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# Exploring cutting edge strategies for the rehabilitation of desiccated histopathological artifacts - A cross sectional observational study

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**Abstract :**

**Background**: Histopathological tissue samples serve as invaluable resources for understanding the intricacies of disease processes and pathological conditions. However, the preservation of these specimens can be challenging, often leading to desiccation and irreversible damage. In this study, we explore various innovative techniques for the recovery and rehydration of intentionally dried histopathological tissue samples and provide recommendations for optimal preservation and recovery protocols for dried tissue samples in research and clinical settings. **Materials and methods:** The study was conducted in a tertiary health care hospital, Chennai, India. A cross-sectional observational study was conducted over a period of one year [March 2023 - March2024]. The tissue samples from various organs were intentionally dried. A standard rehydration protocol was derived based on concentration of solution and time of immersion and the tissue samples were pretreated with four different solutions before treatment with 10% neutral buffered formalin. The pretreated tissue samples were then evaluated for various parameters like color, texture and tissue adherence by naked eye examination. The histological characteristics like tissue morphology, staining quality and presence of artefacts were analyzed by microscopic examination and these features was compared with tissue samples directly treated with 10% neutral buffered formalin.

**Results**: A total of 250 tissue samples subjected to our rehydration protocol were evaluated. Fabric softener demonstrated superior preservation of cellular details and consistent staining quality compared to other rehydration solutions.

**Conclusion** : We found that Fabric softener emerges as a promising alternative to formalin, particularly for maintaining fat tissue integrity and cellular morphology of tissues. These findings underscore the importance of considering rehydration techniques in tissue processing protocols and highlight the potential of fabric softener as a safe and effective alternative in histopathological practice.

Key words: Desiccated tissue, dried tissue, rehydration protocol, fabric softener

## INTRODUCTION:

The histopathological specimens obtained from biopsies, surgical resections, and autopsies provide a snapshot of tissue architecture, cellular morphology, and molecular alterations associated with pathological conditions. [1,2] Despite meticulous preservation efforts, samples are susceptible to desiccation, leading to structural distortion and molecular degradation. Desiccation of tissue samples occurs due to improper storage, prolonged fixation times, or intentional drying for specific research purposes. [3,4] Rehydration techniques for tissues are commonly employed in the restoration of mummified specimens to retrieve the cellular morphology and tissue integrity. [5,6] Recovery and rehydration of dried histopathological tissue samples are critical steps in tissue analysis, essential for preserving tissue integrity and enabling accurate diagnostic and research findings. [7,8,9,10]However, the choice of rehydration method can significantly impact the quality and reliability of the subsequent analysis. In response to the pressing need for efficient tissue rehydration methodologies, this study aims to investigate and evaluate various techniques and provide optimal strategies for the recovery of intentionally dried histopathological tissue samples.

## Materials and Methods:

The study was approved by institutional scientific review board (Approval number 140/04/2024).The study was conducted for a period of one year( March 2023- March 2024) in the department of pathology of a tertiary care hospital.Sample preparation: We obtained a total of 250 tissue samples from already reported histopathological specimens, which were preserved in formalin containers. The tissue samples were retrieved from various organs and intentionally dried for about 48 hours and the tissue samples from each of the specimens were divided into five groups, each containing 50 samples and were subjected to our standard rehydration protocol.

Tissue samples taken from various specimens, immersed in formalin were included in the study, Bone specimens and organs with hard consistency were excluded. Based on method of rehydration dofferent groups were divided as below.

Group I: The first 50 samples were used as controls, which were directly fixed in a 10% neutral buffered formalin solution.

Group II: The next 50 samples were pretreated with a Formol glycerol solution containing a stock solution of 10 ml formaldehyde, 2 gm sodium acetate, and 90 ml tap water. The rehydrating solution is initiated in a ratio of 10 ml of glycerol solution to 90 ml of formol - sodium acetate solution.

Group III: The third 50 samples were pretreated with Sandinsons solution consisting of 20 ml of 5% sodium carbonate, 30 ml of 96% ethanol, and 50 ml of 1% formalin.

Group IV:The next 50 samples were pretreated with a commercial fabric softener, "Comfort," marketed by “Unilever”( HSN code 34029099), containing benzisothiazolinone,mlimonene,cationic surfactants,Hexyl cinnamal,butylphenyl methylpropional, citronellol, and unsaturated fatty acids.

Group V:The last 50 samples were pretreated with a commercial body moisturizer, ’Nivea’ moisturizing lotion marketed by “Unilever (HSN code 33049110), containing aqua,paraffinumliquidum,glycerin,lanolin alcohol,paraffin wax,pathenol,decyl oleate,citric acid, and citronellol.

The group I samples were used as controls. The group II to V samples, after pretreatment for 72 hours in different solutions, were fixed in 10% neutral buffered formalin for 24 hours. Then tissue samples were assessed macroscopically for color and texture and routinely processed by dehydrating in increasing concentrations of ethanol, clarified in xylene, and embedded in paraffin wax. Sections of 4-5µm thick slides were taken from all the groups using a microtome and stained with hematoxylin eosin technique. The special stain, Verhoeff -Van Gieson, was employed using standard staining protocol in one of the tissue sections to demonstrate the staining quality of the solutions over elastic fibers.

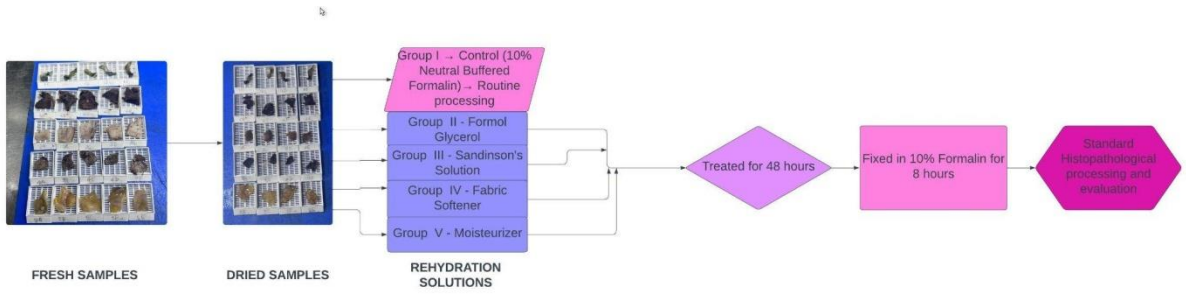


**Figure1 :Flowchart to demonstrate the rehydration protocol used in tissue samples for this study**

The various histological parameters were analyzed and blindly evaluated by two trained observers independently, and any discrepancies were resolved through consensus or by a third party reviewer. The dried tissues after treatment with different rehydration solutions were assessed grossly for color, texture. The histological parameters, such as cellular morphology, tissue artefacts and staining intensity, were analyzed.

**RESULTS:**

**Macroscopic assessment of tissue samples treated with various rehydration solutions:**

In our study involving 250 tissue samples from diverse organs, we conducted macroscopic assessments to evaluate tissue texture and adherence to slides. Upon treatment with groups II,III,IV solutions and in the subsequent formalin fixation phase, we observed a notably soft to firm texture, while pretreatment with group V resulted in a hard texture to the

tissue. Furthermore, during sectioning and staining after pretreatment with these solutions, we noted a significant detachment of tissue over the slide in samples treated with group V, moderate detachment in those treated with group II and III solutions, and minimal detachment in samples treated with group IV.

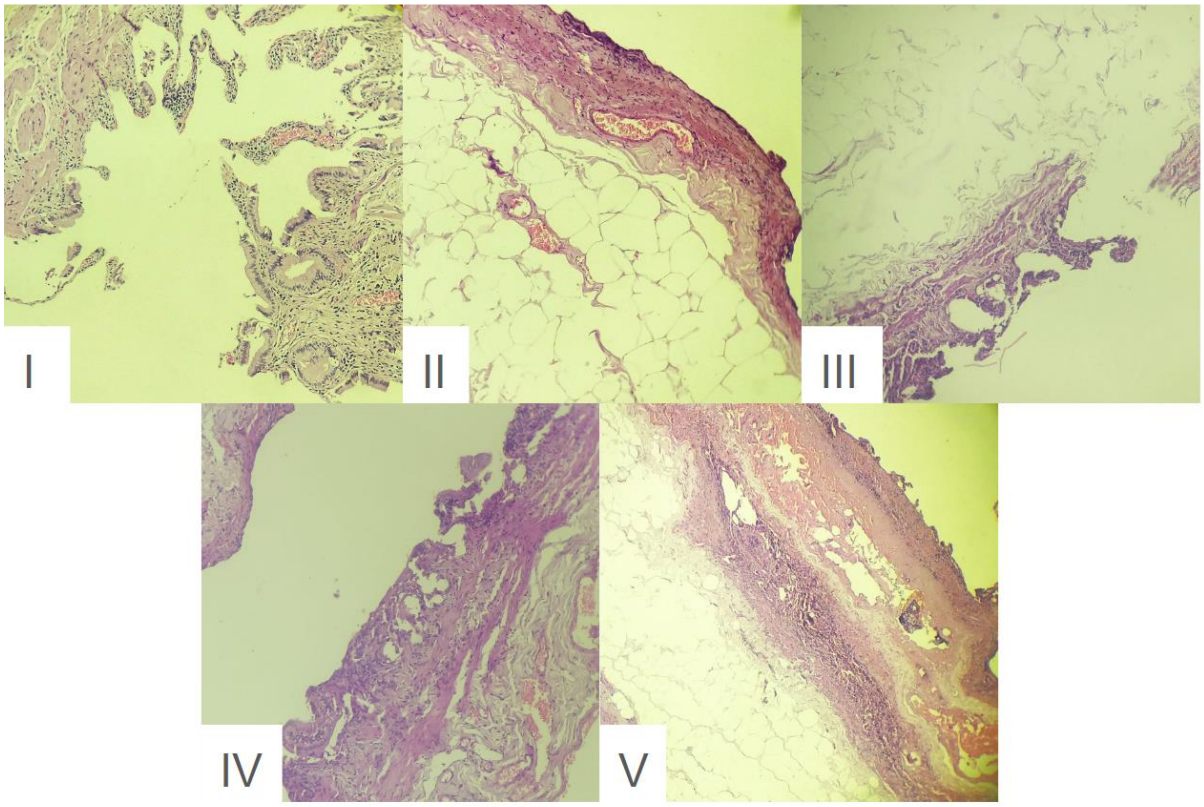


**Figure 1:** I - Group I (Control) showing normal, well preserved gall bladder mucosa. II and V - Groups II and V respectively, showing moderately preserved cell morphology. III and IV - Groups III and IV respectively, showing well preserved cell morphology

**Microscopic assessment of tissue samples treated with various rehydration solutions:**

In our study, we conducted microscopic assessments of tissue samples treated with various rehydration solutions, focusing on cellular morphology, the presence of artefacts and staining quality. We observed well-preserved, clear morphology and intact cell boundaries in samples treated with solutions III and IV, while those in groups II and V exhibited moderately preserved features (Figure 1). In terms of staining quality we observed similar results, with tissue sections from group II and V showing variable and slightly reduced hues, While groups II and IV showed uniform and consistent staining (Figure 1).

Samples treated with solution V displayed noticeable loss of epithelial integrity and tissue distortion. There is severe formation of artefacts in group V, characterized by numerous tissue folds, and also in groups II and III while minimal artefacts were observed in group IV (Figure 2).

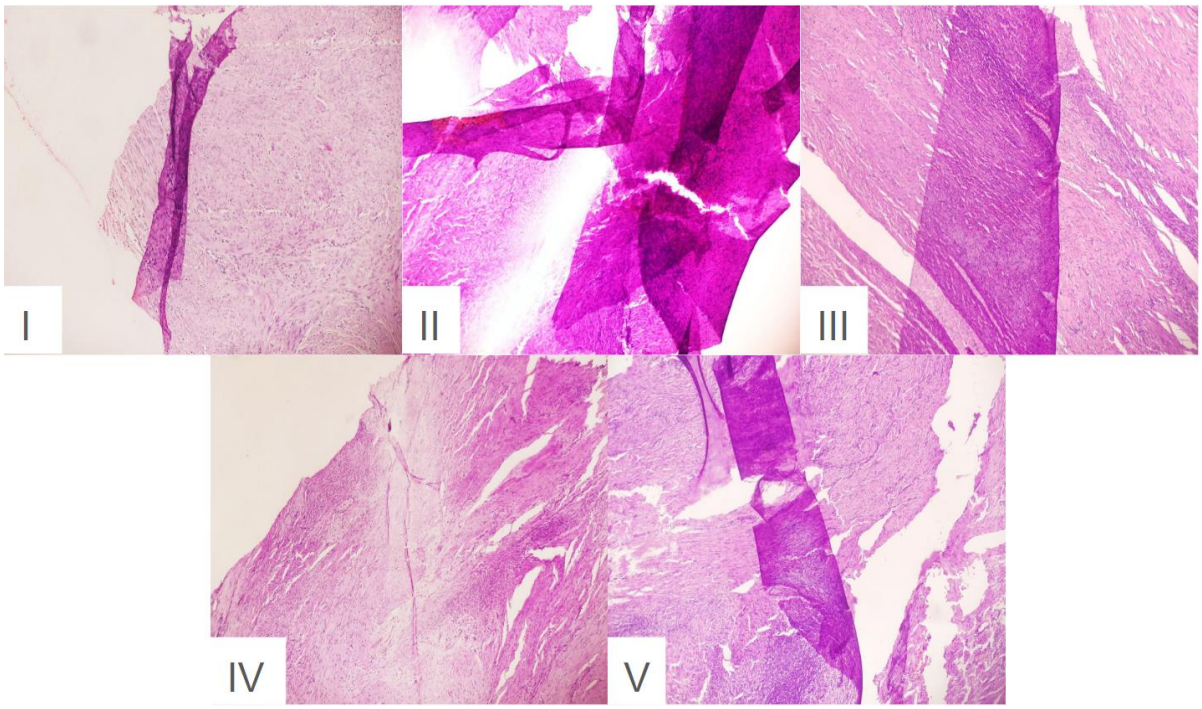


**Figure 2 :** I - Group I (Control) showing folding artefact. II, III and V - Groups II, III and V respectively, showing severe artefactual folding. IV - Group IV, showing minimal tissue folding artefact.

Another significant observation we made pertained to fat tissue is well preserved morphology with sharp nuclear details only in samples treated with group IV, whereas there is poor morphology and loss of tissue in samples treated with solutions II, III, and V (Figure 3).

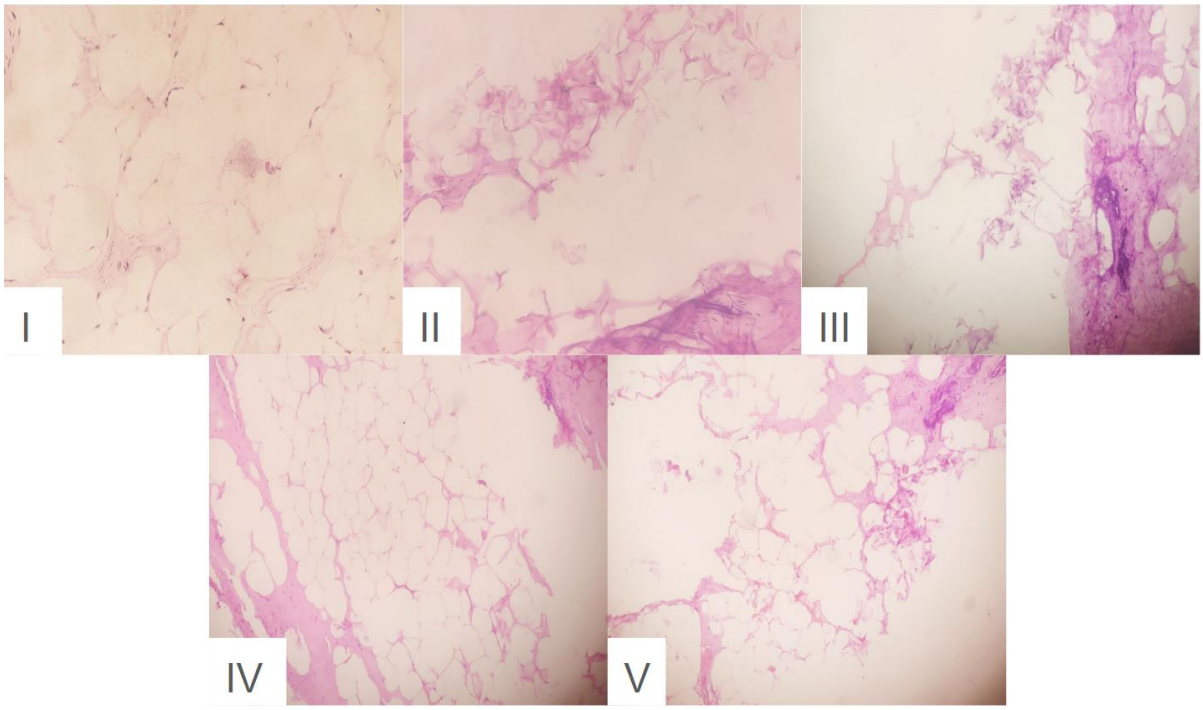


**Figure 3 :** I - Group I (Control) showing well preserved fat morphology. II, III and V - Groups II, III and V showing poor morphology and loss of tissue. IV - Group IV showing well-preserved fat tissue morphology.

We also noted that in tissue samples taken from placental specimens, there is an enhanced staining hue in areas of calcification treated with groups III and IV. The staining intensity in calcified areas is weak and faint in samples treated with solutions II and V (Figure 4).

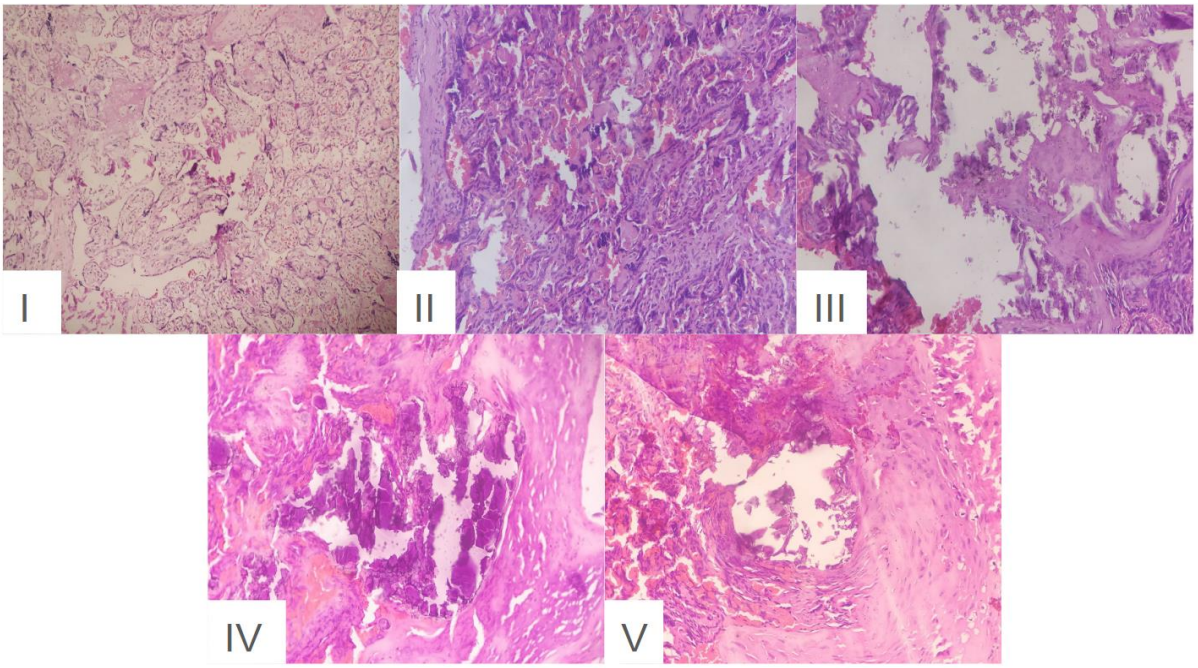


**Figure 4:**I - Group I (Control) showing normal calcification in placental tissue. II and V - Groups II and V respectively, showing weak and faint staining intensity in the calcified areas. III and IV - Groups III and IV respectively showing enhanced staining hue in the areas of calcification.

The special stain, Verhoeff -Van Gieson was incorporated in this study to demonstrate the elastic fibers of the blood vessel. The morphology, fiber density, and distribution were well retained with better staining quality in tissue samples treated with group IV (Figure V).

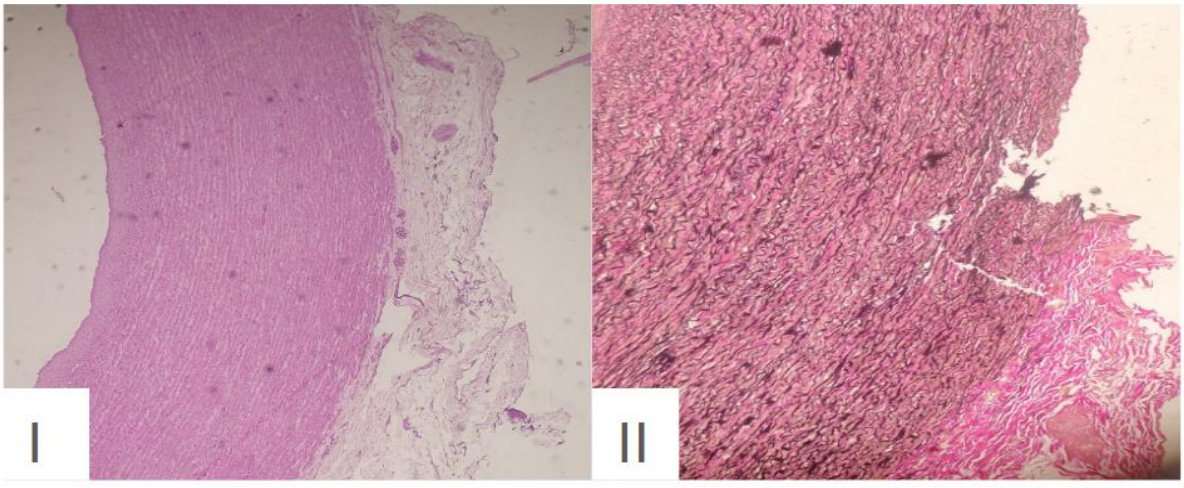


**Figure 5 :** I - Hematoxylin and Eosin stain of vessel wall section treated in Group IV. II - Verhoeff - Van Gieson stain demonstrating the well preserved elastic fibres in sections treated in Group IV.

**Discussion**:

Dessication of tissue samples can occur due to various factors, often stemming from technician or pathologist errors. [11, 12, 13] The common causes are improper sealing of the container, inadequate fixation, delays in processing, and any miscommunication between staff regarding handling procedures. [14,15,16] Using a rehydration protocol for such dried out tissue samples helps in restoring the structural integrity of the tissue and facilitates more accurate pathological assessment. [17,18,19] Our aim was to evaluate which rehydration solution performed best and would be a better alternative to the formalin solution. From the macroscopic evaluation, we found that all the tissue samples pretreated with moisturizer had a hard texture, while the rest had a soft to firm texture. The hardness in tissue samples treated with moisturizer might be due to poor penetration into the tissues or inappropriate concentration.

Microscopically, the samples pretreated with four different rehydration solutions showed an overall improvement in tissue structure and stainability compared to those samples directly treated with 10% neutral buffered formalin. The tissue morphology and cellular details were better in samples treated with Sandinsons solution and fabric softener, while formol glycerol and moisturizer resulted in noticeable tissue distortion. Sandinsons solution is a widely used rehydrating solution in mummified tissues during postmortem analysis and is favored for its ability to produce well-fixed specimens with preserved cellular morphology and tissue architecture. [19,20]. The calcified tissue areas were well enhanced in samples pretreated with Sandinsons solution and fabric softener. Sandinsons solution aids in stabilizing tissue structures through formaldehyde fixation. Fabric softener contributes to tissue hydration and softening, both of which are beneficial for enhancing areas of calcification. The superior preservation of fat tissue was observed in samples treated with fabric softener, which might be attributed to its several chemical properties. Fabric softeners typically contain cationic surfactants that interact with the lipid rich components of fat tissue; furthermore, the presence of quaternary ammonium compounds can easily penetrate and soften tissue structures, thereby maintaining the integrity of the lipid membrane and preventing lipid coalescence and crystallization. [21]We also found that fabric softener exhibited consistent staining quality and minimal artefact formation compared to other solutions,indicating its compatibility with downstream histological analyses.Additionally, our findings highlight the utility of special stains in complementing the evaluation of rehydration techniques, providing valuable insights into tissue architecture and composition.

**Conclusion:**

Our study revealed that the adoption of fabric softener based rehydration protocols holds promise as a rehydration solution for improved preservation of specific tissue

components, such as fat tissue, and reducing reliance on formalin based fixatives, thus enhancing workplace safety and environmental sustainability for pathologists and technicians in their day-to-day practice. Furthermore, this study serves as a valuable resource for postgraduate students, offering insights into innovative techniques and methodologies in histopathology research.

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