

# Natural Alternative to D.P.X (Mounting Medium) - Comparison between Honey and Castor Oil

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## ABSTRACT

**Introduction:** To go organic is the theme of the present day. With added benefits of both honey and castor oil being eco-friendly, easily available, cost effective, nontoxic and non-inflammable, they can also be used as an effective natural alternative to synthetic mounting media as they have good refractive index. Study aimed to evaluate and compare the efficacy of honey and castor oil with D.P.X as a mounting medium.

**Material and Methods:** Six paraffin-embedded blocks with a confirmed histopathological diagnosis of pyogenic granuloma were taken from the archives. Three sections were taken from each block and subjected to routine H & E staining procedures, and later they were mounted with three different mounting media and analyzed for different parameters.

**Result:** The results were tabulated. Kruskal wallis test was used for comparing the results. Statistically significant results were observed for different mounting media and castor oil showed better details of cellular architecture than D.P.X and honey.

**Conclusion:** Both Castor oil and honey as a mounting media showed a superior quality over D.P.X due to their properties but they do not adhere to the slide as well as cover slip with lack of adhesive agents. Both can be used as an alternative to D.P.X with the addition of some adhesive agents because they are having a good refractive index.

**Keywords:** Honey, Castor Oil, D.P.X, Mounting Media, Refractive Index

sections from external environment, thus providing physical protection for the specimen and it will stick the specimen and cover slip to the glass slide with a clear film formation without interruption to histopathological examination procedures.<sup>2</sup>

Most widely used mountant in histopathology laboratories is D.P.X. A mixture of distyrene (a polystyrene), a plasticizer (tricresyl phosphate), and xylene. It was introduced in 1939 and later modified by the substitution of a more satisfactory plasticizer, dibutylphthalate (butyl, phthalate, and styrene-BPS). Its advantages are its ability to preserve stains and ability to dry quickly, at the same time it is a known irritant as xylene is one of the component of it.

In this regard alternative natural substances can be used as mountants such as honey and Castor oil. Honey is a delicious viscous sweetener made naturally by bees for their own nourishment. Its flavor and texture vary with the type of flower nectar from which it was made most. The physical properties of honey vary depending on the specific flora used in its products as well as its water content. Honey is known to have antioxidant, antimicrobial, and soothing properties. It is made up of glucose, fructose and minerals such as iron, calcium, phosphate, sodium, chloride, potassium and magnesium and it is viscid fluid with a refractive index 1.3-1.72.<sup>4</sup>

Castor oil is clear colorless slight viscous oil. Pure castor oil has been used in the preparation of lubricants, dyes, resins, and paints and also used in oil immersion for high power objectives in smear examination procedures. As colorless viscid oil, it produces good cellular details and also preserves

## INTRODUCTION

The histopathological sections should be examined for diagnostic purposes during treatment plan. For this, they have to be preserved for a longer period of time.<sup>1</sup> In the histopathological laboratory procedures, tissue mounting is the last step, and the used mountant should be placed between a cover slip and tissue section glass slide. Ideal mountant should not interrupt the diagnostic procedure at the same time it should not damage the tissue sections to be diagnosed, for this it must have closure or same refractive index of tissue sections as well as glass slide.<sup>2</sup> Refractive index (RI), also called index of refraction, is the measure of the bending of a ray of light when passing from one medium into another and it will help in the proper visualization of structural details of the object.<sup>3</sup>

Generally, mounting media are available in solution form; they can be soluble in water and alcohol. Based on availability it is mainly divided into two classes, which are aqueous media and nonaqueous/ resinous media. The main uses of mounting medium are: sealing the histopathological

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tissue stains. Castor oil has an RI of 1.477-1.48. It is easily removed by clearing agents such as Xylene.<sup>5</sup>

Based on their properties both honey and castor oil are used in nonmedical and medical purposes and also used for treatment purposes like asthma, skin allergies. Both are used in the preparation of edible items and also used as flavoring agents. When compared to honey, castor oil is a clear, colorless fluid and it can be used as a lubricant. Apart from these advantages both honey and castor oil have good refractive index and it will aid in microscopic evaluation of tissues. Study aimed to evaluate and compare the efficacy of honey and castor oil with D.P.X as a mounting medium.

**MATERIAL AND METHODS**

To evaluate the efficacy of mounting media, six paraffin-embedded blocks of histopathologically confirmed pyogenic granuloma cases were taken from the archives of the department of oral pathology of SVS institute of dental sciences. Three sections of 4-5 μm thickness from each block were made with the help of soft tissue microtome. The prepared sections were cleared in xylene for 20mins and later they were subjected to routine H&E staining procedures, all the processing parameters were same for all sections. Finally, first group of sections were mounted with D.P.X, second set were mounted with honey and last set of sections were mounted by pure castor oil. Three observers were asked to give scores from 0-3 for parameters such as adhesion, clarity of cellular details, presence of air bubbles and transparency.

Score 0 stands for No/Absence and 1, 2, 3 for mild, good, and very good for all parameters, respectively.

**Statistical test:** Obtained data was analyzed statistically by using “Kruskal Wallis” with statistical software Graph pad prism version 6.0.

**RESULTS**

According to the median scores given by the three observers, castor oil had better clarity of cellular details and transparency with median score 3 over D.P.X and honey (Table-1, Figure-1 and graph-1). Based on adhesion D.P.X had maximum median score 3 than castor oil and honey (Table-1, Graph-1).

Kruskhal wallis test was used for compare the statistical significance of three different mounting media, where all P value were < 0.05.

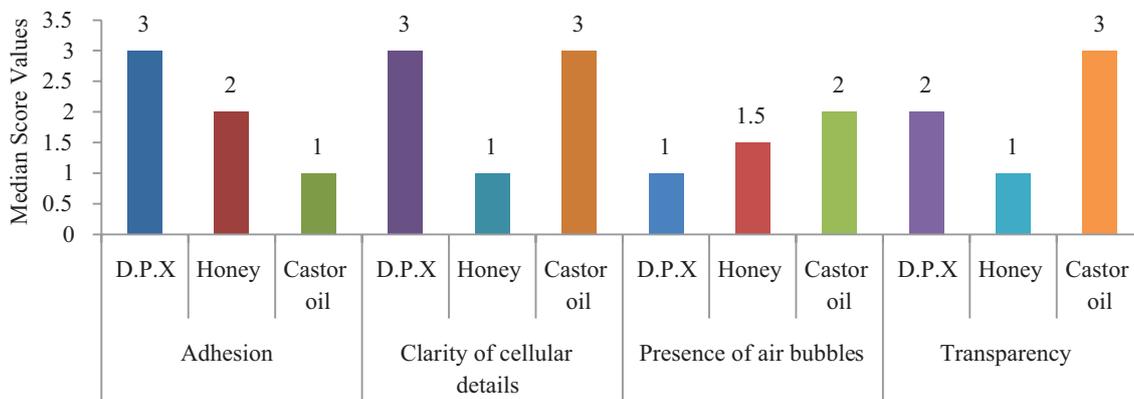
**DISCUSSION**

The mounting medium is the solution in which the specimen is embedded, generally under cover glass. It may be liquid, gum or resinous substance, soluble in water and alcohol. 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 colorless, transparent, dry and must harden relatively quickly.<sup>6</sup>

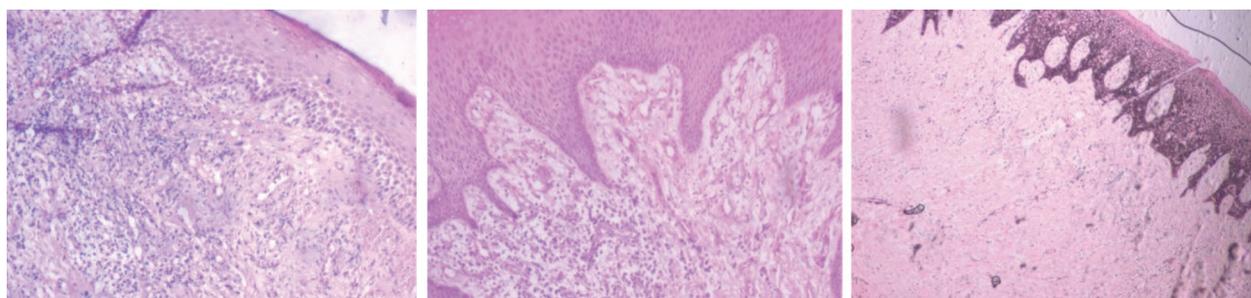
The examination of histopathological sections done by using microscope which is an electrical device. When the target or tissue section is focused, a magnified image can be

Parameters	Mounting media	N	Minimum	Maximum	Median	IQR	P-value
Adhesion	D.P.X	6	3	3	3	3 to 3	0.0004
	Honey	6	1	2	2	2 to 1.75	
	Castor oil	6	1	1	1	1 to 1	
Clarity of cellular details	D.P.X	6	2	3	3	3 to 2	0.001
	Honey	6	1	1	1	1 to 1	
	Castor oil	6	2	3	3	3 to 2.75	
Presence of air bubbles	D.P.X	6	1	1	1	1 to 1	0.003
	Honey	6	1	2	1.5	2 to 1	
	Castor oil	6	2	3	2	3 to 2	
Transparency	D.P.X	6	2	3	2	3 to 2	0.0004
	Honey	6	1	1	1	1 to 1	
	Castor oil	6	3	3	3	3 to 3	

**Table-1:** Showing scores for the different parameters given by three observers



**Graph-1:** Simple mean bar diagram for the different parameters of different mounting media



Castor oil

D.P.X

Honey

**Figure-2:** Photomicrographs of tissue sections mounted with different mounting media

observed through the ocular lens by its illuminated light.<sup>2</sup> The illuminated light energy is nothing but a refractive index. This refractive index should not alter by used substances in histopathological sections such as mounting media.<sup>3</sup> To get proper cellular details refractive index of glass slide, mounting media and tissue sections should be similar. In this study naturally available substance such as honey and pure castor oil were used as an alternative to commonly used synthetic resin such as D.P.X. which is routinely used in histopathology laboratory procedures and which also have similar refractive index.<sup>2,7</sup>

D.P.X is the most commonly used resinous media with several advantages.<sup>1</sup> Although D.P.X satisfies all the requirements for an ideal mountant, it also has some disadvantages such as setting time and hazardous effects due to presence of xylene and dibutyl phthalate as its principal constituents. This drawback is given by OSHA and also by manufacturers themselves in the safety data sheet of D.P.X. The 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, may be harmful if absorbed through skin, may also cause central nervous system depression.<sup>8</sup>

Histopathology lab is the place where the specimen gets processed and stained to view under microscope for interpretation. Exposure to the chemicals used in these processes cause various health hazards to the laboratory technicians, pathologists, and scientists working in the laboratory. Hence, there is a need to introduce healthy and bio-friendly alternatives in the field. There are several natural mountants such as glycerine and also many recipes available to make own mounting medium.<sup>9</sup> The natural ones may be cheaper than commercial products, but shows variable refractive indices. Choosing a right mounting media involves certain factors to be considered, such as, toxicity, refractive index, compatibility with specimen, pigment stability, shrinkage, durability, cost and ease of use.<sup>10</sup>

Present study also carried out with naturally available substances such as honey and castor oil as an alternative to commonly used mountants. We observed that as colorless viscid fluid castor oil produced good clarity of cellular details and transparency over the D.P.X and honey (Table 1, Figure 1 and Graph 1), though honey has refractive index near to the glass slide and tissue section, it produced poor cellular

details and transparency as compared to castor oil and D.P.X (Table 1, Figure 1 and Graph 1). This could be because of its more viscosity.

Kannan et al., 2017 conducted a study to compare the quality of castor oil with D.P.X as a mounting medium and they concluded that Castor oil showed superior quality and characteristics as a mounting media on basis of clarity of cellular characteristics, which is in accordance with our study results.<sup>5</sup>

To our best knowledge present study is the first study conducted with honey as mounting media. Unprocessed honey was used in present study and it exhibited poor quality of cellular details over castor oil and D.P.X on basis of parameters such as clarity and transparency.

## CONCLUSION

Castor oil as a mounting medium showed a superior quality over honey and D.P.X as it produced good clarity of cellular details and transparency, at the same time, it does not adhere to the slide as well as cover slip, but it can be used as an alternative to D.P.X with the addition of some adhesive agents. Honey showed a poor quality of cellular details and it has a good refractive index. It can be also used as an alternative choice to routinely used synthetic resin D.P.X by mixing of some resinous substances and proper purification to lighten its colour and to remove dust particles.

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