every 12 h. Then the tissues were shaken in distilled water for 24 h to remove the detergent. The solution was replaced every 8 h (Fig. 1).

3.4. DNA content

Using a kit from the Iranian company SinaClon, the amount of DNA that remained in the decellularized ovary and native tissue was measured. Tissues were incubated at 55 °C for 3 h after containing 5 μl of protease and 100 μl of protease buffer. After mixing 100 μl of the samples with 400 μl of the lysis solution and 300 μl of the precipitation solution, the mixture was centrifuged for 10 min at 12000g. The pellet is centrifuged at 12,000 g for 30 s after being suspended in 50 μl of solvent buffer. With the use of a NanoDrop spectrophotometer (BioTeK, USA), the amount of DNA in the supernatant was determined [13].

3.5. Biocompatibility (MTT assay)

An indirect test was used to determine the biocompatibility of decellularized ovarian tissue (ISO 10993-5). For a full day, scaffolds were submerged in a culture medium made of Dulbecco's Modified Eagle's Medium (DMEM). After culturing adipose mesenchymal stem cells (AMSCs) (1×10^4 in each well of 96 plates), the media condition was collected and applied. The MTT test was used to check for biocompatibility after 48 and 72 h. After adding 20 μl of MTT solution (5 mg/ml) to the cells, the plate was incubated for 4 h. The purple formazan crystals were dissolved by adding 100 μl of dimethyl sulfoxide (DMSO) to the wells containing the cells after the MTT solution was withdrawn. Lastly, an ELISA reader (Stat Fax 2100, USA) at 570 nm was used to measure the amount of light absorption [14]. The following formula was used to calculate the results: Sample OD/Control OD × 100 = cell biocompatibility (%)

3.6. Histology analysis

The tissues were prepared and then histologically evaluated. After they were fixed, they were extracted, and paraffin molded. Tissues with a 5-μm diameter were cut using a microtome. To identify the nucleus, collagen, and elastin in the tissue, the sections were first deparaffinized using xylene solution, hydrated by ethanol in descending degrees, and then stained with Hematoxylin-Eosin (H-E), Masson's trichrome, and Van Gieson [15].

3.7. Hemocompatibility

Ovary tissues (3 × 3 mm) were incubated in 2 ml of PBS for 30 min (temperature 37 °C). The positive control group received distilled water and the negative control group received PBS. 20 μl of fresh blood containing anticoagulant was added to the groups and incubation continued for 1 h. At the end, centrifugation (10 min at 1500 rpm) was performed, and the supernatant was read at a wavelength of 545 nm (ELISA reader, Stat Fax 2100, USA) [16]. % Hemolysis degree (HD) formula: DS: OD sample, D0: OD negative control, D1: OD positive control. HD (%) = [(DS - D0)/(D1 - D0)] × 100.

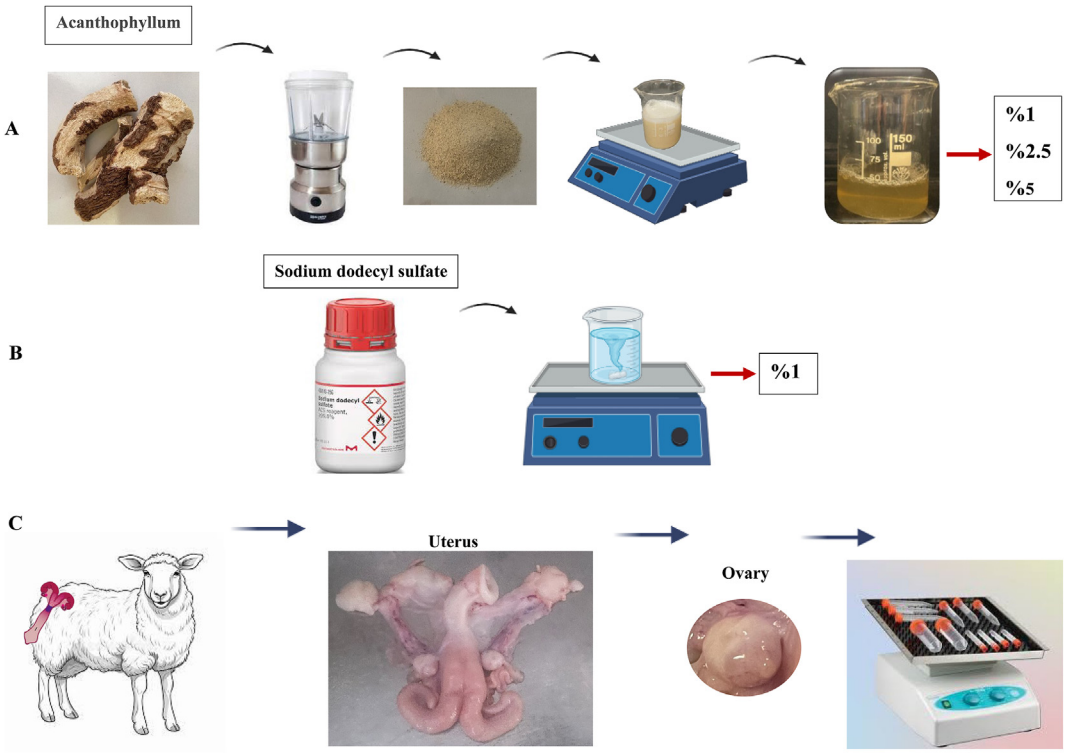

Fig. 1. Preparation steps of detergents and decellularization, A) Preparation of different doses of ACP using plant root powder, B) Preparation of SDS 1 %, C) Ovaries were separated from the uterus of sheep and after washing, they were immersed in various chemical and herbal detergents and subjected to a shaker.

3.8. Attenuated total reflection Fourier transform infrared spectroscopy (ATR-FTIR)

FTIR spectroscopy was utilized to assess the scaffolds' molecular structure, functional group identification, and bond formation. Using the IRPrestige-21 (Shimadzu, Japan) equipped with ATR mode, infrared spectra of the ovarian tissue were acquired over a range of 500–4000 cm-1, with an average resolution of 4 cm-1 over 64 scans [17].

3.9. Cell morphology (SEM)

A scanning electron microscope (SEM) (Philips, The Netherlands) was used to analyze the ultrastructure of ovarian tissues. The samples were first left for 24 h in a fixing solution (2.5 % glutaraldehyde). The samples were dehydrated by increasing the amount of ethanol (40, 50, 60, 70, 80, 90 and 100 %) used. The samples were freeze-dried (−50 °C for 12 h, Christ Alpha 2–4 LDplus), and then gold-palladium was applied to them to enable microscopic inspection [18].

3.10. Antibacterial test

To test the antibacterial effect of ovarian tissue scaffolds, sensitive (ATCC25923) and methicillin-resistant (M30) strains of *Staphylococcus aureus*, the sensitive (ATCC27853) and imipenem-resistant (IMI10) strains of *Pseudomonas aeruginosa*, and the imipenem-resistant (IMI10) *Escherichia coli* strain (ATCC25922) were used. The tested strains were incubated for 6–8 h in BHI enrichment medium in a shaker incubator. After culturing bacteria on blood agar medium and incubating overnight at 37 °C, the suspension of microorganisms was prepared at a concentration of 1.5×10^8 CFU mL^{-1}. Scaffolds were placed in two groups. The first group was decellularized with 1 % SDS and the second group with 5 % ACP. Before culture, the scaffolds were sterilized under UV light for 20 min and then immersed in 100 μL of BHI broth culture medium to prewet. Half McFarland suspension of microorganisms was inoculated on Mueller Hinton agar culture medium. To evaluate the antibacterial activity, scaffolds were placed against the antibiotic discs of imipenem and methicillin [19].

3.11. Statistical analysis

The data was analyzed using GraphPad Prism software (version 8) using the one-way ANOVA and Tukey's post hoc test. $P \leq 0.05$ was used as the significance level.

4. Results

4.1. DNA content and biocompatibility

DNA content was checked to confirm proper decellularization in all decellularized ovarian tissues and compared with the native group. DNA content decreased in all decellularized groups compared to control. A significant difference in DNA concentration was reported between the native ovarian tissue group (907.33 ng/mg) compared to the decellularization groups including 1 % ACP (102.73 ng/mg), 2.5 % ACP (76.28 ng/mg), 5 % ACP (37.31 ng/mg) and SDS 1 % (37.05 ng/mg). The highest removal of DNA from the tissue was related to 5 % ACP and 1 % SDS groups (Fig. 2 A). MTT test was performed to determine the biocompatibility of the scaffolds at 48 and 72 h. In all experimental groups, cell viability was reported to be more than 90 %. There was no significant difference between the groups using chemical and herbal detergents, although the highest biocompatibility was observed in the 5 %ACP group. Therefore, it can be said that different decellularization methods did not cause toxicity in ovarian scaffolds and are biocompatible (Fig. 2 B and C).

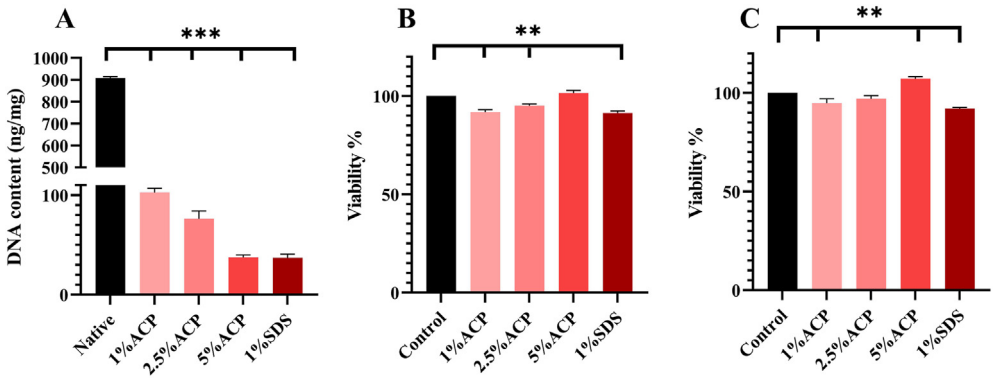

Fig. 2. (A) DNA content in native ovary tissue showed a significant difference with the decellularized group, (B) MTT test in 48 h and (C) MTT test in 72 h, cytotoxicity evaluation after 48 and 72 h shows high cell proliferation for all groups. All experimental groups reported cell viability above 90 %, **($P \leq 0.001$) and ***($P \leq 0.0001$): Significant compared to the control group, the data presented are mean ± SD, n = 3.

- According to the definition of cell decellularization in the 1 % SDS and 5 % ACP methods, the amount of DNA remaining in the tissue was reported to be < 50 ng.

4.2. Histology

Tissue staining was done in all the tested groups. In the native tissue, the main structure of the tissue can be seen. The collagen network is seen. The presence of nuclei and vascular structure in the tissue was reported. In the decellularization method with 1 % ACP, the structural order of the tissue has been preserved and the nuclei have been partially removed from the tissue. In the 2.5 % ACP method, the morphological structure is not preserved as well as in the 1 % ACP method, but the nuclei have been removed in a large amount and the order of the vascular structure is still visible. In 5 % ACP and 1 % SDS, complete decellularization was done. Collagen fibers are still part of the extracellular matrix. In the 5 % ACP method, compared to 1 % SDS, the texture structure is more orderly (Fig. 3).

- According to the definition of decellularization in the 1 % SDS and 5 % ACP methods, no nuclei were observed in the histological images.

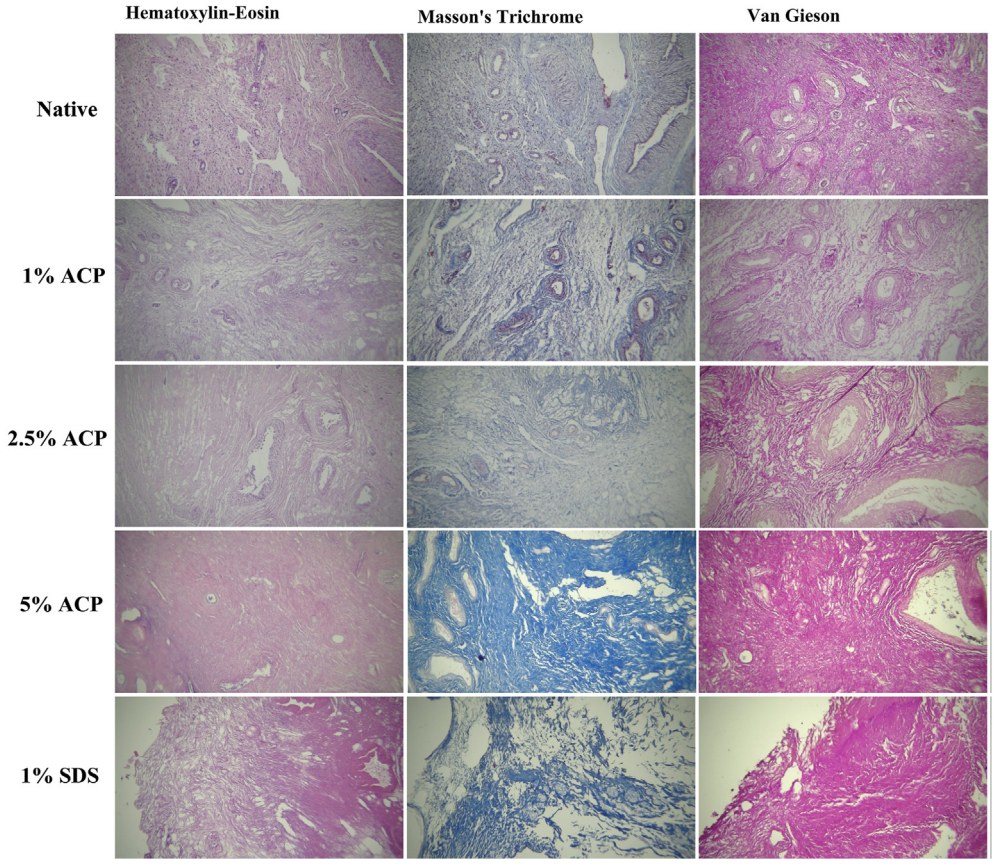

Fig. 3. Histological staining in native and decellularized ovary. magnification 100x.

4.3. Hemocompatibility

The results of the hemolysis test were analyzed. A significant difference was reported in the compatibility of all methods of ovarian decellularization compared to the positive control group. The percentage of hemolysis in the 1 % SDS method was reported to be higher than 2 % (2.5 %), while in decellularization with ACP (all percentages), this value was less than 1 % (Fig. 4).

4.4. ATR-FTIR

ATR-FTIR spectroscopy of native and decellularized ovarian tissue was performed to analyze the structural changes of proteins. Native ovary tissue mainly showed five absorption peaks at 1647.21 cm^{-1}, 1517.98 cm^{-1}, 1224.80 cm^{-1}, 3292.49 cm^{-1}, 1743.65 cm^{-1} consistent with amide I, amide II, amide III, amide B, and carbonyl groups (C═O) respectively. It is necessary to evaluate a decellularized tissue by focusing on the above bands, as it was observed that protein bands were preserved to a large extent in all groups. Carboxyl group and amide B were observed only in decellularized ovaries with 1 % ACP (Fig. 5).

4.5. SEM

According to the histological results, among the decellularized tissues with herbal detergent, the 5 % ACP group was selected. Compared to the images of native ovarian tissue, decellularization with herbal detergent has been able to preserve the structure of the tissue to a large extent and the interconnection of fibers can be seen well. In the 1 % SDS method, fiber breakage is visible and tissue porosity has increased (Fig. 6).

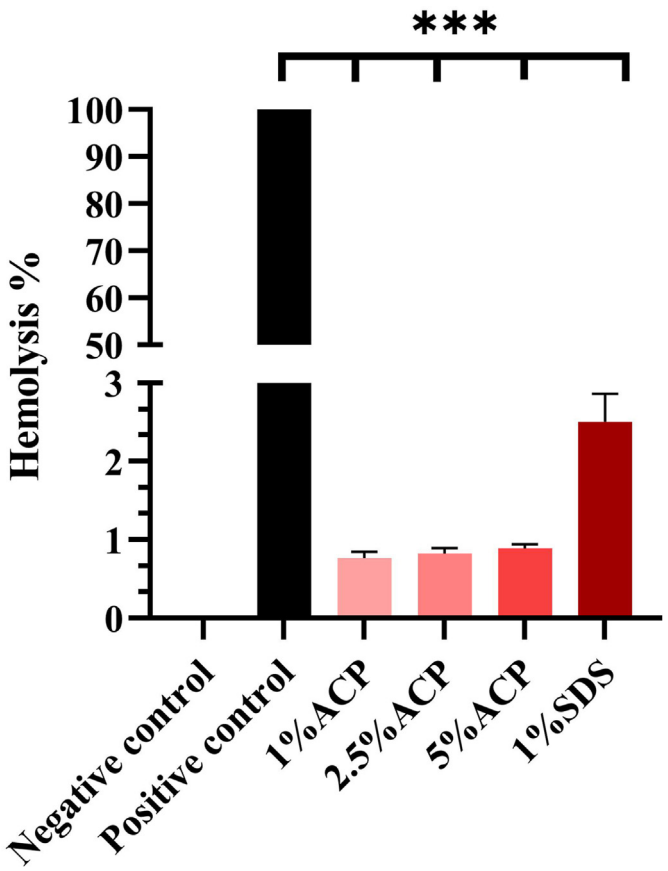

Fig. 4. Hemolysis test, all methods reported significant differences from the positive control group, ***($P \leq 0.0001$): Significant compared to the control group, the data presented are mean ± SD, n = 3.

4.6. Antibacterial

The antibacterial activity of the scaffolds on *S. aureus*, *P. aeruginosa*, and *E. coli* after 24 h was evaluated, and the diameter of the inhibition zone scaffolds was compared with the diameter of the inhibition zone antibiotics. As can be seen, the scaffolds had a very good antibacterial effect. The measurement of the inhibition zone of the scaffolds showed that the diameter of the inhibition zone of the 5 % ACP group scaffold was greater than the inhibition zone of the 1%SDS group scaffold. The diameter of the inhibition zone of studied bacterial strains is shown in Table 1 and Fig. 7. As can be seen, the remarkable effectiveness of the scaffolds was observed against bacterial strains, especially against antibiotic-resistant strains. The results indicate the strong antibacterial activity of the scaffolds against common sensitive and resistant gram-positive and negative bacteria.

5. Discussion

Generally, chemical agents such as SDS have a high power to remove cells, but they may negatively affect the ECM structure. Herbal agents are usually milder and may preserve the ECM structure better. Due to its functional importance for follicular studies, ovarian tissue has been investigated in various studies for decellularization methods. Choosing the right method and detergent can have an effective contribution to the quality of the scaffold obtained in preclinical and clinical studies. The DNA content remaining in the tissue after decellularization is used as an indicator of the efficiency of the decellularization process. If the amount of DNA remaining in the tissue is more than 50 ng/mg of dry weight, the method used does not confirm the quality and efficiency of proper decellularization [11]. In a study, swine ovaries were decellularized with a combination of physical, chemical, and enzymatic methods. The concentration of DNA was reported to be around 44 ng/mg, which according to the mentioned contents, the amount of reduction in DNA content is appropriate [2]. On the other hand, in another study where porcine ovaries were decellularized with different methods, the DNA content was reduced by 94 % compared to the native tissue [20]. In our study, the herbal method at the lowest percentage, i.e., 1%ACP, could not reduce the DNA content to the optimal level (less than 50 ng/mg), but 5 % ACP reduced this amount to 96 % compared to the native tissue. The reduction of turnover provided similar data with 1 % SDS in reducing DNA concentration (37 ng/mg), in ovarian tissue. Therefore, this herbal agent will be able to reduce DNA content with a higher percentage than chemical detergent. These results are confirmed in another way in the histology test. The removal of nuclei from the tissues in the technique of 1 % ACP and 2.5 % ACP has not happened completely, while in % ACP and 1 % SDS percent of cells are not observed in the tissue. Chemical detergents have been very successful in removing cells from the ovary. Although the structure of the tissue is messed up compared to the native tissue [1]. This case is not only specific to ovarian tissue. In studies that used other tissues such as the testis [21] or kidney capsule [22] in decellularization with chemical detergents, the architecture of the tissue is disturbed especially in higher concentrations of chemicals. A decrease in the density of the collagen network, a decrease in the amount of GAG, and the loss of the general appearance of the tissue are observed. Decellularization of goat esophagus with Sapindus mukorossi extract at 5 % compared to 2.5 % and 10 %, in addition to proper removal of cells and reduction of DNA content, also

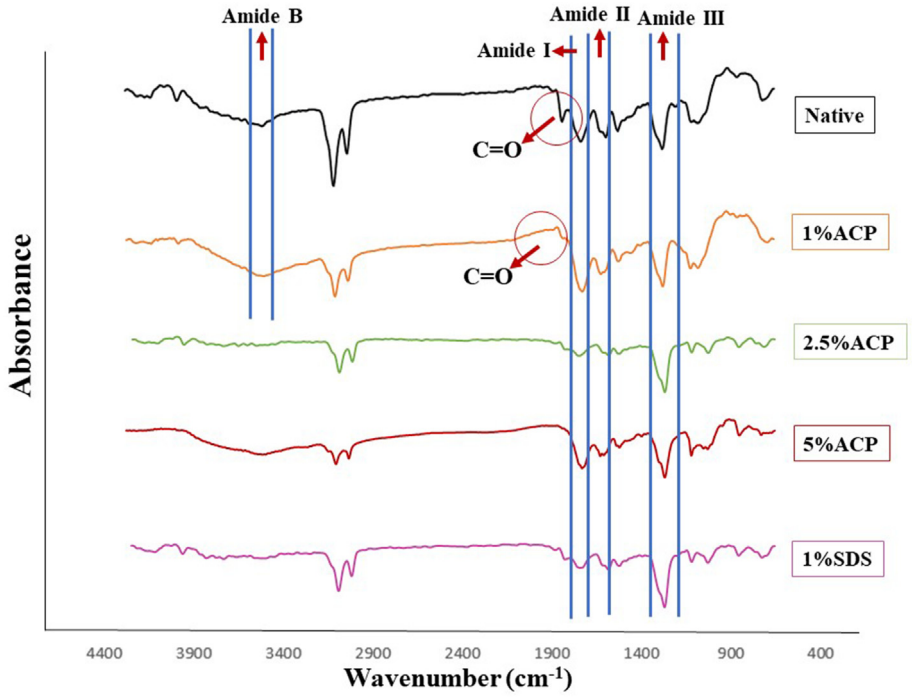

Fig. 5. FTIR spectra of native and decellularized ovarian tissues.

preserved the tissue structure well [6]. In our study, the herbal agent in low concentrations preserved the structure of the tissue, but it was not accepted because it failed in completely removing the cells. Although the chemical detergent has completely removed the cells, it has messed up the structure of the tissue. In the meantime, it seems that 5 % ACP has been successful because in addition to the complete removal of cells, it has preserved the structure of the tissue better than 1 % SDS. Perhaps a combination of lower percentages of chemical detergents-lower percentages of herbal agents can provide suitable results for researchers to use this suggestion.

The MTT test is an efficient tool to evaluate the non-toxicity of engineered tissues and to determine the viability of cells after interacting with decellularized tissues. If the chemical substance or other decellularization agents remain in the tissue, viability decreases [23]. To reduce the toxicity of chemical detergents, a 5 % extract of Sapindus mukorossi was used in a study for the decellularization of goat gall bladder, and it was introduced as a suitable option for decellularization without toxic effects and good biocompatibility [24]. In our study, there was no significant difference between plant and chemical agents in the biocompatibility of decellularization scaffolds. In the 5 % ACP method, cell survival

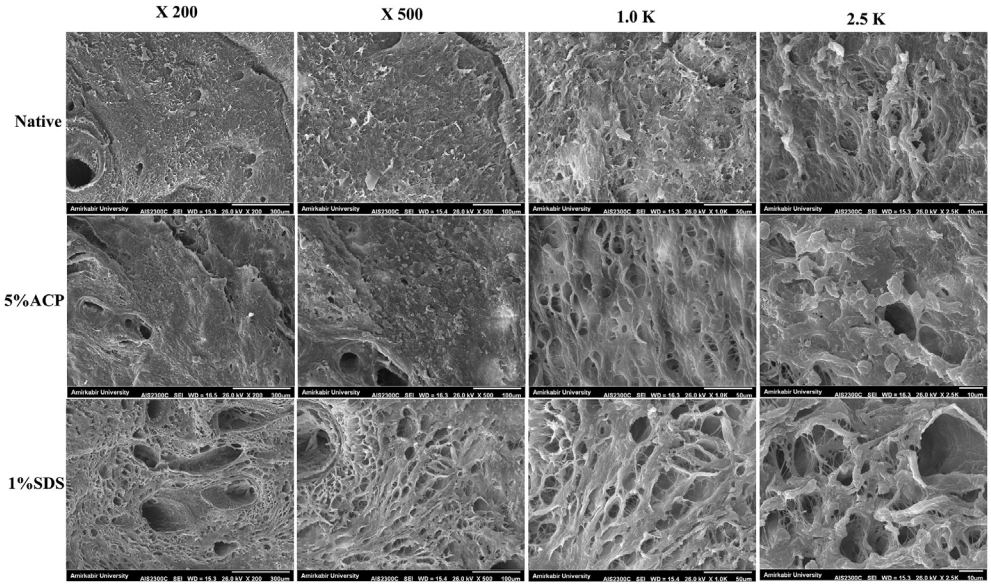

Fig. 6. SEM images, In magnifications of×200, ×500, 1.0K, and 2.5K for native ovarian tissue, decellularization with 5 % ACP and 1 % SDS.

Table 1
The diameter of the inhibition zone of the scaffolds and antibiotics.

Bacteria	*S. aureus* ATCC25923	*S. aureus* resistance	*P. aeruginosa* ATCC27853	*P. aeruginosa* resistance	*E. coli* ATCC25922
The aura diameter of growth inhibition zone M5	25	0	NT	NT	NT
The aura diameter of growth inhibition zone IMI10	NT	NT	15	0	46
The aura diameter of growth inhibition zone O-1	20	19	18	9	19
The aura diameter of growth inhibition zone O-2	23	23	22	12	25

E. coli: Escherichia coli, P. areuginosa: Pseudomonas aeruginisa, S. aureus: Staphylococcus aureus, NT: Not Test, M5: Meticillin 5, IMI: Imipenem 10, O-1: 1%SDS group, O-2: 5 % ACP group.

was higher than in other groups, which may be due to the antioxidant factors present in ACP.

The hemolysis test is one of the important tests in confirming the decellularization scaffold. If the scaffold is to be used in clinical studies, if it undergoes hemolysis, it will cause serious problems in the recipient. If the percentage of hemolysis is reported to be more than 5 %, the scaffold is considered hemolytic [25]. Pig hearts decellularized with chemical detergents had a percentage of hemolysis, which was effective in solving this problem by washing with Triton [26]. In our study, none of the scaffolds reported hemolysis of more than 5 %. The plant agents were all non-hemolytic because they showed the above index of less than 2 %. 1 % SDS showed a number between 2 % and 5 %, which according to ASTM F756 had little hemolytic properties. There is a theory that the detergent on the surface has caused a small percentage of hemolytic, and with the increase in washing time and removal of the detergent, the percentage of hemolysis will decrease.

Observation of Amide I, Amide II, Amide III, and Amide B bands after tissue decellularization shows valuable information about ECM residues and tissue protein structure. The Amide I bond (1600–1700 cm^{-1}) is usually associated with the stretching vibrations of the C=O bond in the peptide backbone of proteins. The presence of this band indicates the preservation of the secondary structure of proteins (such as alpha helix or beta sheet) in the extracellular matrix after decellularization [27,28]. The amide II (1470–1570 cm^{-1}) and amide III (1250–1350 cm^{-1}), bonds are related to the combined vibrations of N–H stretching and C–N bending in proteins [29]. Amide B band (3100–3500 cm^{-1}) is related to N–H stretching vibrations and indicates the presence of amino groups in proteins. The retention of this band indicates that the amino groups present in the extracellular matrix are still present after decellularization [30]. Heart valve decellularization also reported the preservation of amide structures [31]. Decellularization of bovine spinal meninges also showed preservation of proteins and C=O group in ATR-FTIR results, although some of the variation that occurred was attributed to chemical detergent [32]. In our study, the least changes compared to the native tissue were seen in the decellularization group with 1 % ACP.

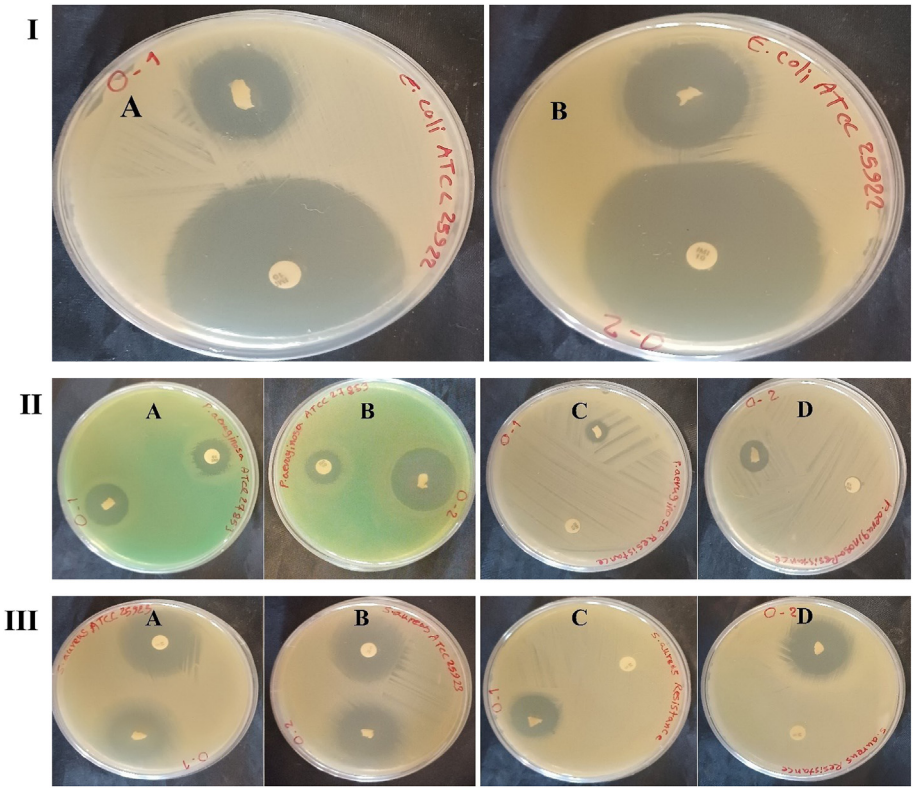

Fig. 7. Antibacterial test, I: Antibacterial assay of scaffolds against the imipenem-resistant (IMI10) *Escherichia coli* strain (ATCC25922) in O-1 (A) and O-2 (B) group scaffold, II: Antibacterial assay of scaffolds against the sensitive (ATCC27853) and imipenem-resistant (IMI10) strains of Pseudomonas aeruginosa in O-1 (A), O-2 (B) group scaffold and in O-1 (C), O-2 (D) group scaffold, III: Antibacterial assay of scaffolds against the sensitive (ATCC25923) and methicillin-resistant (M30) strains of Staphylococcus aureus in O-1 (A), O-2 (B) group scaffold and in O-1 (C), O-2 (D) group scaffold. O-1: 1%SDS group, O-2: 5 % ACP group.

Investigating tissue architecture with an electron microscope can show the exact structure of collagen fibers and follicular position in ovarian tissue. Decellularization of the porcine ovary with the combination of several chemical detergents showed the disintegration of collagen fibers to some extent compared to the native tissue [1]. In the present study, because proper decellularization was possible with two methods of using 5 % ACP and 1 % SDS, these two samples were examined for ultrastructure examination. Both samples were seen somewhat different from the native group, although the disintegration of collagen fibers in 5 % ACP was less than compared to chemical detergent.

Decellularization of the sciatic nerve with a relatively high percentage of chemical detergents showed good antibacterial properties in the obtained scaffolds [33]. The pericardium decellularized with chemical agents has also shown antibacterial properties, but if the same pericardium was loaded with resveratrol as an effective component of herbal agents, its antibacterial properties increased [11]. Also, the decellularized kidney capsule containing vitamin A (15000 U/ml) reported more antibacterial properties than the lower dose (5000 U/ml) of the same vitamin [19]. Therefore, it can be assumed that chemical detergents can have antibacterial properties to some extent. Some factors such as vitamins and plants, due to their antibacterial properties, when they are used in the decellularization process, can be used to reduce infectious agents. In our study, it was also seen that ACP shows more antibacterial properties than SDS.

6. Conclusion

The results of this investigation demonstrate how well 5 % Acanthophyllum (ACP) decellularizes ovarian tissue. The outcomes show that while 1 % SDS and 5 % ACP both efficiently eliminated cellular content, 5 % ACP was better at preserving the ovary's ultrastructure and collagen organization. ACP-treated scaffolds also showed improved blood compatibility, biocompatibility, and antibacterial qualities, which made them a viable substitute for chemical detergents like SDS. For ovarian investigations, ACP might be regarded as a feasible and potentially better herbal agent for decellularization in tissue engineering due to its capacity to decrease cytotoxicity and preserve protein integrity. By providing a safe and efficient process for ovarian scaffold creation, this work advances bioengineering techniques and opens new avenues for future research and possible clinical uses.

Disclosure statement

No potential conflict of interest was reported by the authors.

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Declaration of competing interest

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

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