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Comparative Evaluation of Tea Tree Oil and Carrot Oil as Clearing Agent for Tissue with Xylene

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Abstract

Background: Clearing is a critical step in histopathological tissue processing that facilitates paraffin infiltration after dehydration. Xylene has traditionally been the clearing agent of choice because of its rapid clearing ability and compatibility with paraffin wax. However, its toxic effects and environmental hazards have encouraged researchers to explore safer alternatives. Natural plant-derived oils have been investigated as potential substitutes due to their lipophilic properties and lower toxicity profile.
Aim: To evaluate and compare the clearing ability of tea tree oil, carrot oil for tissue with xylene.
Materials and Methods: Forty-five formalin-fixed soft-tissue specimens were included in this experimental study. Samples were divided into three groups: Group I – Tea tree oil (n=15), Group II – Carrot oil (n=15), and Group III – Xylene (n=15). Tissue sections of 4–5 µm thickness were prepared and stained with hematoxylin and eosin. Physical properties, gross tissue characteristics, and microscopic parameters were evaluated.
Results: Natural oils demonstrated lower volatility and absence of toxic fumes compared with xylene. Carrot oil exhibited translucency and rigidity comparable to xylene, whereas tea tree oil demonstrated satisfactory clearing capability. Microscopic evaluation revealed comparable staining characteristics and preservation of tissue morphology in all groups.
Conclusion: Tea tree oil and carrot oil demonstrated clearing efficiency comparable to xylene and may serve as safer alternatives for histopathological tissue processing.

Introduction:

Histopathological examination plays a crucial role in the diagnosis and management of various diseases. Accurate microscopic evaluation depends on proper tissue processing, which involves a sequence of steps including fixation, dehydration, clearing, infiltration, embedding, sectioning, and staining.[1,2] These procedures ensure adequate preservation of tissue architecture and facilitate optimal visualization of cellular details.

Among these steps, clearing is a critical stage in which the dehydrating agent present within the tissue is replaced by a substance that is miscible with both alcohol and paraffin wax.[3] Clearing agents render tissues translucent and enable effective paraffin infiltration, thereby allowing the preparation of thin sections suitable for microscopic examination.[4]

Traditionally, xylene has been the most commonly used clearing agent in histopathology laboratories because of its rapid clearing ability and compatibility with paraffin wax.[5] However, prolonged exposure to xylene has been associated with several adverse health effects including irritation of the skin and mucous membranes, central nervous system depression, and environmental hazards.[6,7] These concerns have prompted researchers to explore safer and environmentally friendly alternatives to conventional clearing agents.

In recent years, various natural substances and plant-derived oils have been investigated as potential substitutes for xylene due to their lipophilic nature and relatively low toxicity. Several studies have reported that natural oils can effectively replace xylene in tissue processing while maintaining acceptable histological quality. Ravindran et al.[9] demonstrated that vegetable oils could produce satisfactory histological sections comparable to those obtained with xylene. Similarly, Ankle and Joshi[11] reported that coconut oil could serve as an alternative clearing agent with good preservation of tissue morphology and staining characteristics.

Tea tree oil, derived from *Melaleuca alternifolia*, contains terpene hydrocarbons with lipophilic properties that may facilitate penetration into tissues and aid in the clearing process. Carson et al.[24] reported that tea tree oil contains biologically active terpene compounds that contribute to its solvent characteristics. Likewise, carrot oil obtained from *Daucus carota* contains lipid-soluble carotenoids and fatty acids which may enhance its

ability to act as a clearing agent during tissue processing. Khalil and Ramadan[25] described the presence of carotenoids and other lipid compounds in carrot oil that contribute to its biological and solvent properties.

Considering the increasing need for safer laboratory practices and eco-friendly alternatives to conventional clearing agents, the present study was undertaken to evaluate the effectiveness of tea tree oil and carrot oil as alternative clearing agents and to compare their performance with the conventional clearing agent, xylene, in routine histopathological tissue processing.

Materials and Methods: This experimental comparative study was conducted in the Department of Oral and Maxillofacial Pathology to evaluate the efficacy of tea tree oil and carrot oil as substitutes for xylene in routine histopathological tissue processing.

A total of 45 formalin-fixed soft tissue specimens obtained from routine diagnostic biopsies were included in the study. Only specimens adequately fixed in 10% neutral buffered formalin and measuring at least 0.5 cm were selected. Hard tissue specimens and poorly fixed or autolyzed tissues were excluded.

The specimens were randomly allocated into three groups based on the clearing agent used: Group I (tea tree oil, n = 15), Group II (carrot oil, n = 15), and Group III (xylene, n = 15).

All specimens were subjected to routine tissue processing procedures, including dehydration through ascending grades of alcohol, clearing, paraffin infiltration, and embedding in paraffin wax according to standard histological protocols.[2,3] The only variable in the study was the clearing agent used.

Paraffin-embedded tissue blocks were sectioned at 4–5 µm thickness using a rotary microtome. Sections were mounted on glass slides and stained with hematoxylin and eosin following standard procedures.

The processed specimens were evaluated based on physical properties of the clearing agents (odour, volatility, viscosity, health hazards, and ease of disposal), gross tissue characteristics (translucency, rigidity, tissue shrinkage, and ease of section cutting), and microscopic parameters (preservation of cellular architecture, nuclear staining quality, and cytoplasmic staining quality). All slides were independently assessed by two experienced histopathologists to minimize observer bias.

Results: The data obtained were analyzed using Statistical Package for Social Sciences (SPSS) version 20.0. Analysis of variance (ANOVA) and Chi-square tests

were applied to assess statistical significance among the groups.

Statistical analysis revealed no significant difference in microscopic staining quality among the three groups ($p > 0.05$). Gross tissue evaluation demonstrated adequate translucency, rigidity, and ease of section cutting in tissues processed with both tea tree oil and carrot oil, comparable to xylene. **(Table 1-3)**

Table 1: Physical Properties of Clearing agent

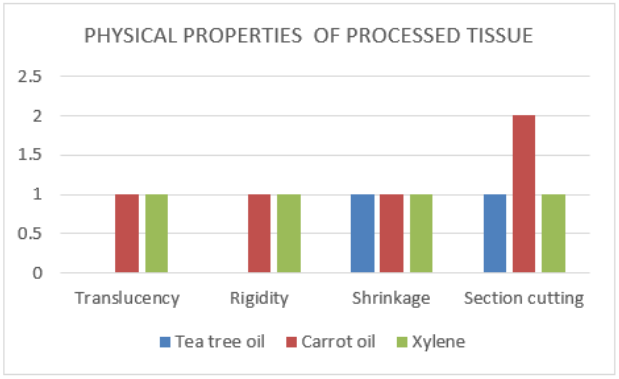
Physical properties	I Tea tree oil	II Carrot oil	III Xylene
Odour	Strong pleasant	Unpleasant	Strong pleasant
Viscosity	Medium	Medium	Less
Volatility - Health hazards	Non-volatile, non-toxic	Non-volatile, non-toxic	Volatile, toxic
Disposal	Recycled	Recycled	Not-recycled

Table 2: Physical Properties of Processed tissue

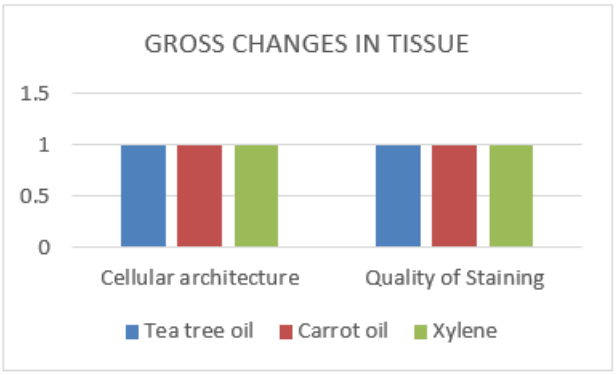
Physical properties	I Tea tree oil	II Carrot oil	III Xylene	P
Translucency	0	1	1	0.010^{S}
Rigidity	0	1	1	0.020^{S}
Shrinkage	1	1	1	0.014^{S}
Section cutting	1	2	1	0.016^{S}

Table 3: Gross changes in tissue

Physical properties	I Tea tree oil	II Carrot oil	III Xylene	p value
Cellular architecture	1	1	1	0.931^{NS}
Quality of staining	1	1	1	0.742^{NS}



Graph 1: Physical Properties of Processed tissue



Graph 2: Gross changes in tissue

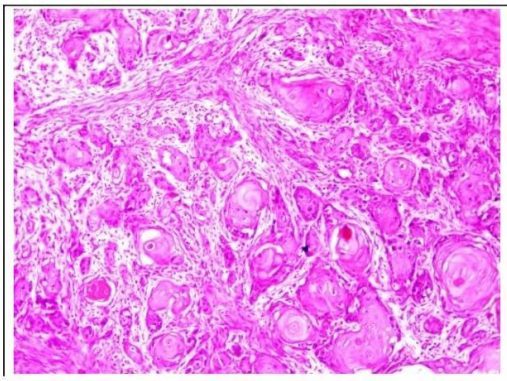


Figure1: Tea Tree Oil (10x view)

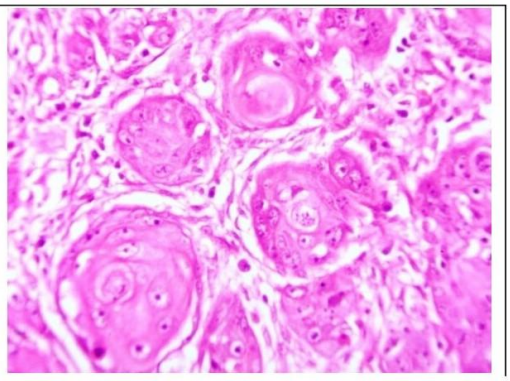


Figure2: Tea Tree Oil (40x view)

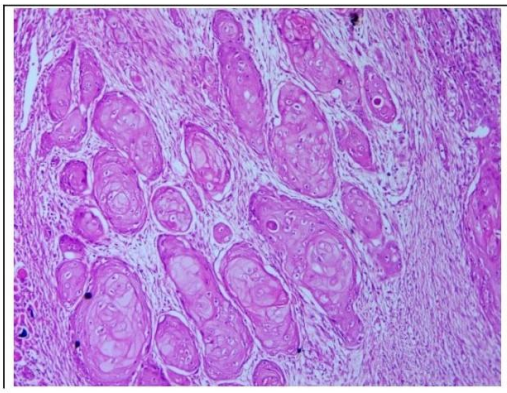


Figure3: Carrot Oil (10x view)

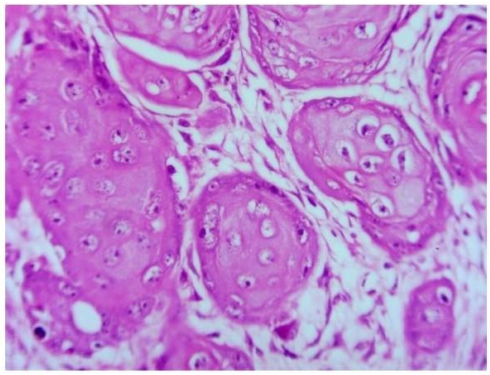


Figure4: Carrot Oil (40x view)

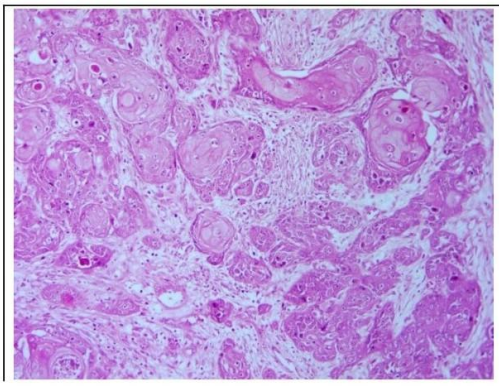


Figure5: Xylene (10x view)

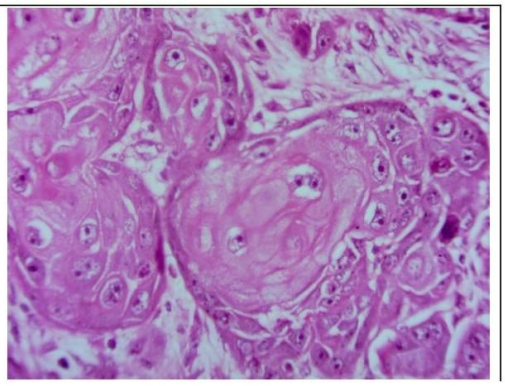


Figure6: Xylene (40x view)

Discussion:

Clearing is a critical step in histopathological tissue processing that renders tissues translucent and facilitates paraffin infiltration. Xylene has traditionally been the most widely used clearing agent due to its rapid clearing ability and compatibility with paraffin wax. However, exposure to xylene has been associated with several health hazards including mucosal irritation, neurotoxicity, and environmental toxicity.[6,7] These concerns have encouraged researchers to explore safer and eco-friendly alternatives.

Several investigators have evaluated the use of natural oils as substitutes for xylene in histopathology. Ravindran et al.[9] evaluated vegetable oils as clearing agents and reported that natural oils were capable of producing satisfactory histological sections comparable to xylene while reducing toxic exposure in the laboratory environment. Similarly, Bordoloi and Goswami[10] demonstrated that vegetable oils could effectively clear tissues and produce acceptable microscopic staining characteristics.

Another study conducted by Ankle and Joshi[11] evaluated coconut oil as a clearing agent and observed that the histological sections produced were comparable to those processed with xylene, with minimal tissue distortion and good staining quality. These findings support the concept that lipophilic natural oils can act as efficient clearing agents.

In the present study, tea tree oil and carrot oil were evaluated as alternative clearing agents. Both oils demonstrated lower volatility and milder odour compared with xylene, indicating improved laboratory safety. Similar observations were reported by Tanwar and Kaur[12], who evaluated eco-friendly substitutes to xylene and found that natural clearing agents significantly reduced occupational exposure to toxic solvents.

Evaluation of gross tissue characteristics in the present study revealed satisfactory translucency and rigidity in tissues processed with carrot oil and tea tree oil. These findings are consistent with the observations of Madhura and Naik[14], who reported that natural oils possess refractive indices close to tissue proteins and therefore can effectively render tissues transparent during clearing.

Microscopic evaluation in the present study showed well-preserved cellular architecture and satisfactory nuclear and cytoplasmic staining in all groups. Comparable findings were reported by Thajudeen and Hameed[13], who observed that natural oil-based clearing agents produced histological sections with

adequate staining quality and preserved tissue morphology.

Tea tree oil, derived from *Melaleuca alternifolia*, contains lipophilic terpene compounds that enhance its solvent properties and facilitate penetration into tissues during the clearing process. Carson et al.[24] reported that tea tree oil contains terpene hydrocarbons that contribute to its lipid-soluble characteristics. Similarly, carrot oil contains lipid-soluble carotenoids and fatty acids which may contribute to its clearing ability during tissue processing, as described by Khalil and Ramadan[25].

Overall, the findings of the present study indicate that tea tree oil and carrot oil can produce histological sections comparable to those obtained using xylene while offering the additional advantages of reduced toxicity and improved laboratory safety.

Conclusion: Within the limitations of the present study, tea tree oil and carrot oil demonstrated clearing efficiency comparable to xylene in routine histopathological tissue processing. Both oils preserved tissue morphology and staining characteristics while exhibiting reduced volatility and fewer health hazards compared with xylene.

However, the study had certain limitations including a relatively small sample size and evaluation limited to hematoxylin and eosin staining. Future studies involving larger sample sizes, different tissue types, and additional staining techniques are recommended to further validate the use of natural oils as routine clearing agents in histopathology laboratories.

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