

## **Tissue processing procedure**

Briefly, the routine sequence of events of tissue processing as follows:

After the target tissues were obtained from sacrificed animals they were fixed with appropriate fixative neutral formalin 10% for more than 24 hours. These tissues were dehydrated through ascending alcohol concentration (70%, 80%, 90% and 100% alcohol) 2 hours for each concentration then the tissues were cleared by using xylene for 1hour. The tissues were infiltrated in paraffin and put in oven at (45-55°C) overnight then embedded in a block of paraffin. Thin sections (5µm-thick) were cut by using rotary microtome, put in water bath to remove the embedded wax and mounted on glass slide. The slide was put on hot plate to remove the water from the slide.

## **Routine staining procedure**

Routine staining procedure include administration of hematoxyline and eosin stain for complete slide as follows:

The complete slide were rehydrated by using descending alcohol concentration (100%, 90%, 80%, 70% alcohol) 2 minutes for each concentration then cleared by xylene for 1minute. After that, the sections were stained with hematoxyline blue (10-15 minutes) and the sections were washed in running tap water for 5 minutes then the sections were stained with 1% eosin stain (5 minutes) and washed with tap water for 5minutes to remove the excess of stains. The sections were dehydrated by ascending alcohol concentration (70%, 80%, 90%, 100% alcohol) 2 minutes for each concentration and cleared by xylene for 1minute to remove alcohol. Finally, DPX was put on the slide for fixing the cover slip on the slide.