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CEREUS PTEROGONUS PLANT CLADODE EXTRACT A NOVEL TISSUE SECTION ADHESIVE IN HISTOPATHOLOGY LABORATORY

Histopathology

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ABSTRACT

Histopathology contributes scientific context information placed in the form of image to analyzing and interpreting type and stage of disease are vastly needed to selection of appropriate treatment procedure in cancer subject. Study of cells normalcy or irregularity need to attach the tissue section on glass slide using Mayer's egg albumin followed by staining and microscopical analysis was carried out. Presently using tissue section adhesives Mayer's egg albumin have a limitation such as background stain noise and narrow application. However, without staining background based tissue section adhesive will be highly appreciated in the histology laboratory. Hence, our study aimed to evaluating Cereus pterogonus plant extract on tissue section adhesiveness and staining against Mayer's egg albumin.

KEYWORDS

Histopathology, adhesive, Cereus pterogonus, egg albumin.

INTRODUCTION

Histopathology play vital role of analyzing and interpreting the morphological and molecular pattern of tissue with the particular clinical background. Hence a scientific context information placed in the form of image to confirmation and stage of disease for selection of appropriate treatment procedure such as chemotherapy, radiotherapy or surgery to giving safe and effective treatment (1). The histopathology specialty technique date back to 1838, first book of histopathology published on the nature and characteristics of cancer by Johannes Miller followed by the paraffin wax supporting section cutting introduced at mid-1800s leads to many techniques and staining methods was developed promptly. Different chemicals was investigated for histology laboratory use as a fixative, adhesive, stain during the year of 1800 to 1983 and till ongoing (2).

Histological study of cells architecture need to attach the tissue section on to the glass slide followed by staining was carried out. Nevertheless, presently using tissue section adhesives have a limitation such as background stain noise and narrow application. Also, histology lab using chemical based tissue section adhesives such as Mayer's egg albumin and gelatin contain preservatives cause environment impurity and human health unwanted effects due to toxicity. However, non-toxic and eco-friendly with no background staining noise based tissue section adhesive will be highly appreciated in the histology laboratory (3-5). Hence, our study aimed to evaluating the plant Cereus pterogonus extract on the tissue section adhesive efficiency in different type of tissues sections and different staining against Mayer's egg albumin.

MATERIALS AND METHODS

Materials

C. pterogonus plant was collected from semi-arid area of Puducherry regions, India. All the chemicals and satins used in the study were purchased from Sigma-Aldrich (St. Louis, MO, USA). Doubled distilled water was used to preparation of reagents.

Methods

Preparation of Plant Extract

Cladodes were removed followed by the cuticular layer at the surface gently removed at sterile condition than chopped into pieces and homogenized in phosphate buffer (pH 7.0) using a waring blendor to prepare 10 % (w/v) plant homogenate. Cladodes homogenate filtered using muslin cloth followed by centrifuged at 8,000 for 30 min in a centrifuge (11). The clear supernatant obtained was used as tissue section adhesive.

Preparation of Slides

Paraffin embedded two types of rat tissue block were prepared followed by tissue section were taken using semi-automated microtome (4). The tissue black were made from Department Of Biochemistry and Molecular Biology, Pondicherry University, Puducherry after obtained approval from Institutional Ethical committee (IAEC/No.01/2011).

Slide Coating

Four groups of adhesive coated slides were prepared. Group I and Group II 10 slides were coated with serial diluted with phosphate buffer and plant extract at 1:1 to 10:0. Group I stained by Haematoxylin and Eosin (H/E) stain and Group II stained by the periodic acid-Schiff (PAS) stain. On the other hand Group III and IV, slides were coated with freshly prepared Mayer's egg albumin at 0.5mL in each slide. In the group III slides stained for H/E stain and group IV slides stained by PAS stain. After the tissue sections taken on to slide than transferred to incubator for 1 hour for drying followed by stained the tissue sections.

Haematoxylin/Eosin Staining

Haematoxylin/Eosin (H/E) staining deparaffinised the sections by Xylene for 15 minutes followed by rehydration carried out by using different concentration 30%, 40%, 60%, 70%, 80%, 90%, 100% of alcohol each stage given 2 minutes than sections washed in running water for 5-10 minutes. The tissue section were stained using haematoxylin for 10 minutes than washed in running tap water, for differentiation slides were dipped in acid alcohol and ammonia solution, each solution slides dipped one time and washed in running tap water for bluing. Slides were placed in eosin solution for 30 seconds to 1 minute. After that slides dehydrated through descending order of alcohol followed by clearing with xylene and mounted in DPX (7-9).

Periodic Acid-Schiff Staining

In the PAS staining deparaffinised the sections by Xylene for 15 minutes followed by rehydration carried out by using different concentration 30%, 40%, 60%, 70%, 80%, 90%, 100% of alcohol each stage given 2 minutes than sections washed in running water for 5-10 minutes. Followed by section are treated with 0.5% Periodic Acid for 5 min and rinsed in distilled water in six times. Than the section treated with Schiff's reagent for 15 min, followed by slides are washed in tap water for 5 min. Slides were counterstained with Mayer's hematoxylin for 1 min. and washed in tap water 10 min then rinsed with distilled water three times. After that slides are dehydrated through descending order of alcohol followed by clearing with xylene and mounted in DPX (7-10).

RESULTS AND DISCUSSION

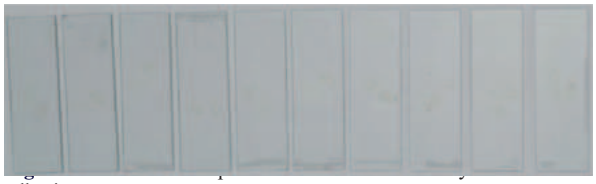
In Group I and II tissue sections were attached with serial dilution of plant extract (Table.1 and Figure 1, 2) and Group III and IV tissues section were attached with Mayer's egg albumin followed by stained with H/E and PAS stain.

Table 1. Preparation of serial dilution plant extract for tissue adhesive

Extract volume (mL)	D.H2O (mL)	% of extract dilution	Total volume (mL)
1	9	1:9	10
2	8	2:8	10
3	7	3:7	10
4	6	4:6	10
5	5	5:5	10
6	4	6:4	10
7	3	7:3	10

8	2	8:2	10
9	1	9:1	10
10	0	10:00	10

adhesive



adhesive

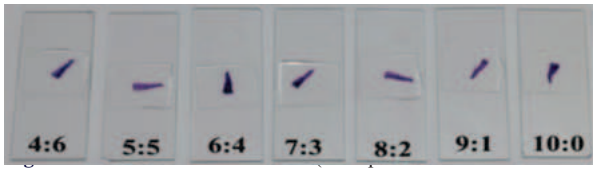
Plant extract are serial diluted with phosphate buffer starting from 1:1%, briefly 1mL plant extract and 1mL distilled water and 10:0%, 10mL plant extract alone taken in the present study. After the tissue sections taken on plant extract coated slides, routine staining procedure continued. In the serial diluted plant extract coated slide at the dilution 4:6% onwards observed tissue section adhesiveness till 10.0% (Table 2 and Figure 3, 4).

Compared with Mayer's egg albumin and plant extract coated tissue section stained with H/E and PAS stain observed that, plant extract coated tissue adhesives shown no stain background and stain residues and any noticeable effects on staining and histological structure changes on the tissue section (Figure 3, 4 and 6). Histological tissues section slides can be permanently prepared inexpensive and simply by applying Xerophytic plant C. pterogonus extract than follow routine procedure to stain the slide (11-13).

Table 2. Adhesiveness Effect Evaluated Using H/E and PAS Staining

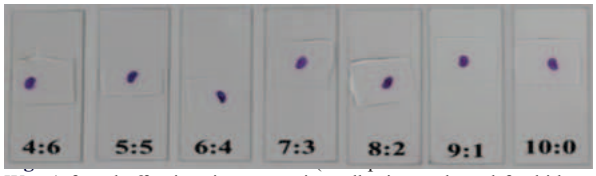
% of extract dilution	Tissues Adhesiveness	Tissues structural changes	Stain background
1:9	Not-effective	-	-
2:8	Not-effective	-	-
3:7	Not-effective	-	-
4:6	Effective	Un-altered	Not found
5:5	Effective	Un-altered	Not found
6:4	Effective	Un-altered	Not found
7:3	Effective	Un-altered	Not found
8:2	Effective	Un-altered	Not found
9:1	Effective	Un-altered	Not found
10:00	Effective	Un-altered	Not found

Figure 3. Adhesiveness effect evaluated using H/E staining in liver tissue



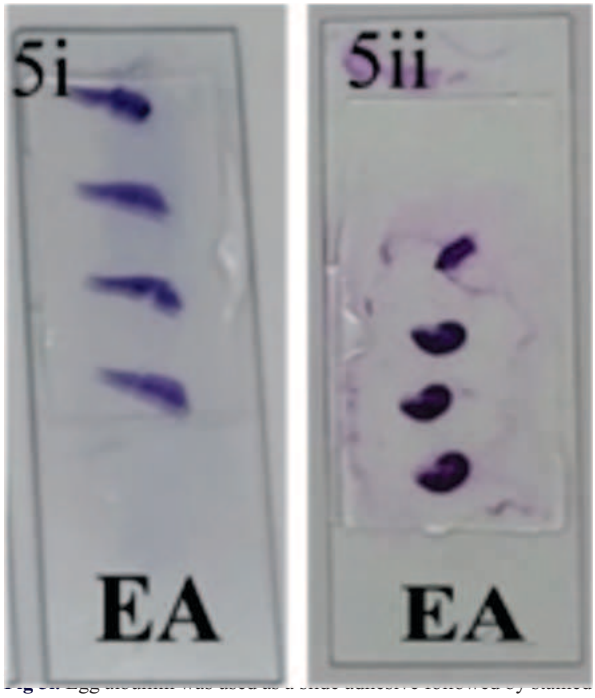
Water) found effective tissues section adhesive and used for liver tissue section adhesive followed by H/E staining performed and observed no stain background and stain resides.

Figure 4. Adhesiveness effect evaluated using PAS staining in kidney tissue



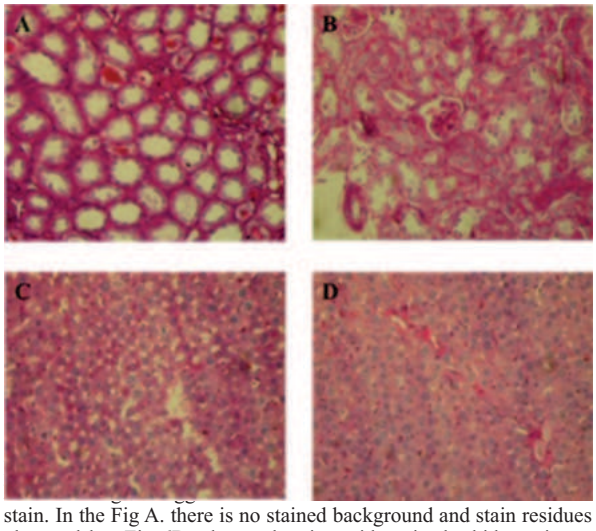
Water) found effective tissues section adhesive and used for kidney tissue section adhesive followed by PAS staining performed and observed no stain background and stain resides.

Figure 5. Egg albumin effect evaluated using H/E and PAS staining in liver and kidney tissue



H/E showed background stain. Fig 5ii. Egg albumin adhesive slide stained PAS stain and showed background stain.

Figure 6. Plant extract adhesiveness and tissues morphological effects.



stain. In the Fig A. there is no stained background and stain residues observed but Fig 6B. observed stain residues in the kidney tissue. Similarly, plant extract used for adhesive and H/E stained liver tissues shown in the Fig 6C. There are no stain residues and morphological alteration but egg albumin used for adhesive Fig 6D. observed stain residues.

Noticeable background coloured residue produced by Mayer's egg albumin likely due to contains different types of protein which react with stains (Figure 5) (5). However, Xerophytic plant C. pterogonus extract protein which may not react with stains, therefore no background stain residues observed in stained slides used plant extract.

CONCLUSION

Our study is the first to use xerophytic plant extract as a tissue section adhesive agent. The commonly using egg albumin gave a background

staining thus to reduce using xerophytic plant extract, absence of background staining compared to egg albumin highly appreciated in histology laboratory.

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