

EVALUATION OF ISOPROPANOL AS A BETTER ALTERNATIVE TO XYLENE IN TISSUE PROCESSING BY THE PARAFFIN WAX METHOD

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ABSTRACT

Aim: The aim of the study was to find a suitable less toxic alternative to xylene in the paraffin wax method of tissue processing.

Methods: Twenty albino rats were randomized into two groups of ten animals each. One group was fed with normal diet while the second group received in addition to the normal diet, 20mg/kg of omeprazole for 7days. They all had water freely. The second group was given 2ml ethanol to induce gastric mucosa injury, and sacrificed two hours later. One group of the tissues were processed by the paraffin wax technique while the second group was processed with replacement of xylene with isopropyl alcohol. Sections were stained with haematoxylin and eosin and examined.

Results: There was no difference between the xylene treated routine paraffin wax method and the isopropanol replacement method in the sectioning, staining and diagnosis of the tissues of stomach, liver and kidney. The isopropanol method was also faster.

Conclusion: xylene in the paraffin wax method should be replaced with a less hazardous isopropanol.

Key words: Isopropanol alcohol, Omeprazole, Tissue processing, Xylene

INTRODUCTION

Xylene, an aromatic hydrocarbon is widely used in industry and medical laboratory as a solvent. It is used as a clearing agent in Histopathology to increase the refractive index of tissue. It is a flammable liquid that requires utmost care during its usage. On exposure, the vapour is rapidly absorbed through the lungs and slowly through the skin. Prolonged exposure to xylene leads to significant amount of solvent accumulation in the adipose and muscle tissue. When tissue biopsies are exposed to xylene for a long time, they become brittle. Blood containing tissue and brain tend to be more brittle in xylene (Avwioro, 2014; Sharada and Malathi, 2014). Xylene is a combination of the three isomers: Ortho, Para and Meta. This combination is referred to as Xylol (Jacobson *et al.*, 2003). The hazardous effect of xylene became indisputable in the 1970s; many potential substitutes became available, some with many if not more hazards (Rene and Maxim, 2009). Some of the demerits of xylene are as follows: 1.) It is associated with vasodilation of the skin of the hand, dryness and scaling of the area and skin erythema of the

hand, toxic eczema of the hands (Engstram *et al.*, 1977; Riihimaki, 1978). 2.) Direct exposure to vapour of xylene causes urticaria-elicitation of erythema and whealing of the skin (Palmer and Rycroft, 1993). 3.) It also causes irritation of the eye (Nelson *et al.*, 1943; Hake *et al.*, 1981; Uchida *et al.*, 1993). Xylene is one of the top 30 chemicals produced in the United States of America in terms of volume. It is used as solvent in the rubber, printing and leather industries. It is also used as a thinner for paints, cleaning agents and in varnishes. A small amount of xylene is also found in airplane fuel and gasoline (U.S Department of Health and Human Services, 1995). Xylene is released from industrial sources, automobile exhaust and consumer products such as cigarette smoke, paints, varnish, rust preventives. The amount of xylene in urine can be analysed using techniques such as High Performance Liquid Chromatography and Gas Chromatography Agency for Toxic substance and disease Registry, {1995}.

Routine Xylene Method of Tissue Processing

Tissues for this method must have been fixed in a fixative of choice such as neutral buffered formalin. Tissues of 3-5mm in thickness are passed through a range of dehydrating alcohols, 70%, 90% and three or four baths of absolute alcohol. The tissues are cleared in two baths of xylene and impregnated with molten paraffin wax (Cook and Hitchkiss, 1977). The automated processing takes 22-48hours depending on the nature of the tissue (Wallington and Drufy, 1980).

Isopropyl Alcohol Processing Method (IPA)

Isopropyl alcohol is miscible with water, ethanol and molten paraffin wax. Therefore, it acts both as a dehydrant and as a clearing agent in the paraffin wax method of tissue processing. It is used in the manufacture of some agricultural chemicals, some pharmaceuticals, and as a solvent for chlorhexidine, a disinfectant (Logsdon *et al.*, 2000). The histology turnaround time is the time in hours from when the tissue is received at the reception to when the result is signed out to the patient (Novis *et al.*, 1998). With IPA method of tissue processing, the processing time for tissue blocks, 3-5mm has been reduced from 22 hours in xylene conventional method to 9 hours.

MATERIALS AND METHOD

Omeprazole was obtained from the Pharmacy of University of Benin Teaching Hospital, Benin City. The drug was dissolved in distilled water and administered to the rats in concentrations of 20mg/kg body weight (2ml/kg).

Animals

The experiments were performed using adult albino rats of either sex weighing 150 to 200g, acclimatized for two weeks in the Animal House of the Department of Anatomy, University of Benin, Benin City, fed with standard pellet diet (Bendel Feeds and Flour Mill tunted, Ewu, Nigeria) and allowed free access to water. They also received human care in accordance with the international guidelines (NRCNA, 2011).

Ethanol-Induced Ulcer Experiment

Rats were randomly assigned to two groups (n=10 per group). The rats in group one were given 2ml/kg of the Omeprazole while rats in 2nd group were given 2ml/kg of distilled water for 7days (Nora *et al.*, 2016). Rats in the two

groups were sacrificed 1hr after administering 2ml/kg of ethanol by using Orogastric tubes to induce ulcer in the gastric walls of the rats. The animals were anesthetized with chloroform vapour in a chamber. The stomach was immediately excised. Tissues of stomach, heart, kidney and liver were fixed in neutral buffered formalin.

Xylene Processing Method

Tissue blocks of 3-5mm thick were dehydrated in 70% alcohol for 3 hours, 90% alcohol for 3hours, two baths of absolute alcohol for 1 hour each, two baths of alcohol for 2 hours each. Clearing was done in two baths of xylene for 1½ hours each. Impregnation was done in two baths of cell path wax at 56% for three hours each. The tissues were embedded in metals moulds on Thermo Scientific Tek II embedding centre, Microm EC 350-1 and Microm EC 350-2.

IPA- Processing Method

The fixed tissue specimens were processed in Thermo Scientific Spin Tissue Processors STP 120. The tissues were treated for half hour each in three baths of 50% alcohol. They were transferred into three baths of 80/20 Ethanol/IPA for half hour each and later treated in three baths of neat Isopropyl alcohol (IPA) for 1 hour each. The samples were allowed to drain for 2 hours before they were immersed in two baths of cell path wax at 56°C for 1½ hours each.

Sectioning

The tissue blocks were trimmed at 10µm to expose the surface of the section. The sectioning was done on LEICA RM2125 RTS at 3-5µm, floated on Raymond A Lamb Circular Paraffin section mounting bath about 45°C and dried on a histology hotplate at 58°C

Staining

The slides were stained with heamatoxylin and eosin (H&E) as prescribed by Avwioro (2014). The sections were dewaxed, hydrated and stained in Erlich's heamatoxylin for 15minutes. They were rinsed in water, differentiated in 1% HCl in 70% alcohol for 1minute, rinsed and blued in tap water for 10minutes. They were counterstained with 1% eosin for 2 minutes, rinsed in water, dehydrated, cleared and mounted (Avwioro, 2014).

RESULTS

The gross appearance of the gastric mucosa in the test group revealed severe lesions while rats pre-treated with 2ml/kg omeprazole (20mg/kg) had their stomach protected. The histopathological results showed severe

ulcerations in all animals in test group as compared with omeprazole group. The H&E staining reactions of the xylene processed tissue were not different from the IPA processed tissue. The ease of sectioning was much better in IPA treated slides.

MACROSCOPY

Gross Appearance of Mucosal Gastric Lesions



Fig 1a: Mucosal gastric lesions in test group



Fig 1b: Mucosal gastric lesions in test group



Fig 2a: Omeprazole treated group



Fig 2b: Omeprazole treated group

MICROSCOPY

OMEPRAZOLE TREATED RATECTIONS

Isopropyl Alcohol-Paraffin Wax Method

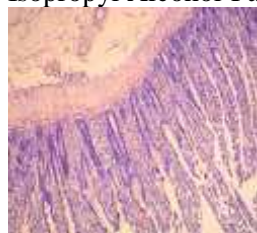


Fig 3a: Small intestine: Mild tissue edema



Fig 3b: Kidney: Engorged blood vessels, fresh interstitial haemorrhage



Fig 3c: Liver: Fresh parenchymal hemorrhage

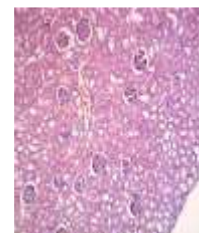


Fig 3d: Kidney: Normal histology

Xylene -Paraffin Wax Method

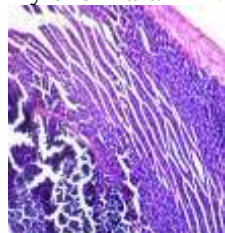


Fig 4a: Small intestine: Mild tissue edema

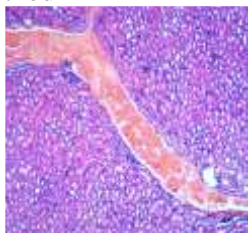


Fig 4b: Kidney: Engorged blood vessels, fresh interstitial haemorrhage

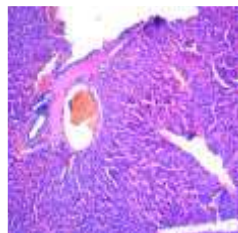


Fig 4c: Liver: Fresh parenchymal hemorrhage

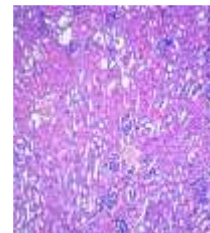


Fig 4d: Kidney: Normal histology

MICROSCOPY

Isopropyl Alcohol-Paraffin Wax Method

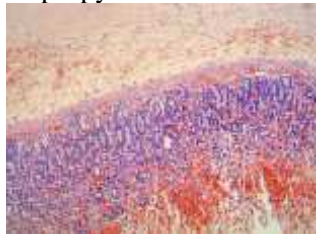


Fig 5a Gastric: Extensive ulceration, hemorrhage and dense infiltration by mixed inflammatory cells

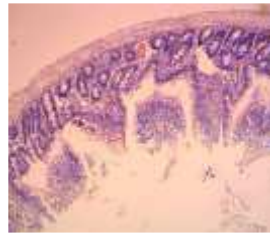


Fig 5b: Small intestine: edema with some scanty inflammation

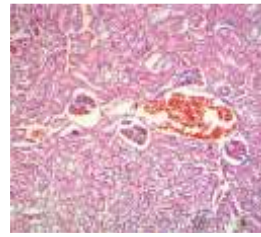


Fig 5c: Kidney: acute tubular necrosis, fresh interstitial hemorrhage

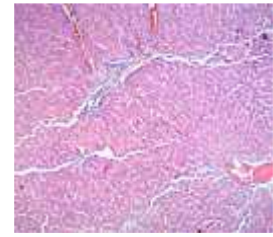


Fig 5d: Liver: Ballooning degeneration

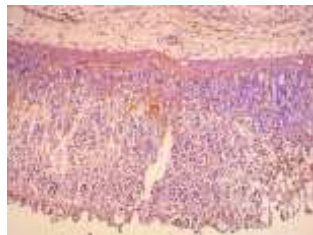


Fig 5e: Stomach: Extensive ulceration of the epithelium with mixed inflammatory



Fig 5f: Small intestine: Tissue edema, No ulceration

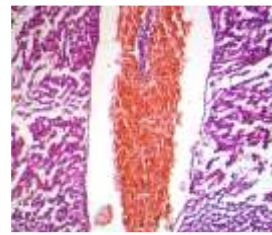


Fig 5g: Liver: Some fresh haemorrhage with the liver parenchymal

Xylene -Paraffin Wax Method

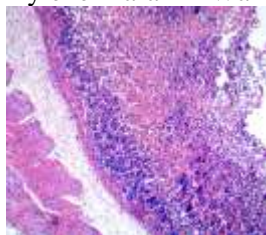


Fig 6a: Gastric: Extensive ulceration, hemorrhage and dense infiltration by mixed inflammatory cells

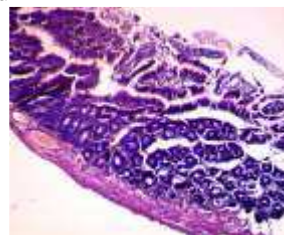


Fig 6b: Small intestine: edema with some scanty inflammation

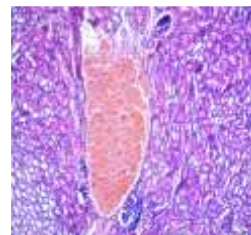


Fig 6c: Kidney: acute tubular necrosis, fresh interstitial hemorrhage

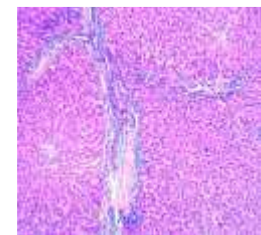


Fig 6d: Liver: Ballooning degeneration

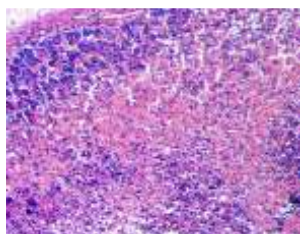


Fig 6e: Stomach: Extensive ulceration of the epithelium with mixed inflammatory



Fig 6f: Small intestine: Tissue edema, No ulceration

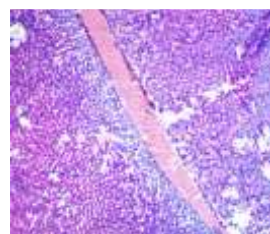


Fig 6g: Liver: Some fresh haemorrhage with the liver parenchymal

DISCUSSION

Most histopathology laboratories in Nigeria still employ the xylene method of tissue processing despite the demerits and hazards involved in its use. Isopropanol gives a better turn-around time. The staining and sectioning in IPA paraffin processed tissue are comparable with the xylene method in all details. Diagnosis of gastric extensive ulceration, edema with scanty inflammation of small intestine, acute tubular necrosis of the kidney and ballooning degeneration in liver were all exemplified in both methods of processing. There was a reduction in turn-around time. The staining and ease of sectioning increased significantly, $p < 0.01$. The result of this work is in tandem with the previous authors who confirmed that isopropanol can serve as an alternative clearing agent in place of xylene. Andrew Betting, (2011) found isopropanol as a potential alternative to xylene with a limited number of quality H&E slides produced showing no significant objective or subjective differences to the xylene processed materials. Rene and Maxim, (2009) confirmed that isopropanol alone or mixed with molten paraffin is a technically acceptable and cost-effective substitute for xylene. Geoffrey Roll, a histology consultant to Leica biopsy stems confirmed on his unpublished work that isopropanol can be used instead of xylene especially at higher wax temperatures

CONCLUSION

In view of the better turn-around time, less hazardous effects of IPA method and precise diagnosis, it is strongly recommend that the xylene method of tissue processing be replaced with isopropanol.

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