Chapter – 6

Acute and subacute oral toxicity Study
CHAPTER – 6
Acute and subacute oral toxicity study of Cycas circinalis and Ionidium suffruticosum

6.1. INTRODUCTION:

“For every disease there is a plant that cures it” - Hippocrates.

The herbs are safe to consume when its toxic dose is determined. The determination of biosafety level of the drugs, the harmful effect of a compound and its level are very much important for its safe usage. Herbal medicines have been popular from ancient times among people and in recent years, a multilateral approach has emerged on using medicines which is of herbal origin (Mohajera et al., 2008). Medicinal herbs may cause some irretrievable tissue damage through its unwanted side effects. Evaluating toxic effects of medicinal herbs by performing experimental tests on animal models will have an effective advantage on identification and recognition of medicine’s harmful effects on humans (Mirhadi et al., 2011). WHO has advocated for the proper identification, sensible exploitation, scientific development and appropriate utilization of herbal medicines which provide safe and effective remedies in medicare (Wambebe 1998). However, information about several medicinal plants to the consumers does not have any scientific data support. The use of herbal medicine as a phytomedicine is based simply on a traditional folk utilization that has been perpetuated along several generations. Pharmacological and toxicological evaluations of medicinal plants are essential for drug development (Ibarrola et al., 2000; Ahmed et al., 2006; Perera et al., 2010).

The present research was done to evaluate the acute and subacute toxicity of two medicinal herbs such as Cycas circinalis and Ionidium suffruticosum. The acute oral toxicity study was done as per the guidelines of Organization for Economic Cooperation and Development (OECD) guideline 425 and subacute toxicity study was done as per OECD guidelines 407.

6.2. MATERIALS AND METHODS:

A total of 54 male Wistar rats weighing of 150 to 200 gm were selected randomly for the toxicity study (Table 6). The animals were maintained under standard environmental conditions and had free access to standard rodent pellet diet and water ad libitum. The protocol of the toxicity study was approved by the Institutional Animal Ethics Committee of Saveetha University (IAEC. NO. ANAT.005/2012). The experiments were performed in accordance with the CPCSEA guidelines. Male rats were selected because the literature survey of conventional LD$_{50}$ tests shows that usually there is only little difference in sensitivity between sexes (Panunto et al., 2011). As the herbs were used for treating male infertility by traditional practitioners for years together, the toxicity study was done in male albino rats.
Table – 6: Distribution of male Wistar rats for toxicity study

<table>
<thead>
<tr>
<th>S.no</th>
<th>Groups</th>
<th>Acute Toxicity Study</th>
<th>Sub-Acute Toxicity Study</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Normal Control</td>
<td>6</td>
<td>6</td>
</tr>
<tr>
<td>2</td>
<td>C.circinalis (Exp I)</td>
<td>6</td>
<td>18</td>
</tr>
<tr>
<td>3</td>
<td>I.suffruticosum (Exp II)</td>
<td>6</td>
<td>18</td>
</tr>
</tbody>
</table>

Normal control – administered sterile water (Same control of acute toxicity study was used for sub-acute toxicity study after rehabilitation, Experimental I – administered C.circinalis extract, Experimental II – administered I.suffruticosum extract.

6.2.1. Evaluation of Acute toxicity:

The animals were kept under fasting for overnight but allowed water *ad libitum* before drug administration. The weight of the fasting animal was taken and drug dosages were calculated. The *C.circinalis* and *I.suffruticosum* extracts were orally administered in a single dose by oral gavage for the experimental animals (Table 6). Single animal was dosed in sequence, usually at 48 hrs intervals. The dosage was initiated at 175mg / kg, then 550 mg / kg, and preceded with 2000 mg/kg body weight as recommended in OECD Guidelines 425. Signs of toxicity and mortality were observed for the first 30 minutes followed by 1, 2, 4, 6 and 24 hours and thereafter twice daily until 2\textsuperscript{nd} -14\textsuperscript{th} day (Panunto et al., 2011). The wellness parameters and mortality were recorded for each animal. The wellness parameters such as skin looked for pigmentation, discoloration, fur loss, nasal and oral mucous membrane for any ulceration, respiratory rate, heart rate, salivation, lacrimation, lethargy, pilo erection, urinary incontinence, defecation, sleep, gait, tremors, convulsion and mortality were all observed, recorded for each animal and compared between control and experimental groups for assessment of individual organ system (Patrick Iwuanyanwu et al., 2012). The hematological parameters and biochemical were analysed at the end of 2 weeks.

6.2.2. Evaluation of sub-acute toxicity:

Thirty six rats were randomly divided into six groups of 6 animals in each group for 3 dosages for a period of 28 days (Table 6) and each group was administered 250 mg / kg, 500 mg / kg, 1000 mg / kg body weight of *C.circinalis* and *I.suffruticosum* extracts respectively. The control rats were administered sterile water simultaneously. The mortality in each treatment group was recorded during the course of the 28 days drug administration.

6.2.3. Collection of blood and tissue samples:

On the 29\textsuperscript{th} day all the rats were anesthetized and shaved on the ventral aspect of the neck and the skin was incised to about 2 cm in length and opened. The jugular vein was traced out and using 21 gauze needle and a 3 ml disposable syringe, 2 ml of blood was withdrawn. Serum was separated by centrifugation for about 3000 rpm after the coagulation of blood for 40 minutes.
The serum was collected using micropipette from the samples and the hematological parameters such as haemoglobin, red blood cell count, white blood cell count, platelet count, and total protein were done and analysed. Some of the biochemical markers such as urea, aspartate aminotransferase, alkaline phosphatase and cholesterol were estimated and analyzed. The procedures for all the above haematological and biochemical markers were done according to the standard procedure (Satya et al., 2012).

The rat was cut open by midline thoraco-abdominal incision and followed by transcardial perfusion using 4% paraformaldehyde in 0.1 M phosphate buffered saline on the left ventricle and the right atrium was cut open. Flickering of limbs, blanching of tissues with consequent hardening of the whole body of the animal indicates that perfusion made was complete. The vital organs such as brain, liver, kidney, lungs, heart, stomach, intestine, testes, and skeletal muscles were collected carefully by fine dissection as per standard procedure (Gandhare et al., 2013). The collected organs were washed with cold saline solution and weighed immediately followed by fixation in formalin (10% formaldehyde solution) for 48 hrs. Samples tissues were collected from each organ, processed and stained as per the general procedure explained under material and methods (Chapter – 4).

6.2.4. Histo-pathological analysis of tissues from vital organs:

The routine haematoxylin and eosin stain was used for staining all the tissue samples. The histopathological analysis was done for all the tissues of the above collected organs. The lining epithelium, interstitial spaces, blood capillaries, sinusoids, supporting connective tissues, infiltrations, architecture and focal lesions were carefully observed and secondary opinion was taken from pathologist to avoid bias in analysis. Slides were examined using various objectives. The toxicant-induced changes, if any was noted down for drug infused animals. The slides were photographed using binocular virtual compound microscope. The stained slides were preserved for future use.

6.2.5. Statistical analysis:

The values are expressed as Mean ± SEM. The data’s were analysed using one-way ANOVA. Significance was considered at values of p < 0.05.

6.3. RESULTS:

6.3.1. Acute toxicity study of C. circinalis and I. suffruticosum:

C. circinalis and I. suffruticosum treated animals did not exhibit any mortality until 2000 mg/kg body weight (Table 7). The wellness parameters were found to be normal in all the rats (Table 7). The heart rate and respiratory rate was found to be normal when compared between control and experimental rats. The lethal value of both C. circinalis and I. suffruticosum were determined to be higher than 2000 mg/kg body weight and also regarded as safe within the dose.
Acute and subacute oral toxicity study of *C.circinalis* and *I.suffruticosum*

**Distribution of Wistar Rats into groups**

**Acute Toxicity Study**
- NC – 6 rats
- EI – 6 rats
- EII – 6 rats

**Sub-acute Toxicity Study**
- NC – 6 rats
- EI – 6 rats (3 groups)
- EII – 6 rats (3 groups)

**Control – Sterile water**
- Exp I (175 mg/kg, 550 mg/kg, 2000 mg/kg BW) (Cc)
- Exp II (175 mg/kg, 550 mg/kg, 2000 mg/kg BW) (Is)

**Oral Administration**
- (Single dose)
- 48 hrs interval
- OECD 425

**Blood Collection**

**Mortality & wellness parameters was observed**

**Haematological & biochemical parameters was analyzed**

**Control – Sterile water**
- Exp I (250mg/kg, 500mg/kg, 1000mg/kg BW) (Cc)
- Exp II (250mg/kg, 500mg/kg, 1000mg/kg BW) (Is)

**Oral Administration**
- (28 days)
- OECD 407

**Blood**

**Haematological & biochemical parameters was analyzed**

**Viscera’s**

**Histo pathological analysis was done**
Table 7: Wellness parameter observation for acute toxicity study

<table>
<thead>
<tr>
<th>Parameters</th>
<th>30 min</th>
<th>4hrs</th>
<th>24hrs</th>
<th>1st Week</th>
<th>2nd Week</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>C</td>
<td>EI</td>
<td>EI</td>
<td>C</td>
<td>EI</td>
</tr>
<tr>
<td>Skin &amp; Fur</td>
<td>N</td>
<td>N</td>
<td>N</td>
<td>N</td>
<td>N</td>
</tr>
<tr>
<td>Mucous Membrane</td>
<td>N</td>
<td>N</td>
<td>N</td>
<td>N</td>
<td>N</td>
</tr>
<tr>
<td>Respiratory rate</td>
<td>N</td>
<td>N</td>
<td>N</td>
<td>N</td>
<td>N</td>
</tr>
<tr>
<td>Heart rate</td>
<td>N</td>
<td>N</td>
<td>N</td>
<td>N</td>
<td>N</td>
</tr>
<tr>
<td>Salivation &amp; Lacrimation</td>
<td>N</td>
<td>N</td>
<td>N</td>
<td>N</td>
<td>N</td>
</tr>
<tr>
<td>Lethargy</td>
<td>Nil</td>
<td>Nil</td>
<td>Nil</td>
<td>Nil</td>
<td>Nil</td>
</tr>
<tr>
<td>Piloerection</td>
<td>N</td>
<td>N</td>
<td>N</td>
<td>N</td>
<td>N</td>
</tr>
<tr>
<td>Urinary incontinence</td>
<td>Nil</td>
<td>Nil</td>
<td>Nil</td>
<td>Nil</td>
<td>Nil</td>
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<tr>
<td>Defecation</td>
<td>N</td>
<td>N</td>
<td>N</td>
<td>N</td>
<td>N</td>
</tr>
<tr>
<td>Sleep &amp; Gait</td>
<td>N</td>
<td>N</td>
<td>N</td>
<td>N</td>
<td>N</td>
</tr>
<tr>
<td>Tremors &amp; Convulsion</td>
<td>Nil</td>
<td>Nil</td>
<td>Nil</td>
<td>Nil</td>
<td>Nil</td>
</tr>
<tr>
<td>Mortality</td>
<td>Nil</td>
<td>Nil</td>
<td>Nil</td>
<td>Nil</td>
<td>Nil</td>
</tr>
</tbody>
</table>

N – Normal, Nil – not observed,
Normal control (C) - administered sterile water, E I (Cc) – administered C.circinalis and E II (Is) - administered I.suffruticosum. Control group compared to experimental groups.

Table 8: Hematological and biochemical parameters of acute toxicity study

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Normal Control</th>
<th>Exp I - C.c</th>
<th>Exp II - I.s</th>
<th>F</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hb (mg/dl)</td>
<td>13.62 ± 0.56</td>
<td>13.78 ± 0.61</td>
<td>14.21 ± 0.74*</td>
<td>1.36</td>
<td>0.29</td>
</tr>
<tr>
<td>RBC (x10^6/µl)</td>
<td>8.31 ± 0.72</td>
<td>8.29 ± 0.34</td>
<td>8.42 ± 0.73*</td>
<td>0.08</td>
<td>0.93</td>
</tr>
<tr>
<td>WBC (x10^3/µl)</td>
<td>3.77 ± 0.48</td>
<td>3.68 ± 0.34</td>
<td>3.98 ± 0.82*</td>
<td>0.42</td>
<td>0.67</td>
</tr>
<tr>
<td>Platelet (x10^5/µl)</td>
<td>4.98 ± 0.37</td>
<td>5.71 ± 0.41</td>
<td>5.43 ± 0.32*</td>
<td>0.34</td>
<td>0.06</td>
</tr>
<tr>
<td>Total Protein (g/dl)</td>
<td>5.77 ± 0.71</td>
<td>5.94 ± 0.72</td>
<td>5.64 ± 0.64*</td>
<td>0.28</td>
<td>0.75</td>
</tr>
<tr>
<td>Urea (mg/100ml)</td>
<td>32.64 ± 0.33</td>
<td>38.82 ± 0.45</td>
<td>29.63 ± 0.21*</td>
<td>0.94</td>
<td>0.001</td>
</tr>
<tr>
<td>Creatinine (mg/dl)</td>
<td>0.32 ± 0.04</td>
<td>0.41 ± 0.07</td>
<td>0.39 ± 0.41*</td>
<td>0.04</td>
<td>0.96</td>
</tr>
<tr>
<td>AST (U/l)</td>
<td>39.41 ± 4.31</td>
<td>42.81 ± 5.27</td>
<td>41.68 ± 4.84*</td>
<td>0.13</td>
<td>0.88</td>
</tr>
<tr>
<td>ALP (U/l)</td>
<td>36.42 ± 2.21</td>
<td>32.6 ± 2.28</td>
<td>33.72 ± 1.28*</td>
<td>0.98</td>
<td>0.01</td>
</tr>
<tr>
<td>Cholesterol (mg/dl)</td>
<td>42.4 ± 0.60</td>
<td>47.71 ± 0.91</td>
<td>41.91 ± 0.54*</td>
<td>20.97</td>
<td>0.001</td>
</tr>
</tbody>
</table>

Hb (mg/dl) - Haemoglobin, RBC (x10^6/µl) - Red Blood Cells count, WBC (x10^3/µl) - White Blood Cells count, AST (U/l) - Aspartate aminotransferase, ALP (U/l) - Alkaline Phosphatase. Normal control - administered sterile water, Exp I (Cc) – administered C.circinalis and Exp II (Is) - administered I.suffruticosum. Values are expressed as Mean ± SEM, n = 6. * – non significant, * - significant, P value * P<0.05. Control group compared to experimental groups. Statistical analysis – One Way ANOVA.
6.3.2. Sub-acute toxicity study:

The haematological and biochemical parameters of subacute toxicity study were tabulated and analysed as below,

**Table 9: Hematological and biochemical parameters**

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Normal Control</th>
<th>Exp I (C.circinalis)</th>
<th>Exp II – (I.suffruticosum)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>250 mg/kg</td>
<td>500 mg/kg</td>
<td>1000 mg/kg</td>
</tr>
<tr>
<td>Hb (mg/dl)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>13.6±0.6</td>
<td>13.1±0.5</td>
<td>12.9±0.5</td>
<td>14.2±0.8*</td>
</tr>
<tr>
<td>RBC (×10⁶/µl)</td>
<td>8.31±0.72</td>
<td>8.51±0.8</td>
<td>8.39±0.6</td>
</tr>
<tr>
<td>WBC (×10³/µl)</td>
<td>3.77±0.48</td>
<td>3.71±0.4</td>
<td>3.54±0.3</td>
</tr>
<tr>
<td>Platelet (×10⁵/µl)</td>
<td>4.98±0.37</td>
<td>4.7±0.31</td>
<td>4.84±0.4</td>
</tr>
<tr>
<td>Total Protein (g/dl)</td>
<td>5.77±0.71</td>
<td>5.21±0.6</td>
<td>5.98±0.6</td>
</tr>
<tr>
<td>Urea (mg/100ml)</td>
<td>32.6±0.33</td>
<td>32.2±0.3</td>
<td>31.8±0.28</td>
</tr>
<tr>
<td>Creatinine (mg/dl)</td>
<td>0.32±0.04</td>
<td>0.31±0.03</td>
<td>0.38±0.04</td>
</tr>
<tr>
<td>AST (U/l)</td>
<td>39.4±4.31</td>
<td>38.9±3.29</td>
<td>39.4±4.1</td>
</tr>
<tr>
<td>ALP (U/l)</td>
<td>36.4±2.21</td>
<td>35.7±1.9</td>
<td>35.9±1.6</td>
</tr>
<tr>
<td>Cholesterol (mg/dl)</td>
<td>42.4±0.60</td>
<td>42.2±0.6</td>
<td>42.8±0.58</td>
</tr>
</tbody>
</table>

Hb (mg/dl) - Haemoglobin, RBC (×10⁶/µl) - Red Blood Cells count, WBC (×10³/µl) - White Blood Cells count, AST (U/l) - Aspartate aminotransferase, ALP (U/l) - Alkaline Phosphatase. Normal control - administered sterile water, Exp I (Cc) – administered C.circinalis and Exp II (Is) - administered I.suffruticosum. Values are expressed as Mean ± SEM, n = 6, * - non significant, * - significant, P value *P<0.05. Control group compared to experimental groups. Statistical analysis – One Way ANOVA.

6.4. DISCUSSION:

6.4.1. Acute toxicity study:

The wellness parameters such as skin looked for pigmentation, discoloration and fur loss, nasal and oral mucous membrane for any ulceration, respiratory rate, heart rate, salivation, lacrimation, lethargy, pilo erection, urinary incontinence, defecation, sleep, gait, tremors, convulsion and mortality were all observed, recorded for each animals and compared between control and experimental groups and found to be normal in all the animals (Table 7).

The Hematological parameters such as haemoglobin concentration, red blood cell count, white blood cell count, platelet count of all rats, were found out. Data’s were tabulated as Mean ± SEM (Table 8) and compared between control and experimental rats by one way ANOVA, p value for all hematological parameters were insignificant and show only less difference among the groups and the values are approximately within same range to the study done by Akila et al., (2012) for some Siddha formulations. The biochemical parameters such as total protein, creatinine, alkaline Phosphatase, were within same ranges with a slight difference between control and experimental rats. P value for all markers...
was not significant among the groups. Urea, aspartate amino transferase and cholesterol were highly significant among the groups but within normal laboratory values (Table 8).

6.4.2. Sub-acute toxicity study:

The hematological and biochemical parameters when compared between the control and experimental groups, only slight variations were noted and which was found to be within the normal limits of laboratory values (Table 9) and statistical analysis of the parameters showed insignificance as the values are within the same range except hemoglobin and platelets, which was found to be significant. Transaminases and ALPs are good indices of liver damage (Preeja et al 2011). There were no deleterious changes found in the level of transaminases and ALPs in serum of drug treated groups when compared with control animals. The level of transaminases and ALP usually rises in acute liver damage. AST is also present in red blood cells, cardiac muscles, skeletal muscles, kidney and brain tissue, and may be elevated due to damage to these sources as well. AST is defined as a biochemical marker for the diagnosis of acute myocardial infarction. Equally, there also was no marked change in creatinine, when compared to the control, creatinine is known as an effective indicator of renal function and any rise in creatinine levels is observed if there is marked damage to functional nephrons. Thus, the results recorded in this study suggest that extract of *C. circinalis* (Cc) and *I. suffruticosum* (Is) did not affect the renal function. The liver is the site of cholesterol disposal or degradation and the major site of synthesis. Since, no significant changes were observed in cholesterol levels in this study, it suggests that Cc and Is extract had no effects on the cholesterol metabolism of the rat (Preeja et al., 2011).

The histopathological examination of different organs such as Brain (Fig. 24), Liver (Fig. 25), Kidney (Fig. 26), spleen (Fig. 27), Lung (Fig. 28), Stomach (Fig. 29), Large intestine (Fig. 30), Testes (Fig. 31), Heart (Fig. 32) and skeletal muscle (Fig. 33) of both control and experimental groups were compared and no abnormal architecture, morphological disturbances, desquamation, disorganization, infiltration and destruction of these tissues were observed. Brain tissue (cerebrum) showed normal architecture without any infiltration and cerebral oedema. Neuron cell bodies were displayed on a loose fibrillary background. Prominent perineuronal and perivascular halos or clearing (Virchow Robbins spaces) were seen in both the control and drug treated rats brain (Fig. 24). The liver of drug treated animals showed normal histological feature at 250, 500 and 1000 mg/kg with preserved hepatic architecture, hepatocytes arranged as radial plates. There was no degeneration of hepatocytes, no cytoplasmic inclusions in hepatocytes, focal steatosis and sinusoidal spaces did not show any vascular congestion. The central vein did not show any congestion and no
inflammation of portal tract when compared with control animals (Fig. 25). The kidney of drug treated rats showed normal glomerular tufts displayed on a background containing tubules and there is no necrosis of tubular epithelium in the kidney, no evidences of focal tubular casts and no glomerular hyperaemia in kidney when compared to the control rats kidney (Gandhare et al., 2013) (Fig. 26).

The spleen appears to consist of discrete lymphatic nodules called the white pulp, embedded in a red matrix called the red pulp. The splenic stroma showed engorgement of sinusoids by red blood cells and showed normal white pulp with a central arteriole surrounded by periarteriolar lymphoid sheath and red pulp which is permeated by an interconnected network of sinuses, lined by endothelial cells resting upon a basement membrane with numerous narrow slits (Fig. 27). The lung tissues were normal in all the drug treated groups. The alveolar wall consists of three tissue components, surface epithelium (Type I and II pneumocytes), supporting tissue (reticular, collagenous, elastic fibres) and blood vessels. The alveolar air spaces were surrounded by interstitium containing few blood vessels and inflammatory cells and showed no focal lesions, alveolar epithelium were normal (Fig. 28).

The mucosa of stomach is thrown into prominent folds and consists of gastric glands, the submucosa made up of connective tissues and large blood vessels. There were no mucosal erosions observed in stomach of drug treated rats when compared to control (Fig. 29). The mucosa of large intestine lined by simple columnar epithelium with goblet cells, the mucosal linings were normal in both control and drug treated group (Fig. 30). Testis showed cut sections of seminiferous tubules with normal germinal epithelium in various stages of development and spermatozoa in the lumen of the tubules. Leydig cells were seen in the interstitium (Fig. 31). The heart was normal in the drug treated group with cardiac myocytes, arranged in interlacing and parallel array and their nuclei were spindle shaped and elongated (Fig. 32). The skeletal muscle fibres were elongated, unbranched cylindrical cells, with multiple nuclei arranged at the cell periphery. The skeletal muscle showed normal histological presentation in all drug treated groups when compared to control groups (Fig 33).

The study was concluded stating that Cc and Is does not produce any toxic effect in male albino rats with no adverse histological presentations. The above haematological, biochemical and histological findings of the acute and sub-acute toxicity tests suggest that *C.circinalis* and *I.suffruticosum* is practically non-toxic when administered orally for an extended period at therapeutic doses. The drugs at the above doses showed potential for boosting components of the immune system and protecting the liver, kidney and cardiovascular system.
These findings provide a justification for specially designed studies to further investigate the possible beneficial activities of the herbs.

6.4.3. Dosage selection for fertility study:

The acute toxicity study shows that 2000 mg/kg body weight of both *C. circinalis* and *I. suffruticosum* were proved to be safer. The drug dosage for the fertility study was taken from the acute toxicity study, about 1/10th of the dose was selected (200 mg/kg body weight) for administration to the experimental group of rats.

The acute toxicity study shows that the lethal value of both *C. circinalis* and *I. suffruticosum* were determined to be higher than 2000 mg/kg and also regarded as safe within the dose.

The sub-acute toxicity study proves that ethanolic extract of *C. circinalis* and *I. suffruticosum* when given orally at concentration of 1000 mg/kg body weight, did not exhibit any toxicological effect and proved to be safe until 1000 mg/kg.
Fig. 24: Histology of brain tissue H & E Stain (10x)

- Neurons with perineuronal spaces in cerebrum.

Fig. 25: Histology of liver H & E Stain (10x)

1 – Control, 2 – Exp I (250 mg/Kg BW), 3 – Exp I (500 mg/Kg BW), 4 – Exp I (1000 mg/kg BW)
5 – Exp II (250 mg/Kg BW), 6 – Exp II (500 mg/Kg BW), 7 – Exp II (1000 mg/kg BW)
Fig. 26: Histology of kidney H & E Stain (10x)

![Histology of kidney H & E Stain](image)

1 – Glomerulus, 2 – Proximal and distal convoluted tubules

Fig. 27: Histology of spleen H & E Stain (10x)

![Histology of spleen H & E Stain](image)

1 – White pulp, 2 – Red pulp of spleen

1 – Control,
2 – Exp I (250 mg/Kg BW), 3 – Exp I (500 mg/Kg BW), 4 – Exp I (1000 mg/kg BW)
5 – Exp II (250 mg/Kg BW), 6 – Exp II (500 mg/Kg BW), 7 – Exp II (1000 mg/kg BW)
Control – administered sterile water, Exp I - administered *C.circinalis* and Exp II - *I.suffruticosum.*
**Fig. 28: Histology of lung H & E Stain (10x)**

- Alveoli lined by simple squamous epithelium

**Fig. 29: Histology of stomach H & E Stain (10x)**

- Mucosa lined by simple columnar epithelium and shows gastric glands

1 – Control,
2 – Exp I (250 mg/Kg BW), 3 – Exp I (500 mg/Kg BW), 4 – Exp I (1000 mg/kg BW),
5 – Exp II (250 mg/Kg BW), 6 – Exp II (500 mg/Kg BW), 7 – Exp II (1000 mg/kg BW)

Control – administered sterile water, Exp I - administered *C.cirrhosis* and Exp II - *I.suffruticosum.*
Fig. 30: Histology of large intestine H & E Stain (10x)

Mucosa lined by simple columnar epithelium

Fig. 31: Histology of testes H & E Stain (10x)

Seminiferous tubule lined by germinal epithelium

1 – Control,
2 – Exp I (250 mg/ Kg BW), 3 – Exp I (500 mg/Kg BW), 4 – Exp I (1000 mg/kg BW)
5 – Exp II (250 mg/ Kg BW), 6 – Exp II (500 mg/Kg BW), 7 – Exp II (1000 mg/kg BW)

Control – administered sterile water, Exp I - administered *C.circinalis* and Exp II - *L.suffruticosum.*
Fig. 32: Histology of Heart H & E Stain (10x)

Myocytes with centrally placed nucleus

Fig. 33: Histology of skeletal Muscles H& E Stain (10x)

Myocytes with peripherally placed nucleus

1 – Control,
2 – Exp I (250 mg/ Kg BW), 3 – Exp I (500 mg/Kg BW) , 4 – Exp I (1000 mg/kg BW)
5 – Exp II (250 mg/ Kg BW), 6 – Exp II (500 mg/Kg BW), 7 – Exp II (1000 mg/kg BW)

Control – administered sterile water, Exp I - administered *C.circinalis* and Exp II - *I.suffruticosum*. 