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A NEW USAGE OF SOFT PLASTINATION TECHNIQUE TO PREPARE HISTOLOGICAL SLICES OF ONE HUMPED CAMEL KIDNEY (*CAMELUS DROMEDARIUS*)

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ABSTRACT

The soft plastination technique is primarily used for preserving anatomical specimens by replacing water and fat in the tissues with polymer, it is a novel technique which is applied as an anatomical teaching aid and can be modified to prepare thin tissue sections. A modified technique was used to transform the plastinated specimens into microscopic sections for histology and pathology education. In this study, the soft plastination technique was used to identify the advantages and disadvantages of the technique. Tissue samples of plastinated kidneys were obtained for histological study. De-plastination refers to the process of removing the plastinated medium, which is typically a plastic resin. Acetone is commonly used as a solvent in the de-plastination process. De-plastination is the reverse process of plastination, where the polymer is dissolved to reveal the preserved specimen. After de-plastination, the samples were embedded in a mold of paraffin wax. The wax was allowed to solidify into blocks. The block was then cut into thin sections using a microtome. The microtome settings were adjusted to obtain sections of the desired thickness (typically around 5-10 micrometers for histological studies). The histological sections were then mounted onto glass microscope slides. Appropriate staining techniques were applied, such as hematoxylin and eosin (H&E), to enhance cellular details and highlight specific structures within the kidney tissue. The plastinated sections were compared to de-plastinated and formalin-fixed specimens. The soft plastination technique was found to be a helpful method for the examination of the microscopic specimens.

Keywords: *Soft Plastination, camel, kidney*

INTRODUCTION

Plastination is a technique used for preserving biological tissues by replacing water and lipids in the tissues with curable polymers. The process was developed by Gunther Von Hagens in the late 1970s. Plastination involves several steps to create a durable, dry, odorless and non decomposing specimen with preserved anatomical structures (Ottone *et al.*, 2018). The process allows long-term preservation of tissues while maintaining their natural color, texture, and flexibility. Soft plastination, on the other hand, aims to maintain the natural

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flexibility and pliability of soft tissues, allowing for a more realistic representation of anatomical structures. This method is particularly modified by (Elnady, 2016), that focuses on preserving soft tissues and organs, such as muscles, nerves, and organs, in a flexible and lifelike state. The conventional plastination process involves impregnating the tissues with a glycerin and corn_starch. Glycerin and cornstarch can be used to create a simple, non-toxic, and biodegradable polymer known as "oobleck. The advantage of soft Plastination characterized by its cheap cost, and allows for flexibility in creating custom specimens tailored to specific educational needs. Different anatomical structures can be isolated and preserved individually or combined to demonstrate specific anatomical relationships. Specimens are commonly used in medical education, research, and museum exhibitions, to provide a detailed and realistic representation of human and animal anatomy (Grondin *et al.,* 1994). In addition to cryopreservation, other techniques like chemical fixation, formalin fixation, and paraffin embedding are used to preserve tissues for histological examination and research. These techniques involve treating tissues with fixatives to stabilize and preserve their cellular structures, enabling further analysis, staining, and microscopic examination (Bouchet *et al.,* 2003). While plastination allows for detailed examination of anatomical structures, traditionally it has not been suitable for observation using a light microscope due to the lack of transparency of the polymerized material (Pereira-Sampaia *et al.,* 2011).

By studying de-plastinated kidneys, the researchers aimed to provide a more detailed understanding of the anatomical structures within the organ. This knowledge can be valuable for various purposes, including medical education, research on kidney diseases, surgical planning, or improving surgical techniques related to the kidney.

It is worth noting that since there are limited data for de-plastination of kidneys, this study may contribute to filling some gaps in the existing knowledge and provide insights into the morphological structures of de-plastinated kidneys.

MATERIALS AND METHODS

Ten freshly collected camel kidneys which were obtained from Basra slaughterhouses were used in this study. The samples were then fixed in 10% formaldehyde to preserve their structure and prevent decomposition.

The samples in the control group underwent a routine histological tissue processing protocol. This protocol involved dehydration, clearing, and then embedding the tissue samples in paraffin wax. These processes help prepare the samples for microscopic examination and analysis (Kim *et al.,* 2019).

In the plastinated group, the necessary dissection procedures were performed, and excess connective and fatty tissues were removed from the kidneys.

The kidneys were then immersed in a 10% formalin solution for one week to fix the tissue.

Dehydration

After fixation, the kidneys were dehydrated by using acetone. The dehydration process involved gradually replacing the fluid in the kidney tissue with acetone, over a period of fifteen days. After the dehydration process, the kidneys were kept in acetone for an additional five days at room temperature to remove fat from the tissue.

Impregnation

The kidneys were placed in a glycerin bath for fourteen days at room temperature. After impregnation with glycerin, the kidneys were kept in corn starch powder for six days to allow the starch powder to penetrate inside the kidney and interact to form a soft

polymer. Following that, the kidneys were removed from the corn starch, and excess corn starch was removed from the surface.

After the completion of these steps, the soft plastination process of the camel kidneys was complete (Elnady, 2016).

De-plastination method

De-plastination is the reverse process of plastination, where the polymer is dissolved to reveal the preserved specimen.

Acetone is often used as the solvent to remove the polymer from the specimen. Acetone is a powerful organic solvent capable of dissolving many polymers used in plastination. The plastinated specimen, which had undergone plastination and has the polymer material preserving it, was immersed in a container of acetone. The acetone will gradually dissolve and break down the polymer material, allowing it to leach out of the specimen. Depending on the size and complexity of the specimen, multiple changes of acetone may be required during the de-plastination process. This helps ensure efficient removal of the polymer material. The de-plastination process can take about 4-6 days (Rahul *et al.*, 2020).

Sectioning

Once the polymer had fully cured, the kidney was set in a paraffin block for microtome sectioning. The microtome settings were adjusted to obtain slices of the desired thickness (typically around 5-10 micrometers for histological studies).

Mounting and Staining

The histological sections were collected and mounted on glass slides. The slides were then stained with hematoxylin and eosin (H&E), to distinguish cellular details and highlight specific structures within the kidney tissue. Finally, the effects of plastination on the structure and properties of the camel kidneys were investigated, by using a light microscope.

RESULTS

Anatomical results: The outer shape of the plastinated kidney closely resembled its natural appearance. The plastination technique enabled the preservation of external features, such as the size, shape, and overall contour of the kidney. This allows for a realistic representation of the organ, making it a valuable tool for anatomical study, surgical training, and public education (Fig. 1).

The fibrous capsule retained its standard structure during the plastination process. This means that the fibrous capsule surrounding the kidney was preserved and maintained its typical appearance, while the adipose capsule was dissolved.

In addition, the adipose capsule, which is the layer of fatty tissue surrounding the kidney, was also preserved during the plastination process. This suggests that the plastination process was successful in retaining the structure and appearance of the adipose capsule in that particular sample (Fig. 2).

The color difference range between the renal cortex and renal medulla was found to be less evident in the plastinated kidneys compared with the de-plastinated (non-preserved) kidneys. This could be due to the specific properties of the polymer used in the plastination process, which may have resulted in a more uniform appearance of the renal cortex and medulla in terms of color (Fig. 3).

The observation of the renal sinus appeared to be following the renal hilum. It could indicate a normal anatomical arrangement where the renal sinus occupies the region around the hilum (Fig. 4).

Histological results: this study compared the histological structures of the camel kidney sections between a control group and a plastination group. The study used light microscopic micrographs to examine the

kidney tissues (Fig. 5). In the control group, the kidney sections from camel showed typical histological structures. Various components such as proximal and distal convoluted tubules, cortical and juxtamedullary glomeruli, and renal vascular structures were observed. The arterioles at the vascular pole of the renal corpuscles appeared normal. The renal glomeruli exhibited a normal morphology, including a glomerular capillary network, mesangial stroma, mesangial cells, and podocytes. Bowman's space was observed in all glomeruli. The renal pyramids, loop of Henle, and collecting tubules also appeared normal, with longitudinal collecting tubules observed at the medullary level (Fig. 6).

When the stained sections from the plastinated group were evaluated under a light microscope, cortical structures such as glomeruli and proximal and distal tubules were visible. However, medullary structures could not be observed. The cortical structures appeared tightly packed. The H&E-stained sections showed visible cell nuclei of glomerular, tubular, and capillary cells, and distinguishing between proximal and distal tubules without considering morphological differences was observed (Fig. 7).

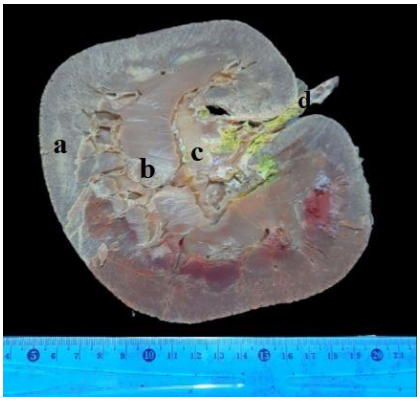

Figure 1. Section of camel soft plastinated kidney; a-cortex. b-medulla, c-renal pelvis, d-ureter

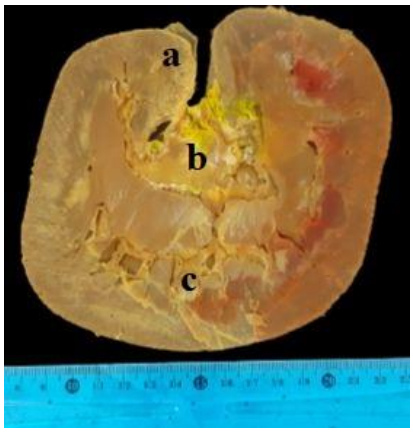

Figure 2. Section of camel soft plastinated kidney; a-renal fibrous capsule, b- renal pelvis, c-renal cortex

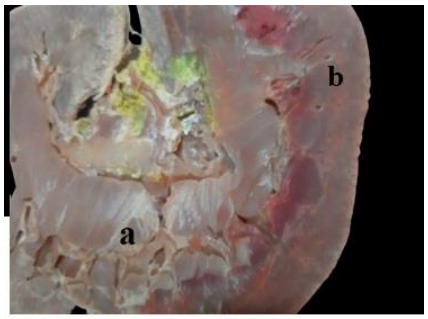

Figure 3. Section of camel soft plastinated kidney; a-renal medulla, b-renal cortex

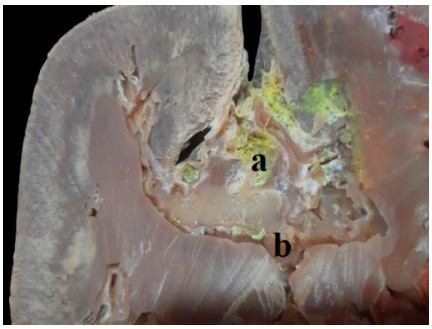

Figure 4. Section of camel soft plastinated kidney; a- renal pelvis, b-renal sinus

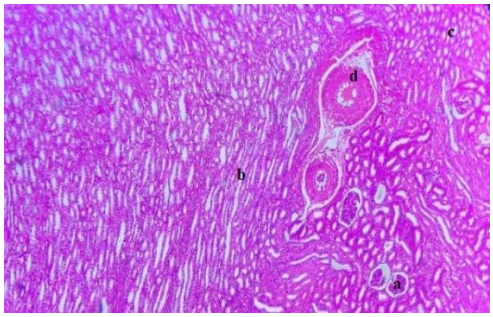

Figure 5. Histological section of the control camel kidney: a- glomerulus, b -distal convoluted tubules, c-proximal convoluted tubules, d- blood vessel (100X, H&E)

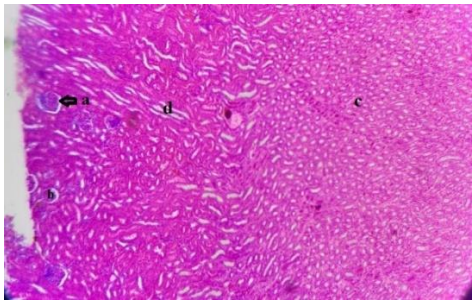

Figure 6. Histological section of soft plastinated camel kidney: a-Bowman's space, b-glomerulus, c- proximal convoluted tubules, d- distal convoluted tubules. (100X, H&E)

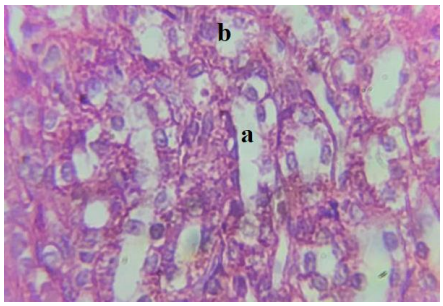

Figure 7. Histological section of soft plastinated camel kidney: a-distal convoluted tubules, b-proximal convoluted tubule (400X, H&E.)

DISCUSSION

Plastination offers several advantages in terms of tissue preservation. It allows for the long-term storage of specimens while maintaining their natural morphological structure. One disadvantage of plastination is the hardening of the tissues. The polymers used during the plastination process result in a firm and rigid texture which can make the specimens less flexible and more challenging to manipulate (Reiderer, 2014). The process results in dry, odorless, and durable specimens that can be used for

educational and scientific purposes (Monteiro *et al.*, 2021)

There are many articles on the morphological and histological applicability of plastination for the kidney (Ramos et.al., 2008; Pendovski *et al.*, 2008). Plastination is a preservation technique that involves impregnating biological tissues with a curable polymer. The decision to use acetone for de-plastination was based on previous successful results, that in agreement with (Rahul *et al.*, 2019) who used alcohol and methylbenzene for de-plastination of heart tissue using these chemicals. alcohol and methylbenzene are commonly used in histology and laboratory settings for various purposes, including tissue processing and de-paraffinization (Baygeldi *et al.*, 2020).

The plastinated kidneys in the plastinated and de-plastinated groups were found to be soft, displaying natural morphological structures that were deemed suitable for educational purposes. This study, which aimed to perform histological examination on de-plastinated kidneys by paraffin embedding, differs from the method which is described by Rahul *et al.* in (2020), which reported successful histological sectioning of plastinated oral carcinoma after de-plastinating it with 5% sodium methoxide dissolved in methanol.

The study found different results to the study conducted by Ravi and Bhat in 2011, which observed a delay in staining of de-plastinated histological sections. We were able to capture images under the light microscope after de-plastination using normal eosin staining.

According to these differences, we suggest that the pale eosin staining described by Ravi and Bhat (2011) may be related to the staining delay observed in de-plastinated sections.

In conclusion, the plastination-de-plastination technique shows promising findings in allowing researchers to examine the morphological structures of organs even after an extended period. Studies have demonstrated remarkable success in histological examination following de-plastination using the paraffin embedded method.

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