EXPERIMENT NO. 2

Aim: To study about various kinds of microscopic preparations and the steps involved in microscopy

Materials required: Glass slide, coverslip, forceps, brush, blade, microscope, sample, safranin and glycerine

Theory: ***Preparation of microscopic slides***

There are three types of microscopic preparations used in the undergraduate section of pharmacy for understanding microscopic identification of features:

1. **Surface preparation:** These microscopic preparations involve study of surface morphology like epidermis, trichomes, palisade ratio, stomata, etc. These types of preparation do not require sectioning as well as staining. Usually the epidermal layer is torn/peeled and mounted using mountants, e.g. epidermis, stomata, leaf constants, etc.
2. **Temporary preparation:** These are general microscopic preparations used for tissue as well as for dermal observation, but these are mounted with glycerine. These preparations can be used upto 48 hours of preparation at room temperature. Sometimes microtomic preparation is also observed in the lab using temporary slide. For temporary preparation usually general staining reagent like HCl and phloroglucinol, safranin, etc is used after clearing with chloral hydrate or alcohol, e.g. all laboratory T.S. for day-to-day study.
3. **Permanent preparation:** These preparations can be stored and used for years. Soft tissue and hard material are plant materials which are tough to prepare microscopic slide are and these usually prepared for permanent preparation. These preparations require sophisticated steps and usually sectioning is done by microtome using wax block, e.g. organs, hard and soft tissues, etc.

   Microscopic preparation of slides involves seven steps as given in Table 1.

General procedure for sectioning and laboratory staining

1. Hold a small piece of wet sample in your left hand
2. Cut transverse section using a razor blade and put in a watch glass containing water.
3. Place the finest section on the glass slide using a brush
4. Add few drops of chloral hydrate for clearing
5. If required heat by taking the slide near the flame after the addition of chloral hydrate
6. Add a drop of safranin/phloroglucinol with HCl on the section and wait for 30 s to 1 min and remove the section from the reagent
7. Use a mounting reagent (glycerine) to mount the stained section with coverslip
8. Observe under microscope at 10×, 25×, 40× as per the need.