



EXPLORING THE POTENTIAL OF MORINGA OLEIFERA EXTRACT AS A NATURAL TISSUE FIXATIVE FOR HISTOPATHOLOGICAL APPLICATIONS

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ABSTRACT

Histopathology, crucial for disease diagnosis, heavily relies on effective tissue fixation to maintain cellular architecture. However, the carcinogenic nature of formalin has spurred interest in safer alternatives. Moringa oleifera, renowned for its medicinal properties, presents a promising natural fixative candidate. This study aimed to assess Moringa oleifera extract's viability as a fixative for histopathological applications, specifically focusing on its compatibility with subsequent histological analyses. Distilled water and normal saline solutions of Moringa extract were prepared at varying concentrations. Kidney tissue samples from euthanized rats were treated with these solutions alongside formalin. Tissue sections underwent manual processing, embedding, sectioning, and staining using Hematoxylin and Eosin (H&E) technique. Microscopic examination revealed varying degrees of tissue preservation across Moringa concentrations and solvents. Higher concentrations (15%) in distilled water exhibited optimal preservation, akin to formalin-fixed tissues, while lower concentrations and normal saline solutions showed suboptimal fixation. These findings highlight Moringa oleifera's potential as a tissue fixative, particularly at higher concentrations in distilled water. Further research is recommended to isolate active components and explore alternative Moringa extracts for enhanced tissue preservation. Comparative studies would elucidate the efficacy of Moringa-derived fixatives and inform their utilization in histopathological practice.

Keywords: Moringa oleifera extract, Tissue fixation, Histopathological applications, Alternative fixatives, Maiduguri

INTRODUCTION

Histopathology stands at the forefront of diagnostic medicine, providing critical insights into disease pathology through the meticulous examination of tissue samples. Central to this discipline is the process of tissue fixation, which plays a pivotal role in preserving cellular architecture and morphology for accurate interpretation (Gianni, 2022). For decades, formalin has served as the gold standard fixative in histopathological practices, facilitating the study of tissues with remarkable fidelity (Ajileye and Esan, 2022). However, its longstanding reign is overshadowed by concerns over its carcinogenic properties and potential harm to both laboratory personnel and the environment, prompting a pressing need for safer and more sustainable alternatives (Carmela et al., 2022). In recent years, the spotlight has turned towards Moringa oleifera, a plant indigenous to tropical and subtropical regions, renowned for its diverse medicinal properties (Mmabatho et al., 2023). Among its notable attributes are its antioxidant and antimicrobial effects, making it a subject of increasing interest in various fields of research (Mmabatho et al., 2023). Of particular significance to histopathology is Moringa oleifera's chemical composition, which boasts polyphenols and flavonoids known for their tissue-preserving properties (Sangeeta and Vrunda, 2016). Moreover, studies have revealed its inherent cross-linking capability, a feature critical to the fixation process, similar to that of formalin (Lavanya et al., 2023).

Despite these promising characteristics, the incorporation of Moringa oleifera extract as a tissue fixative in histopathological applications remains relatively unexplored. Despite its demonstrated tissue crosslinking properties, research into its efficacy and practical utility lags behind (Kenneth et al., 2007; Elizabeth et al., 2015). As such, there exists a compelling need to delve deeper into the potential of Moringa oleifera as a natural alternative to formalin, not only to mitigate the environmental and health hazards associated with conventional fixatives but also to explore avenues for enhancing the sustainability and efficacy of tissue fixation methods in histopathology.

The objective of this study was to explore the viability of Moringa oleifera extract as a natural fixative for histopathological uses. Additionally, we aimed to evaluate the compatibility of Moringa oleifera extract with subsequent histological analyses, with a specific focus on its compatibility with the Haematoxylin and Eosin staining technique.

MATERIALS AND METHOD

Study Area

The study was carried in Maiduguri, the capital city of Borno State, situated in northeastern Nigeria. Maiduguri is characterized by a semi-arid climate, making it conducive for the growth and cultivation of Moringa trees and is widely distributed across tropical and subtropical regions (Deji and Efe, 2019).

Study Site

The study was conducted at the University of Maiduguri Teaching Hospital (UMTH) in Maiduguri, Borno State. UMTH, as a renowned medical institution in Nigeria, serves as a pivotal referral center not only for the populace within Borno State but also for patients from neighboring states and bordering countries of Niger, Chad, and Cameroon. This designation underscores the critical role of UMTH in providing access to a wide range of tissue samples necessary for conducting histopathological studies.

Study Design

Preparation of Moringa oleifera powder

The Moringa leaves were collected directly from the tree and air-dried before being finely ground into a powder using a mortar and pestle. Following this, the powder underwent sieving with a fine mesh sieve to obtain a fine Moringa powder. Subsequently, this powder was used to create the Moringa solutions.

Preparation of Moringa oleifera Solutions

Six different concentrations of Moringa solution were prepared from the Moringa powder. This included 5%, 10%, and 15% Moringa solutions in distilled water, where 5g, 10g, and 15g of Moringa powder were individually weighed and dissolved in 100ml of distilled water. Additionally, 5g, 10g, and 15g of Moringa powder were also individually weighed and dissolved in 100ml of normal saline to create corresponding 5%, 10%, and 15% Moringa solutions.

Animal Subject/Source of Sample

A mature male albino Wister strain rat, approximately 12 weeks old and weighing 180 grams, was selected as the subject for this study. The rat was in apparent good health prior to its use in the experiment. Following established protocols, the rat was euthanized in a humane manner, and its abdomen was opened to obtain kidney tissues for analysis.

Animal Welfare Provision and Ethical Clearance

The study adhered to the prevailing guidelines for animal welfare outlined by the National Academy of Sciences (NIH, 1985). The protocol was subjected to review by the institutional Animal Care and Use Committee. Ethical clearance for the study was obtained from the Faculty of Veterinary Medicine University of Maiduguri, Borno State.

Sample Tissue Collection and Preparation

Following the extraction of kidney tissues from the euthanized rat, the tissue samples were cut into pieces approximately 2 mm in size. These segments were then placed into different prepared concentrations of Moringa solutions, while others were immersed in a 10% formal saline solution to serve as control samples.

Sample Fixation

The fixation procedure involved submerging tissue samples in specific solutions, with formalin used for the control group and Moringa Oleifera extract for the experimental groups. Careful attention was paid to ensure proper immersion of the samples and clear labeling for identification. The fixation process was then allowed to continue for a period of 7 days to ensure thorough preservation of tissue morphology and cellular structure.

Tissue Processing and Embedding:

The tissue processing procedure was conducted manually following the procedures described by Ochei and Kolhatkar

(2000); the tissues underwent dehydration by immersion in increasing concentrations of alcohol (starting from 70%, then 90%, 95%, and finally two changes of absolute alcohol). Subsequently, they were placed in a hot air oven (DNP – 9052 LAB INCUBATOR) set at 50 degrees Celsius for one hour each to facilitate dehydration. Following dehydration, the tissues were cleared by immersion in two changes of xylene and similarly incubated in the hot air oven for one hour each. They were then impregnated by immersion in two changes of molten paraffin wax and again incubated in the hot air oven for one hour each. The use of the hot air oven hastened the tissue processing. Finally, after tissue processing, the dehydrated tissue samples were embedded in paraffin wax using a Leica embedding stage. The tissue samples were oriented appropriately within the embedding molds to ensure optimal sectioning.

Tissue Sectioning

Once the paraffin blocks solidified, they underwent trimming to remove excess wax, exposing the tissue surface and creating a flat surface suitable for sectioning using Leica rotary microtome – RM2125 RTS – Leica Biosystems, ensuring that the tissue samples were securely positioned for accurate sectioning and then placed on ice with the trimmed surface in contact with the ice.

Next, the paraffin-embedded tissue blocks were mounted onto the Leica rotary microtome. Using a sharp microtome blade, thin sections, typically 3 µm in thickness, were carefully cut. Special attention was paid to maintain uniform thickness and avoid any artifacts during the sectioning process.

Following sectioning, the sections were delicately transferred onto glass slides by gently floating them on a warm water bath (Round Water Bath SKU EQ0519), set at approximately 45°C. This procedure allowed the sections to flatten and adhere to the slides without distortion.

After the sections were affixed to the slides, they were manually labeled appropriately to ensure accurate identification of the tissue samples.

Subsequently, the labeled slides were placed on a hot plate (Item number: GEST_5015

EAN: 4064343361491) set at approximately 60 degrees Celsius to facilitate the adhesion of the tissue sections to the slides.

Staining

The Hematoxylin and Eosin (H&E) staining technique served as the method of choice to evaluate the histological characteristics of the tissue samples. The staining protocol followed a procedure described by John (2014):

The tissue sections, initially mounted on glass slides, underwent deparaffinization to remove the paraffin embedding material. This was achieved by immersing the slides in two changes of xylene for 5 minutes each. Subsequently, the sections were rehydrated by brief immersion in a series of descending concentrations of alcohol, (Absolute, 90%, and 70%).

Next, the tissue sections were immersed in Harris hematoxylin solution for 10 minutes, followed by rinsing with distilled water to eliminate excess stain.

Differentiation of the nuclei was achieved by briefly immersing the slides in acid alcohol, (1% hydrochloric acid in 70% alcohol).

To enhance the contrast of the nuclei, the slides were then blued by getting immersed in running tap water for 5 minutes. Subsequently, the tissue sections were counterstained with eosin Y solution for 1 minute to highlight the cytoplasm and

extracellular matrix, followed by rinsing with distilled water to remove excess stain.

The slides were dehydrated by sequential immersion in ascending concentrations of alcohol (70%, 90%, and Absolute) for brief periods. Finally, they were cleared in xylene, mounted with DPX mountant, and coverslipped using 22x22 glass coverslip.

Microscopic Evaluation

The stained tissue sections were observed under An Olympus light microscope (model number: CX31RBSFA) at both 10X and 40X magnifications to assess the quality of tissue fixation, cellular morphology, and histological features, and a comprehensive comparison between the control group, treated with formalin, and the experimental groups treated with Moringa Oleifera extract of various concentrations.

RESULTS AND DISCUSSION

The result table presents the effects of different concentrations of Moringa solutions on kidney tissue fixation.

Moringa in Distilled Water

At a concentration of 5%, there were regions of autolysis observed on the kidney tissue, indicating degradation of the tissue structure.

When the concentration was increased to 10%, the kidney tissue showed signs of putrefaction and autolysis, particularly evident in the renal glomeruli.

However, at a concentration of 15%, the kidney tissue appeared well-preserved, with fixation comparable to that of the control group fixed with formalin. This concentration demonstrated promising results, suggesting effective tissue preservation.

Moringa in Normal Saline

Similar to the findings in distilled water, the 5% concentration in normal saline also exhibited regions of autolysis on the kidney tissue.

Increasing the concentration to 10% resulted in more pronounced regions of autolysis, as well as putrefaction and autolysis of the renal glomeruli, indicating incomplete fixation.

However, at a concentration of 15% in normal saline, the kidney tissue showed slight preservation, although not as effective as the 15% concentration in distilled water.

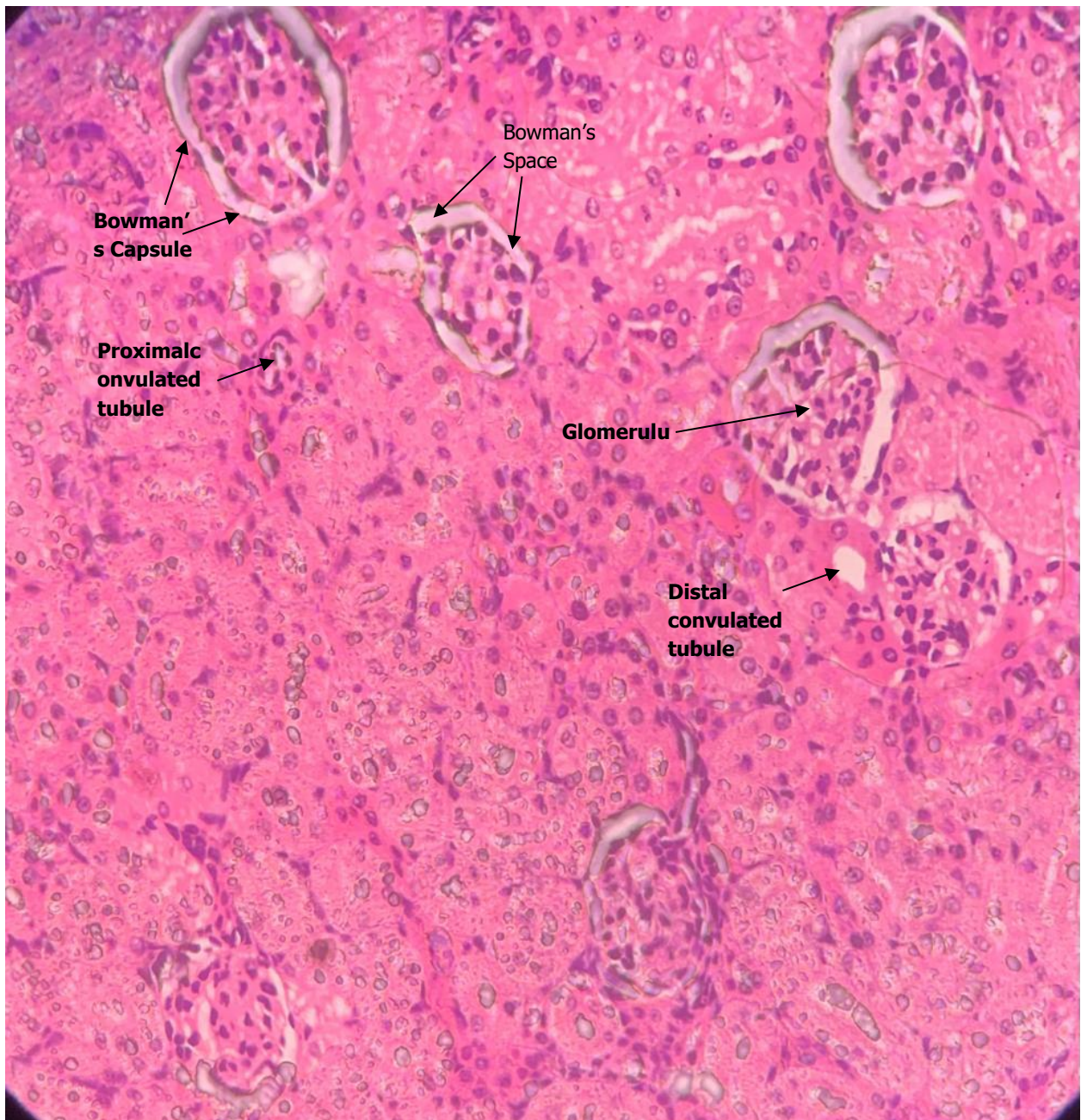


Figure 1: Haematoxylin and Eosin stain, demonstrating Kidney tissue fixed with 10% formal saline as control (bar=50 microns).

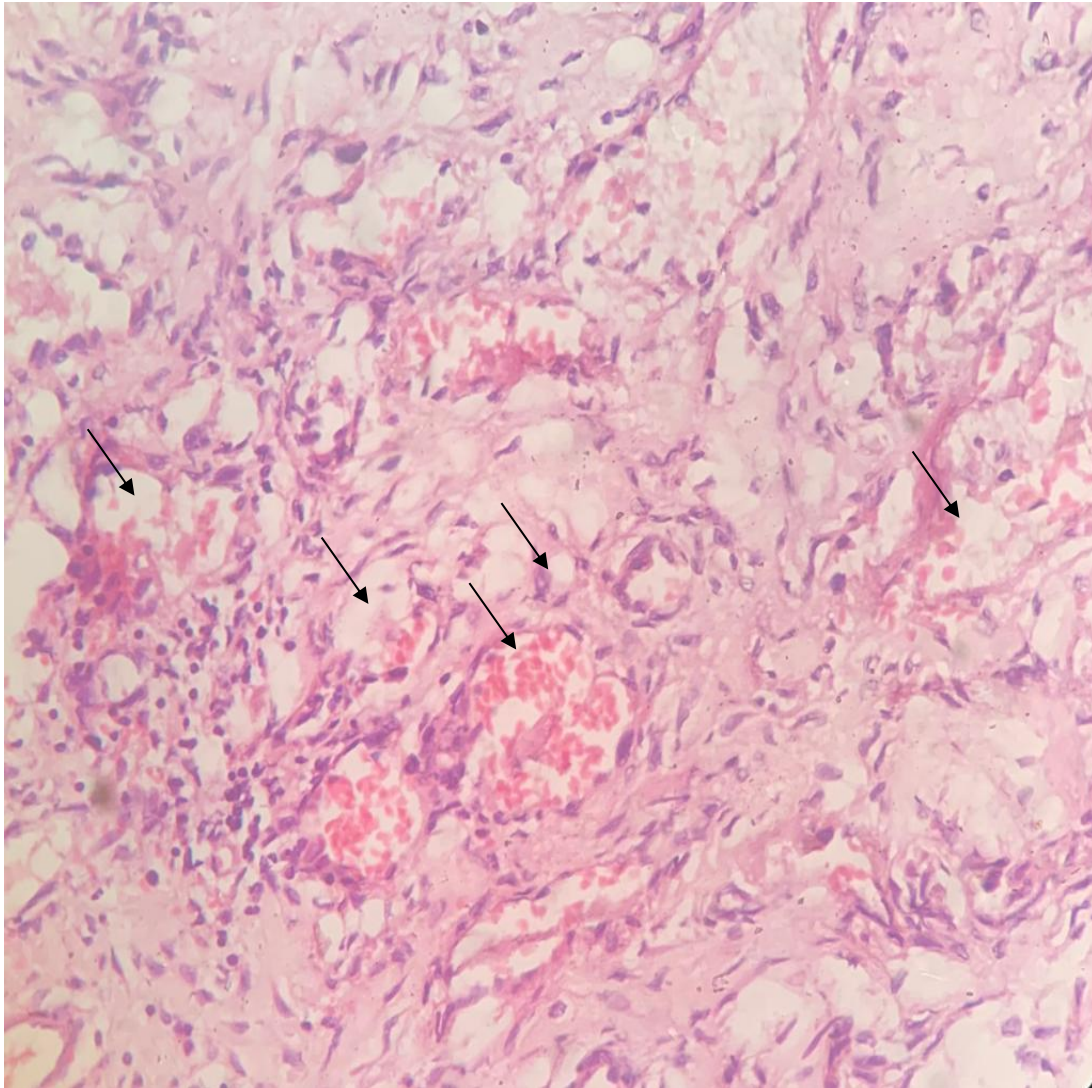


Figure 2: Haematoxylin and Eosin stain, demonstrating regions of autolysis (arrows) on Kidney tissue fixed with 5% moringa in distilled water (bar=50 microns).

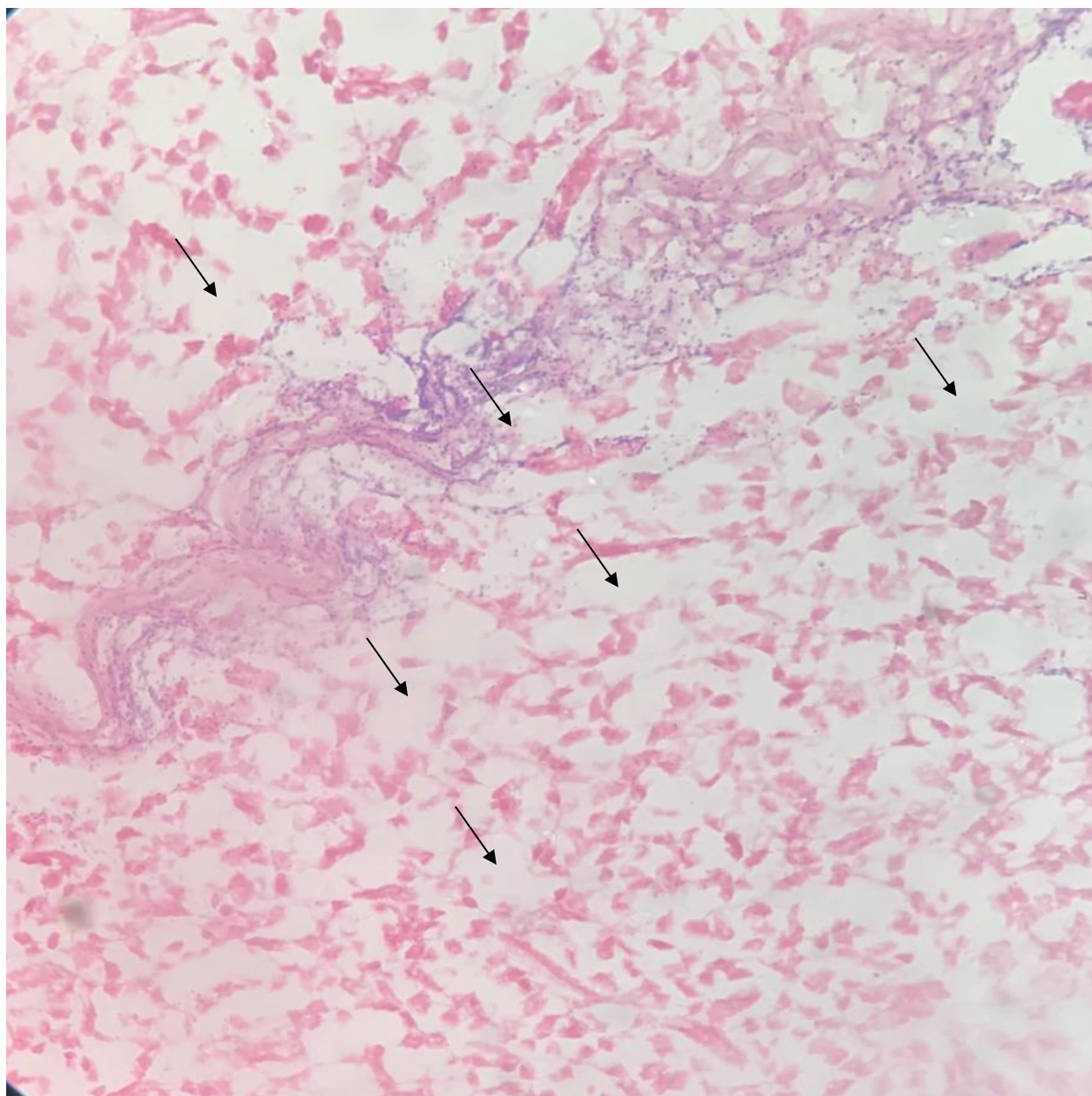


Figure 3: Haematoxylin and Eosin stain, demonstrating regions of autolysis (arrows) on Kidney tissue fixed with 5% moringa in normal saline (bar=50 microns).

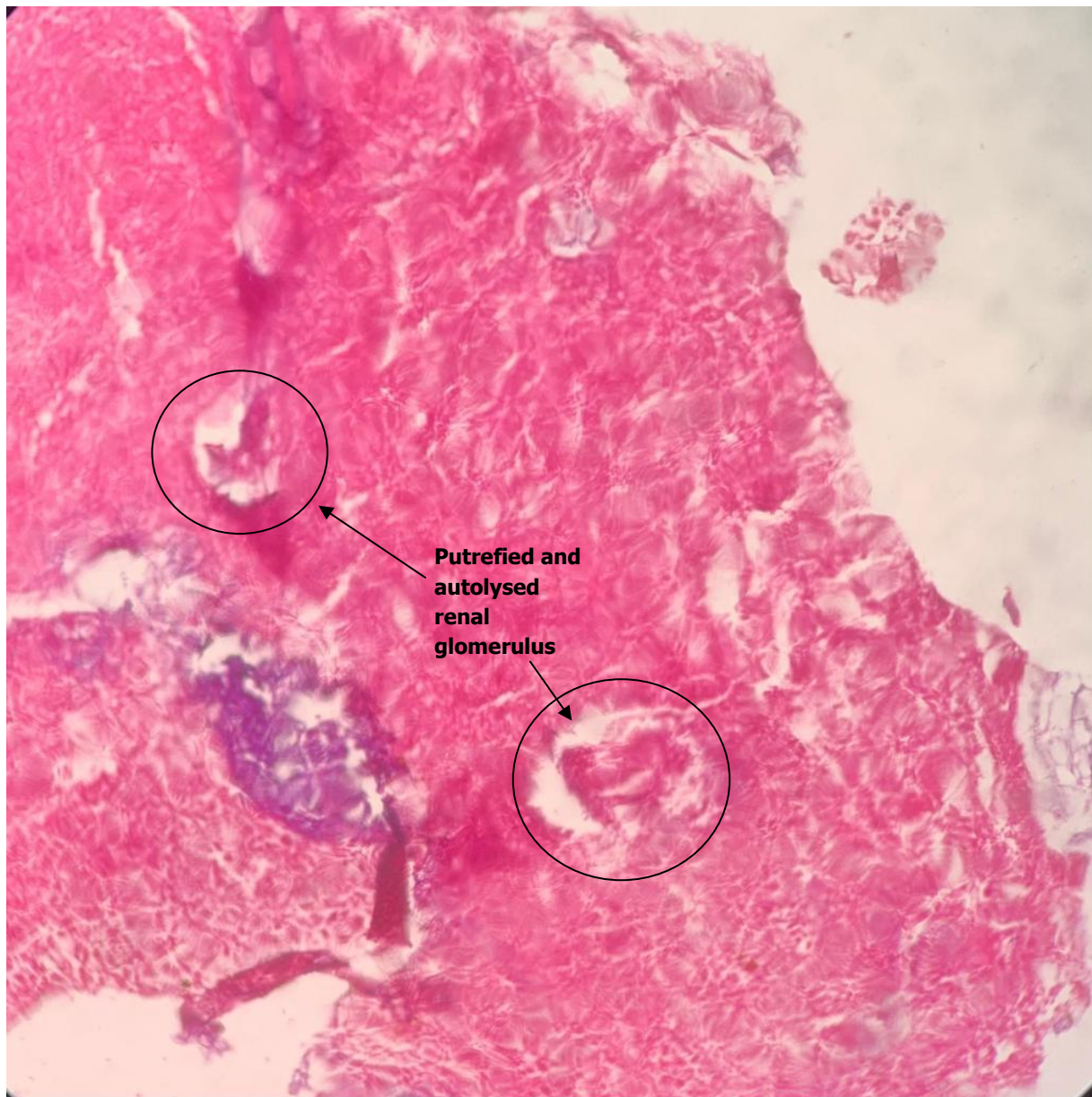


Figure 4: Haematoxylin and Eosin stain, demonstrating Putrefied and autolysed renal glomerulus on Kidney tissue fixed with 10% moringa in distilled water (bar=50 microns).

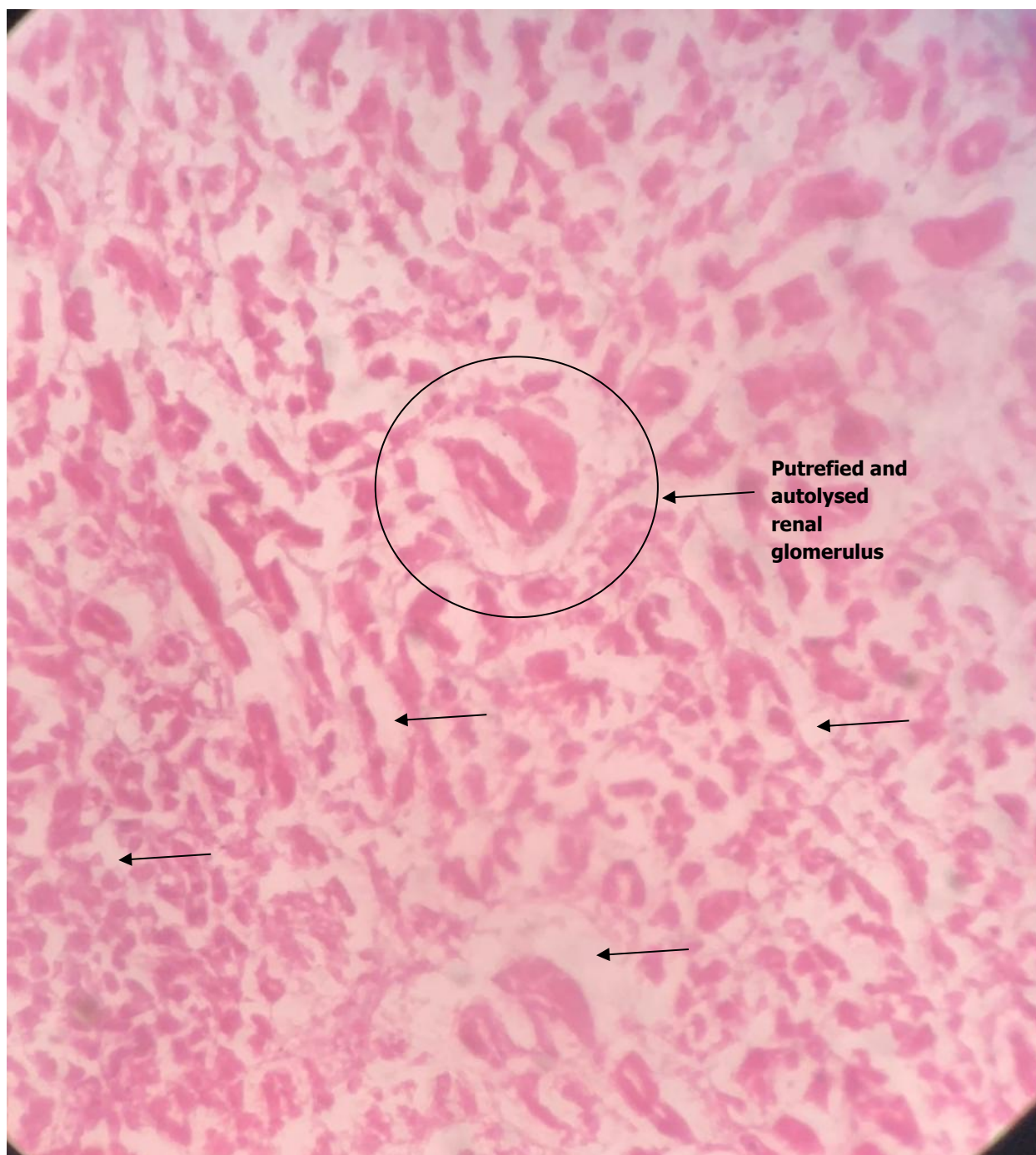


Figure 5: Haematoxylin and Eosin stain, demonstrating regions of autolysis (arrows) as well as Putrefied and autolysed renal glomerulus on Kidney tissue fixed in 10% moringa in normal saline (bar=50 microns).

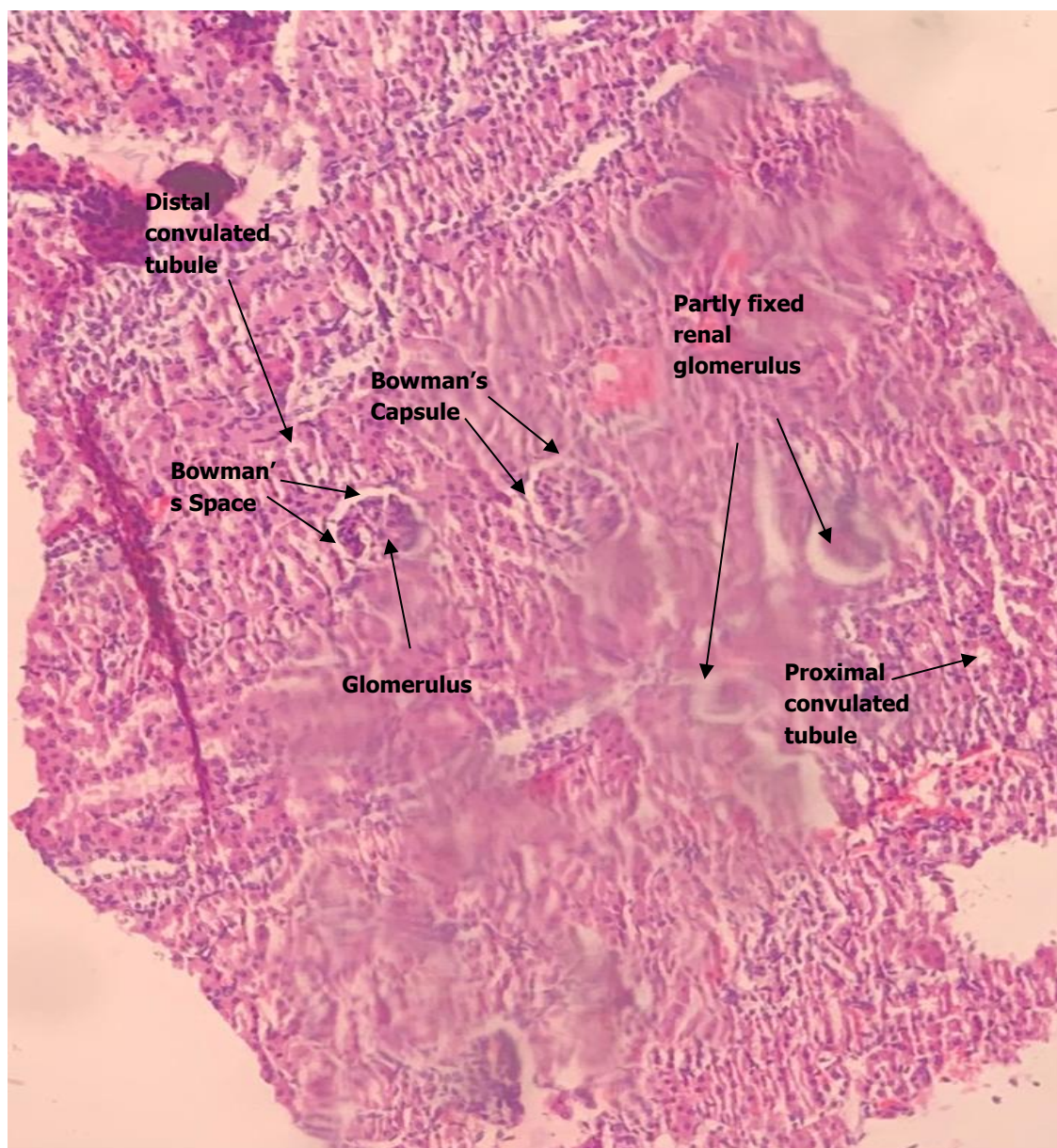


Figure 6: Haematoxylin and Eosin stain, demonstrating well preserved kidney tissue fixed with 15% moringa in distilled water. Fixation almost close to that of the control. (bar=25 microns).

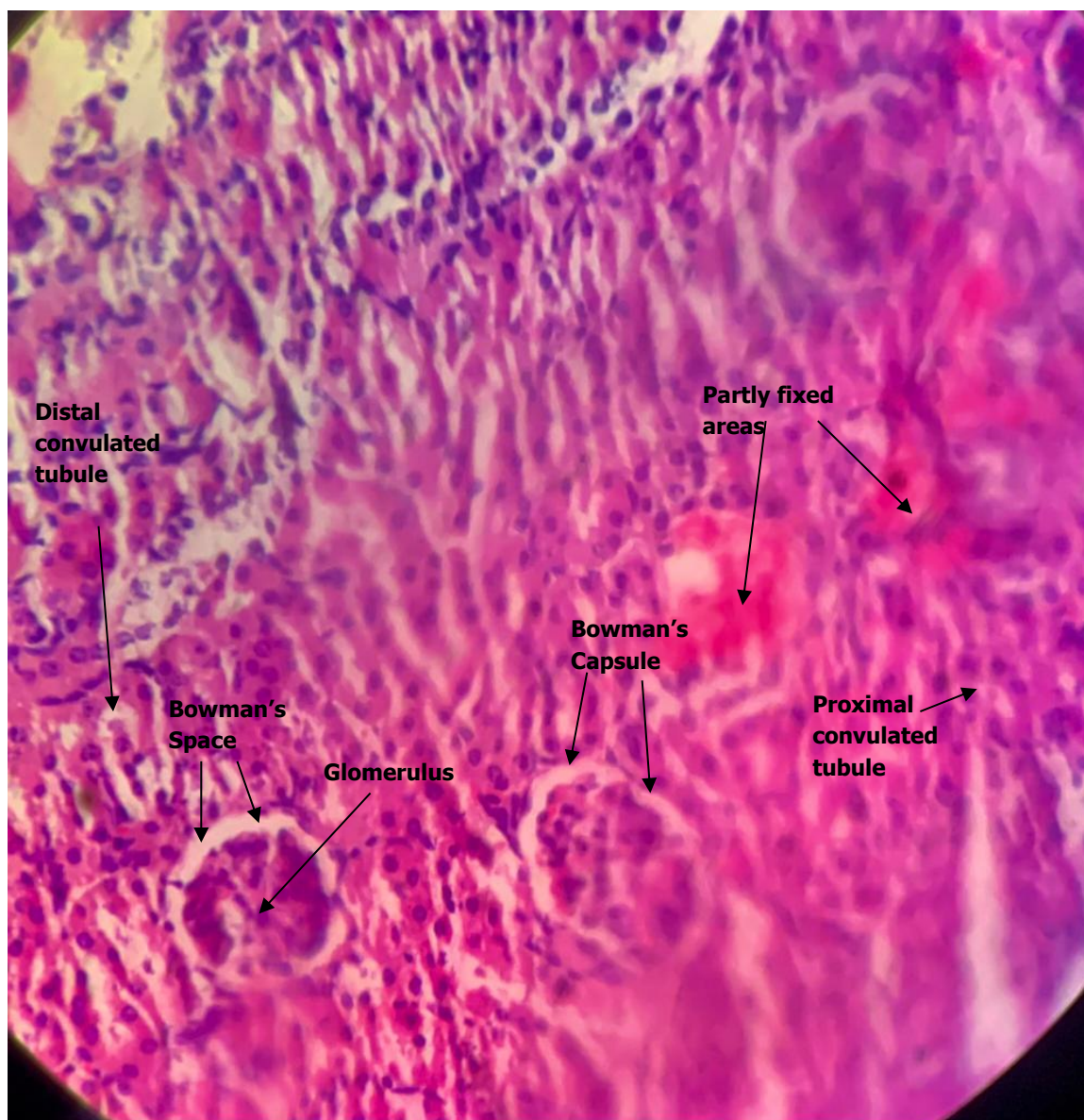


Figure 7: Haematoxylin and Eosin stain, demonstrating well preserved kidney tissue fixed with 15% moringa in distilled water. Fixation almost close to that of the control. (bar=50 microns).

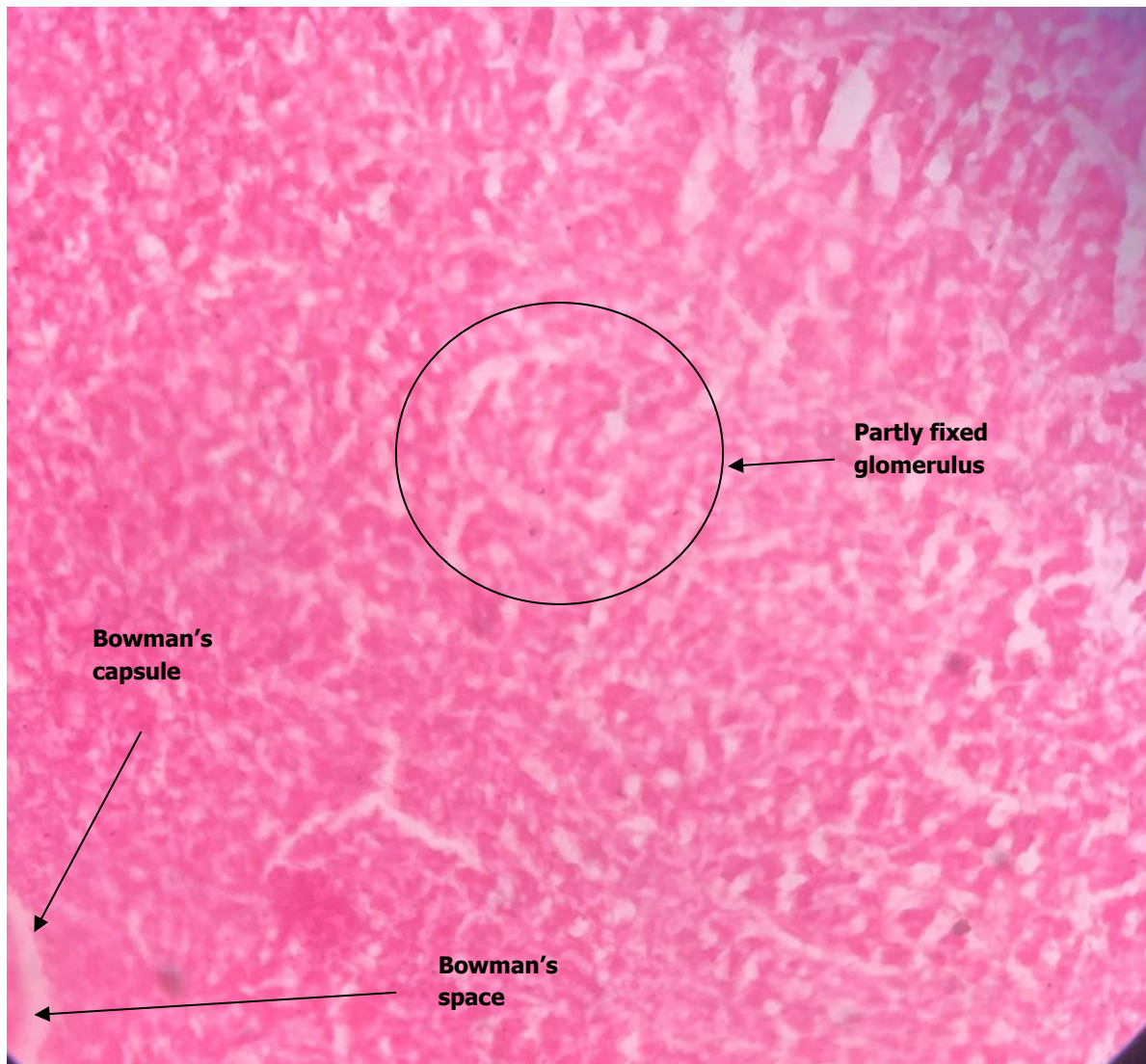


Figure 8: Haematoxylin and Eosin stain, demonstrating slightly preserved kidney tissue fixed with 15% moringa in normal saline. (bar=50 microns).

Discussion

The results presented in this study indicate the effects of different concentrations of Moringa solutions, prepared in distilled water or normal saline, on kidney tissue fixation.

Regarding Moringa solutions prepared in distilled water, the 5% concentration demonstrated regions of autolysis on the kidney tissue, as indicated by the presence of deteriorated tissue areas. Similarly, the 10% concentration showed putrefied and autolysed renal glomeruli, suggesting suboptimal fixation. However, the 15% concentration of Moringa in distilled water resulted in well-preserved kidney tissue, with fixation almost comparable to that of the control (10% Formal saline).

Conversely, when Moringa solutions were prepared in normal saline, the 5% and 10% concentrations exhibited regions of autolysis on the kidney tissue, resembling the observations seen with Moringa in distilled water. Additionally, the 10% concentration in normal saline also demonstrated putrefied and autolysed renal glomeruli, indicating incomplete fixation. However, the 15% concentration in normal saline showed slightly preserved kidney tissue, suggesting improved fixation compared to lower concentrations but still falling short of the optimal preservation achieved with 15% Moringa in distilled water.

These findings align with previous studies that have highlighted the potential of *Moringa oleifera* extract as a natural fixative for histopathological applications (Lavanya *et al.*, 2023). The varying degrees of tissue preservation observed with different concentrations of Moringa solutions underscore the importance of optimizing fixation conditions to achieve optimal results in histopathological analysis.

CONCLUSION

This study suggests that higher concentrations of Moringa solutions, particularly in distilled water, offer preservation potential. Further investigations may be warranted to explore the underlying mechanisms and optimize the use of *Moringa oleifera* extract as a tissue fixative for histopathological applications. However, lower concentrations and solutions prepared in normal saline exhibited regions of autolysis and incomplete fixation, indicating suboptimal preservation of the kidney tissue. Further research should focus on isolating and characterizing the active components of *Moringa oleifera* extract to develop targeted fixative formulations, conduct comparative studies to determine the most effective Moringa-derived fixatives, and assess their biocompatibility and safety for clinical use in histopathological practice.

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