A Systematic Review on the Efficacy of Coconut Oil as a Clearing Agent in Histopathology

Hannah Beatrice T. Dalayoan[1] Jilliane Daniele S. Cagadoc [1], Ma. Katrina Anne T. Dela Cruz[1], Hadi Adrianne B. Malinao[1] Kristine Lorraine L. Rivera[1], John Paul C. Soliven[1], Reymund M. Mabbagu[1]

[1]*School of Medical Laboratory Science, Far Eastern University- Nicanor Reyes Medical Foundation (NRMF), Regalado Ave., West Fairview, Quezon City, Metro Manila, Philippines.*

Corresponding Author: hannah.dalayoan@astra.feu-nrmf.edu.ph

Abstract: - Xylene, the most widely used clearing agent, is applied in the histopathological section for tissues. However, Xylene has toxic effects that may harm laboratory personnel and the environment. This systematic review aims to determine the efficacy of coconut oil as an alternative medium for Xylene. Six research studies were included in this systematic review retrieved from PubMed, ScienceDirect, EBSCO, and Research Gate. The researchers have discovered that coconut oil-treated specimens demonstrate the same characteristics with Xylene-treated specimens without affecting its histopathological details. Based on the data gathered, coconut oil showed increased performance in translucency compared to Xylene and an equal performance after completing impregnation. Similar characteristics were also noticed in coconut oil-treated specimens and Xylene-treated specimens in terms of staining. In the process of sectioning, coconut oil-treated specimens appear to be softer than Xylene which makes it slightly difficult to thinly cut the tissues. Specimens treated with coconut oil also appeared to be less rigid than the specimens treated with Xylene. However, coconut oil still demonstrates comparable characteristics to Xylene, making it an efficient alternative as a clearing agent in histopathology.

Key Words: — ***Coconut Oil, Clearing Agent, Xylene, Histopathology.***

I. INTRODUCTION

Tissue processing involves different stages including fixation, dehydration, clearing, impregnation, embedding, and orientation. Clearing, also known as dealcoholization, is the process of removing alcohol from tissues prior to infiltration of the embedding material. Before a substance can be used as a clearing agent, it must satisfy the following criteria: (1) able to form homogenous mixture with alcohol and paraffin wax, (2) can be eliminated by a melted paraffin wax, (3) does not cause tissue shrinkage and damage, (4) does not diffuse out aniline dyes, (5) can resist evaporation when placed in water bath, (6) and can produce transparent tissues8.

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Xylene has been routinely used as an agent of choice in dealcoholization. In most routine histologic processing, Xylene is appropriate for schedules of less than 24 hours. Its action is rapid with a clearing time of 30 minutes to 1 hour, and it also makes tissue transparent. It shows miscibility with alcohol and paraffin and does not interfere with staining3. However, Xylene is highly flammable and makes the soft tissues quite brittle with prolonged use[1].

In addition, Xylene has toxic effects that may harm laboratory personnel and the environment. Hence, Medical Laboratory Scientists are routinely exposed to Xylene during performing histopathological techniques in the laboratory [2].

II. MATERIALS AND METHODOLOGY

The researchers used systematic review to evaluate the efficacy of coconut oil as a clearing agent in Histopathology. The parameters used to assess the outcome are translucency, rigidity, impregnation, sectioning, and quality of staining. Furthermore, the researchers used PRISMA as a guideline for

assessing and analyzing literature.

2.1 Search Methods for Identification of Study

Online databases such as PubMed, Science Direct, EBSCO, and ResearchGate were used to find numerous articles, journals, and research regarding the target variables. The keywords and terms used were associated with coconut oil, Xylene, clearing agent, and histopathology. The search terms used in the following databases were presented, as well as the use of Boolean operators "AND" and "OR" to further eliminate unnecessary articles. Afterwards, the researchers added limiters such as "2011 - August 2021" to limit the studies by date.

2.2 Study Selection

For the data collection and analysis, online databases such as PubMed, Science Direct, ResearchGate, and EBSCO were used for the selection of articles, journals, and research that fit the eligibility criteria. The researchers created a spreadsheet to categorize the eligibility criteria in terms of the PICO (Population, Intervention, Comparison, and Outcome) method and the research design of the review.

For the data extraction, the researchers utilized Google spreadsheets. The data was extracted by gathering data that focuses on the efficacy of coconut oil as a clearing agent in histopathology, as well as other relevant studies. For the analysis, pattern tracing and coding was used. It consists of several processes including data organization, preliminary conduction of database scanning, theme organization and coding, data representation, and formulation of final interpretation.

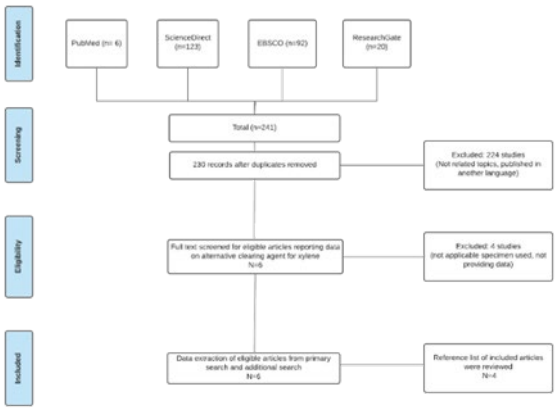

Fig.1. PRISMA flow diagram for the literature search and study selection.

2.3 Exclusion Criteria

The studies that are not related to coconut oil as a clearing agent were excluded. Also, the population did not include any animal tissue specimen. Studies that are non-experimental, not cross-sectional, and not written in English were also excluded.

III. Results And Discussion

3.1 Results

There are a total of six (6) studies included in this systematic review. The following studies below were obtained in a manner that presents the information in categories of PICO (Population, Intervention, Comparison, and Outcome). The intervention material (pure coconut oil) and comparison characteristics (coconut oil- and Xylene- treated specimens) used are similar to all the studies. All the researches included used a tissue specimen as a sample, but each study differs on the number of specimens used. Moreover, the studies included also vary in research design; for instance, some of the authors have utilized cross sectional comparative study, experimental, and others, comparative (Table 1). The studies gathered have used different outcomes in assessing the efficacy of coconut oil as a clearing agent. This systematic review focuses on the most common outcomes namely: Translucency, Rigidity, Impregnation, Sectioning, and Quality of Staining. Upon the intervention between xylene and coconut oil in tissues, comparison was made.

Table.1. Characteristics of Studies Included

Authors & Date	Study	Types of Study	Population	Intervention	Comparison	Outcome
Ashitha (2018)	Comparing the Efficiency of Coconut Oil and Palm Oil with Xylene as a Clearing Agent in Conventional Hematoxylin and Eosin Histopathological Staining Procedure	Cross sectional comparative study	90 gingival tissues with size measuring not less than one centimeters (cm)	Coconut Oil and Palm Oil	Coconut Oil-treated specimens, Palm Oil-treated specimens and Xylene-treated specimens	1. Rigidity - same as Xylene 2. Translucency - Coconut oil is superior to Xylene while Palm oil is same with xylene 3. Impregnation - Same as Xylene 4. Sectioning - Inferior to Xylene 5. Nuclear Staining with H&E - Good 6. Cytoplasmic Staining with H&E - Good performance with Palm Oil 7. Clarity Staining with H&E - Good performance with Palm Oil 8. Nuclear, Cytoplasmic and Clarity Staining with H&E - Good

Authors & Date	Study	Types of Study	Population	Intervention	Comparison	Outcome
Chandraker, R., Rathod, V., Pundir, S., Dixit, S., Chandraker, N., Desai, V (2018)	Comparison Between Xylene and Coconut Oil in Tissue Processing	Experimental/ Comparative	2 equal halves of 25 oral soft tissue specimens which were stained with H&E	Pure coconut Oil	Coconut oil-treated specimen and Xylene-treated specimens	1. Translucency • Coconut oil - Less translucent • Xylene - translucent 2. Rigidity • Coconut oil - Soft • Xylene - Hard 3. Impregnation • Coconut oil - Soft • Xylene - Hard 4. Sectioning • Coconut oil - Difficult • Xylene - Easy 5. Quality of staining • Coconut oil - Same • Xylene - Same

Authors & Date	Study	Types of Study	Population	Intervention	Comparison	Outcome
Digala, Prathapavarma & Bollu, Dushyanth & Karthicka, C & Vincy, Sherafin & Selvam, Robert & Kandaswamy, Selvakumar. (2019)	Alternative To Reduce Occupational Hazards for Paramedical Staffs in Histopathology Department	Experimental	3 random tissue section from appendix, gallbladder, and hemorrhoid measuring approximately 0.5cm x 0.8cm and 1.5mm-3mm thick	Pure coconut Oil	Coconut oil-treated specimen and Xylene-treated specimens	1. Translucency: Coconut oil has similar translucency to Xylene. 2. Rigidity: Rigidity scored better in coconut oil than xylene. 3. Sectioning: There was no difference observed in the tissue bits after impregnation and ease of sectioning was observed in both coconut oil and xylene 4. Shrinkages: There was less shrinkage of tissue with coconut oil than xylene. 5. Quality of Staining (Nuclear Vs Cytoplasmic): There is no difference between coconut oil and xylene.

Authors & Date	Study	Types of Study	Population	Intervention	Comparison	Outcome
Prema, V., Prasad, H., Srichinthu, K. K., Kumar, S. S., Rajkumar, K., & Marudhamani, C. (2020)	Biofriendly Substitutes for Xylene in Deparaffinization	Experimental	15 tissue sections stained with xylene-free H&E stain using 100% coconut oil	Pure coconut Oil	Coconut oil-treated specimen and Xylene-treated specimens	1. Nuclear staining: Coconut oil is equivalent to xylene 2. Cytoplasm staining: Coconut oil is equivalent to xylene 3. Uniformity of staining: Coconut oil is inferior to xylene 4. Clarity of stain: Coconut oil is inferior to xylene 5. Crispness of stain: Coconut oil is equivalent to xylene 6. Wax retention: Coconut oil is inferior to xylene 7. Adequacy for diagnosis: Coconut oil is inferior to xylene

Authors & Date	Study	Types of Study	Population	Intervention	Comparison	Outcome
Saravanakumar, P., Bharanidharan, R., Ramadoss, R., Aravind & Kumar A. (2019)	Efficacy of "groundnut oil" and "coconut oil" as a substitute for "xylene" in clearing tissues samples - A comparative study	Comparative	45 specimens were used, 15 were subjected to Xylene, 15 in Coconut oil and 15 on another oil. These were stained using H&E	Pure coconut Oil	Coconut oil-treated specimen and Xylene-treated specimens	1. Cellular architecture • Cytoplasm - Coconut oil and Xylene have same discrete architecture and excellent nuclear-cytoplasmic contrast • Nucleus - Coconut oil and Xylene have same discrete architecture and excellent nuclear-cytoplasmic contrast 2. Staining quality: Coconut oil and Xylene both exhibits satisfactory staining quality

Authors & Date	Study	Types of Study	Population	Intervention	Comparison	Outcome
Sermadi, W., Sudeendra, P., Acharya, S., & Javali, SB (2014)	Comparing the efficacy of coconut oil and xylene as a clearing agent in the histopathology laboratory.	Experimental	60 Specimens were used. Stained by H&E Stain and PAS (for salivary glands)	Pure Coconut Oil	Coconut oil-treated specimen and Xylene-treated specimens	1. Gross Analysis: • Rigidity - Coconut Oil is Inferior to Xylene • Translucency - Coconut oil is Superior to Xylene • Impregnation - Coconut oil is Equivalent to Xylene • Sectioning - Coconut oil is Equivalent to Xylene 2. Shrinkage- There was no significant shrinkage in the tissue after clearing in coconut oil 3. Cellular architecture and Staining - Coconut oil is Equivalent to Xylene

3.2 Gross Analysis

3.2.1 Translucency

In a study done by Sermadi, et. al (2014), coconut oil-treated specimens are more translucent than those of Xylene-treated specimens. Digala, et.al (2017) presented those tissues subjected to coconut oil and Xylene have the same translucency. In the study conducted by Ashitha (2018), tissue specimens showed that the translucency of coconut oil-treated specimens is equivalent to the Xylene-treated specimens. However, in the study of Chandraker et.al (2017) and Prema et.al (2020), translucency is observed in specimens treated with xylene rather than those with coconut oil. Based upon the data gathered from the six experimental studies, the translucency of coconut oil-treated specimens is comparable and has increased translucency than Xylene treated specimens. Both xylene and coconut oil share a close refractive index, hence exhibiting similar performance in terms of translucency.

3.2.2 Rigidity

Results showed that the specimens have more rigidity in Xylene and less rigidity in coconut oil as stated in the studies performed by Sermadi, et al (2014) and Digala, et al (2017). Chandraker et. al (2017) has also presented this evaluation in

their study stating that tissue specimens were soft in coconut oil. Based on the 30 specimens prepared, coconut oil-treated tissues exhibited the same rigidity characteristics with Xylene in the study of Ashitha (2018).

3.2.3 Impregnation

According to the studies of Sermadi, et al (2014), Ashitha (2018), and Digala et al. (2014), Xylene and coconut oil as clearing agents have an equal performance after complete impregnation of tissues. There was no change in rigidity in all of the specimens used. However, none of the specimens treated with coconut oil showed superior characteristics with Xylene. In comparison with Xylene, coconut oil has shown high performance among other oils in terms of wax retention as per the study of Prema et al (2020). Meanwhile, the study of Chandraker et. al (2017) showed that specimens treated with coconut oil produced softer outcomes compared to Xylene.

3.2.4 Ease of Sectioning

In the study conducted by Digala et.al (2017), tissues treated with Xylene and coconut oil have been both graded as good in sectioning. Specimens cleared by coconut oil showed a similar sectioning characteristic with Xylene in the study performed by Ashitha (2018). On the contrary, Chandraker's study (2018) reported that sectioning with microtome was difficult in coconut treated specimens, whereas it was easy in case of Xylene specimens.

3.3 Nuclear and Cytoplasmic Staining

Specimens that used coconut oil as a clearing agent showed a better staining quality in terms of nuclear staining, cytoplasmic staining, and staining clarity (Ashitha, 2018). In the study conducted by Sermadi et.al (2014), no difference was presented in cellular, nuclear, and cytoplasmic staining when Xylene and coconut oil were compared among the specimens stained with Periodic Acid-Schiff (PAS). Chandraker et. al (2017) stated that Xylene and coconut oil exhibited an equal performance in staining the tissue specimens. As a support, Digala et.al (2014) concluded that there is no difference between tissues with coconut oil and Xylene. Based on the study of Prema et al (2020), the individual performance of 100% coconut oil presents adequate nuclear and cytoplasmic staining as well as uniformity, clarity, and crispness of staining in H&E staining. Additionally, the study performed by Saravanakumar, et. al (2019) highlighted that coconut oil exhibited a satisfactory result in the quality of staining.

3.4 Discussions

Xylene is known to be a hazardous chemical that can bring harmful effects not only to the laboratory workers but also to the environment. The goal of this systematic review is to examine various studies conducted over the last ten years to determine the efficacy of coconut oil as a clearing agent in comparison to Xylene, which is the clearing agent of choice in ordinary laboratories. Out of 241 published researches, only six (6) were eligible enough to be part of this systematic review. Following the collection of data, the intervention of coconut oil-treated specimens is evaluated using the following criteria: Translucency, Rigidity, Impregnation, Sectioning, and Quality Staining. Coconut oil is a viable and efficient option, but it should be used with caution because its effects on the tissues may impact the processing results. However, there are some limitations that the researchers encountered during the process such that there are no consistent results in measuring the efficacy of coconut oil as a clearing agent across the studies included in the data collection. The performance of coconut oil as a clearing agent may be affected by complications from in-between procedures of preserved tissue examination. Some authors did not mention other processes of tissue examination in their methodologies. There are some studies that do not contain criteria of a good clearing agent such as the physical property while some studies also discussed the properties of other oils and solutions as an alternative to Xylene. The authors recommended that to validate better results, more research with a larger sample size and more parameters can be used. The researchers also suggested that a consistent set of characteristics or criteria be used to determine whether alternative bio-friendly oils may be used as a substitute for Xylene.

IV. CONCLUSION

Clearing in histopathological process is mainly used to remove alcohol and contribute to the clarity or translucency of the tissue. This is an essential step for the histological technique since paraffin wax is not miscible with alcohol. Coconut oil can be used as an efficient alternative to Xylene in routine histopathological techniques. It permits less shrinkage to the tissues, it is colorless, it has high optical activity, and it has a long shelf life. All the studies stated that Xylene and coconut oil have attained the same characteristics in nuclear and cytoplasmic staining, translucency, and impregnation. While in terms of rigidity, most of the studies showed that softer tissue specimens were obtained using

coconut oil compared to Xylene.

Coconut oil is an efficient substitute for Xylene considering that it is not toxic, poses no serious health risk, and causes less shrinking of tissue. It is proven that using coconut oil, as a clearing agent, has a good result in terms of rigidity, translucency, and quality of staining in tissue specimens, which is important in tissue analysis. The observed tissue findings of coconut oil demonstrate efficacy as a clearing agent in histopathology laboratory. The only disadvantage with coconut oil as a clearing agent is that it solidifies at a lower temperature. To prevent this, the researchers suggest the clearing process be performed by setting the temperature at 60℃. By doing so, it can also lower its viscosity which hastens the penetration to the tissue.

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