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Experimental Insights into Rehydration and Reprocessing of Biopsy Samples: Remedial Tissue Processing

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Abstract

Background: The preservation of tissue morphology relies on proper processing techniques in histopathology. The tissue quality suffers when processing errors result from inadequate dehydration or inadequate infiltration of solutions. This study investigates how processing mistakes affect tissue quality and tests a reprocessing method for recovering proper histological characteristics.

Materials and Methods: The research used eighteen rats assigned to three distinct groups namely Group C (control) and Group TX (processing error) and Group T (retreatment). The heart and kidney tissues received fixation with 10% formalin solution. The standard procedures guided Group C but Group TX received drying errors and Group T required rehydration and reprocessing. The cellular details including cytoplasm appearance, nuclear structure and staining quality were assessed using tissue score system.

Results: The tissue quality of Group C reached optimal levels (4–5 scores) yet Group TX displayed poor features (2–3 scores). The tissues treated in Group T demonstrated substantial enhancement (mean scores: 3–4) which brought them near the quality level of the control group.

Conclusion: Processing errors damage tissue quality but developers increase the need for protocols development to recover tissue structure effectively and enhance the diagnostic quality together with tissue maintenance.

Keywords: Tissue Processing; Histology; Retreatment; Paraffin Embedding; Quality Control; Formalin Fixation

1. Introduction

Histological examination requires proper tissue processing which consists of a sequence of systematic steps that transform fresh or fixed tissues into microscopic-ready thin stained sections (Gurina and Simms, 2020). The entire processing protocol requires exact execution during fixation as well as final paraffin wax embedding to preserve cellular structure and tissue integrity (Fischer et al., 2008a). Diagnostic utility of tissue together with its compatibility for additional tests such as immunohistochemistry and molecular diagnostics may become compromised by errors during any point of the sample processing steps (Grizzle et al., 2011, Bancroft and Gamble, 2008). While tissue processing method starts with fixation as an essential first step that stops autolysis and putrefaction through stabilization of protein structures and tissue cells (Ganjali et al., 2013). The preferred solution for tissue preservation and penetration is 10% neutral buffered formalin. However, quality of diagnosis suffers from artifacts that include nuclear distortion and poor staining when fixatives are used incorrectly (Fischer et al., 2008b). The tissue dehydration is crucial step in tissue processing that requires graded alcohols while agents such as xylene help clear the tissue before infiltration with molten

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paraffin. The proper duration of each reagent transition is essential because extended exposure or improper transitioning may harm cell structures which leads to tissue stiffness and vacuoles formation while causing incomplete infiltration (Suvarna et al., 2019).

The prevalence of technical processing errors increases within laboratories that process high numbers of samples and when attempting to work with small or fatty tissue biopsies. Research shows that standard processing errors stem from tissue drying that goes beyond appropriate levels and inadequate dehydration, high xylene exposure and improper paraffin embedment temperature control (Titford, 2009, Dholabhai et al., 2024). The problems result in substantial modifications of tissue structure which impedes sectioning processes and lowers staining effectiveness. Tissue specimen processing requires additional protocols when pathologists and histotechnologists determine them to be poorly managed. The remedial procedures start with tissue rehydration followed by re-dehydration and then clearing and reinfiltration steps. The use of formolglycerol as a softening agent prior to reprocessing has been reported to improve section quality and restore staining characteristics (Chong et al., 2012, Mubarak and Pathology, 2023). While retreatment cannot entirely eliminate all artifacts, it often yields structurally interpretable sections that are suitable for diagnostic or research use (Taqi et al., 2018).

Evaluating the efficacy of retreatment protocols requires systematic comparison of tissue integrity across different groups. Quantitative scoring methods assessing cellular details, cytoplasmic clarity, nuclear morphology, and staining intensity offer a reproducible framework for such evaluations (Klatt, 2014). In this context, this study investigates the effectiveness of a structured tissue retreatment protocol in inadequately processed tissue biopsies.

This study hypothesized that properly retreatment procedures can significantly restore tissue morphology and staining properties to a level comparable with adequately processed controls. Furthermore, the application of standardized retreatment protocols may thus offer an essential quality control measure in diagnostic histopathology.

2. Materials and Methods

Table 1 Tissue processing for the different group types

Group	**C-group**		**TX-group**		**T-group**	
Steps	**Tissue processing steps**	**Time (hour)**	**Tissue processing steps**	**Time (hour)**	**Tissue processing steps**	**Time (hour)**
1	10 % formalin	1	10 % formalin	1	10 % formalin	1
2	40% Alcohol	1	40% Alcohol	1	40% Alcohol	1
3	70% Alcohol	1	70% Alcohol	1	70% Alcohol	1
4	90% Alcohol	2	90% Alcohol	2	90% Alcohol	2
5	Absolute Alcohol (I)	1	Absolute Alcohol (I)	1	Absolute Alcohol (I)	1
6	Absolute Alcohol (II)	1	Absolute Alcohol (II)	1	Absolute Alcohol (II)	1
7	Absolute Alcohol (III)	2	Absolute Alcohol (III)	2	Absolute Alcohol (III)	2
8	Xylene (I)	1	Empty station (dry out station)	10	Empty station (dry out station)	10
9	Xylene (II)	2	Xylene (II)	2	Recovery in 10% formolgylecerol	10
10	Xylene (III)	2	Xylene (III)	2	Reprocess from step 2 to 12	
11	Melted paraffin (65 ^{o}C)	2	Melted paraffin (65 ^{o}C)	2		
12	Melted paraffin (65 ^{o}C)	2	Melted paraffin (65 ^{o}C)	2		

A total of 18 male and female rats (250 – 300 grams) were used in this study. All animals were housed and maintained on normal diet. Animals were divided into 3 groups (n = 6/group). At the day of experiment, all animals were deeply anesthetized using chloroform then decapitalized. After that, heart and kidney tissues were collected in freshly prepared 10% formalin and kept for overnight. Tissues from the first group (C-group) were processed following the normal tissue processing protocol in our lab. In the second group (TX-group), the tissues dried out during tissue processing (i.e. tissue processing troubleshoot). The third group (T-group), treatment of dried out tissues during tissue processing. After collecting the tissues, tissue processing completed as in **Table 1**. All slides were evaluated using blind evaluation system by Professor in histopathology techniques.

3. Results and discussion

The control group (Group C) (**Table** 2 and **Figure 1**) exhibited excellent preservation of tissue architecture, as evidenced by uniformly high scores across all histological parameters evaluated. Kidney samples had a mean score of 4 in cellular details, cytoplasm, and nuclear morphology, with an outstanding score of 5 for staining. Heart tissues scored slightly lower in cellular detail (mean: 3.5) but maintained high scores in cytoplasmic clarity and nuclear morphology (4 each) and strong staining quality (4.5).

Table 2 Score of structural features of group C

Group C					
		Score of Structural Feature changes			
Tissue type	**Sample No.**	**Cellular details**	**Cytoplasm**	**Nuclear Morphology**	**Staining**
Kidney	18	4	4	4	5
	14	4	4	4	5
	9	4	4	4	4
	2	3	3	4	5
	1	4	4	4	5
	20	3	4	4	4
Mean		4	4	4	5
Heart	18	4	4	4	4
	14	4	4	4	5
	9	4	4	4	4
	8	3	4	4	5
	2	3	3	4	4
	1	3	3	4	5
Mean		3.5	4	4	4.5
P-value (ANOVA)		0.5994	0.5490	Anova can’t be computed due to zero variance	0.5994

1= Poor; Tissue details destructed, presence of vacuoles, nuclear shape change, no cytoplasm, staining is pale; 2= Not Bad; diffuse tissue destruction, vacuoles, nuclear change and staining variation; 3= Good; Focal structural destruction, most of nuclei are good, no staining variation; 4= Very Good; There is few artefacts due to tissue fold, good staining; 5= Outstanding; All details are good

These findings affirm that the standard tissue processing protocol employed in Group C maintained morphological integrity with minimal processing artifacts. Such outcomes reflect adherence to established best practices in formalin fixation, alcohol dehydration, and paraffin infiltration, which are critical to achieving high-contrast HandE staining and nuclear-cytoplasmic differentiation (Bancroft and Gamble, 2008, Kondo et al., 2021). Differences between kidney and heart samples in cellular detail scores may be attributed to tissue-specific variables such as vascularization and matrix density, which influence reagent penetration and fixation efficiency (Mishra et al., 2021). Proper tissue orientation and sufficient exposure to graded dehydrants and clearing agents are also crucial for optimal morphology retention, especially in dense muscular tissues like the heart. Recent studies have emphasized the importance of real-time quality assurance steps, including visual assessment of tissue firmness and coloration after fixation, as predictors of eventual section quality (Boyle and Ganesan, 2021). The staining scores of 4.5–5 across both tissues support that optimal fixation and consistent reagent timing were achieved, minimizing under- or over-processing effects. Thus, the Group C profile serves as a gold-standard comparator for assessing retreatment outcomes in compromised samples. The figures taken of tissues are in **Figure 2**.

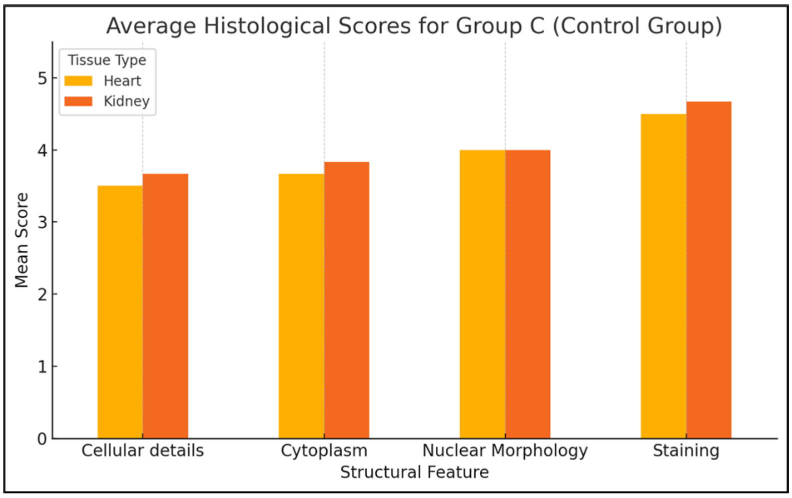

Figure 1 Comparison of mean histological scores in kidney and heart tissues of Group C (Control Group)

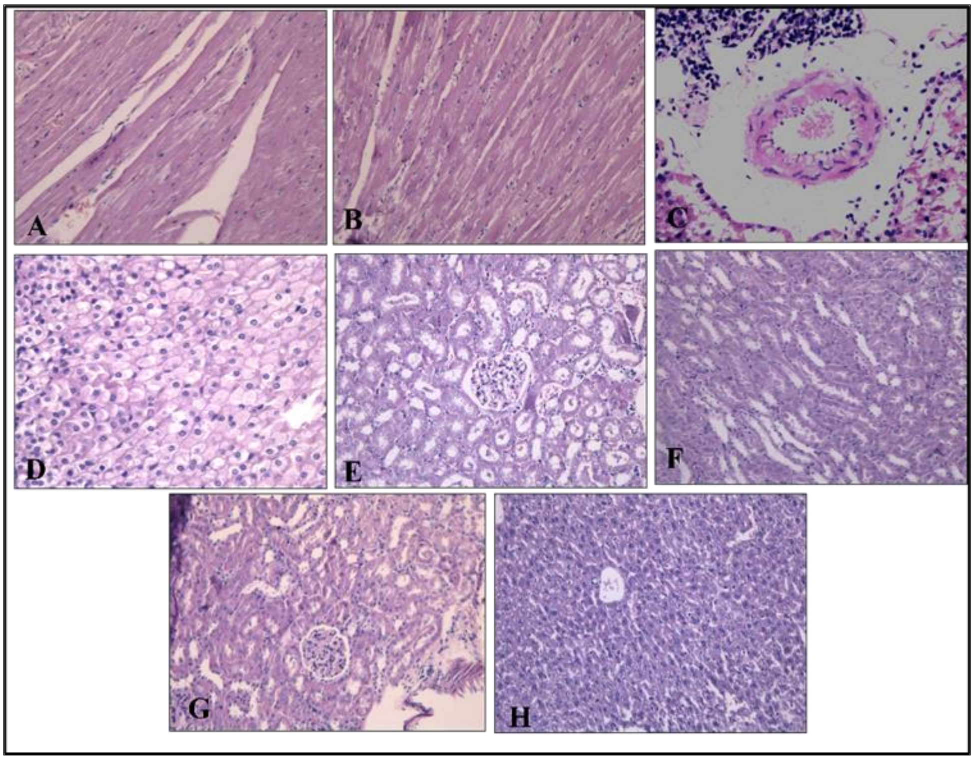

Figure 2 Tissues of control group (A) Heart 14; (B) Heart 18; (C) Kidney blood vessel; (D) Kidney epithelial cells; (E) Kidney cortex; (F) Kidney medulla; (G) Kidney 20 ;(H) Liver

Table 3 (**Figure 3**) presents the histological quality scores of kidney and heart tissues in Group TX, which underwent suboptimal tissue processing. In this group, kidney samples exhibited average scores of 2.5 for cellular detail, 2 for cytoplasm, 3 for nuclear morphology, and 3 for staining, indicating mild to moderate tissue distortion. Heart tissues demonstrated even greater compromise, with mean scores of 2 for cellular details, cytoplasm, and staining, and 3 for nuclear morphology. Processing errors involving inadequate dehydration along with insufficient clearing and improper paraffin infiltration result in these values which lead to cytoplasmic disruption and poor nuclear visibility and staining inconsistency. The study's results match previous research which shows that pre-analytical aspects strongly affect tissue section quality and staining results (Chlipala et al., 2021). The improper execution of dehydration and clearing steps during histology procedures leads to the creation of three main artifacts: vacuolization alongside poor contrast and tissue fragmentation (Rao et al., 2016). The combination of tissue-specific factors like density and vascularization adds more challenges to poor processing outcomes because dense tissues such as the heart need exact clearing and dehydration requirements (Maldonado and Rapini, 2024). Rapid tissue processing innovations, such as microwave-assisted fixation, have been suggested as potential alternatives to minimize such artifacts, especially in time-sensitive diagnostic settings (Mishra et al., 2021). The TX group demonstrates how tissue processing mistakes create harmful outcomes which proves that histology laboratories need strict quality control systems. The research data presented in **Figure 4** depicts the examined tissues.

Table 3 Score of structural features of group TX

Group TX					
		Score of Structural Feature changes			
Tissue type	**Sample No.**	**Cellular details**	**Cytoplasm**	**Nuclear Morphology**	**Staining**
Kidney	15	3	3	3	3
	13	2	2	3	3
	12	2	1	3	2
	11	3	2	3	4
	10	3	3	3	2
	5	2	2	3	3
Mean		2.5	2	3	3
Heart	15	2	2	3	2
	13	2	2	2	2
	12	2	3	3	2
	11	2	2	3	2
	10	3	2	3	2
	7	2	2	3	2
Mean		2	2	3	2
P-value (ANOVA)		0.2595	1.0	0.3408	0.0218

1= Poor; Tissue details destructed, presence of vacuoles, nuclear shape change, no cytoplasm, staining is pale
2= Not Bad; diffuse tissue destruction, vacuoles, nuclear change and staining variation
3= Good; Focal structural destruction, most of nuclei are good, no staining variation
4= Very Good; There is few artefacts due to tissue fold, good staining
5= Outstanding; All details are good

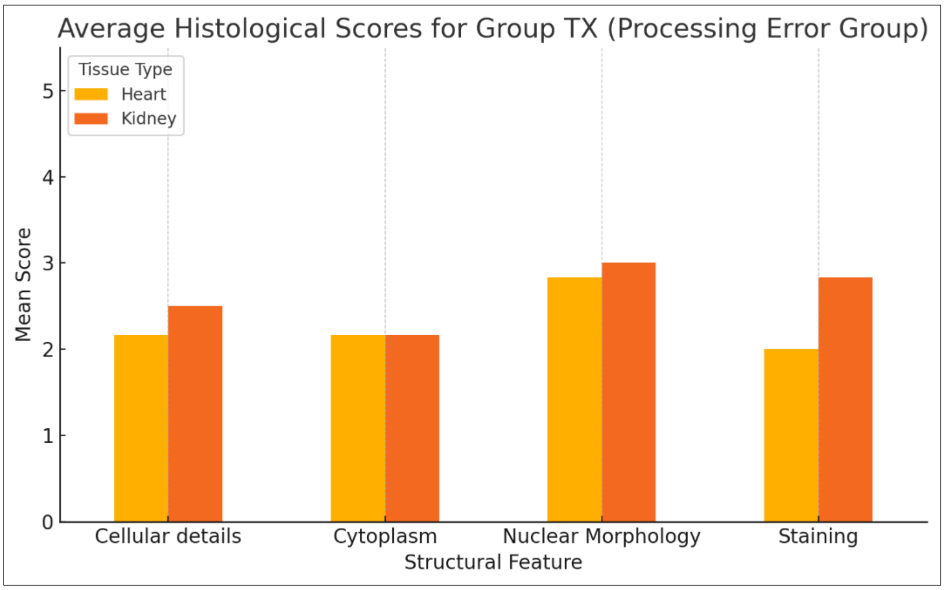

Figure 3 Mean histological scores in kidney and heart tissues of Group TX (Processing Error Group)

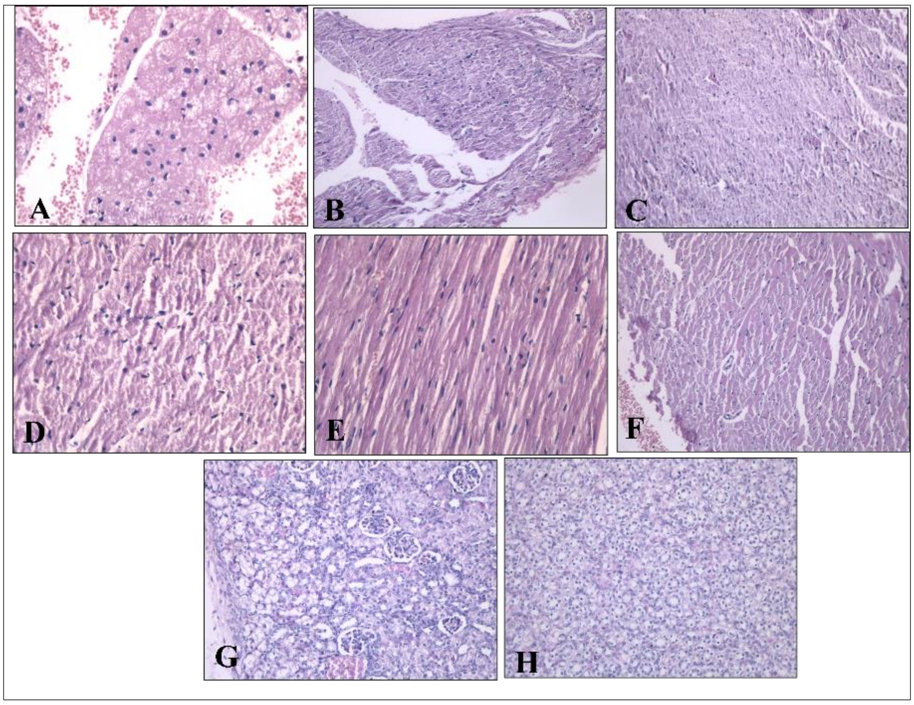

Figure 4 Tissues of TX group (A) Heart vacuoles; (B) Heart basophilic and destruction; (C) Heart myofibril; (D) Heart destructdemy fibril; (E) Heart mycard; (F) Heart myofibril; (G) Kidney subcapsular vacuoles; (H) Kidney vacuolization

The histological quality scoring of kidney and heart tissues in Group T is shown in **Table 4 (Figure 5)** following their retreatment after processing errors. The kidney tissue specimens obtained mean scores of 3 for cellular details along with 4 for cytoplasm and 4 for nuclear morphology and staining. Heart tissues obtained similar quality ratings to kidney samples where cellular details scored 3 and cytoplasm received 4 points along with nuclear morphology and staining quality achieving both 4 points. The tissue retreatment method demonstrated success in restoring both tissue structural integrity and staining quality so they matched performance levels of optimal samples. Modern studies demonstrate that standardized tissue processing protocols will result in such observed improvements. The preservation of tissue morphology depends heavily on three essential procedures which also result in consistent staining outcomes. Broken procedures during tissue processing can generate diagnostic-impairing artifacts which affect diagnostic precision (Da Silva et al., 2023). Quality assurance strategies with regular monitoring and processing step validation help reduce the mentioned issues while improving histopathological evaluation reliability (Boktor et al., 2024). Additionally, advancements in tissue processing technologies, including automated systems and real-time monitoring tools, have shown promise in reducing artifacts and improving overall tissue quality (Tweel et al., 2024). The successful treatment in Group T indicates that these methods offer great promise to fix processing mistakes and recover tissue specimens for precise diagnostic evaluations. The images of tissues appear in **Figure 6**.

Table 4 Score of structural features of group T

Group T					
		Score of Structural Feature changes			
Tissue type	**Sample No.**	**Cellular details**	**Cytoplasm**	**Nuclear Morphology**	**Staining**
Kidney	4	2	2	2	4
	6	3	4	4	4
	16	3	3	4	4
	19	3	3	4	4
	17	2	2	2	4
	21	3	3	4	4
Mean		3	4	4	4
Heart	4	4	4	4	3
	6	4	4	4	4
	16	3	4	4	4
	19	3	3	4	4
	17	3	4	3	4
	21	3	3	4	5
Mean		3	4	4	4
P-value (ANOVA)		0.04933	0.04933	0.29593	1.0

1= Poor; Tissue details destructed, presence of vacuoles, nuclear shape change, no cytoplasm, staining is pale
2= Not Bad; diffuse tissue destruction, vacuoles, nuclear change and staining variation
3= Good; Focal structural destruction, most of nuclei are good, no staining variation
4= Very Good; There is few artefacts due to tissue fold, good staining
5= Outstanding; All details are good

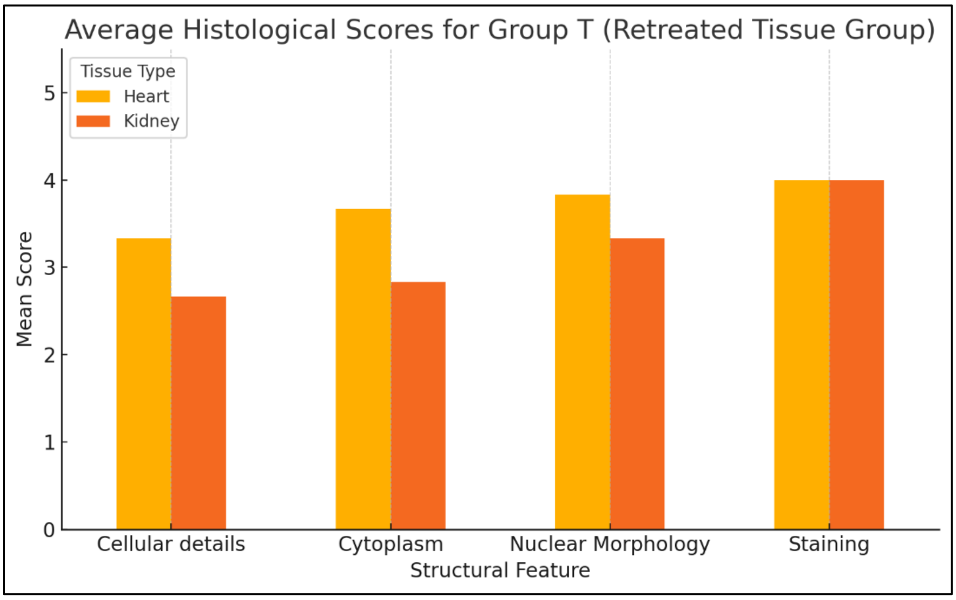

Figure 5 Mean histological scores in kidney and heart tissues of Group T (Retreated Tissue Group)

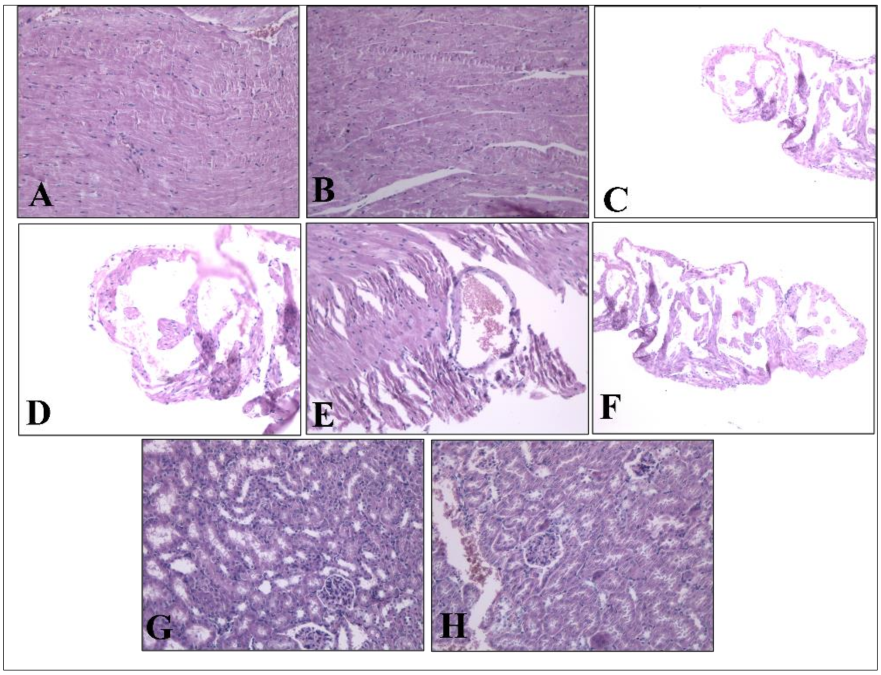

Figure 6 Tissues of T group (A) Heart T; (B) Heart T scatter; (C) Heart tif; (D) Heart 20; (E) Sweet hearts; (F) T heart; (G) 21 kidney T; (H) 21 kidney T scatt

4. Conclusion

This study directly evaluated the impact of tissue processing errors on histological quality and the effectiveness of a standardized remedial protocol in restoring diagnostic integrity. The comparative assessment across three groups—control, processing error, and retreatment demonstrated that histological features such as cellular morphology and staining clarity are significantly compromised by suboptimal processing. However, rehydration and reprocessing methods markedly improved structural preservation and staining outcomes. These findings establish the practical relevance of integrating retreatment protocols into histopathology workflows, particularly in cases where tissue quality is impaired. The approach enables recovery of diagnostic value in compromised samples, minimizing material loss and reducing the need for repeat biopsies. Further research into automated and precision-guided retreatment strategies may enhance reproducibility and efficiency in diagnostic and research-based histology.

Compliance with ethical standards

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Disclosure of conflict of interest

There is no conflict of interest.

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