CHAPTER – VI
HISTOPATHOLOGY

6.1 INTRODUCTION

Histopathological alteration can be used as indicators for the effect of chemicals on organisms. Histological analysis appears to be a very sensitive parameter and is crucial in determining cellular changes that may occur in target organs (Datta et al., 1996 and Priya Kannappan and Krishnakumari Shanmuga Sundaram, 2009).

Immune system is vital to survive, because a hyperactive immune system may cause fatal disease due to overwhelming allergic reaction leading to series of derangements, loss of normal capacity to distinguish self from non-self resulting in immune reactions against one’s own tissues and cells called autoimmune diseases. These autoimmune changes are receiving increased attention in drug discovery and development as the progress has been made in understanding immune and inflammatory processes. Several autoimmune diseases including myasthenia gravis, serum sickness, pernicious anaemia, Pemphigus vulgaris, SLE, reactive arthritis, etc. are the severe concern of medical and pharmaceutical community because of unknown etiology. Rheumatoid arthritis (RA) is one of the most common inflammatory condition of unknown etiology characterized by symmetric erosive synovitis and in some cases extra articular involvement (Harris, 2004).
Herbal medicine is the roof of various traditional medicine systems around the world. Herbal medicines yielding about 25% of currently used crude drugs with another 25% derived from chemically altered natural products (Huxtable, 1992). In ancient texts about 500 plants have been indicated in the treatment of arthritis, however only few number of plants have been evaluated scientifically (<50). As the result of the inherent problems associated with current non steroidal as well as steroidal anti-inflammatory agent, there is a continuous search especially from natural source. Recently there is a greater global interest in non synthetic, natural drugs derived from plant sources due to better tolerance and minimum adverse drug resistance (Kimmatkar et al., 2003). Herbal drugs used in Indian system are claimed to be effective and safe for treatment of inflammation. Considering the medicinal values of plants the acute toxicity and histopathology of extracts was evaluated in mice to assess its safety or otherwise, since the findings are important considering the usage of the plants by human beings (Abdullahil, 2007 and Bytul, 2007).

The liver is an important organ performing vital functions including bio transformation, migration of lipids, glycogen storage and release of glucose (Holt et al., 2008). The Spleen is a dark red to blue – black organ located in the left cranial abdomen. It is adjacent to the greater curvature of the stomach and within the omentum. The ratio of splenic weight to body weight remains fairly constant regardless of age and in rats is typically around 0.2% (Losco 1992). The functions of the spleen are centered on the systemic circulation. As such, it lacks afferent lymphatic vessels. It is comprised of 2 functionally and morphologically distinct compartments. The spleen is also the largest secondary lymphoid organ containing
about one–fourth of the body’s lymphocytes and initiates immune responses to blood borne antigens (Kuper et al., 2002; Nolte et al., 2002 and Balogh et al., 2004).

Muscle tissue on the other hand comprises in major percentage in weight and is significant for the structural integrity of the animals. Moreover, muscles are involved in contraction and relaxation and the animal under arthritic condition underwent muscular changes and so, there is a need to study the structural integrity of muscle. Therefore, the present study was carried out with an objective to evaluate anti-arthritic activity of tender fruit extract of *A. esculentus* prepared by successive solvent extract on the liver, spleen and muscle tissue of rheumatoid antigen treated Swiss albino mice. Further, to test the toxic effect of test plant on the histology of the animal model is also imperative. Hence this study was carried out in this line.

6.2 MATERIALS AND METHODS

The specimens of various organs (liver, spleen and muscle) from Swiss albino mice treated with lone effect of Rheumatoid Antigen, butanol and ethanol extract of *A. esculentus* and in combination of RA individually with butanol and ethanol were fixed in 10% buffered formalin solution then processed for histopathological studies in ascending grades of ethyl alcohol, cleared in xylene, then embedded in paraffin wax. Sections of about 5-7 microns thickness were taken and then stained with hematoxylin and eosin. (Drury and Willington, 1967) The tissues were examined by a Nikon 600 light microscope and photographed by a Nikon Dxm 1200 digital camera (Humason, 1972 and Bancrifft et al, 1996).
6.3 RESULTS AND DISCUSSION

Liver

Mice maintained as control showed normal structural organization of cells with granulated cytoplasm and uniform nuclei (Plate 6.1a). Mice treated with rheumatoid antigen total loss of hepatic architecture with abnormal hepatocyte nuclei, hepatic necrosis, cytoplasmic vaculization and conjunction of sinusoids were observed (Plate 6.1b). Similar observations were reported by Ahsan et al. (2009) in mice due to calcium tetrachloride. Rheumatoid antigen treated mice after administering with ethanol extract of A. esculentus, the liver showed mild hepatic necrosis, cytoplasmic vacuolar degeneration (Plate 6.1c). The lone effect of ethanol extract of A. esculentus in liver of the mice showed similar histological structure as in mice maintained as control but with a very few hepatic necrosis (Plate 6.1d). Similar result of histological features due to leaf extracts of various plants were reported (Zambessicus Ofusori et al., 2008, Rajkapoor et al., 2008 and Thamizh Selvam et al., 2010).

6.3.2 SPLEEN

The mice maintained as control the lymphoid areas of spleen were found to be normal (Plate 6.2a). In mice treated with rheumatoid antigen congestion and free apoptotic debris were observed in spleen (Plate 6.2b). Whereas in mice treated with rheumatoid antigen after administering with A. esculentus a mild congestion and infiltration of cells were noticed (Plate 6.2c). In mice treated with rheumatoid antigen after administering with ethanol extract of A. esculentus the normal histology of spleen was limbing back. The present study revealed the repairing efficiency of the ethanol extract of A. esculentus in mice administered with rheumatoid antigen. A
moderate congestion was noticed in mice treated with ethanol extract of \emph{A. esculentus} (Plate 6.2d). The congestion observed due to the various treatments in the spleen of mice was coincided the observations made by Priya Kannappan and Krishnakumari Shanmugasundaram (2009) in mice due to methanol extract of \emph{Cyathula prostrate}. The study of Okokon \textit{et al.}, (2010) due to the administration of ethanol extract of \emph{Croton zambesicus} showed no variation in the structures of spleen. From the discussion it was obvious that degree of change in histology of spleen in mice varied by the administration of different extracts of various plants.

\subsection*{6.3.3 MUSCLE}

On the histology of muscle of mice maintained as control, RA treated, RA treated in combination with \emph{A. esculentus} and lone effect of \emph{A. esculentus} were observed.

Mice maintained as control and extract treated the gross muscle mass was found to be stable (Plate 6.3a and 6.3d). In mice treated with RA muscular atrophy and high inflammatory cellular infiltration and vacuolization were observed (Plate 6.3b). In RA treated mice after the administration of ethanol extract of \emph{A. esculentus} regenerative myogenic events were observed (Plate 6.3c). In mice treated with ethanol extract of \emph{A. esculentus} showed no much variation, as in the case of mice maintained as control (Plate 6.3d).

The myogenic changes observed in mice due to RA treatment were in accordance with observation made in the rat due to Freund’s complete adjuvant (Havagiray \textit{et al} 2009). The anti-arthritic effect of ethanol extract of \emph{A. esculentus} was
evidenced from the result of the present study and that can be correlated with the report of Havagiray et al. (2009) in mice due to indomethacin treatment. They reported that the treatment of indomethacin significantly inhibited infiltration of inflammatory cells and synovitis. Histological recovery of various tissues of rheumatoid mice after administering with ethanol extract of A. esculentus may be due to the protective effect of different phytoconstituents reported in the present study (Table 1.1).