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Irisan yang Sesuai untuk Pemeriksaan Mikroskopis Hepar dan Ginjal pada Mencit dengan Fiksasi BNF 10%

Appropriate Slice for Microscopic of Hepar and Kidney Mice Fixed With BNF 10%

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Abstrak

Latar Belakang: Pemrosesan histologis melibatkan pengambilan jaringan segar, mengawetkannya dengan fiksasi untuk mempertahankan tampilan aslinya, memotongnya menjadi bagian-bagian kecil, memasang bagian-bagian tersebut pada *slide* kaca, dan kemudian menodainya. Hal ini memungkinkan pemeriksaan elemen histologis yang berbeda di dalam jaringan dengan menggunakan mikroskop. Tujuan dari penelitian ini adalah untuk mengetahui gambaran mikroskopis sediaan heparin dan ginjal mencit (*Mus musculus*) dengan cara memfiksasinya dengan larutan buffer formalin.

Metode: Jenis penelitian ini termasuk penelitian eksperimental. Penelitian ini menggunakan metodologi sampel purposif dalam desain penelitiannya. Temuan penelitian menunjukkan bahwa preparat yang menggabungkan gambar secara akurat mencerminkan inti sel dan sitoplasma, dan secara konsisten menampilkan warna yang sesuai pada berbagai ketebalan pemotongan. Ketebalan konvensional untuk membelah jaringan hati tikus menggunakan mikrotom adalah 5 µm.

Hasil: Ketebalan sampel menghasilkan hasil mikroskopis yang baik, menghasilkan 25 lapangan pandang dari 5 sediaan. Tingkat keberhasilan memperoleh gambar mikroskopis berkualitas tinggi pada ketebalan khusus ini adalah 100%. Analisis statistik menunjukkan perbedaan substansial ($p<0,05$) antara kelompok dengan ukuran partikel 2 µm dan 5 µm, serta antara kelompok dengan ukuran partikel 5 µm dan 8 µm. Tidak ada perbedaan yang signifikan secara statistik ($p> 0,05$) yang ditemukan antara kelompok 2 µm dan 8 µm dalam hal banyak faktor, seperti struktur, nukleolus, sitoplasma, keseragaman warna, dan skor struktur total.

Kesimpulan: Ketebalan pemotongan pada penelitian ini terbaik pada 5 µm. Diperlukan analisis lebih lanjut untuk mempelajari mikroskopis sampel jaringan dengan membat variasi lama pewarnaan pada sampel.

Abstract

Background: *Histological processing encompasses the steps of acquiring fresh tissue, preserving it through fixation to maintain its natural appearance, slicing it into thin sections, placing the sections on glass slides, and subsequently staining them. This enables the observation of various histological components within the tissue using a microscope. The objective of this study was to ascertain the microscopic characteristics of heparin preparations and the kidneys of mice (Mus musculus) through fixation using a formalin buffer solution.*

Method: *This kind of research encompasses experimental research. The study employed a purposive sample methodology in its research design. This study concludes that preparations containing images provide a representative depic-*

tion of the cell nucleus, cytoplasm, and exhibit consistent coloration with different cutting thicknesses. The standard thickness for cutting the mice hepatic tissue using a microtome is 5 µm.
Result: *This thickness yields good microscopic results overall, with 25 field views from 5 preparations. The percentage of high-quality microscopic images obtained at this thickness is 100%. The statistical analysis revealed significant differences ($p< 0.05$) between the 2 µm and 5 µm groups, as well as between the 5 µm and 8 µm groups. There was no statistically significant difference ($p> 0.05$) observed between the 2 µm and 8 µm groups in terms of many aspects including structural, nucleolus, cytoplasm, color uniformity, and total structure scores.*
Conclusion: *The optimum thickness in this research was 5 µm. Additional investigation is required to examine the microscopic characteristics of tissue samples by varying the duration of staining on the samples.*

INTRODUCTION

Histological processing encompasses the steps of acquiring fresh tissue, preserving it through fixation to maintain its natural appearance, slicing it into thin sections, placing the sections on glass slides, and subsequently staining them. These stained sections are then examined under a microscope to distinguish various histological elements present in the tissue.[1,2] The thickness of a histological section has a significant impact on the contrast, sharpness, and capacity to recognize details in the microscopic image of the slide.[3,4] A moderate level of variation in the thickness across different sections is permissible as long as the overall quality remains satisfactory. This will ensure that the morphological characteristics of the tissue being studied can be accurately assessed.[3] Some methods for determination of histological section thicknesses calculate the average thickness of sections from a series of a known number of consecutive sections by measuring the reduction of the length of the tissue block during the sectioning process at the microtome.[3] This study aimed to show that the images represent the cell nucleus cytoplasm and exhibit consistent colouration with different cutting thicknesses.

METHOD

This research is a type of experimental research with descriptive research criteria. This study used two organs, the kidney and hepar. These two organs get to organs processing and cutting in the difference thickness 2, 5 and 8µm. Then we compared the microscopic quality. The study employed a purposive sampling method to obtain 5 tissue preparations with a cutting thickness of 5µm. These preparations were preserved with formalin buffer solution and observed in 5 fields of view for each preparation of hepar and kidney tissues from mice (*Mus musculus*). Observations were conducted using a light microscope at a magnification of 400x. A microscopic quality assessment was conducted on each field of view, focusing on the cell nucleus, cytoplasm, and color uniformity in the preparations of hepar and mice (*Mus musculus*). The weight of the microscopic quality score was then calculated for each preparation, based on five fields of view. This calculation was done using a tissue histology microscopic quality scoring model that was developed.[5,6]

The research was conducted between May and July 2023. The research was conducted at the Poltekkes Health Analyst Laboratory under the Ministry of Health in Semarang. The equipment used in this study were tweezers, scalpels, pencil needles, steroforms, ovens, tissue processors, microtomes, water baths, trays, a set of painting tubes, timers, a cross-section of preparations, microscopes, beker cups, tissue cassettes, object glass, deck glass, measuring instruments, microtome knives.

Table 1. Microscopic Quality Assessment Criteria of Preparations

No.	Structure	Description	Scale Nominal
1.	Cell nucleus	The core cannot be identified	0
		Unclear cell nucleus	1
		The cell nucleus is less clear	2
		Clear cell nucleus	3
2.	Cytoplasm	Cytoplasm cannot be identified	0
		Cytoplasm and connective tissue are not clear	1
		Cytoplasm and connective tissue are less pronounced	2
		Cytoplasm and connective tissue are clear	3
3.	Uniformity of colours in preparations	Colour uniformity cannot be identified	0
		The colour of the preparation is not uniform	1
		Colour uniformity in preparations is less	2
		Colour on uniform preparations	3

Source: This criterion was developed from (Ariyadi & Suryono.[7])

Table 2. Microscopic Quality Scoring of Preparations

No	Description	Value
1	Bad	0-3
2	Not Good Enough	4-6
3	Good	7-9

Source: This criterion was developed by Prasetiawan E., Sabri E., & Ilyas S.[5]

RESULTS and DISCUSSION

Results

Table 3. The Results of The Total Observation Of Microscopic Images of Hepar Tissue Preparations and Kidney Mice (Mus Musculus) that Have Been Fixed with Formalin Buffer Solutions

Microscopic picture	Animal organs	
	Liver n (%)	Kidney n (%)
Bad	0 (0)	0 (0)
Not Good Enough	0 (0)	5 (20)
Good	25 (100)	20 (80)

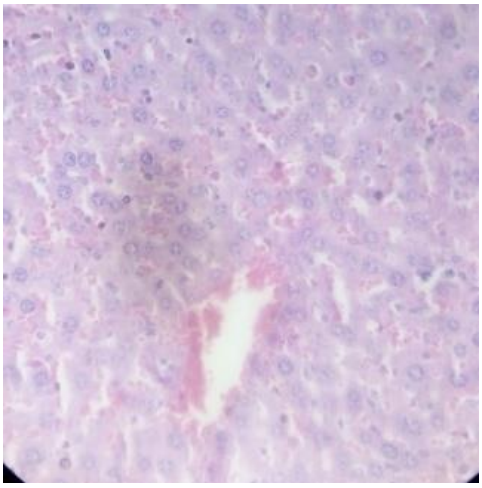

Figure 1. Microscope Pictures A Tissue of Hepar Mice Fixed with Formalin Buffer Solution

Discussion

The statistical test for normality achieved a significance level of less than 0.05, indicating that the data did not follow a normal distribution. The test persisted in identifying the disparities among the groups. The significance level (Sig) is less than 0.05 for the comparison between the groups with 2 μm and 5 μm, as well as between the groups with 5 μm and 8 μm. No statistically significant difference ($p > 0.05$) was observed between the 2 μm and 8 μm groups in terms of various measures, including structural, nucleolus, cytoplasm, colour uniformity, and total structure scores.

According to the research, the microscopic pictures of hepar mouse tissue preparations (Mus Musculus) are preserved using a formalin buffer solution, ensuring excellent preparation quality. Observing requires thorough and adequate preparations, leaving no room for insufficiency or bad circumstances. The average value of the total tissue preparations was calculated by taking the proportion of values obtained from the microscopic assessment criteria in Table 2.1. This assessment was based on 25 field views and 5 hepar tissue preparations.

The preparation shows the best results, as in the medical laboratory technology teaching material book. Formalin buffer fixation alignment for 24 hours generally offers a good and detailed cytoplasmic and core condition. The use of formalin buffer will mostly be perfectly fixed within 24 hours. The formalin buffer fixation solution utilized has a pH of 7, considered neutral. According to Alwi Muhammad, if the pH of the formalin buffer falls outside the range of 6-8, it can significantly alter the tissue structure and cause damage due to the impact on the ions in the tissue.[8]

Under microscopic examination, several snags and folds are evident at a tiny level. In the book "Learning Sitohistotechnology for beginners," Didik Sumanto elucidated that the tissue pieces may break because of the heat generated by the friction between the paraffin block and the blade.[9] Consequently, it is necessary to cool the network block multiple times. Strategically immersing the tissue band in warm water will cause the formation of creases in the band. Holes in the hepatocyte tissue may also result from the excessive duration of the fixation or hardening process, leading to inadequate tissue cutting. Furthermore, suboptimal embedding or residual seeding fluid can lead to crystallization, rendering the tissue more susceptible to tearing during cutting.[9]

According to Hasanah, Rusny & Masri [10], mice (Mus musculus) are a subset of animals classified inside the Muridae family, a family of rats. The standard thickness for cutting the mice hepatic tissue

using a microtome is 5 µm. This thickness yields good microscopic results, with 25 field views from 5 preparations. The percentage of high-quality microscopic images obtained at this thickness is 100%. Furthermore, the central region, cytoplasm, and consistent colour can be observed in 5 field views of the preparations. This thickness also exhibits excellent transparency in distinguishing cell borders.

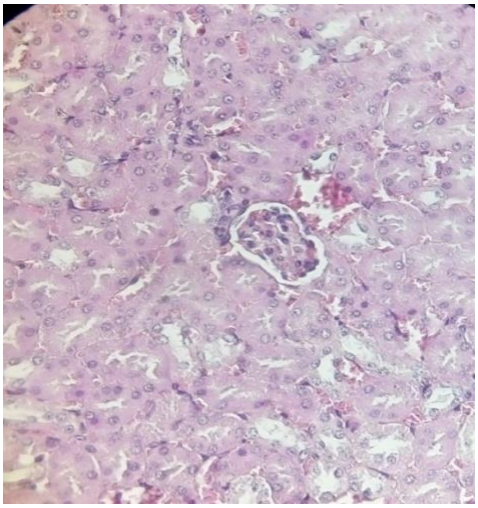

Figure 2. Microscope Pictures of kidney tissue Mice fixed with formalin buffer solution

According to the research, while examining microscopic pictures of kidney tissue samples from mice (Mus Musculus) treated with formalin buffer solutions, 80% of the preparations were found to be of high quality, while 20% were of poor quality. This assessment was made after examining 25 different field views. Upon examining kidney tissue preparations of formalin-fixed mice (Mus Musculus), it was seen that the colouration was insufficient, specifically the pale purple of the cell nucleus. This is due to hematoxylin's inadequate staining of the nucleus, which functions as a basic dye. The paleness of the Cytoplasm is attributed to the insufficient control over the painting process. This occurrence will likely transpire for a novice recently conducting the necessary investigation. Suprianto Abang states that insufficient hematoxylin staining results from an imprecise fixation technique, leading to autolysis.[11] Additional factors contributing to the issue include the incomplete elimination of paraffin, inadequate duration of staining, very intense or excessive discolouration procedures, thin slicing, and inappropriate pH levels.[12] Eosin functions as an acidic dye that specifically stains the cytoplasm. Due to inadequate fixation, the Cytoplasm undergoes a reduction in colour intensity and becomes less distinct. This is due to the indistinct and challenging nature of cell borders. The absence of eosin-stained cytoplasms can also result from excessive alcohol dehydration, contamination during alcohol dehydration, too thin cutting, and incorrect staining duration.[13] This occurrence will likely happen to an inexperienced individual who has recently conducted their research.

Nevertheless, adhering to time criteria does not ensure favourable outcomes. The timing in this colouring process is crucial as it is affected by the characteristics of each network processed, resulting in the potential

for time utilization to vary based on specific requirements.[14]

Formalin buffers generally yield satisfactory outcomes when examining microscopic images of hepar tissue and kidneys in mice (Mus Musculus). Jusuf states that appropriately treated tissues will yield high-quality microscopic preparations.[15] The tissue process in question involves a series of steps: fixation, dehydration, clearing, embedding, blocking, sectioning, mounting, and labelling.[16] The resulting image may be incomplete or inaccurate if the techniques employed in creating histological tissue preparations are flawed.[16,17] Furthermore, it is imperative to consider both the knife's age and its blade's cleanliness. A contaminated microtome knife can lead to visible irregularities in the tissue sections. Additionally, it is crucial to ensure that all parts of the knife are utilized evenly from one end to the other.[9,18] The heating temperature should be maintained at the precise melting point of paraffin without exceeding it. Elevating the temperature can result in alterations to the tissue's structure.

CONCLUSION

The findings of this study indicate that the microscopic examination of hepar tissue and kidney in mice (Mus musculus) reveals a consistently favourable depiction of the cell nucleus, cytoplasm, and colour consistency.

SUGGESTION

The study's findings indicate the need for additional research on the microscopic quality of tissue preparations. This research should explore the effects of different staining times on the preparation process.

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