

Efficacy Of WrighteriaTinctoria Oil As A Natural Alternative To DPX Mounting Media

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ABSTRACT:

Background: Mounting is a series of histological slide preparation. Most commonly used mountant is DPX, but owing to its hazardous nature, organic alternatives should be sought for. The aim of the present study is to evaluate and compare the efficacy of wrighteriatinctoria oil (WT oil) as an alternative to gold standard DPX mounting media

Material and methods: 19 histopathologically confirmed cases of OKC blocks were retrieved from the Department of Oral Pathology and four section were made from each block. Sections were subjected to routine H& E staining and four different mountants and a cytological assessment was made. Kruskal – Wallis ANOVA, was done to compare the groups. $p < 0.05$ was considered to be statistically significant.

Result: WT oil and DPX(2.63) had similar clarity of cellular details. Based on the adhesion DPX had a maximum median score of 2.95 compared to other natural alternatives.

Conclusion: WT oil showed superior quality as a mountant over castor and clove oil. It can be used as an alternative temporary mounting medium to DPX with the addition of some adhesive agents because of its excellent refractive index.

Keywords: Wrighteriatinctoria oil, clove oil, castor oil, DPX, Mounting media, refractive index

INTRODUCTION:

In the era of molecular pathology, many sophisticated, immunological and molecular biological techniques have been introduced which aids in precise diagnosis and treatment planning[1]. However, hematoxylin and eosin (H and E) stained paraffin sections still remain the most widely used technique to arrive at the proper diagnosis of the disease condition as in whether it is benign or malignant. For this reason, histopathological sections have to be preserved for a long period of time[2]. Mounting is the last procedure in the series of histological slide preparation. Mounting media is generally placed between tissue section and cover slip of the slide. The main purpose of mounting media is that it physically protects the specimen; the mounting medium bonds specimen, slide and coverslip together with a clear durable film[3]. The medium is important for the image formation as it affects the specimen's rendition[4].

Ideal requisites for the mounting media should be free flowing nature, colourless, free of air bubbles and it should have refractive index equal to protein(1.53), shouldn't diffuse or fade stained tissues and harden relatively quickly[5]. A mounting medium with an refractive index close to that of the fixed tissue will therefore render it transparent, with only the stained tissue elements visible[6]. Generally, the mounting media can be broadly classified into resinous and aqueous media.

Most commonly used mountant in routine histological preparation is DPX, a mixture of distyrene (a polystyrene), a plasticizer (tricresyl phosphate), and xylene. DPX is a colourless synthetic, non aqueous, mounting medium for microscopy. It preserves stains and dries quickly. Although it is considered as ideal it possesses a lot of drawbacks like toxicity of reagents used particularly xylene, cost containment, problem of disposal of hazardous substances and polluted work environment[7]. Due to its teratogenicity many natural alternatives have been sought out like castor oil, honey and clove oil.

Wrighteriatinctoria oil is natural, easily available, cost effective, nontoxic, noninflammable which is used in medical field for its antimicrobial properties, as a galactagogue to treat abdominal pain, skin diseases and wounds, as an antipyretic, anti-diarrheal- and anti-hemorrhagic agents, and as an antidote for snake poison[8]. The aim of the present study was to evaluate and compare the efficacy of wrighteriatinctoria oil as an alternative to gold standard DPX mounting media

MATERIAL AND METHODS:

Tissue Preparation: An Ex-Vivo comparative pilot study was done to compare and evaluate the efficacy of mounting media. The total sample size of 19 cases of formalin-fixed paraffin-embedded blocks of histopathologically confirmed cases of odontogenic keratocyst were retrieved from the archives of the department of oral pathology. Four sections of 3 μ m thickness from each block were prepared using a soft tissue rotary microtome (RM - 2245) and mounted on positively charged slides. The sections were deparaffinized after incubation, with xylene for 20 min, then they were subjected to routine H& E staining procedure. The slides were cleaned after staining and ready for mounting. The sections were labelled into four groups A, B, C and D.

Mounting Media:

Group A sections mounted with DPX

Group B sections mounted with Wrighteriatinctoria oil

Group C sections mounted with castor oil

Group D sections mounted with clove oil.

Evaluation of slides:

Two observers who were blinded to the choice of mounting media examined the slides under light microscopy (Olympus CG20i) and scored the slides based on the scoring criteria such as clarity of cellular details, the presence of air bubbles and adhesion of cover slip to the glass slide.

Scoring Criteria:

Clarity of cellular details and adhesion: Score 0 - no, 1 - mild, 2-good, 3- very good

Air bubbles: Score 1 - presence, 0- absence of air bubbles.

Any disagreements between the observers were resolved by asking them to evaluate the slides again.

Statistical Analysis:

The scores obtained were tabulated in excel sheets and assessed for the statistical significance using IBM SPSS Statistics software. Version 20. The scores of the samples between the groups were compared using Kruskal Wallis Anova. p value <0.05 was considered to be statistically significant.

RESULT:

According to the average mean score given by the two observers, Wrighteria tinctoria oil and DPX(2.63) had similar clarity of cellular details when compared with clove oil and castor oil with mean scores of 2.47 and 2.53. The kruskalwallis test results showed p value=0.9324 (p<0.05 statistically not significant). Based on the adhesion DPX had a maximum median score of 2.95 compared to Wrighteria tinctoria oil and castor oils with a mean score of zero. The difference was found to be statistically significant (p value= 0.0001). There was presence of air bubbles in all the three groups of mountant except clove oil used with an average score of 1. However the results were not found to be statistically significant (p value=0.0053). The results are represented in table 1 and figure 1.

Table 1: Comparison between DPX, castor oil, clove oil and Wrighteria tinctoria oil using Kruskal-Wallis test

Parameter	Mounting media	N	Mean	p- Value
CLARITY OF CELLULAR DETAILS	DPX	19	2.63	0.9324
	CASTOR OIL	19	2.53	
	CLOVE OIL	19	2.42	
	WT OIL	19	2.63	
ADHESION	DPX	19	2.95	0.0001
	CASTOR OIL	19	0	
	CLOVE OIL	19	0.32	
	WT OIL	19	0	
AIR BUBBLES	DPX	19	0.42	0.00553
	CASTOR OIL	19	0.42	
	CLOVE OIL	19	1	
	WT OIL	19	0.58	

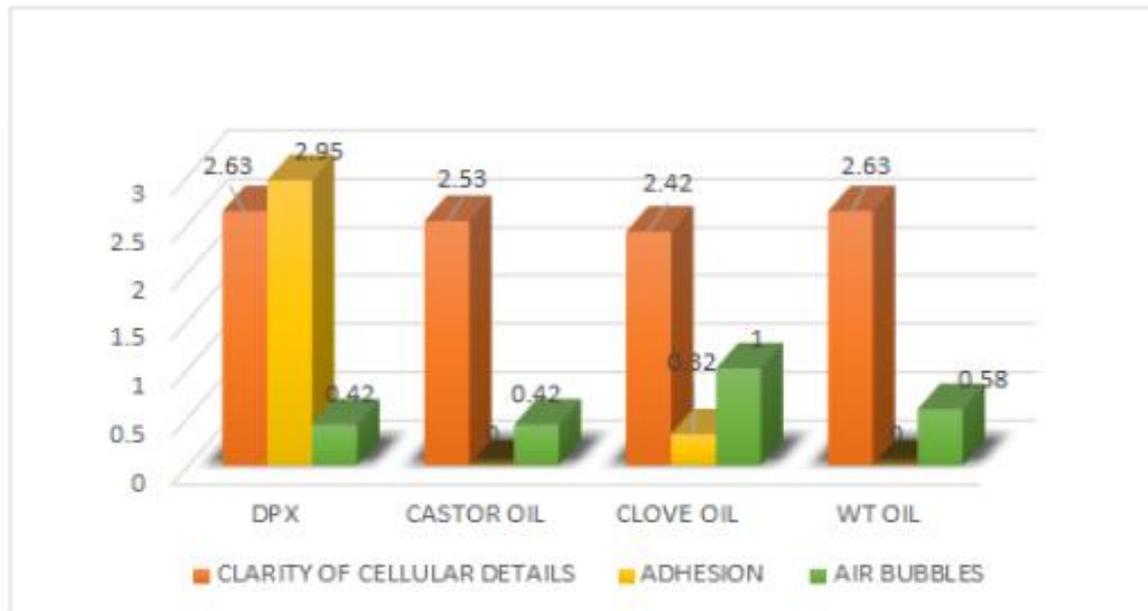


Figure 1: Bar chart depicting the comparison of means of different mounting medium

DISCUSSION:

Histopathology is the study of tissues where the tissue is fixed, processed and stained to view under microscope, such that the tissue structures resemble life-like conditions. Attempts to make confirmatory diagnosis are made possible only after the advent of histotechniques. Mounting is the last procedure in the series of histological slide preparation.

The mounting medium is the solution in which the specimen is embedded, generally under cover glass. The main purpose of mounting media is to physically protect the tissue sections. It should not shrink or cause the stain to diffuse or fade and it should be colourless, transparent, dry and must harden relatively quickly [4]

D.P.X is the most commonly used resinous media which satisfies all the requirements for an ideal mountant, yet it has its own set of disadvantages. According to Occupational Safety and Health Administration (OSHA), hazardous effects of D.P.X includes eye, skin and respiratory tract irritation, teratogenic, aspiration hazard if swallowed, can also enter lungs and cause damage and may cause nervous system depression [9].

This necessitates the need to introduce healthy and bio-friendly alternatives in the field of histotechnology. Hence many studies were carried out to seek a natural alternative to DPX because natural alternates are cheaper, renewable resources and also have low toxicity when compared to synthetic agents. Kannan et al.,2017 and Shylaja et al.,2019 had already studied the effect of castor oil and honey as an alternative to DPX mounting media [10]^[11]. Choosing a right mounting media involves certain factors to be considered, such as refractive index, compatibility with specimen, shrinkage, durability, cost and ease of use [10].

The present study is carried out with easily available, cheap and natural substance *WrighteriaTinctoria* oil as an alternative to DPX. Slides mounted with *WrighteriaTinctoria* oil showed superior quality of cellular details over other natural oils (Figure 2-5).

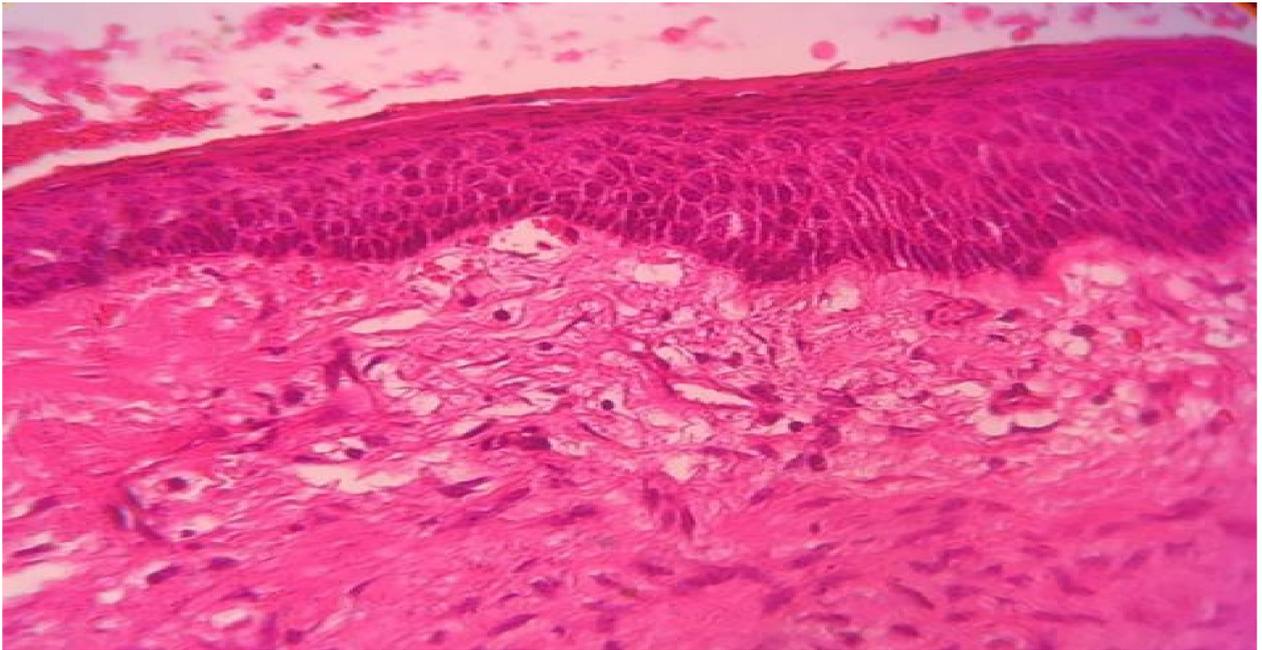


Figure 2: Photomicrograph showing the H&E staining of tissue section mounted with Wrighteriatinctoria oil (Mag: 10x)

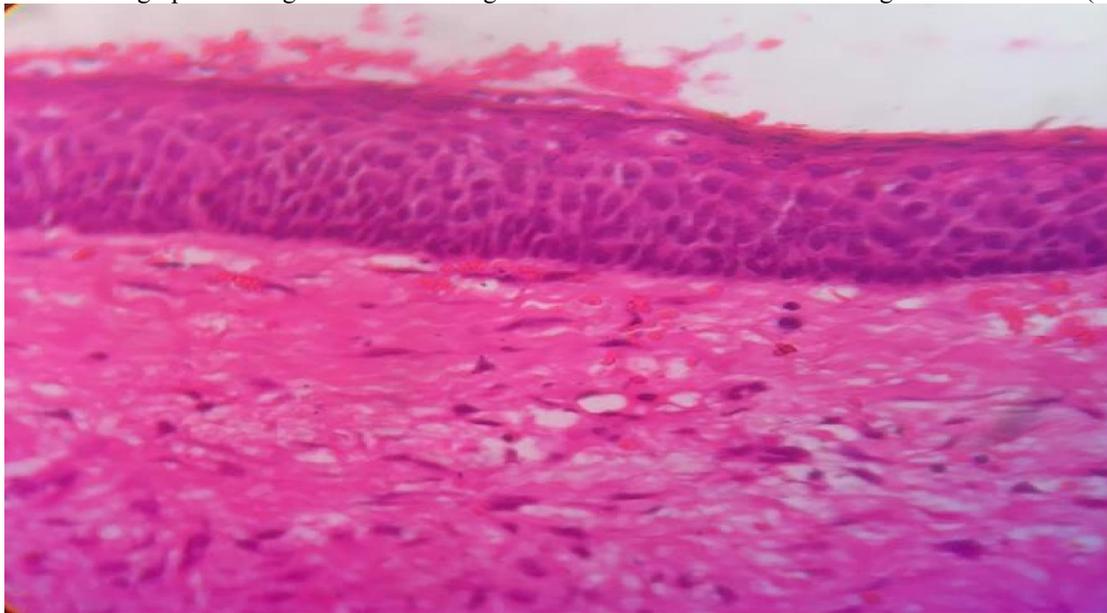


Figure 3: Photomicrograph showing the H&E staining of tissue section mounted with DPX (Mag: 10x)

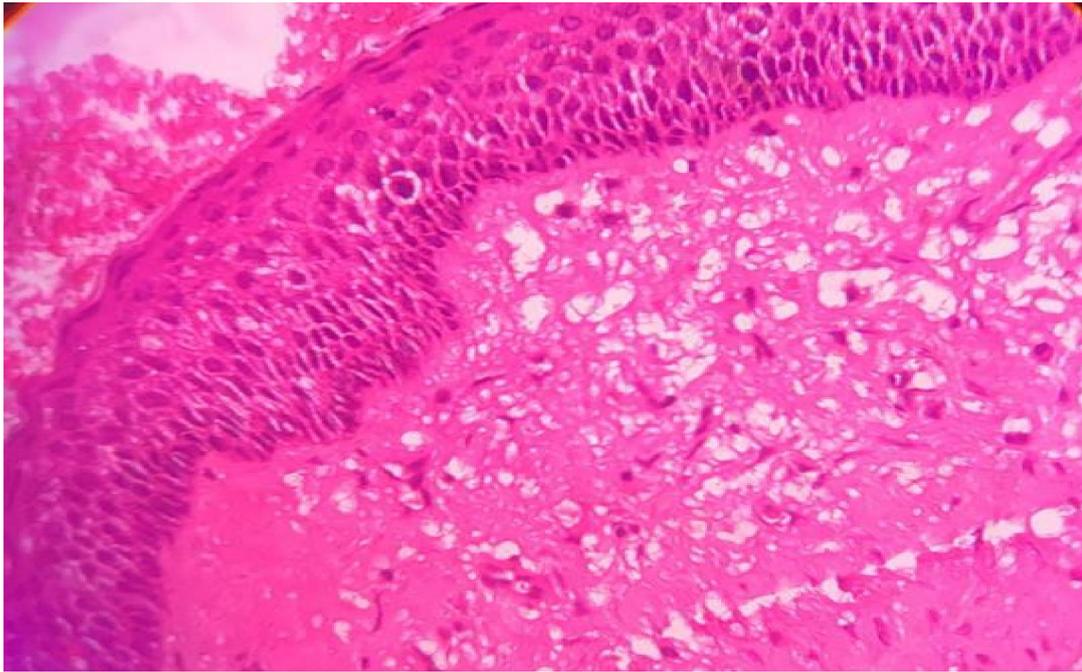


Figure 4: Photomicrograph showing the H&E staining of tissue section mounted with castor oil (Mag: 10x)

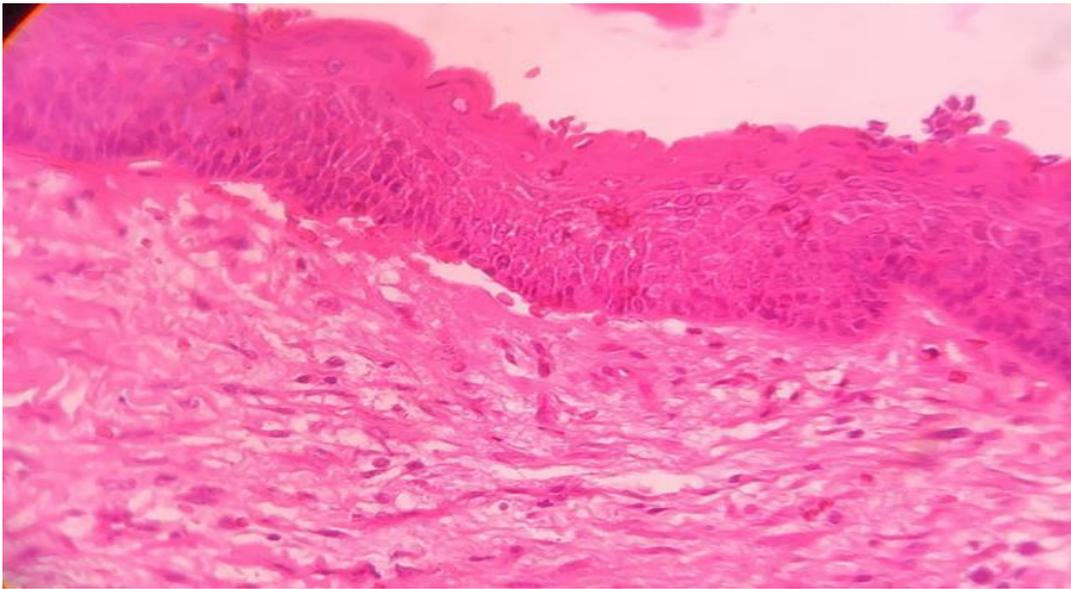


Figure 5: Photomicrograph showing the H&E staining of tissue section mounted with clove oil (Mag: 10x)

The refractive index of Wrighteria tinctoria oil is almost equal to glass and has good transparency over the tissue section. Though castor oil has a refractive index near to the glass slide and tissue section, it produced poor clarity of cellular details compared to other natural oils as a mountant.

Based on adhesion, the slides mounted with castor oil, clove oil and Wrighteria tinctoria oil showed only cohesiveness and no adhesion of the cover slip to the slide was observed. The coverslip slid when pushed with fingers or when in physical contact with an object. If not there was no movement of the coverslip and the slides with DPX as mounting media showed maximum adhesion to the glass slide. The natural oils used as a mountant didn't harden or shrink which makes it easier for the coverslip to be removed and remounted when air bubbles are encountered.

According to Kannan et al., 2017 & Shylaja et al., 2019 castor oil shows superior quality as a mountant compared to DPX and honey respectively, but in the present study Wrighteria tinctoria oil is more superior than castor oil and clove oil in terms of clarity and transparency [10]^[11].

Mounting slides with Wrighteria tinctoria oil is an innovative attempt. This attempt of ours is the first of its kind with no existing literature on the usage of Wrighteria tinctoria oil as DPX substitute. Among the natural mounting media investigated, the humble Wrighteria tinctoria oil has all the novel qualities which could be used extensively as an alternative to DPX. However, further studies with large sample sizes and parallel studies focusing on increasing the adhesive nature of Wrighteria tinctoria oil using additives needs to be conducted to determine whether the properties that influence mounting are universal.

CONCLUSION:

Wrighteria tinctoria oil as a mountant shows superior quality of cellular architecture compared to DPX and castor oil. At the same time, it lacks adhesive property. So it can be used as a bio safe temporary alternative to DPX. This pioneer eco-friendly idea needs further research to expand its application.

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