

## Alternative To Reduce Occupational Hazards For Paramedical Staffs In Histopathology Department

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### Abstract:

**Introduction:** Xylene (Xy) is an aromatic hydrocarbon known for its wide usage in tissue processing on human anatomical samples which has numerous health hazards. **Objective:** Use of edible oil such as coconut oil (Co) and groundnut oil (Go) has been experimented successfully as a substitute for xylene in tissue processing. **Materials and Methods:** To compare the ability of conventional tissue processor with Co and Go have been studied. 3 sections of each tissue of appendix, gallbladder, haemorrhoid, were processed using, Co and Go and compared with Xy. **Results:** Slides were scored for physical properties, gross changes, cellular details and morphometric analysis. The physical properties of Co and Go shows the pleasant odour, more viscous and non-volatile while compare with Xy, the translucency, rigidity, shrinkages and section cutting of Co and Go were enhanced while compare with Xy. The cellular details and morphometric analysis of Co and Go were also observed better while compare with Xy. **Conclusion:** The present study revealed better results with edible oil (Co and Go) in tissue processing and suggests its use as an alternative to Xy to avoid occupational health hazards.

**Key words:** Coconut oil, Groundnut oil, Occupational Health hazards, Tissue processing, Xylene

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### I. Introduction

To categorize a disease or deficiency is generally identified by the clinical laboratory diagnosis (Rai R et al., 2016). Among many departments in diagnostic services, histopathology plays a vital role and major focus in recent trends. Xylene (Xy) is one of the major chemical which widely used in the histopathology departments both in hospitals and stand-alone laboratories. (Xy) is C<sub>6</sub>H<sub>4</sub>(CH<sub>3</sub>)<sub>2</sub> are different forms are known as ortho xylene, meta xylene, and para xylene. Mixed Xy is a mixture of 3 isomers it contains 6-15% of ethylbenzene. It appears colorless liquid, volatile in nature with sweet odour. Agency for toxic substance and disease registry(ATSDR)describes about the adverse effect of xylene and its wide usage in industrial side printing rubber and leather industries. Human expose to Xy by different ways especially in the pathology laboratory technicians have higher risk. These technicians are long term exposed to Xy on the tissue processing as clearing agent. (Rajan T S et al., 2014) assessed the workers in the histopathology Laboratory proved that inhalation of Xy causes irritation of nose, throat it leads to gastric discomfort, head ache, vomiting and nausea during long term exposure. Xy shows in different problems such as drying scaling kidney injuries and some fatal blood syscrasis.(Kandyala R et al., 2010 and ATSDR, August 2007).

(Uchida Y et al., 1993) studied that the human exposed to Xy for 8 hours/ day for 7 years found the defect in cognition, anxiety, and inability of concentrate. (Xiao et al., 2001) analyzed the equality of semen on workers of who exposed to the organic solvents like benzene toluene and xylene. The activity of acrosin, motility, and sperm viability found decreased. Xylene, benzene and toluene were found in blood and semen, it shows that it can cross blood-testis barrier which adversely effect on sperm. (Xiao G et al., 2001)These chemical compounds transfer male to female so that Xy can ultimately cause infertility. Gestational exposure of workers on organic solvents and the outcome of pregnancy shows increased risk of major malformation and more miscarriages. (Khattak S et al.,1999) low birth weight in newborn babies.(Chen et al., 2000)lab technicians are also a one of the category for this assessment of workers.

A pilot study was done by (Sravya T et al., 2013) used sesame oil and limonene oil as an alternative for Xy to deparafinize. The sections were stained with Haematoxylin & Eosin (H & E). (Sermadi W et al., 2014) studied with the coconut oil for the soft tissue processing such as skin, lymphnode, salivary gland, muscle. The quality of cellular architecture, morphometric analysis and Quality of staining were found equal competence to

that of Xy. (Udonkang M et al., 2015) studied in 15 different types of tissues from bird and used bleached palm oil as a substitute to xylene. The beached palm oil used for tissue processing and deparaffinizing after heated at 60° C to reduce the viscus nature and helps to penetrate easily into the tissue. The results were showed satisfactory by exhibit the properties of similar to that of xylene. Since palm oil is one of the food stuff which found no health hazards. Carrot oil, olive oil, pine oil, and rose oil also used as a clearing agent instead of Xy (Swamy SR et al., 2015). He also studied the stability and longevity of H & E stain for one year which shows no significant difference in the quality of stain. Liver, lung and small intestine samples were collected from Wistar rats and the tissues were processed with ground nut oil as a clearing agent. The groundnut oil has special kind of property it can miscible with xylene and paraffin wax and it cannot miscible with alcohol.(Esan EO et al., 2015) It was observed the refractive index in tissue looks more transparent.

In recent years the researchers were used the natural oil for histopathological techniques as clearing agent in the tissue processing and also used in deparaffinizing of tissues. As a clinical laboratory professional we found may laboratory technicians were involved in the histopathology process. The study is to evaluate the efficacy of Coconut oil (Co) and Ground nut oil (Go) in the human tissue as a clearing agent which found a lacunae in the literature.

## II. Materials And Methods

### Reagents:

10% Formalin, isopropyl alcohol, acetone, xylene, coconut oil, groundnut oil, paraffin wax, egg white (adhesive), Hot water, Ice cubes, Hematoxylin & Eosin, 1% acid alcohol, ammonia water, butanol, D.P.X mountant,

### METHODS

#### Sample Collection and fixation

Samples were randomly collected from the operation theatre of Billroth Hospitals and the biopsy and surgical specimen were fixed with neutral 10% buffered formalin for 24 Hrs. Specimens were selected after the grossing made for the patient by the pathologist for official reporting. Since it is a pilot study the few bits were selected for this study from the anatomical structures from appendix, gallbladder, and hemorrhoid. The study proposal was approved by the IEC / BH/Lab/2016/003.

**Grossing and labelling of the specimens:** The gross description of an anatomic pathology specimen is essential for its complete pathologic evaluation and is complementary to the microscopic description. Randomized selections of different tissue (a. Appendix, b. gallbladder, c. Hemorrhoid) samples were taken from the fixed 10% formalin and grossing was done. Each tissue specimen was cut into 3 equal sections and each measure approximately 0.5cm x 0.8cm and thickness ranging from 1.5mm-3mm and then each section was labelled and placed in cassettes. First bit was placed in Xy, second in Co and the third in Go respectively.

**Tissue Processing:** The principle of tissue processing is to remove the extractable water from tissue specimens and replace it with a medium that solidifies to allow sectioning. It consists of 3 stages which are dehydration (Isopropyl alcohol for 30 min, Acetone-I for 30 min and Acetone-II for 30 min), clearing (Xylene-I for 30 min and Xylene-II for 30 min) and infiltrating (liquid paraffin wax for 1 Hr). The purpose of dehydrating is to remove water from the tissue using graded alcohols from a lower to a higher concentration. Clearing is to remove alcohol from the tissue with a solvent that is miscible with paraffin wax such as Xylene. Infiltrating is to infiltrate the tissue with paraffin wax to allow sectioning of tissues.

## III. Study Design:

**Table: 1: STANDARD AND EXPERIMENTAL PROCEDURES I AND II FOR TISSUE PROCESSING**

Standard Procedure	Experiment procedure - I	Experimental procedure - II	Time Duration
Isopropyl alcohol	Isopropyl alcohol	Isopropyl alcohol	30 min
Acetone-I	Acetone-I	Acetone-I	30 min
Acetone-II	Acetone-II	Acetone-II	30 min
Xylene-I	Coconut oil-I	Groundnut oil-I	30 min
Xylene-II	Coconut oil-II	Groundnut oil-II	30 min
Liquid paraffin wax	Liquid paraffin wax	Liquid paraffin wax	1 hr

Labelled sample cassettes were placed in a glass bottle containing Isopropyl alcohol and it was incubated for 30 min at 60°C. The sample cassettes were removed from isopropyl alcohol and placed in Acetone I and incubated for 30 min at 60°C. The sample cassettes were removed from Acetone-I and placed in the Acetone II and incubate for 30 min at 60°C. Total sample cassettes were separated according to the labelled (Xy-

A to C), (Co – A to C) and (Go– A to C). All samples were measured for the gross changes such as translucency rigidity, and shrinkage. Then samples were processed for further steps. Samples (Xy-A to C) were transferred into the Xy - I for 30 min and incubated at 60°C. Samples (Co–A to C) were transferred into the Co for 30 min and incubated at 60°C and samples (Go– A to C) were transferred into the Go for 30 min and incubated at 60°C. The total samples were again checked for gross changes rigidity, translucency and shrinkage in compare with before and after the experimental process. All samples were transferred into liquid paraffin wax for infiltration to remove the clearing agent.

**Embedding:** Embedding is the method in which the tissue or the specimens are enclosed in a mass of the embedding medium using a mold. Embedding is carried out after complete impregnation of the tissue. The tissue is sliced in micrometer thickness to allow the passage of light through them for microscopic examination. Since it is very thin they need a supporting medium in which the tissue sections are entangled. The cleaned dry big steal tray was taken for embedding. Small amount of liquid was poured in the tray 1/3 of the tray. Each cassette was opened and transferred the tissue into the tray according to the labelled number with appropriate distance. Tray was filled with the samples and poured liquid wax allowed to convert into solid wax. Tray was separated from the melted wax and cut the sample pieces with melted wax around it. Each sample piece was trimmed in appropriate size for sectioning.

**Block Making:** Steel knife was heated and then it was placed on the wood piece. Then paraffinized sample was placed on hot knife. It was melted slightly and allowed liquid wax on wood surface. Block was attached to the wood surface while slightly pressed it.

**Section Cutting:** Through the motion of the sample holder, set the thickness gauge to 15 µm. The sample is cut by the knife in which the fresh section remains on the knife. At the highest point of the rotary motion the sample holder is advanced by the same thickness size (5µ) as the section that is to be made allowing the next section to be made. The section was transferred into water bath at 40-45°C. Section was taken on the slide by dipping into the hot water bath. Adhesive were taken on glass road and applied on the slide. The adhesive was spread throughout the side simultaneously, sections were made fixed on it. Later it was kept in hot air oven for 15 min to deparaffinization.

**Staining:** Deparaffinised to distilled water. Slide was washed with Xy I and II for 3 min respectively. Slide was washed with Isopropyl alcohol I and II for 2 min each later slide was rinsed in tap water for 2 min. Then slide was stained with Hematoxylin for 5 min and kept under running water for 1 min. Then slide was dipped once in 1% acid alcohol and kept under running water for 2 min. Again slide was dipped in 1% ammonia solution for 60 sec and kept in running water for 2 min. Slide was dipped once in 1% aqueous eosin and dipped 15 times in Butanol, 15 times in Butanol & Xylene finally dipped 15 times in Xylene and allow them to dry using blotting paper

**Mounting:** Dry sterile coverslip was taken and placed on blotting paper. Two to three drops of DPX was dispensed on the coverslip with the help of glass road. Mounted the cover slip on the stained area of slides and it became ready to observed under the microscope.

#### IV. Results

##### 1. Comparative assessment of physical examination on Edible oils (Co, Go) Vs xylene (Xy) (Table 2)

PROPERTIES	COCONUT OIL (Co)	GROUNDNUT OIL (Go)	XYLENE (Xy)
Odour	Pleasant	Pleasant	Strong unpleasant
Viscous	More	More	Less
Volatility	No	No	Volatile
Cost	Less compare with Xylene	Less compare with Xylene	High
Health hazards	No	No	Yes

Promising results were found on Edible oils (Co, Go) Vs Xylene (Xy) on the comparison of nature and physical properties such as odour, viscosity, volatility cost and health hazards. While comparing with odour of Edible oils Vs Xylene, Xy found strong unpleasant, less Viscous, volatile and high is cost. Above all Xy was reported as potential health hazard.

**2. Comparative assessment of gross tissue specimen examination on Edible oils (Co, Go) Vs xylene (Table 3)**

After clearing was made using Edible oils and Xy the following features were assessed such as

- a. translucency of a tissue (surface translucency when viewed for reflected light),
- b. Rigidity of a tissue (change in the rigidity because of infiltration of wax).
- c. Shrinkage of sections
- d. Section Cutting patterns

S. No.	GROSS CHANGES	COCONUT OIL (Co)	GROUNDNUT OIL (Go)	XYLENE (Xy)	
1.	<b>Translucency</b>	<b>1</b>	<b>2</b>	<b>1</b>	
2.	<b>Rigidity</b>	<b>0</b>	<b>0</b>	<b>1</b>	
Scoring was given while comparing the parameters with Edible Oil Vs Xylene: Translucency was scored equal while compare with Co and high on compare with Go, Among rigidity scored good in Edible oil while compare with xylene					
3.	<b>Shrinkages</b>	<b>Clearing</b>	<b>Mean value</b>	<b>Standard deviation</b>	<b>p. value</b>
	Xylene	Before	1.54	1.004	0.049
		After	1.15	0.753	
If $p < 0.05$ then we can reject null hypothesis (Ho) therefore, there is no significant difference between before shrinkage of Xylene and after shrinkage of Xylene					
	Coconut oil	Before	1.54	1.004	0.995
		After	1.51	1.002	
If $p > 0.05$ then we can accept null hypothesis (Ho) therefore, there is no significant difference between before shrinkage of coconut oil and after shrinkage of coconut oil					
	Groundnut oil	Before	1.54	1.004	0.995
		After	1.51	1.002	
If $p > 0.05$ then we can accept null hypothesis (Ho) therefore, there is no significant difference between before shrinkage of groundnut oil and after shrinkage of groundnut oil					
4.	<b>Section Cutting :</b>				
	Comparison with edible oils (Coconut oil and Ground nut oil), the section cutting found good in Groundnut oil while compare with Xylene and Coconut oil.				

Tissues cleared in different solvents showed similar gross changes compared to that of Xy. However, Translucency of the tissues cleared in Go was superior with that of Xy and Co has similar translucency to Xy (table 3). There was no difference observed in the tissue bits as far as rigidity after impregnation and ease of sectioning was observed (Figure 1), in all the groups. There was no significant shrinkage in the tissue bits after clearing made with edible oils ( $P = 0.9958$ ) However, with respect to Xy-S, the specimen shrank significantly, when compared with the measurements taken before clearing ( $P = 0.049537$ ). On other hand Co, Go were showed less shrinkage of tissue compared to Xy.

**3. Cellular Description**

Clearing Solutions	Cytoplasm	Nuclear	Quality of staining
Coconut oil	1	1	2
Groundnut oil	1	1	2
Xylene	1	1	2

**Cytoplasmic scoring:** Score 0 (Indistinct nucleus cytoplasm), Score 1 (Distinct nucleus cytoplasm). **Nuclear scoring:** Score 1 (Distinct chromatin condensation, prominent nuclear membrane and crisp staining of the nucleus, Score 0 (Indistinct smudging and psychosis of the nuclei). **Quality of Staining:** Score 0 (Poor), Score=1 (Satisfactory), Score=2 (Good). (Sermadi W et al., 2014)

Tissue cellular description was preserved in all the sections cleared with the three different groups and a clear distinction was observed between nucleus and cytoplasm. The overall staining quality was almost equivalent with that of Xy (**Figure 1-3**). The microscopic observation shows similar cellular details in three different solvents Go (**Figure 1-3**) and Xy (**Figure 1-3**) and Co(**Figure 1-3**) **Morphology Analysis:** After reviewing, the sections were further subjected to morphometric analysis. The images were captured using Nikon eclipse 80i microscopic attached to a binocular microscope with a 100X objective (Figure)

## **V. Discussion**

Considering the toxicity of Xy and its hazards, various substitutes, including vegetable oils and mineral oils, have been tried in the past. However, most of them showed an inconsistent outcome in research tissues, which motivated us to take up this study in the health care providers. Edible oils (Co & Go) were selected for this study as these are profusely available in India. These are edible, cost effective and non-hazardous, when compared with hazardous Xy. These are not harmful to the environment. Based on Bernoulli's principle of fluid dynamics, the edible oils works in viscosity of the fluid is indirectly proportional to temperature, as temperature increases viscosity of the fluid decreases and penetration of fluid increases in the cells.

The present study was proved that edible oils (Co & Go)after clearing was apparently more translucent compared to Xy. The Co and Go are less in rigidity shrinkage in contrast to Xy. It did not adversely affect impregnation and section cutting. Morphometrically, the shrinkage was relatively less in Co and Go when compared with Xy. However, there were no changes in cellular architecture, nuclear and cytoplasmic staining, when both groups were compared to that of Xy. (Esan E O et al., 2015) used Go as a clearing agent instead of Xyon the tissues of Wistar rats and tried different types of special stains which showed results no significant difference from tissues cleared with Xy. Go increases the refractive index of the tissue and make the tissue looks more transparent.

(Buesa R J et al., 2009) used a mixture of ethanol, isopropyl alcohol and mineral oil as an alternative for Xy and found the mixture to be as efficient as Xy in dealcoholization. Instead, the study conducted in environment friendly, readily available alternative, edible oils (Co & Go), to prevent occupational health hazards. A mixture of coconut oil and olive oil was tried by (Rasmussen B et al., 1992) and they noted incomplete impregnation, leading to problems in the cutting sections and therefore, they concluded that this mixture was ineffective as a clearing agent. In contrast to their observation, these study edible oils found that effective as Xy, without interfering with further impregnation and cutting.

(Andre G G et al., 1994) substituted Xy with a mixture of peanut oil, soyabean oil, coconut oil and cotton oil and concluded that it was a poor alternative, as the quality of sections with respect to Xy were better. The present study showed the cellular arrangements, morphology, structure and stain in good quality. Even the staining found satisfactory for the pathologist to report, proving no interference by both the oils with the tissue composition and it used as a transient media. As the result of our study showed less shrinkage was found in Co and Go while, compared to Xy and Go shows high translucency among all the three (Co, Go, Xy), in morphometric studies. The only drawback associated with coconut oil, is its tendency to get solidified at a lower temperature. However, this can avoid by using this procedure using incubator, to maintaining the temperature. This research study is unique, as we have tried to assess the efficacy of two clearing agents with Xy at different stages of the histopathological procedure, such as, processing, impregnation, and sectioning, staining and microscopic evaluation. It also plays a major role to avoid occupational health hazards to the paramedical staffs.

## **VI. Conclusion**

As the edible oil is equality contribute its significance in the tissue processing, which revels the pathologic difference as such while compare with Xy. Though, both have no difference in quality of the stain, cell morphology, structure and results. This study suggest to use reduce the occupational hazard to the paramedical health care providers. Since it is less expensive than the Xy, edible oils can be used as an alternative in the histopathology department for tissue processing as a clearing agent.It can also be confirmed with the further research on all stains and advanced histological procedures like immunohistochemistry which should not affect the patient's results.

**Conflict Of Interest: Nil**

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**Fig . 1.** Arrangement of tissue and the section cutting

**Fig 1a.**



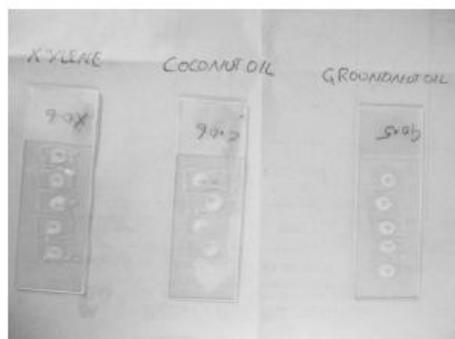
**Fig.1a :** Arrangement of Tissues in the Histopathology department

**Fig 1b.**

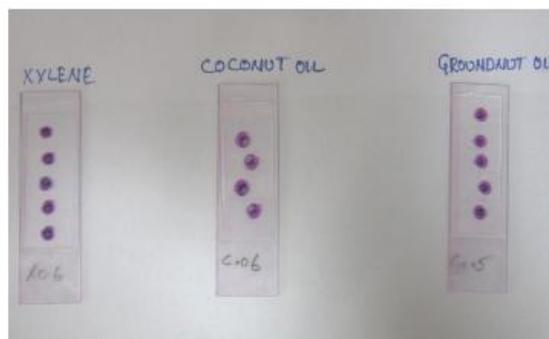


**Fig 1b:** shows good section cutting in three different solvents Xylene, coconut oil and groundnut oil.

**Figure 2: Sectioning and the staining of the tissues in the Edible oils and Xylene**



**Fig. 2a.** shows good sections on the slide in three different solvents Xylene, coconut oil and groundnut oil.



**Fig. 2b.** Slides shows good staining quality in three different solvents after mounting

**Fig. 3. Microphotograph of the appendix processed using Edible oil and the Xylene and stained with H & E**

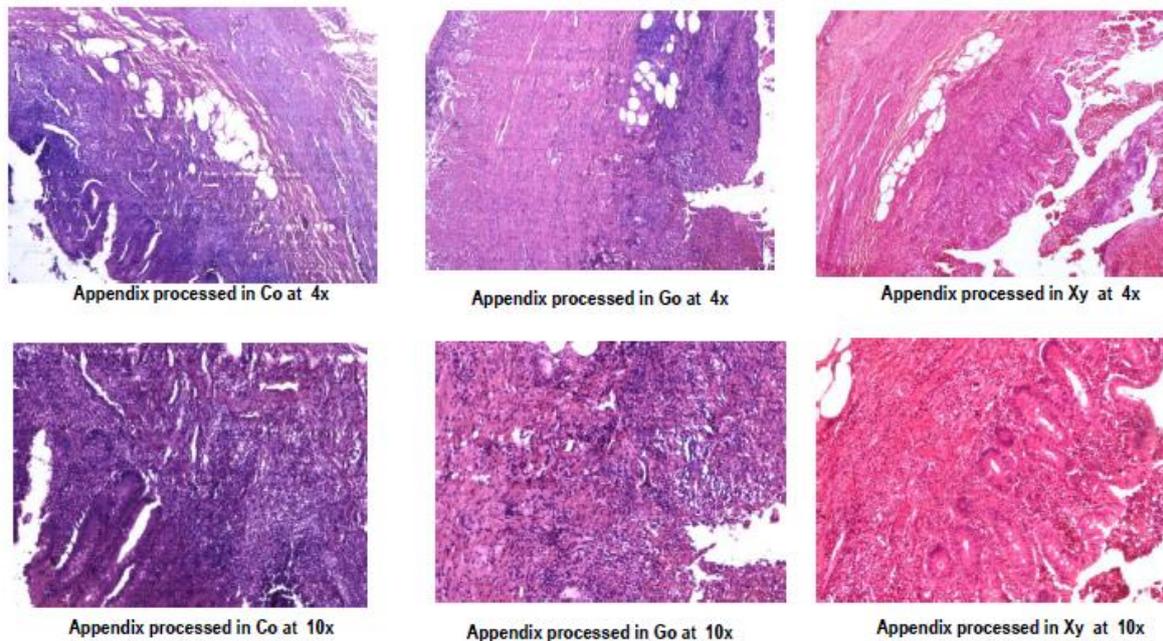


Fig.3. Representative picture of appendix of 3 individual bits. Microphotograph of the appendix processed using Co, Go, Xylene and stained with H & E

**Fig. 4. Microphotograph of the gall bladder processed using Edible oil and the Xylene and stained with H & E**

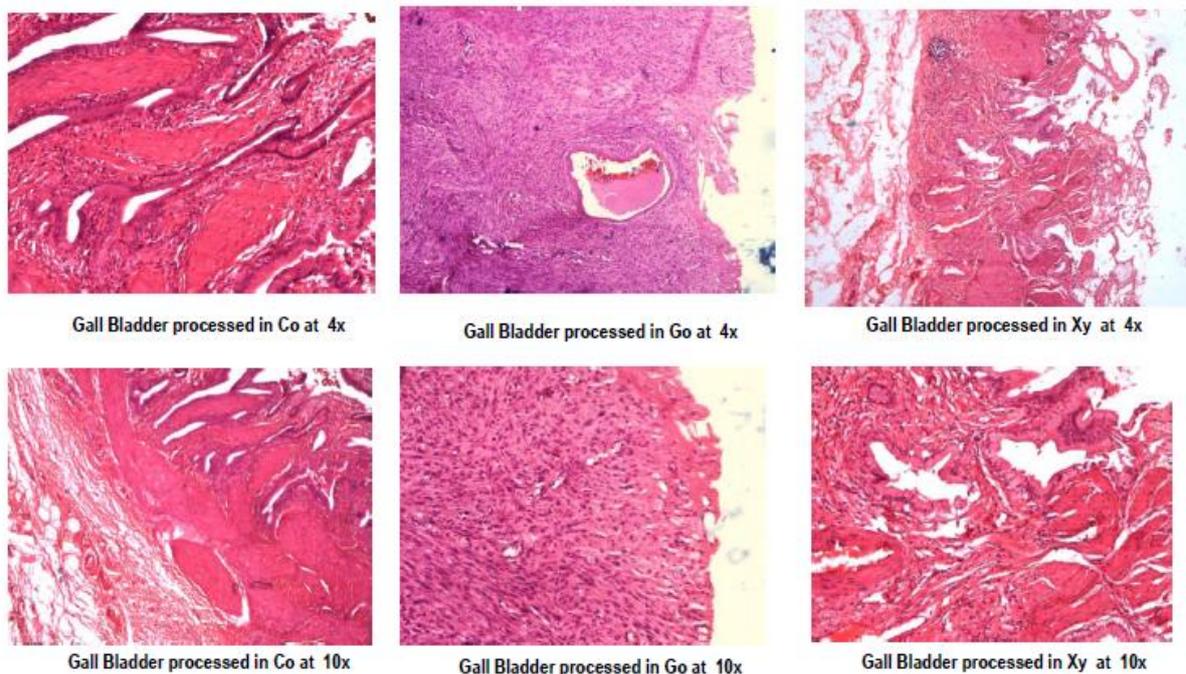


Fig.4. Representative picture of gall bladder of 3 individual bits. Microphotograph of the appendix processed using Co, Go, Xylene and stained with H & E

**Fig. 5. Microphotograph of the haemorrhoid processed using Edible oil and the Xylene and stained with H & E**

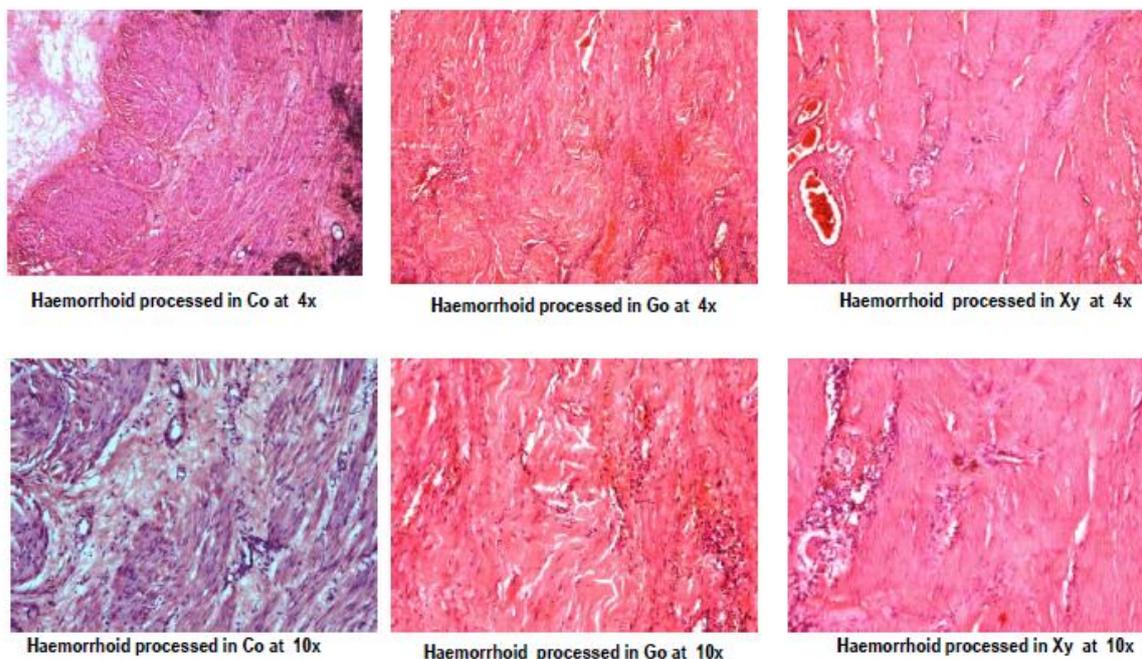


Fig.5. Representative picture of haemorrhoid of 3 individual bits. Microphotograph of the appendix processed using Co, Go, Xylene and stained with H & E

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