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Enhancing Animal Histology Through Eosin and Haematoxylin Staining Preparations

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Abstract
Histology is a field of biology focused on examining tissue structure in detail using a microscope to analyse thinly sliced tissue samples. These sections reveal a variety of shapes, sizes, and layers, including cellular, fibrous, and tubular structures. Histology plays a crucial role in studying the normal tissue architecture of organs, addressing both anatomical and physiological aspects. This type of research employs a descriptive qualitative approach, utilizing secondary data primarily derived from literature such as journals and supporting articles. The literature review serves as an essential tool alongside contextual assessment, offering valuable insights and enhancing the understanding of the subject matter. Histological preparation involves the application of haematoxylin-eosin (HE) staining, which includes several key steps: tissue fixation, tissue selection (trimming), dehydration, clearing and tissue impregnation, tissue block creation, tissue sectioning, staining, and tissue examination.

1. Introduction

Histology is very closely related to the science of anatomy and physiology, because in various aspects of the sub -knowledge in the field requires histology as a translator. Why? Because histology is a branch of biology that studies tissue structure in detail by using a microscope in tissue preparations with thin slices. This slices will then show various shapes, sizes and layers including cellular, fibrous and tubular structures (Zulham, 2009).

Histology is needed to study the normal tissue structure of another organs or organs, both anatomical and physiological. The recognition of pathological conditions caused by disease is important, and cellular changes also help in the diagnosis of the disease, because one of the diagnostic considerations is to observe the suspected tissue disrupted by sampling (Suntoro, 1983).

Normal or abnormal tissue structure can be studied under a microscope in the form of tissue preparations. This preparation is carried out by tissue processing until the color composition is obtained. Histological structure is clearly visible, facilitating tissue reading. Making histology preparations is carried out in several stages, namely preparation, processing, slicing and tissue staining (Suntoro, 1983; Leeson, 1996).

The definition and exposure above becomes the reason why it is very important to understand histology as well as the way in its implementation. A student who has an obligation to explore histology knowledge in the laboratory has the task to present well so that the results of the preparation can provide accurate results and the problems under study can be resolved. Understanding begins with preparation before making preparations such as anesthesia, euthanasia, surgery, formation for pruning or pruning. Tissue maintenance ranging from dehydration, clarification, paraffin infiltration, detention, cutting specimen preparations, staining for binding. The steps in making tissue preparations must be considered so that damage does not occur due to treatment errors such as tear, scratched, wrinkled, colored, and filtering inadequate solutions that can cause diagnosis errors.

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2. Method

The methodology used in this study includes the use of qualitative methods with secondary data methods, especially through literature studies obtained from journals or supporting articles. (Afifuddin 2012), Literature Study is a tool that is as important as contextual assessment, where literary works are very helpful and helpful in providing context and meaning to the writing and through the assessment. Knowing why what you want to learn is a problem to be examined, both in terms of research subjects and the environment that is of concern to the research. The relationship between the research and other related research. It is hoped that this understanding can be used as a guideline in its implementation.

3. Result and Discussion

Anatomy or microscopic histology is a careful and detailed study of organs or parts of the animal or plant's body. Efforts or means to be able to observe, study and examine certain tissues from an organism can be done by preparing histological specimens (Gunarso 1986 in Perceka, 2011). The steps in making animal histology preparate in general are the same, animal sampling involves the same steps, but there are differences in the way they handle it if the structure is different. For example, write the current research using gouramy samples.

Sample preparation

Gouramy samples will be tested in the pathology laboratory due to clinical changes. Clinical changes occur in pale body shape and gills. Therefore, greedy organs are released and immediately preserved to be brought to the pathology laboratory for histological preparation. The purpose of the gouramy used for histology is to make a more comprehensive diagnosis than the clinical changes that occur.

Work on making histology preparations

Preparation of Tools and Materials Purpose Preparation of tools and materials to facilitate the next steps in making histology preparations.

Tissues fixation

The fixative material used to preserve the cucurbital tissue is a bouin immobilization solution. Bouin breakdown is used for Gouramia because it is quickly absorbed and uniform but can cause a little shrinkage. Fixation with bouin solution is carried out for 24 hours. According to Jusuf (2009), if the tissue is soaked for too long, the tissue can become fragile and cannot be cut properly with a microtome.

Tissue selection (trimming)

The tissue remains cut with a sharp and sterile surgical ran so that the tissue is not damaged in the process. After the cutting process is complete, the cut cloth is placed on a cassette. Cassettes containing tissue are then immersed in distilled water for one minute to prevent tissue from shrinking due to too long air exposure.

Dehydration of cleaning and tissue impregnation

Tissue dehydration is done with the aim of removing all the liquid contained in the immobilized tissue and then filling it with paraffin or other substances used to make bulk preparations. This is necessary because water cannot be mixed with liquid paraffin or other substances used to make fatty preparations. The removal of water from cells / tissue is carried out by soaking tissue in chemicals that are dehydrated (absorbing water) in increasing concentration, namely alcohol.

Stages of dehydration, clearing and impregnation are carried out by the process of alcohol dilution using four alcohol concentrations namely 70%alcohol, 80%alcohol, 96%alcohol and absolute alcohol (100%). The multilevel alcohol used in this process aims to gradually remove water in the test organs. As for the stages of the dehydration process, clearing and impregnation presented in:

Table 1. The process of dehydration, clearing and impregation.

Process	**Time**
NBF 10%	1 hour
NBF 10%	1 hour
Dehydration	
Alcohol 70%	1,5 hours
Alcohol 80%	1,5 hours
Alcohol 96%	1,5 hours
Alcohol 100%	1 hour
Alcohol 100%	1 hour
Alcohol 100%	1 hour
Clearing	
Xylene	1.5 hours
Xylene	1.5 hours
Impregnation	
Parafin	2 hours
Parafin	2 hours

Manufacturing tissue blocks

According to Kurniasih (2008), tissue massage is carried out to prevent each part of the tissue from changing as in the initial cutting phase using a tool called tissue embedding. In this process, anti -printed molds or basic molds are used to make paraffin blocks. In this process, soaking material is used, namely hot liquid paraffin at 70 ° C.

Tissue slicing

Tissue slicing is the process of slicing tissue blocks using a microtome. Microtome is a device used to slice thin of tissue. Candle tissue samples are moved manually towards the knife according to the desired thickness of the slices. The results of this tissue resection in the form of thin ribbons, which is important because this thin slice will help more accurate diagnosis (Kurniasih, 2008).

Tissue staining

Almost all tissues in the human body are colorless. To observe the structure, cells and tissues must be colored first. There are many types of dyes. Staining is the process of tissue staining that is cut so that the components become contrast and can be recognized/observed under a microscope. The process of the appearance of colors is related to the emergence of certain intermolecular bonds found in certain tissue regions and structures. Light with a certain wavelength contained in light from sunlight or microscope lamps that illuminate the stained plate absorbed (absorbed) or continued. Dyes attached to the fabric will absorb light with a certain wavelength to give color to the fabric.

Staining is a tissue coloring process. Staining is intended to facilitate microscopic observation and to distinguish the desired tissue parts such as nucleus, cytoplasm and others (Ellyawati, 2018). Tissue under a microscope. Dyes commonly used regularly are dyes that can be used for nucleus and cytoplasmic staining and its connective tissue, especially hematoxylin-eosin (HE) staining. In the staining he used 2 dyes, namely hematoxylin functions to color the cell nucleus and produce blue (basophils) and eosin is an antistain of hematoxylin, used to tarnish cytoplasm and connective tissue and for pink with different patterns (Jusuf, 2009). In street vendors, hematoxylin used is Mayer's Hematoxylin and the reagent used is Eosin. This color is commonly used in all types of fish, including gourmet fish. Description of the stages of when the color is carried out as follows: 1. Deparahization, 2. Rehydration, 3. Staining I (Hematoxylin), 4. Differentiation, 5. Blueing, 6. Staining II (Eosin), 7. Dehydration, 8. Mounting.

If described in a way in the staining process HE, as follows: Container 1: xylol in the process = 5 minutes, Container 2: xylol in the process = 5 minutes, Container 3: xylol in the process = 5 minutes, Container 4: alcohol 96% in the process = 2 minutes, Container 5: alcohol 96% in the process = 2 minutes, Container 6: alcohol 80% in the process = 2 minutes, Container 7: water flows with a small flow = 5 minutes, Container 8: Paint Hematoxylin in the process = 5 minutes, Container 9: water flows with small flow = 10-20 minutes, Container 10: Cat Eosin in the process = 0.5 minutes/30 seconds, Container 11: alcohol 80% in the process = 2 minutes, Container 12: alcohol 96% in the process = 2 minutes, Container 13: Alcohol 96% in the process = 2 minutes, Container 14: alcohol 96% in the process = 2 minutes, Container 15: empty on the drying process = 10 minutes, Container 16: xylol in the process = 5 minutes, Container 17: xylol in the process = 5 minutes, Container 18: xylol in the process = 5 minutes, Container 19: empty in the drying process = 10 minutes.

Preparate observations

The colored preparations are then observed under a microscope. The microscope used is a binocular microscope. With the results of this preparate can clearly identify tissues in the organs that are used as samples.

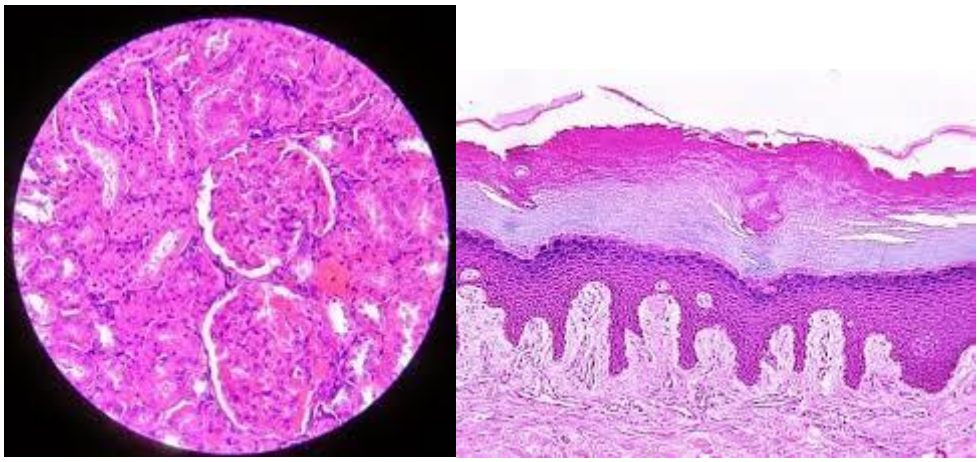

Figure 1. Examples of histology preparations using Hematoxylin-Eosin (HE) staining techniques

In the picture above, we can observe the preparate with HE staining, there are two blue -in contrasting colors caused by Hematoxylin to color the cell nucleus and pink nuclei from Eosin are antistifits of hematoxylin, used to tarnish cytoplasm and connective tissue with different patterns.

4. Conclusion

Histology is a branch of biology that studies a detailed tissue structure using a microscope in tissue preparations with thin slices. This slices will then show various shapes, sizes and layers including cellular, fibrous and tubular structures. Making histology preparations using hematoxylin-eosin (HE) staining with steps: tissue fixation, tissue selection (trimming), dehydration of clearing and tissue impreging, Making tissue blocks, tissue slicing, nets and observation.

Author Contributions

Made Karma Maha Wirajaya: Conceptualization, Methodology, **Eunike Sri Puspaningsih**: Data curation, Writing- Original draft preparation**, Juniarti Wulan Lestari**: Visualization, Investigation, **Baiq Farista**: Supervision. **Yuventi Nenometa**: Collecting data.

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