TOXICOLOGICAL EVALUATION OF ALOE VERA EXTRACT
3. TOXICOLOGICAL EVALUATION OF ALOE VERA EXTRACT

3.1 INTRODUCTION

Toxicology tests, which includes acute, sub-acute, and chronic toxicity. Acute toxicity is studied by using a rising dose until signs of toxicity become apparent. Sub-acute toxicity is where the drug is given to the animals for four to six weeks in doses below the level at which it causes rapid poisoning, in order to discover if any toxic drug metabolites build up over time. Testing for chronic toxicity can last up to two years.

In screening drugs, determination of LD$_{50}$ (the dose which has proved to be lethal (causing death) to 50% of the tested group of animals) is usually an initial step in the assessment and evaluation of the toxic characteristics of a substance. It is an initial assessment of toxic manifestations (provides information on health hazards likely to arise from short-term exposure to drugs) and is one of the initial screening experiments performed with all compounds.

Data from the acute study may:

(a) Serve as the basis for classification and labeling

(b) Provide initial information on the mode of toxic action of a substance

(c) Help arrive at a dose of a new compound

(d) Help in dose determination in animal studies

(e) Help determine LD$_{50}$ values that provide many indices of potential types of drug activity

3.1.1 Aim of acute toxicity test

To determine the therapeutic index, i.e. ratio between the lethal dose and the pharmacologically effective dose in the same strain and species (LD$_{50}$/ED$_{50}$). The greater the index, safer is the compound. LD$_{50}$ with confidence limits is to be
established on one common laboratory species such as mouse/rat using the standard method. The LD₅₀ dose thus found was administered to guinea pigs, rabbits, cats or dogs on weight basis (on basis of relative surface area gives better results). Because of species variation, several species of animals (one rodent and one non-rodent) were used to determine LD₅₀. When a clearly different response was observed in any of these species, a larger number of that species needs to be tested to establish the approximate LD₅₀ value (Ghosh, 1984).

The use of plants for healing purposes is getting increasingly popular as they are believed as being beneficial and free of side effect (Leonardo et al., 2000). *Aloe vera* gel has been used medicinally for several thousands of years with a long and illustrious history. The gel of *Aloe vera* contains about 99 to 99.5% water with pH in the range of 4.4 to 4.7. The potency of the gel with respect to its capability in reducing chemically induced toxicity is also reported (Anilakumar et al., 2010). As this plant has potential health benefits, it can be better utilized for nutraceutical and functional food formulations. Hence a study was conducted to evaluate methanol extract of the gel for its toxicity up to the level of 16g/kg body wt.

**3.2 MATERIALS AND METHODS**

**3.2a MATERIALS**

All the biochemicals employed in these investigations were of highest purity and procured from Sigma company USA, Merck Germany, Sisco Research Laboratory, Mumbai, Qualigens Mumbai, Across Organics Mumbai, Spectrochem, Mumbai or S.D. Fine chemicals Mumbai. All the organic solvents were of AR grade.

Spectrophotometer (Schimatsu model uv1601) double beam, spectroflouro meter (Elico model), refrigerated superspeed centrifuge (Sorvall RC-5B model), light microscope (Lynx, Lawrence and Mayo), were used for the preparation and estimation of biological samples.
3.2b METHODS

3.2b.1 Treatment of experimental rats

Inbred male rats (*Rattue norvegicus*) of Wistar strain reared in the Defence Food Research Laboratory animal facility, Mysore in the weight range 110-150 g were used in this study. The rats before attaining the required weight were kept on 20% standard casein protein diet for stabilizing the semisynthetic diet for a period of 7 days before commencement of experiments. The casein diet was used as control diet in the experiments (Table 5). The various solid ingredients of the diet were powdered before mixing. The diet was stored in the walk-in- cooler maintained at 4±1ºC during the course of the experiments. Diets were weighed out into feeding cups and slight water was added to the diet and mixed into a paste; spillage of diet was largely prevented by this method. Food and water were allowed *ad libitum*. The animals were kept at ambient temperature and exposed to light-dark cycle of 12 hours each. The left over diet was collected, dried and weighed to determine the food intake. Weekly food intake and weight gain were monitored.

Table 5. Composition of control diet (g/kg)

<table>
<thead>
<tr>
<th>Ingredients</th>
<th>Quantity (g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Casein</td>
<td>240</td>
</tr>
<tr>
<td>Ground nut oil</td>
<td>40</td>
</tr>
<tr>
<td>Mineral mixture USP XIV *</td>
<td>40</td>
</tr>
<tr>
<td>Vitamin mix**</td>
<td>10</td>
</tr>
<tr>
<td>Shark liver oil +</td>
<td>10</td>
</tr>
<tr>
<td>Methionine</td>
<td>2</td>
</tr>
<tr>
<td>Corn starch</td>
<td>658</td>
</tr>
</tbody>
</table>

* Purchased from Sisco Research Laboratory, Mumbai, India.
** Prepared as per Indian Standards I.S. 7481(1975)
+ Provides 1500 IU vitamin A and 100 IU vitamin D per g; 0.1 g α-tocopherol acetate per kg diet was added.
3.2b.2 Acute toxicity study

The acute oral toxicity was evaluated following the World Health Organization (WHO) guideline (WHO, 2000) and the Organization of Economic Cooperation and Development (OECD) guideline for chemical testing (OECD, 2001). Rats were divided into four groups. The treated group was orally given the methanol extract with a single dose of 8, 16, 20 g/kg b.wt. while the control group received only water vehicle. The animals were monitored for apparent signs of toxicity for 14 days.

3.2b.3 Sub acute toxicity study

The method was performed following the WHO guideline (WHO 2000) and the OECD guideline (OECD, 1981). Rats were divided into six groups. The treated group was orally given the extract at a dose of 1(dose 1), 2(dose 2), 4(dose 3), 8(dose 4), and 16(dose 5) g/kg body weight daily for 42 days, while the control group received the vehicle at the same volume. All the rats were observed for apparent signs of toxicity or behavioral alterations during the experimental period.

3.2b.4 Liver and kidney function tests

Weekly blood was drawn from the orbital plexuses; blood samples were taken in clean sterile tubes and left till clotting occurred. Serum was collected after centrifugation at 3000rpm for 15 min. Serum was kept at -20°C until used. Chemical analysis carried out on serum to assay the state of liver and kidney. All the parameters are determined with Agappe diagnostics kits. This includes creatinine, urea-B, albumin, total protein, total bilirubin and direct bilirubin, alkaline phosphatase (ALP), glutamic oxaloacetic transaminase (SGOT) and glutamic pyruvic transaminase (SGPT) measured. Weekly body weighted was also noted. At the end of the experiment, all the rats were fasted for 12 hrs, sacrificed under anesthetic condition.

3.2b.4a Creatinine

Creatinine reacts with picric acid to produce a colored compound, creatinine alkaline picrate. The change in absorbance is proportional to the creatinine concentration. Creatinine in serum is determined by modified Jaffe’s method. The
reagents includes working reagent is prepared by mixing 1 volume of reagent1 with 1 volume of reagent 2. Reagent 1 is creatinine dye reagent contains 8.73 mmol/L of picric acid and surfactant; Reagent 2 is creatinine base reagent contains 300 mmol/L of sodium hydroxide and 25 mmol/L of sodium phosphate. A standard creatinine is also used having a concentration 2 mg/dL. The reagent is linear up to 24 mg/dL (Allen et al., 1982). Pipette out 1ml of working reagent in a cuvette. Add 0.1 ml of standard/ sample to it. Mix well and read the optical density (T₁) at 505 nm. Exactly after 60 sec reading (T₂) is taken. The creatinine in samples/ standard is expressed as,

\[
\text{Creatinine Conc. (mg/dL)} = \frac{(T₂ - T₁)}{(T₂ - T₁) \text{ of sample}} \times 2
\]

3.2b.4b Urea – B

Enzymatic determination of urea is by following reaction

\[
\text{Urea} + \text{H}_2\text{O} \xrightarrow{\text{Urease}} 2\text{NH}_3 + \text{CO}_2
\]

\[
\text{NH}_3 + \text{salicylate} \xrightarrow{\text{Nitroprusside}} 2,2\text{-Diacroxy indophenol} \xrightarrow{\text{Hypochlorite}} \]

Urea - B is determined by modified Berthelot methodology. The regents include urea – B colour reagent R₁ contains 80 nmol/L of sodium salicylate, 4 mmol/L of sodium nitroprusside and 45 mg/dL of sodium hypochlorite. Urea – B reagent R₂ contains 60 mmol/L phosphate buffer pH 6.9 and 20 KU/L urease. A standard urea B is also used having a concentration 40 mg/dL. The reagent is linear up to 200 mg/dL. R₂ is used as working reagent by mixing on vial of R₂ with known volume of distilled water. Pipette out 1ml of working reagent in a test tube. Add 0.01 ml of standard/ sample, mix and incubate at 37° C for 5 min. 1 ml of colour reagent R₁ is added to it, mix and incubate at 37° C for 5 min. to the above mixture add 1ml of distilled water (Wheatherburn, 1967). Mix well and measure the absorbance at 600 nm of sample and standard against the reagent blank. Urea – B in standard/ samples is expressed as,
Absorbance of sample
Absorbance of standard

\[ \text{Urea conc. (mg/dL)} = \frac{\text{Absorbance of sample}}{\text{Absorbance of standard}} \times 40 \]

### 3.2b.4c Albumin

The reaction between albumin from serum or plasma and the dye bromocresol- green produces a change in colour that is proportional to the albumin concentration. Albumin is determined by bromocresol green methodology. The reagents includes albumin reagent contains 75 mmol/L of succinate buffer, pH 4.2 and 0.14 g/L of bromocresol green. A standard albumin concentration 3gm/dL is also used. The reagent is linear up to 6 g/dL (Doumas et al., 1971). 1ml working reagent is mixed with 0.01 ml of standard/samples. Incubate the reaction mixture at 37° C for 1 min. Measure the absorbance of standard and samples at 630 nm against reagent blank. The albumin of standard/ sample is expressed as,

\[ \text{Albumin conc. (gm/dL)} = \frac{\text{Absorbance of sample}}{\text{Absorbance of standard}} \times 3 \]

### 3.2b.4d Total protein

Protein in plasma or serum samples forma a blue colored complex when treated with cupric ions in alkaline solution. The intensity of the blue colour is proportional to the protein concentration. Total protein is estimated by direct Biuret method. The reagent composition includes 6 mmol/L of potassium iodide, 21 mmol/L of sodium potassium tartarate, 6 mmol/L of copper sulphate and 58 mmol/L of sodium hydroxide. Total protein standard concentration of 6 g/dL (Layne, 1957). 1ml working reagent is mixed with 0.02 ml of standard/samples. Incubate the reaction mixture at 37° C for 10 min. Measure the absorbance at 546 nm against reagent blank. Total protein of standard/sample is expressed as,

\[ \text{Total protein conc. (gm/dL)} = \frac{\text{Absorbance of sample}}{\text{Absorbance of standard}} \times 6 \]
### 3.2b.4e Bilirubin (total and direct)

Sulfanilic acid reacts with sodium nitrite to form in the presence of diazotized sulfanilic acid to form azobilirubin. In the absence of dimethyl sulfoxide, only the direct bilirubin reacts to give azobilirubin. Bilirubin is determined by modified DMSO method. The reagents includes total bilirubin reagent contains 28.9 mmol/L of sulfanilic acid, 165 mmol/L of hydrochloric acid and 7 mmol/L of dimethyl sulfoxide (Walter and Gerard, 1980). Direct bilirubin reagent contains 28.9 mmol/L and 165 mmol/L hydrochloric acid, total bilirubin and direct bilirubin activator (Annino, 1960). A total bilirubin standard concentration of 10 mg/dL and direct bilirubin standard concentration of 7.7 mg/dL also is used. The reagent is linear upto 20 mg/dL. 1ml of total bilirubin/direct bilirubin is taken in a cuvette. 0.02 ml of activator total/direct respectively and 0.05 ml of standard/samples are also added. Mix well and incubate at 37° C for exactly 5 min. Absorbance of the standard/samples are measured against reagents blanks. For total bilirubin absorbance is at 546 nm and direct bilirubin absorbance is at 532 nm. Total bilirubin and direct bilirubin of standard/samples are expressed as,

\[
\text{Total bilirubin} = \frac{\text{optical density of sample test (T)} - \text{optical density of sample blank (T)}}{\text{optical density of standard}} \times 10
\]

\[
\text{Direct bilirubin} = \frac{\text{optical density of sample test (D)} - \text{optical density of sample blank (d)}}{\text{optical density of standard}} \times 7.7
\]

### 3.2b.4f Alkaline phosphatase (ALP)

Alkaline phosphatase is determined by the following reactions

\[
\text{alkaline phosphatase} \quad \text{p-nitrophenyl phosphate} + \text{H}_2\text{O} \rightarrow \text{p-nitrophenyl} + \text{inorganic phosphate}
\]
The reagent composition includes alkaline phosphatase reagent 1 (R\textsubscript{1}) contains 125 mmol/L of diethanolamine buffer pH 10.2 and magnesium chloride 0.625 mmol/L, alkaline phosphatase reagent 1 (R\textsubscript{2}) contains p-nitrophenyl phosphate 50 mmol/L. The reagent is linear up to 700 U/L (Schlebusch et al., 1974). The working reagent is prepared by mixing 4 volume of reagent 1 (R\textsubscript{1}) with 1 volume of reagent 2 (R\textsubscript{2}). 1 ml working reagent is mixed with 0.02 ml of samples. Incubate the reaction mixture at 37° C for 1 min. The rate of increase in absorbance at 405 nm was monitored every 60 sec for 3 min against a reagent blank. Alkaline phosphatase in samples were expressed as,

Alkaline phosphatase activity (U/L) = (▲optical density/min x 2750)

3.2b.4g Glutamic oxaloacetic transaminase (SGOT)

Kinetic determination of SGOT activity according to the following reaction.

\[
\text{aspartate aminotransferase} \quad \text{L-aspartate} + \alpha\text{-ketoglutarate} \rightarrow \text{oxaloacetate} + \text{L-glutamate} \\
\text{malate dehydrogenase} \quad \text{Oxaloacetate} + \text{NADH} + \text{H}^+ \rightarrow \text{L-malate} + \text{NAD}^+ 
\]

The reagent composition includes SGOT reagent 1 (R\textsubscript{1}) contains 88 mmol/L of triss buffer pH7.8, 260 mmol/L of L-aspartate, ≥ 1500 U/L lactate dehydrogenase, ≥ 900 U/L malate dehydrogenase. SGOT reagent 2 (R\textsubscript{2}) contains 12 mmol/L of α-ketoglutarate and 0.24 mmol/L of NADH. The reagent is linear upto 350 U/L (Thefeld et al., 1974). The working reagent is prepared by mixing 4 volumes of reagent 1 (R\textsubscript{1}) with 1 volume of reagent 2 (R\textsubscript{2}). 1 ml working reagent is mixed with 0.01 ml of samples. Incubate the reaction mixture at 37° C for 1 min. The rate of increase in absorbance at 405 nm was monitored every 60 sec for 3 min against a reagent blank. SGOT of samples is expressed as,

SGOT activity (U/L) = (▲optical density/min x 1745)

3.2b.4h Glutamic pyruvic transaminase (SGPT)

Kinetic determination of SGPT activity according to the following reaction.
The reagent composition includes SGPT reagent 1 (R1) contains 110 mmol/L of triss buffer pH7.5, 600 mmol/L of L-alanine, ≥ 1500U/L lactate dehydrogenase. SGPT reagent 2 (R2) contains 16 mmol/L of α-ketoglutarate and 0.24 mmol/L of NADH. The reagent is linear upto 350 U/L (Thefeld et al., 1974). The working reagent is prepared by mixing 4 volumes of reagent 1 (R1) with 1 volume of reagent 2 (R2). 1 ml working reagent is mixed with 0.01 ml of samples. Incubate the reaction mixture at 37° C for 1 minute. The rate of increase in absorbance at 340 nm was monitored every 60 sec for 3 min against a reagent blank. SGPT of samples is expressed as,

\[ \text{SGPT activity (U/L)} = (\text{▲ optical density/min} \times 1745) \]

### 3.2b.5 Histopathological studies

All the sacrificed rats were necropsied. Specimens viz. liver, kidney, small intestine, heart were collected from different organs and fixed in 10% neutral buffer formalin. Paraffin sections (6-8 microns) were prepared and stained with Harris haematoxylin and eosin (Carleton and Drury, 1967) for microscopic examination.

### 3.3 RESULTS AND DISCUSSION

#### 3.3a Acute toxicity study

The present study reports the acute and sub-acute toxicity studies of Aloe vera up to the maximum dose of 16 g/kg body wt. of rats. The rats were orally given a multiple dose of the methanol extract from the gel of Aloe vera at 4, 8, 16g/kg neither the signs of toxicity nor death of rats were observed during the 14 days of the acute toxicity study. It is known that the alterations of body weight gain and organ weights of rats would reflect the toxicity of the substance (Carol, 1995). The significant
difference in organ weights between treated and untreated (control) animals may also occur in the absence of any morphological changes (Bailey et al., 2004). In the study, the body weights were recorded as shown in Fig 12 and no significant difference was noticed as compared to control group.

3.3b Sub-acute toxicity study

The sub-acute toxicity study of methanol extract of Aloe vera with the above doses did not reveal any toxicity symptoms as revealed in body weights (Fig 13) and organ weights of rats (Fig 14). The body weights of experimental and control rats were increased throughout the duration of oral feeding. There was also no change in organ weights during the oral feeding.

In sub-acute toxicity study, creatinin, urea-B, total protein, albumin, and bilirubin (total and direct) level of treated rats of all dose groups shows no changes in comparison to control group rats (Figs 15, 16, 17, 18, 19 and 20). The normal values of the renal biochemical parameters, including urea-B and creatinine suggest that the extract does not produce any sort of disturbance in the renal function, as has been found in case of various plant extracts and hence is safe on its chronic use in various diseases. Urea and creatinine are waste products of protein metabolism that need to be excreted by the kidney, therefore marked decrease in serum urea and creatinine, as noticed in this study, confirms an indication of protection to the kidney. Normal creatinine concentration of serum should be below 1.5 mg/100 ml. In renal failure serum creatinine value rises and attains very high values. Urea level can be increased by many other factors such as dehydration, antidiuretic drugs and diet, whilst creatinine is, therefore, more specific to the kidney, since kidney damage is the only significant factor that increases serum creatinine level (Nwanjo et al., 2005).
Fig 12. Body weight of rats in the acute toxicity study of the methanol extract of *Aloe vera*

Values are expressed as Mean ± SD (n=6)

Fig 13. Body weight of rats in the sub-acute toxicity study of the methanol extract of *Aloe vera*

Values are expressed as Mean ± SD (n=6)
Fig 14. Effect of methanol extract of *Aloe vera* on the relative organ weights

Values are expressed as Mean ± SD (n=6)

Fig 15. Effect of methanol extract of *Aloe vera* on creatinine

Values are Mean ± SD for 6 rats (p<0.05)
The increase in serum protein, particularly albumin, could be attributed to changes in protein and free amino acid metabolism and their synthesis in the liver. The protein level suppression may be due to loss of protein either by reduce in protein synthesis or increased proteolytic activity or degradation (Yeragi et al., 2003). Bilirubin is formed from degeneration of hemoglobin by the action of reticulo endothelial systems throughout the body. Increased bilirubin level reflects the depth of jaundice (Tedong et al., 2008). In obstructive jaundice serum bilirubin value shoots up greatly but in hepatic failure due to portal cirrhosis serum bilirubin rise may be only marginal. A low concentration of bilirubin is found in normal plasma, almost all of which is indirect. The sum of the direct and indirect forms (or conjugated and unconjugated) is termed total bilirubin. Routine analytical procedures exist for the determination of total bilirubin and for the measurement of direct bilirubin. The indirect fraction is obtained by subtracting the direct value from the total value. The determination of direct as well as total bilirubin is used in differentiating certain types of jaundice. The normal value of the hepatic biochemical parameters reveals the safety profile of the extract on liver function even on its chronic use.
Fig 16. Effect of methanol extract of *Aloe vera* on urea-B

Values are Mean ± SD for 6 rats (p<0.05)

Fig 17. Effect of methanol extract of *Aloe vera* on total protein

Values are Mean ± SD for 6 rats (p<0.05)
Fig 18. Effect of methanol extract of *Aloe vera* on albumin

![Graph showing the effect of methanol extract of *Aloe vera* on albumin levels.](image)

Values are Mean ± SD for 6 rats (p<0.05)

Fig 19. Effect of methanol extract of *Aloe vera* on total bilirubin

![Graph showing the effect of methanol extract of *Aloe vera* on total bilirubin levels.](image)

Values are Mean ± SD for 6 rats (p<0.05)
Fig 20. Effect of methanol extract of *Aloe vera* on direct bilirubin

![Graph showing effect of methanol extract of *Aloe vera* on direct bilirubin](image)

Values are Mean ± SD for 6 rats (p<0.05)

Fig 21. Effect of methanol extract of *Aloe vera* on glutamic oxaloacetic transaminase activity (SGOT)

![Graph showing effect of methanol extract of *Aloe vera* on glutamic oxaloacetic transaminase activity](image)

Values are Mean ± SD for 6 rats (p<0.05)
Also, serum enzymes viz. SGOT, SGPT and ALP in treated groups of rats did not show any change in comparison to the control groups (Figs 21, 22 and 23). Liver cell damage is characterized by a rise in serum enzymes viz. SGOT, SGPT, ALP etc. (Brautbar and Williams, 2002). Generally, SGPT concentrations are consistently higher than SGOT levels and this is expected as body cells generate more SGPT than SGOT (Mayne, 1996). Usually, about 80% of SGPT is found in mitochondria whereas SGOT is a purely cytosolic enzyme. Therefore, SGPT is found in mitochondria whereas SGOT is a purely cytosolic enzyme.

Therefore, SGPT appears in higher concentrations in a number of tissues (liver, kidneys, heart and pancreas) and is released slowly in comparison to SGOT. But since SGOT is localized primarily in the cytosol of hepatocytes, this enzyme is considered a more sensitive marker of hepatocellular damage than SGPT and within its limits can provide a quantitative assessment of the degree of damage sustained by the liver (Al Mammary et al., 2002). Since SGOT and SGPT activities are not showing any significant difference with the treatment of extracts, it does not indicate damage to liver cells.

The histology revealed evidence of normal hepatocytes (Table 6). The interpretation of the histology results was done with the support of a Pathologist. The markers of renal functions viz. urea and creatinine levels were not changed in the group of rats treated with *Aloe vera* extracts. This view strengthened by the fact that the relative weight of the kidneys did not show any evidence of toxicity (Fig 12) and also histologically determines normal glomeruli and tubules (Fig 24). The relative body weight gain determines any toxicity of all doses administered in rats (Fig 12).
Fig 22. Effect of methanol extract of *Aloe vera* on glutamic pyruvic transaminase activity (SGPT)

Values are Mean ± SD for 6 rats (p<0.05)

Fig 23. Effect of methanol extract of *Aloe vera* on alkaline phosphatase activity (ALP)

Values are Mean ± SD for 6 rats (p<0.05)
<table>
<thead>
<tr>
<th>Treatment dose (g/kg)</th>
<th>Liver</th>
<th>Kidney</th>
<th>Small intestine</th>
<th>Brain</th>
<th>Heart</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>normal histology</td>
<td>normal histology</td>
<td>normal histology</td>
<td>normal histology</td>
<td>normal histology</td>
</tr>
<tr>
<td>dose 1</td>
<td>normal hepatocytes</td>
<td>normal glomerulii, tubules</td>
<td>normal villi with lining columnar epithelium, mucosal glands, serosa</td>
<td>normal histology</td>
<td>normal histology</td>
</tr>
<tr>
<td>dose 2</td>
<td>normal hepatocytes</td>
<td>normal glomerulii, tubules</td>
<td>normal villi with lining columnar epithelium, mucosal glands, serosa</td>
<td>normal histology</td>
<td>normal histology</td>
</tr>
<tr>
<td>dose 3</td>
<td>normal hepatocytes</td>
<td>normal glomerulii, tubules</td>
<td>normal villi with lining columnar epithelium, mucosal glands, serosa</td>
<td>normal histology</td>
<td>normal histology</td>
</tr>
<tr>
<td>dose 4</td>
<td>normal hepatocytes</td>
<td>normal glomerulii, tubules</td>
<td>normal villi with lining columnar epithelium, mucosal glands, serosa</td>
<td>normal histology</td>
<td>normal histology</td>
</tr>
<tr>
<td>dose 5</td>
<td>normal hepatocytes</td>
<td>normal glomerulii, tubules</td>
<td>normal villi with lining columnar epithelium, mucosal glands, serosa</td>
<td>normal histology</td>
<td>normal histology</td>
</tr>
</tbody>
</table>
Fig 24. Histopathological observations of rat organs

i) Section of kidney – control rat

ii) Section of kidney - rat treated with Aloe vera methanol extract (1g/kg b.wt)

iii) Section of kidney - rat treated with Aloe vera methanol extract (2g/kg b.wt)

iv) Section of kidney - rat treated with Aloe vera methanol extract (4g/kg b.wt)

v) Section of kidney - rat treated with Aloe vera methanol extract (8g/kg b.wt)

vi) Section of kidney - rat treated with Aloe vera methanol extract (16g/kg b.wt)
vii) Section of liver - control rat

viii) Section of liver - rat treated with *Aloe vera* methanol extract (1g/kg b.wt)

ix) Section of liver - rat treated with *Aloe vera* methanol extract (2g/kg b.wt)

x) Section of liver - rat treated with *Aloe vera* methanol extract (4g/kg b.wt)

xi) Section of liver - rat treated with *Aloe vera* methanol extract (8g/kg b.wt)

xii) Section of liver - rat treated with *Aloe vera* methanol extract (16g/kg b.wt)
xiii) Section of small intestine - control rat

xiv) Section of small intestine - rat treated with *Aloe vera* methanol extract (1g/kg b.wt)

xv) Section of small intestine - rat treated with *Aloe vera* methanol extract (2g/kg b.wt)

xvi) Section of small intestine - rat treated with *Aloe vera* methanol extract (4g/kg b.wt)

xvii) Section of small intestine - rat treated with *Aloe vera* methanol extract (8g/kg b.wt)

xviii) Section of small intestine - rat treated with *Aloe vera* methanol extract (16g/kg b.wt)
xix) Section of brain - control rat

xx) Section of brain - rat treated with *Aloe vera* methanol extract (1g/kg b.wt)

xi) Section of brain - rat treated with *Aloe vera* methanol extract (2g/kg b.wt)

xii) Section of brain - rat treated with *Aloe vera* methanol extract (4g/kg b.wt)

xiii) Section of brain - rat treated with *Aloe vera* methanol extract (8g/kg b.wt)

xiv) Section of brain - rat treated with *Aloe vera* methanol extract (16g/kg b.wt)
xv) Section of heart - control rat
xvi) Section of heart - rat treated with Aloe vera methanol extract (1g/kg b.wt)

xvii) Section of heart - rat treated with Aloe vera methanol extract (2g/kg b.wt)
xviii) Section of heart - rat treated with Aloe vera methanol extract (4g/kg b.wt)

xix) Section of heart - rat treated with Aloe vera methanol extract (8g/kg b.wt)
xxx) Section of heart - rat treated with Aloe vera methanol extract (16g/kg b.wt)