Chapter-V

Studies on histopathological changes during *in vivo* antifungal and toxicity effects of hexane extract of *Dillenia indica* in albino rats
5.1 Introduction:

Histopathology (from the Greek *kistos* (tissue) and *pathos* (suffering) refers to the microscopic examination of tissue in order to study the manifestations of disease. Specifically, in clinical medicine, histopathology refers to the examination of a biopsy or surgical specimen by pathologists, after the specimen has been processed and histological sections have been placed onto glass slides. This is the most important tool of the anatomical pathologist in routine clinical diagnosis of wounds, cancer and other diseases.

5.2 Skin:

The skin is the outer covering of the body. It is the largest organ of the integumentary system made up of multiple layers of epithelial tissues, and guards the underlying muscles, bones, ligaments and internal organs. The adjective cutaneous literally means "of the skin" (from Latin *cutis*, skin). Because it interfaces with the environment, skin plays a very important role in protecting (the body) against pathogens. Its other functions are insulation, temperature regulation, sensation, synthesis of vitamin D, and the protection of vitamin B folates. Severely damaged skin will try to heal by forming scar tissue. This is often discolored and depigmented.

5.3 Liver:

Liver is considered to be consisting of large number of hexagonal lobules. Each lobule consists of a central vein, from which cords or rows of liver cells radiate like spokes of a wheel. Each lobule is delineated by a connective tissue. The liver is the largest organ in the body, ordinarily weighing about 3.5lbs and measuring about 8-9 inches in width. It is located in the abdominal area in the body, just beneath the diaphragm of the stomach. The liver is connected to the diaphragm and to the anterior walls of the abdomen by five ligaments, one of which is a fibrous cord resulting from the atrophy of the umbilical vein of intrauterine life. Blood vessels connected to the liver include hepatic artery, the portal vein, and the hepatic veins, as well as many capillaries. The liver is composed of lobules, chains of hepatic cells held together by connective tissues. Each lobule contains blood vessels in close connection with secretary cells and ducts by which secretions are carried away. Blood comes to the liver from stomach, spleen, pancreas and intestines. Flow of blood through the liver is estimated to be about 800-1000 ml per mi, the greater portion of which comes from the portal vein. The liver can hold up to a point of blood at a time, an amount equivalent to about 13% of the body's total blood supply.
Liver is metabolically the most complex organ and serves numerous vital functions. These include energy balance regulation, blood protein synthesis and immune modulation. Efficient liver function is necessary for the processing and excretion of endotoxic and exotoxic chemicals (hormones, drugs, chemicals etc). Which are commonly referred to as “xenobiotic” chemicals. Inefficient liver function can lead to “metabolic poisoning” which a nondescript term is referring to the build up within cells, tissues and organs of metabolites which have not been processed by the liver and excreted. These metabolites alter the pH gradient and electrolyte profile within cells and serve as competitive enzyme inhibitors that ultimately interrupt bioenergetics within the cell. The symptoms of metabolic poisoning at the elevated level are reflective of poor energy dynamics and include fatigue, hypotonia and brain biochemical disturbances. Recent studies have reported a relationship between impaired detoxification capability, mitochondrial dysfunction and chronic fatigue syndrome (CFS). These reports suggest that oxidation damage due to mitochondria and the detoxification process is itself a fundamental mechanism in the development of CFS.

5.4 Kidney:

Kidney is covered by a capsule, below which there is a cortex, occupying upper 4/5th of the kidney. Inner to cortex there is medulla. In the cortex circular structures called renal capsules are present, surrounding which are tubules in various shapes. The dark rounded thick walled tubules are parts of the proximal convoluted tubule. The lumen of the proximal convoluted tubule is small and indistinct. The light thin wall tubule with distinct lumen is called distal convoluted tubule.

5.5 Histopathological Studies (Raphael, 1976):

The tissues (Skin, liver, and kidney) preserved in neutral buffered formalin were used for the study of histopathological changes. Tissues were processed, which involves dehydration, clearing and infiltration of the tissue with paraffin and then the tissues were sectioned. The sections are mounted on glass slides and smeared with a drop of Mayer’s egg albumin. The slides are dried on a hot plate at about 50°C for 30 minutes. The sections are then stained with Mayer’s Hematoxylin-Eosin stain and observed under light microscope.
PAS (periodic acid staining) is a special stain used for fungi detection. The PAS stain is a histochemical reaction in that the periodic acid oxidizes the carbon to carbon bond forming aldehydes which react to the fuchsin-sulfurous acid which form the magenta color.

5.6 Results and Discussion:

5.6.1 Skin:

Figure 5.1 represents the skin of rat infected with *Candida albicans*. In this degeneration of cells, tissue keratinization, macrophages is large in number; hair follicle regeneration sites are lost. Fig 5.2 represents the skin of rat infected with *Candida albicans* and treated with hexane extract of *Dillenia indica* at a concentration of 10mg/rat. In this regeneration of cells, hair follicle regeneration sites, complete wound healing, epithelialization were observed. Fig 5.3 represents the skin of rat (rats are immunosuppressed) infected with *Candida albicans* and treated with hexane extract of *Dillenia indica* at a concentration of 10mg/rat. In this regeneration of cells, hair follicle regeneration sites, wound healing, epithelialization were observed. Figure 5.4 represents the dorsum part of the tongue, infected with *Candida albicans*. Figure 5.5 represents the tongue of rat infected with *Candida albicans* and treated with hexane extract of *Dillenia indica* at a concentration of 10mg/rat.

Fig 5.1: Rats infected with *C. albicans*-Cutaneous candidiasis. *C. albicans* seated in dermal layers.

Fig. 5.2: Infected rats treated with hexane extract of *D. indica* at 10 mg/rat concentration showing no culture growth and hair follicle regeneration sites, 100% wound healing.

Fig 5.3: Infected rats (Immunosuppressed) treated with hexane extract of *D. indica* at 10 mg/rat concentration showing no culture growth and hair follicle regeneration sites.

Fig 5.4: Rats infected with *C. albicans*-Oral candidiasis.

Fig 5.5: Immuno suppressed rats treated with hexane extract of *D. indica* at 10 mg/rat concentration.
Table 5.1: Quantitative histopathological findings of wounds of rats infected with Candida albicans after topical application of ointment.

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<thead>
<tr>
<th>Groups</th>
<th>Fibrosis</th>
<th>Necrosis</th>
<th>Hypertrophy of Sub-cutaneous muscle fibre</th>
<th>Infiltration of neut's</th>
<th>MC</th>
<th>Macroph's</th>
<th>Epithelization</th>
<th>Presence of fungal spores ***</th>
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<td>DS (+++)</td>
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Neut's = Neutrophilis; MC = Mast cells; Macro's = Macrophages

The following parameters were marked as follows:

- **Severity**: "-" indicates absent; + indicates scanty; ++ indicates mild; +++ indicates moderate; +++ indicates severe
- **Distribution**: D=Dermal; DS=Dermal and Sub-dermal; LE=Locally Extensive (Dermal and Sub-dermal).
- **Epithelization**: C = Complete

*** indicates presence of fungal spores in dermal layers.
5.6.2 Liver:

Figure 5.6 represents control rat liver section showing normal architecture normal hepatocytes, sinusoids and normal centrilobular space. Figure 5.7 represents the 250 mg/kg b. wt. of hexane extract of *Dillenia indica* treated rat showing normal hepatocytes, central cirrhosis and regenerative sinusoids. Figure 5.8 represents the liver section of CCl₄ treated rat showing highly conjugated blood vessels and hepatocytes with numerous vacuoles. Figure 5.9 represents liver section of CCl₄ + 500 mg/kg b. wt. of hexane extract of *Dillenia indica* treated rat showing regenerative central vein, sinusoids and hepatocytes.

5.6.3 Kidney:

Figure 5.10 represents Control rat kidney section showing normal architecture showing normal glomeruli and tubular epithelial cells. Figure 5.11 represents kidney section of 500 mg/kg b. wt. of hexane extract of *Dillenia indica* treated rat showing regenerative renal tubular epithelial cells. Figure 5.12, kidney section of CCl₄ treated rat showing glomeruli with congestion, blood vessel and RBC damage and damaged renal tubules. Figure 5.13, kidney section of CCl₄ + 500 mg/kg b. wt. hexane extract of *D. indica* treated rat showing regenerative glomeruli and renal tubular epithelial cells.

Fig 5.6: Liver section of normal rat.

Fig 5.7: Liver section of 250 mg/kg b. wt. of hexane extract of *Dillenia indica* treated rat.

Fig 5.8: Liver section of CCl₄ treated rat.

Fig 5.9: Liver section of CCl₄ and 500 mg/kg b. wt. of hexane extract of *Dillenia indica* treated rat.

Fig 5.10: Kidney section of normal rat.

Fig 5.11: Kidney section of 500 mg/kg b. wt. of hexane extract of *D. indica* treated rat.

Fig 5.12: Kidney section of CCl₄ treated rat.

Fig 5.13: Kidney section of CCl₄ and 500 mg/kg b. wt. hexane extract of *D. indica* treated rat.