APPENDIX

APPENDIX A Histological analysis

1. Fixation by excised samples and fixed in buffered neutral formalin solution at least 72 hrs.

2. After that the samples were passed automated tissue process by washed, dehydrated, cleared, and infiltrated.

2.1 Washing: The tissues were washed in tap water to remove the fixative.

2.2 Dehydration: The tissues were dehydrated by immersion in a series of solutions containing increasing concentrations of ethanol.

2.3 Clearing: The clearing reagent, xylene, was used to remove the alcohol used to dehydration and to make the tissues receptive to the infiltration medium.

2.4 Infiltration: The embedding medium, paraffin, was used to immerse the tissues.

3. The samples were embedded in a paraffin block.

4. The paraffin blocks were cut into 5μm thick using rotary microtome.

5. Deparaffinization and rehydration for eliminate paraffin which coat samples sections as follows:

5.1 Slide were immersed in xylene (5 min x 2 times)

5.2 Dip in absolute alcohol (5 min x 2 times)

5.3 Dip in 95% v/v alcohol (3 min X 2 times)

5.4 Wash slide in tap water (5 min)

5.5 Immerse in hematoxylin (5 min)

5.6 Wash slide in tap water (5 min)

5.7 Dip in eosin (12 sec)

5.8 Dip in 95% v/v alcohol (3 min X 2 times)

5.9 Dip in absolute alcohol (5 min x 2 times)

5.10 Immerse slide in xylene, all slides were mounted with cover slips using permount

APPENDIX B List of solutions

1. 10% neutral buffered formalin (1000 ml)

100 ml	40% formalin
900 ml	Distill water
4 g	Sodium dihydrogen phosphate monohydrate
6.5 ml	Disodium hydrogen phosphate anhydrous

2. EDTA-G solution pH 7.3 (100 ml)

14.5 g	EDTA
1.25 g	Sodium hydroxide
15 ml	Glycerol

3. The list of solutions and the times used for processing

70% alcohol	30 min
80% alcohol	30 min
85% alcohol	30 min
90% alcohol	30 min
95% alcohol	30 min
95% alcohol	30 min
100% alcohol	30 min
100% alcohol	30 min
Xylene	1 hr
Xylene	2 hrs
Paraplast	2 hrs
Paraplast	2 hrs