Catechu Stain for Cerebrum and Peripheral Nerve

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Abstract :

Aims and Objectives : Staining of the different components of nervous tissue is in a single preparation is very difficult. Different types stains were used for it but results are not encouraging. Catechu which is routinely known as Kattha is used for staining nervous tissue and observed that different components of nervous tissue are stained in a single preparation. Aim of the present study was to statin the sections of cerebrum and peripheral nerve by using catechu stain.

Material and methods: We used 2% ferric ammonium sulphate as mordent and tincture catechu as principle stain, for paraffin sections of cerebrum and peripheral nerve. Sections were treated with mordant: 2% ferric ammonium sulphate at room temperature for one hour. After one hour, sections were washed in tap water followed by rinsing in distilled water. Sections were stained with tincture catechu for 18 hours at 600 C in an oven. Sections were washed in tap water, followed by rinsing in distilled water then, dehydrated in graded alcohols. Cleared in Xylol and mounted in DPX. **Results :** We observed differential staining of various layers of cerebral cortex and in peripheral nerve axon, epineurium and endoneurium were well defined. **Conclusion :** Various components of cerebral cortex and peripheral nerve are stained in single preparation by this method which is easy, cost effective and can be used by inexperienced person.

Key words : Catechu, Cerebrum, Peripheral nerve, Nervous tissue, Ferric Ammonium sulphate.

Introduction : It is very challenging to stain the nervous tissue. Different types of stains are used by workers for staining of nervous tissue but these stains are either costly or time consuming and most of time expected results are not obtained. Also, different components of nervous tissue are not stained in a single preparation [1, 2]. In neuro-histology metallic impregnations methods are very commonly used. Tincture Catechu is the dried extract of leaves of young shoots of Uncaria gambier and it usually occur as pale grayish brown to dark reddish brown cubes or powder. It contains 7 to 33 % of catechin and 22 to 50 % catechu tannic acid [3]. Commonly, it is known as Kattha and it is used with betal leaves which stain the oral mucosa [2,3]. Tannic acid is used as histological reagent as it has many staining properties [4].

Gaikwad and Kolte [2] used catechu for staining of nervous tissue. They used catechu and calcium chloride in proportion of 2:1 and obtained good results. Tannic –Oxalic acid mixture was used by Lee in 1913 [5] for staining of axis cylinder. Tincture catechu was used by Victor et al. [3] to stain human nervous tissue. They used 2% ferric ammonium sulphate as mordent and obtained differential staining of various components of neurons. We used the procedure which was described by Victor et al. for staining of parasympathetic ganglia (Aurbach plexus) [6] , cerebellum and spinal cord [7] and same method we used for staining cerebrum and peripheral nerve.

Material and methods: For the present work, we used 2% ferric ammonium sulphate as mordent and tincture catechu as principle stain, for paraffin sections of cerebrum and peripheral nerve. Catechu stain was obtained by the procedure which was described by Victor et al. [3] . Catechu (Kattha) was obtained from provision store then powder of it was obtained by grinding. One gram of fine powder of catechu was dissolved in 5gm of cinnamon oil. For the preparation of tincture catechu, One ml of prepared solution of catechu and cinnamon oil was added to twenty ml of 45% of alcohol. Catechu stain was prepared by thorough mixing of equal parts of Tincture catechu and 50% alcohol.

Material: Human cerebrum and peripheral nerve was used for this study. Formalin fixed tissue was processed for paraffin sections and 5 to 7 micron thick sections were obtained.

Technique:

1. Sections were dewaxed and taken to water through graded alcohol.
2. Sections were treated with mordant: 2% ferric ammonium sulphate at room temperature for one hour.
3. After one hour, sections were washed in tap water followed by rinsing in distilled water.
4. Sections were stained with tincture catechu for 18 hours at 600 C in an oven.
5. Sections were washed in tap water, followed by

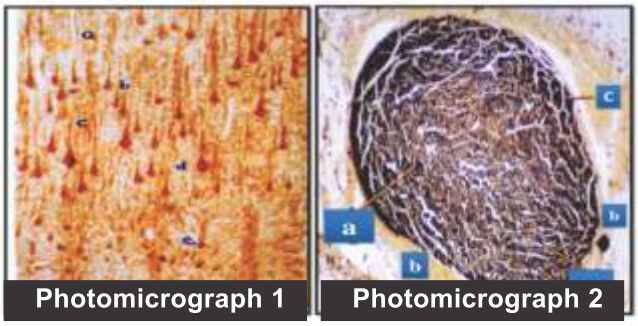
rinsing in distilled water. Then dehydrated in graded alcohols.

6. Cleared in Xylol and mounted in DPX.

Results : We observed differential staining of various layers of Cerebrum in photomicrograph 1

1. Plexiform layer not appreciated very well.
2. Outer granular layer- a: small pyramidal cells.
3. Outer pyramidal layer- b: medium sized pyramidal cells.
4. Inner granular layer- c: small Stellate cells
5. Inner Pyramidal layer- d: large pyramidal cells
6. Polymorphic layer-

Photomicrograph 2 shows a- axon, b- epineurium and c-endoneurium.



Photomicrograph 1 Photomicrograph 2

Discussion : It is difficult to stain various components of nervous tissue in a single preparation and many researchers used verities of staining technique for it. But these methods requires critical differentiation which affects the staining and double and triple staining procedure are used so they a costly and trained person is required to carry out these procedures[8,9] . Gaikwad and Kolte[2] used catechu for nervous tissue and obtained good differential staining of various components of neuron.

In 1987 Victor et al.[5] used catechu along with use of mordant 2% ferric ammonium sulphate. Use of mordant was on the basis of tannic acid-iorn reaction which has been used in neuropathological techniques. Also, they confirmed that the mordant is an essential element in their staining technique by using tincture catechu in different combinations to stain nervous tissue. We used the same technique to stain Aurbach plexus of human small intestine and observed good differential staining of various components of the neuron[7]. Also, we used same technique for the cerebellum and spinal cord and obtained good differential staining of various components of cerebellum and spinal cord.[8] The pioneers of neuroanatomy such as Cajal and Golgi used heavy metal impregnation techniques with gold and silver for study of neuron morphology but, these techniques are very costly. [10]

Various components of nervous tissue are stained in single preparation and doesn't required differentiation. The disadvantage of this method is that it requires higher temperature for staining and time consuming.

Conclusion : Catechu along with use of mordant gives good differential staining of various components of nervous tissue in single preparation and easily available, low cost.

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