2. MATERIALS AND METHODS:

2.1 FOR BOSWELLIA SERRATA PLANT EXTRACTS

2.1.1 Procurement of material: The Bark, Leaves and Gum of the plant Boswellia serrata were collected from Narsapur Forest, Medak District (A.P.) and authenticated at CENTRAL RESEARCH INSTITUTE OF UNANI MEDICINE, HYDERABAD. Voucher no-CRIUM-114/2004. The plant materials were air dried at room temperature and powdered.

2.1.2 CHEMICALS AND DRUGS USED:

STANDARD DRUG:

Liv-52, an ayurvedic multi herbal formulation was used as a Standard drug\(^{(100-102)}\). It is a product of Himalaya Pharmaceuticals Ltd. Bangalore, India. It is a reputed hepatoprotective drug procured from market. It is given at a dose of (100mg/kg) body weight.

Liv-52 is a unique all natural complex multi ingredient formula \(^{(103)}\) containing the following:-

- Capparis spinosa
- Cichorium intybus
- Solanum nigrum
- Terminalia arjuna
Cassia occidentalis
Achillea millefolium
Tamarix gallica

**TOXICANT DRUG:**

The toxicant selected was paracetamol obtained from S.D. Fine Chemicals, Mumbai, India. It is optimally used for inducing liver damage at a dose of 3 gm/kg body weight\(^{104-105}\).

**CHEMICALS:**

1. Solvents Petroleum ether, Chloroform, Ethyl alcohol were obtained from S.D. fine chemicals, Mumbai, India, and used for the extraction purpose. Distilled water was used for the aqueous extraction.
2. Diethyl ether used as an anaesthetic was obtained from the S.D. fine chemicals, Mumbai, India.
3. Alcohol spirit is used for surgical purpose.
4. Enzymes kits (SGOT, SGPT, ALP, BILIRUBIN, and TPA) were obtained from Med Source Ozone Biomedicals Pvt. Ltd.

**2.1.3 METHODOLOGY:**

**SOLVENT EXTRACTION PROCEDURE**

The dried powdered plant materials are extracted in soxhlet assembly\(^{106-107}\) in order of increasing polarity with petroleum ether, chloroform and ethanol\(^{108}\). Following successive extraction, each time before extracting
with the next solvent the powder was air dried and used. For aqueous extract, dried and coarsely powdered plant materials were extracted separately with distilled water for 48 hours by cold maceration at room temperature and filtered. The extracts were concentrated with the help of vacuum evaporator and kept in a dessicator\textsuperscript{[109-111]}. The colour and consistency of extracts are noted.

\textbf{Table 2.1}: Percentage yield of Bark extracts of Boswellia serrata Plant.

<table>
<thead>
<tr>
<th>S.No</th>
<th>Extract</th>
<th>Colour</th>
<th>Consistency</th>
<th>Wt. of Sample before extraction</th>
<th>Wt. of Sample After extraction</th>
<th>Theoretical yield (in gm)</th>
<th>Practical yield (in gm)</th>
<th>% Yield</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>PBE</td>
<td>Golden yellow</td>
<td>Semi solid</td>
<td>142</td>
<td>114</td>
<td>28</td>
<td>8.31</td>
<td>29.6</td>
</tr>
<tr>
<td>2</td>
<td>CBE</td>
<td>Black</td>
<td>Semi solid</td>
<td>114</td>
<td>85</td>
<td>29</td>
<td>2.43</td>
<td>8.2</td>
</tr>
<tr>
<td>3</td>
<td>EBE</td>
<td>Brown</td>
<td>Solid</td>
<td>85</td>
<td>73</td>
<td>12</td>
<td>2.8</td>
<td>23.3</td>
</tr>
<tr>
<td>4</td>
<td>ABE</td>
<td>Light brown</td>
<td>Solid</td>
<td>73</td>
<td>50</td>
<td>23</td>
<td>5.61</td>
<td>24.3</td>
</tr>
</tbody>
</table>
Table 2.2: Percentage yield of Leaves extracts of Boswellia serrata Plant.

<table>
<thead>
<tr>
<th>S.No</th>
<th>Extract</th>
<th>Colour</th>
<th>Consistency</th>
<th>Wt. of Sample before extraction</th>
<th>Wt. of Sample After extraction</th>
<th>Theoretical yield (in gm)</th>
<th>Practical yield (in gm)</th>
<th>% Yield</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>PLE</td>
<td>Dark Green to black</td>
<td>Solid</td>
<td>140</td>
<td>115</td>
<td>29</td>
<td>9.2</td>
<td>31.7</td>
</tr>
<tr>
<td>2</td>
<td>CLE</td>
<td>Black</td>
<td>Semi solid</td>
<td>122</td>
<td>103</td>
<td>32</td>
<td>12</td>
<td>37.5</td>
</tr>
<tr>
<td>3</td>
<td>ELE</td>
<td>Light brown</td>
<td>Semi solid</td>
<td>96</td>
<td>75</td>
<td>16</td>
<td>9.1</td>
<td>56.8</td>
</tr>
<tr>
<td>4</td>
<td>ALE</td>
<td>Dark brown to black</td>
<td>Solid</td>
<td>98</td>
<td>55</td>
<td>21</td>
<td>7.1</td>
<td>33.8</td>
</tr>
</tbody>
</table>

Table 2.3: Percentage yield of Bark extracts of Boswellia serrata Plant.

<table>
<thead>
<tr>
<th>S.No</th>
<th>Extract</th>
<th>Colour</th>
<th>Consistency</th>
<th>Wt. of Sample before extraction</th>
<th>Wt. of Sample After extraction</th>
<th>Theoretical yield (in gm)</th>
<th>Practical yield (in gm)</th>
<th>% Yield</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>PGE</td>
<td>Golden yellow</td>
<td>Semi solid</td>
<td>116</td>
<td>98</td>
<td>18</td>
<td>10</td>
<td>55.5</td>
</tr>
<tr>
<td>2</td>
<td>CGE</td>
<td>Golden</td>
<td>Semi solid</td>
<td>85</td>
<td>72</td>
<td>13</td>
<td>9</td>
<td>69.2</td>
</tr>
<tr>
<td>3</td>
<td>EGE</td>
<td>Dark Brown</td>
<td>Semi Solid</td>
<td>68</td>
<td>55</td>
<td>17</td>
<td>11</td>
<td>64.7</td>
</tr>
<tr>
<td>4</td>
<td>AGE</td>
<td>Golden Brown</td>
<td>Semi Solid</td>
<td>76</td>
<td>48</td>
<td>9</td>
<td>6</td>
<td>66.6</td>
</tr>
</tbody>
</table>
Preliminary Phytochemical evaluation\(^{112-113}\):

All the extracts were screened for the presence of various secondary metabolites like Tannins, Alkaloids, Glycosides, Terpenoids, flavonoids, amino acids and proteins using standard methods.

**HPTLC PROFILE**

Chromatography was performed on high performance thin layer chromatography (HPTLC) plates coated with 0.25mm layer of silica gel 60F254 (Merck, Munchen, Germany). Before using the plates were activated at 110\(^0\)C for 5min. Samples were applied as 4mm wide bands and 6mm apart by using of DESAGA Sarstedt Gruppe applicator equipped with 100µL syringe. A constant application rate of 5µL/second was used. The mobile phase was Toluene: Ethyl acetate (9:1v/v) and chromatograms were monitored at 366nm.

**2.1.4 Procurement of male albino rats & mice:**- The male albino rats weighing between 150-180gms. were procured from Mahaveer Enterprises (Reg.No. 146/1999/CPCSEA) Amberpet, Hyderabad, India. They were maintained at standard housing conditions at a room temperature of 24\(^+\) 1\(^0\)C, relative humidity 45-55% with 12:12 hour light/dark cycle\(^{114-116}\). The feeding was done with commercially available rat feed pellets and water was given adlibitum during the experiment\(^{117}\). The organ protection activity was evaluated after taking approval of ethical committee Reg. No.1330/ac/10/CPCSEA.
2.1.5 ACUTE TOXICITY STUDIES:

ANIMALS: Albino mice

SEX: Males

BODY WEIGHT: 22-30gm.

VEHICLE: Rice Bran Oil.

STEP-1: TO FIND OUT THE LETHAL DOSE

Acute toxicity was carried out as per CPCSEA and OECD/OCDE Guidelines. Groups of 3 mice were selected weighing between 22-30 gm and kept for 3-4hrs fasting with free access to water. Dose is calculated according to body weight and extracts to be studied are dissolved in rice bran oil, and administered via oral route. The starting dose of 2000mg/kg was administered orally in 3 mice and observed for 24 hours.

BARK EXTRACT:

Petroleum ether bark extract (PBE):

After the dosing of 2000 mg/kg, it was observed that 1 mice died after 24 hours and the dosing was again repeated at 2000mg/kg. It was observed that 1 mice died. According to CPCSEA rule, lethal dose falls under category-5 of annex 2d (OECD/OCED rule) for petroleum ether bark extract with the lethal dose of 2500mg/kg body weight.
STEP-2: TO FIND OUT THE EFFECTIVE DOSE:

The $1/10^{th}$ of the lethal dose was taken as an effective dose (E.D)\(^{118-120}\).

Therefore the E.D. was found to be 250 mg/kg body weight.

**Chloroform bark extract (CBE):** Dose of 2000mg/kg was administered orally in 3 mice, 1 mice died after 24hrs, Dosing was again repeated at 2000mg/kg, 1 mice died. According to CPCSEA rule, lethal dose falls under category-5 of annex 2d (OECD/OCDE rule) with the lethal dose of 2500mg/kg body weight. The $1/10^{th}$ of the lethal dose was taken as an effective dose i.e., 250 mg/kg body weight.

**Ethanolic bark extract (EBE):** Dose of 2000mg/kg was administered orally in 3 mice, no mice died after 24hrs thus, dosing was again repeated at 2000mg/kg, no mice died. According to CPCSEA rule, lethal dose falls under category-5 of annex 2d (OECD/OCED rule) with the lethal dose of 5000 mg/kg body weight. The $1/10^{th}$ of the lethal dose was taken as an effective dose i.e; 500 mg/kg body weight.

**Aqueous bark extract (ABE):** Dose of 2000mg/kg was administered orally in 3 mice, 1 mice died after 24hrs, Dosing was again repeated at 2000mg/kg, 1 mice died. According to CPCSEA rule, lethal dose falls under category-5 of annex 2d (OECD/OCED rule) with the lethal dose of 2500mg/kg body weight. The $1/10^{th}$ of the lethal dose was taken as an effective dose i.e; 250 mg/kg body weight.
2.1.6 EFFECTIVE DOSE OF BOSWELLA SERRATA BARK EXTRACTS:

The effective dose of Petroleum ether bark extract (PBE) extract was found to be \textbf{250mg/kg body wt.} body weight.

The effective dose of Chloroform bark extract (CBE) extract was found to be \textbf{250mg/kg body wt.} body weight.

The effective dose of Ethanolic bark extract (EBE) extract was found to be \textbf{500 mg/kg body wt.} body weight.

The effective dose of Aqueous bark extract (ABE) extract was found to be \textbf{250 mg /kg body wt.} body weight.

Similar procedures were followed to find out the lethal dose and effective dose of leaf anf gum extracts respectively.

2.1.7 EFFECTIVE DOSE OF BOSWELLA SERRATA LEAF EXTRACTS:

The effective dose of Petroleum ether leaf extract (PLE) was found to be \textbf{250 mg/kg body wt.}

The effective dose of Chloroform leaf extract (CLE) was found to be \textbf{200 Mg/kg body wt.} body weight.
The effective dose of *Ethanolic leaf extract (ELE)* was found to be 250 mg/kg body wt.

The effective dose of *Aqueous leaf extract (ALE)* was found to be 500 mg/kg body wt.

### 2.1.8 EFFECTIVE DOSE OF BOSWELLIA SERRATA GUM EXTRACTS:

The effective dose of *Petroleum ether gum (PGE)* extract was found to be 250 mg/kg body wt.

The effective dose of *Chloroform gum extract (CGE)* was found to be 100 mg/kg body wt.

The effective dose of *Ethanolic gum extract (EGE)* was found to be 250 mg/kg body wt.

The effective dose of *Aqueous gum extract (AGE)* was found to be 250 mg/kg body wt.
2.2 HEPATOPROTECTIVE ACTIVITY:

2.2.1 In Vitro: Fasting Albino male rats weighing 130-160 g were used. Liver cells were isolated by using a modified procedure\textsuperscript{(121-122)}. The animal was cleaned thoroughly using rectified alcohol and anaesthetized with ether. Dissection of the animal was carried out under aseptic conditions using sterilized instruments. A midline incision was made on the abdomen of the anaesthetized animal. The portal vein was canulated with needle no. 25 connected to an infusion set. The needle was tied in place and the inferior vena cava was cut below the renal vein. Perfusion of the liver was started immediately with Ca\textsuperscript{2+} - Mg\textsuperscript{2+} free Hanks buffer salt solution (pH 7.4 at 37\textdegree C). When the liver was thoroughly perfused (i.e has turned white), the flow of HBSS was stopped and the needle was removed. The liver was transferred to a sterile Petri dish containing Ca\textsuperscript{2+}-Mg\textsuperscript{2+} free HBSS and minced into small pieces, which were transferred to a conical flask containing 10 mL of 0.075% collagenase in HBSS. This was placed on a magnetic stirrer at 37\textdegree C for 10 min. The cell suspension thus obtained was centrifuged at 50 g for 10 min. The supernatant was aspirated and the cells Suspended in the Ca2+-Mg2+ free HBSS. The cells were washed twice and counted in the presence of trypan blue dye. Viability of the cells in each of the experiment performed was found to be 90%. The isolated hepatocytes were cultured in Eagles MEM, supplemented with 10% inactivated serum
at density of 0.5 x 10⁹ cells / L in sterile disposable culture bottles and incubated in a humified incubator at 37°C under 5% CO₂. The viability of hepatocytes was studied after 6, 12, and 24 hrs. The hepatocytes which settled down were observed for their growth. Cytotoxicity of *Boswellia serrata* extracts was tested in primary hepatocytes monolayer cultures. Neither of the extracts caused significant enzymes release or were cytotoxic.

Hepatocytes were prepared from male Wister rats by the collagenase perfusion technique \(^{(123)}\). Cells were purified by several centrifugations and inoculated at density of 0.5 x 10⁸ cells / L on collagen coated plates. One day after the isolated rat hepatocytes were plated, cells were exposed to medium containing 7 mmol /L Paracetamol with or without the sample to be tested for the hepatoprotective activity. After the exposure to Paracetamol for 1 hr. the culture medium was collected and used for the determination of different parameters. Lipid peroxidation was assessed as TBA – reactive substances using malondialdehyde (MDA) as reference. Rat hepatocyte 1 x 10⁹ cells / L were incubated in a final volume of 1.0 mL HBSS buffer containing test materials in presence of 200 mL FeSO₄ + 100 µmol / LH₂O₂. The biochemical estimations were carried out by using the usual technique.
2.2.2 In vivo:- For the in-vivo evaluation of hepatoprotective activity of *Boswellia serrata* Bark extracts the animals were starved overnight and divided into seven groups consisting of 5 rats each, which are as follows:

1) Group 1: Control (vehicle rice bran oil) group

2) Group 2: Paracetamol (PCM) or Toxicant group

3) Group 3: PCM + Liv-52 or Standard group

4) Group 4: PCM + PBE (petroleum ether bark extract)

5) Group 5: PCM+CBE (chloroform bark extract)

6) Group 6: PCM+EBE (ethanol bark extract)

7) Group 7: PCM+ABE (aqueous bark extract)

Group 1 or control group was administered rice bran oil orally for a period of 9 days. Group 2 or toxicant group was administered 3gm/kg paracetamol as acute toxic dose orally only on the 9th day of study. Group 3 or Standard group was given Liv-52 at a dose of 100 mg/kg body weight for a period of 9 days. The groups from 4-7 received the oral dose of extracts respectively for a period of 9 days.
The groups from 3-7 were administered oral dose of 3 gm/kg paracetamol on 9th day of study, 3hrs after the administration of the extracts to the respective groups.

After 48 hrs all the rats were sacrificed under light ether anaesthesia\(^{124}\) and blood samples were collected by puncturing the retro-orbital plexus\(^{125-126}\)) and was allowed to clot for 45 min at room temperature, serum was separated by centrifugation at 2500 rpm for 15 min. and used for investigation of various biochemical parameters\(^{127-128}\).

### 2.2.3 ASSESMENT OF LIVER FUNCTIONS:

#### 1. Bio-chemical Investigations (Liver Function Tests)

On 11th day all the rats were sacrificed and blood samples (about 3-5ml) were collected by puncturing the retro-orbital plexus from all animals and serum was prepared for estimation of liver enzymes like SGOT, SGPT, and ALP and their levels were measured \(^{129}\). The other tests, Direct bilirubin, Conjugated bilirubin, Albumin, Globulin, and total proteins were also determined\(^{130-131}\). Serum Albumin is a marker of synthetic function of liver and is a valuable guide to the severity of disease\(^{132}\).

#### 2. Histopathological examination

After collecting blood, the rat livers were excised quickly and washed with normal saline and fixed in a 10% buffered neutral formalin solution \(^{133}\), they were processed for paraffin embedding following the microtome
Sections were stained with haematoxylin and eosin and were observed microscopically for histopathological examinations and later these microscopic slides were photographed.

2.2.4 BIOCHEMICAL ANALYSIS:

Serum samples obtained after centrifugation were stored at 2-8°C for serum analysis.

Method of Estimation:

**SGPT (ALAT):** Mix 1 part of R2 (reagent 2 from enzyme kit) with 4 parts of R1 as per the requirement. This is called working reagent. Mix 1 ml of working reagent with 100µlt of serum. Mix well and after 1 min incubation, measure the change in optical density per 60sec during 180 sec against distilled water at 340 nm.

**SGOT (ASAT):** Mix 1 part of R2 (reagent 2 from enzyme kit) with 4 parts of R1 as per the requirement. This is called working reagent. Mix 1 ml of working reagent with 100µlt of serum. Mix well and after 1 min incubation, measure the change in optical density per 60sec during 180 sec against distilled water at 340 nm.

**ALKALINE PHOSPHATASE (ALP):** Mix 1 part of R2 (reagent 2 from enzyme kit) with 4 parts of R1 as per the requirement. This is called working reagent. Mix 1 ml of working reagent with 20µlt of serum. Mix
well and exactly after 1 min incubation, measure the change in optical
density per 60sec during 180 sec against distilled water at 405 nm.

TOTAL PROTEIN: Pipette into test tubes labeled blank(B), standard(S)
and test(T) as follows.

**Table 2.4: Estimation of Total Protein**

<table>
<thead>
<tr>
<th>Reagent</th>
<th>B</th>
<th>S</th>
<th>T</th>
</tr>
</thead>
<tbody>
<tr>
<td>Biuret reagent(1)</td>
<td>1.0 ml</td>
<td>1.0 ml</td>
<td>1.0 ml</td>
</tr>
<tr>
<td>TPS 3</td>
<td>-</td>
<td>50 µlt</td>
<td>-</td>
</tr>
<tr>
<td>Specimen</td>
<td>-</td>
<td>-</td>
<td>50 µlt</td>
</tr>
<tr>
<td>Distilled water</td>
<td>2.05 ml</td>
<td>2 ml</td>
<td>2 ml</td>
</tr>
</tbody>
</table>

Mix well and incubate at 37°C for 5 min. Read absorbance of std and
test against blank at 555nm.

ALBUMIN[^138]:

Pipette into test tubes labeled blank (B), standard(S) and test (T) as
follows.

**Table 2.5: Estimation of Albumin**

<table>
<thead>
<tr>
<th>Reagent</th>
<th>B</th>
<th>S</th>
<th>T</th>
</tr>
</thead>
<tbody>
<tr>
<td>BCG reagent(2)</td>
<td>1.0 ml</td>
<td>1.0 ml</td>
<td>1.0 ml</td>
</tr>
<tr>
<td>STD 4</td>
<td>-</td>
<td>10 µlt</td>
<td>-</td>
</tr>
<tr>
<td>Specimen</td>
<td>-</td>
<td>-</td>
<td>10 µlt</td>
</tr>
<tr>
<td>Distilled water</td>
<td>2 ml</td>
<td>2 ml</td>
<td>2 ml</td>
</tr>
</tbody>
</table>
Mix well read absorbance of std and teat against blank at 630 nm.

**BILIRUBIN (TOTAL & DIRECT):**

Pipette into test tubes labeled as T1 and T2 for total bilirubin and D1 and D2 for direct bilirubin.

**Table 2.6: Estimation of Bilirubin**

<table>
<thead>
<tr>
<th>Reagent</th>
<th>T1(ml)</th>
<th>T2(ml)</th>
<th>D1(ml)</th>
<th>D2(ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Diazo A</td>
<td>0.5</td>
<td>0.5</td>
<td>0.5</td>
<td>0.5</td>
</tr>
<tr>
<td>Diazo B</td>
<td>0.05</td>
<td>-</td>
<td>0.05</td>
<td>-</td>
</tr>
</tbody>
</table>

Mix thoroughly and then proceed.

<table>
<thead>
<tr>
<th>Activator</th>
<th>0.5</th>
<th>0.5</th>
<th>-</th>
<th>-</th>
</tr>
</thead>
<tbody>
<tr>
<td>Distilled water</td>
<td>1</td>
<td>1</td>
<td>1.5</td>
<td>1.5</td>
</tr>
<tr>
<td>Serum</td>
<td>0.1</td>
<td>0.1</td>
<td>0.1</td>
<td>0.1</td>
</tr>
</tbody>
</table>

Mix well and read the absorbance of D1 & D2 exactly after 1 min on colorimeter at 540 nm.

Mix well and keep the tubes T1 & T2 in dark at temperature for 5 min then read absorbance of T1 & T2 and of artificial Std. at 540 nm.
Plate: 2.1: Soxhlet extractor
Plate: 2.2: ALBINO RATS UNDER STUDY FOR HEPATO PROTECTIVE ACTIVITY
Plate: 2.3 DISSECTION OF RAT LIVER
Plate: 2.4 SEMI AUTO ANALYSER FOR SERUM ANALYSIS

Plate: 2.5 COLORIMETER FOR ANALYSIS OF RATS SERUM
Plate: 2.6 ENZYME KITS OF SGPT, SGOT, ALP, & BILIRUBIN
2.3 ANTI-ULCER ACTIVITY

2.3.1 Methodology:
Peptic ulcer is one of the major ailments effecting humans and believed to develop because of imbalance between aggressive factors (acid, pepsin, H.pylori, bile salts) and defensive factors (mucous, bicarbonate, blood flow, epithelial cells restoration and prostaglandins)[139]

2.3.2 PYLORIC LIGATION MODEL [140-141]

The animals were divided into six groups (control, standard, and four test groups) of five animals in each. To each animal under ether anesthesia, a midline incision was made and the pylorus was ligated, care being exercised that neither damage to blood vessel nor traction on the pylorus occur. Careful suturing of the abdominal wall was done. Standard drug selected was Ranitidine (20mg/kg) orally. Control was given Rice bran oil- 1ml/kg. The remaining four groups received oral administration of Boswellia serrata plant extracts in calculated effective dose according to body weight of animals. The animals were deprived of food and water post operatively. The animals were sacrificed by euthanasia four hours after the pyloric ligation. The stomach were dissected out along the greater curvature and examined. Various parameters such as spot ulcer, haemorrhagic streak, ulcers and their numbers have been observed and scoring is given for all groups. Before that the gastric juice was collected and centrifuged for the determination
of gastric volume, gastric pH, Free acidity and total acidity\textsuperscript{(142)}. The stomachs were then taken for histopathological Examinations.

\textbf{2.3.3 ETHANOL INDUCED ULCER MODEL (143)}

The animals were divided into six groups (control, standard, and four test groups) of five animals in each group for evaluation of anti-ulcer activity of Bark, Leaves and Gum extracts. Standard drug selected was Omeprazole 20mg/Kg. Control received 1ml/kg rice bran oil, the remaining four groups received calculated effective doses of \textit{Boswellia serrata} plant extracts orally according to body weight of animals. After one hour the animals were administered with 1ml of 99.5% ethanol orally. One hour after ethanol administration the animals were sacrificed by euthanasia. Stomachs were dissected and examined for ulcer. The stomachs were then taken for histopathological studies.

\textbf{2.3.4 ASPIRIN INDUCED ULCER MODEL}

The animals were divided into six groups (control, standard and four test groups) of five animals in each. Standard drug selected was Ranitidine 20mg/kg, orally. Control received 1ml/kg rice bran oil orally. The remaining four groups received calculated effective dose of \textit{Boswellia serrata} plant extracts orally according to body weight of animals. After 8 days of dosing, animals were fasted for 24 hours and later oral administration of aqueous suspension of Aspirin in a dose of 200mg/Kg \textsuperscript{(144)} was given. The animals were then sacrificed by euthanasia four hours later of Aspirin administration. The stomach was dissected and
examined for ulcers. The stomachs were then taken for histopathological studies.

**2.3.5 CALCULATION OF ULCERATIVE SCORE – ULCERATIVE INDEX**

Open the stomach along the greater curvature and wash it slowly under running tap water. Put it on a glass slide and observe under 10 x magnifications for ulcers. Score the ulcer as below

*Table 2.7:* The mean of ulcer score is *ulcer index* (mm)

<table>
<thead>
<tr>
<th>OBSERVATIONS ON STOMACH</th>
<th>ULCER SCORE (mm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal coloured stomach</td>
<td>0</td>
</tr>
<tr>
<td>Red colouration</td>
<td>0.5</td>
</tr>
<tr>
<td>Spot ulcers</td>
<td>1</td>
</tr>
<tr>
<td>Haemorrhagic streaks</td>
<td>1.5</td>
</tr>
<tr>
<td>Ulcers $\geq 3 \leq 5$</td>
<td>2</td>
</tr>
<tr>
<td>Ulcers $&gt; 5$</td>
<td>3</td>
</tr>
</tbody>
</table>

Mean ulcer score of each group were calculated, which was designated as Ulcer index and Percentage was calculated\(^{145-146}\) as

\[
\% \text{ Protection} = \frac{(C-T/C) \times 100}{C}\]

Where C= Ulcer index in Control group  
T= Ulcer index in Treated group

**2.3.6 DETERMINATION OF GASTRIC VOLUME:**

Centrifuge the gastric content at 1000 rpm for 10 min. The volume of the supernatant liquid was noted using a micro pipette for the determination of gastric volume.
2.3.7 DETERMINATION OF pH, FREE ACIDITY & TOTAL ACIDITY:
Stomach contents from all the screening models were taken in centrifuge tube (1 tube for each animal contents) Centrifuge the gastric contents at 1000 rpm for 10 min. Volume of supernatant was noted. Then 1ml of supernatant liquid was pipette out and diluted it to 10ml with distilled water. The pH of the solution was noted with the help of pH meter. The solution was then titrated with 0.01N sodium hydroxide using Topfer’s reagent as indicator (it is dimethyl-amino-azo-benzene with phenolphthalein and used for detection and determination of HCl and total acidity in gastric fluid). The end point is seen as Canary yellow colour. The volume of NaOH was noted which corresponds to free acidity. Titration was carried further adding few drops of phenolphthalein indicator and end point was noted as solution turned pink in colour. The total volume of NaOH was noted which corresponds to the total acidity.
2.4 DIURETIC ACTIVITY

2.4.1 METHODOLOGY:-

6-Groups containing 5 male Albino rats in each group, weighing between 150-180 gm were selected and is kept for overnight fasting but is not deprived of water. 5 ml/kg of water was given orally to rats before administration of drug or extracts. Control received rice bran oil (1ml/kg), standard group received Furosemide (10 mg/kg). The extracts were given respective effective doses orally and kept in metabolic cages specially designed to separate urine and faeses and kept at room temperature of 25±0.5°C\textsuperscript{[148]}. Urine was collected and measured after 5 and 24 hours in graduated measuring cylinder. During this period no water and food was available to animals \textsuperscript{[149]}. The bladder was emptied by pulling the base of tail of each rat \textsuperscript{[150]}. 

Diuretic activity of the plant extracts was evaluated by measurements of various parameters such as urine volume, excretion of electrolytes i.e., sodium (Na\textsuperscript{+}), potassium (K\textsuperscript{+}), chloride (Cl\textsuperscript{-}), Osmolarity, Density, and Sp. gravity. Acid-Base balance was noted by measuring Bicarbonate (HCO\textsubscript{3}\textsuperscript{-}) level and pH of urine.

The organ protection activity of the extracts on the kidneys was evaluated by estimating the BUN level and serum creatinine level. Uric acid excretion was evaluated for Anti-gout activity.
Urine volume was measured using a measuring cylinder. The electrolytes were analyzed using easilyte analyzer and the concentration was determined in milli moles eq/wt. The pH of urine was determined. The Sp. gravity was determined using Bayers multistrips. Density was determined by taking mass and volume as measures.

Plate: 2.7: Albino Rats kept in Metabolic cages

2.4.2 BLOOD ANALYSIS:
Rats were anaesthetized and blood was drawn from retro orbital plexus of eye using capillary tube. The following steps involved are:
Plate: 2.8 Anaesthetized Rat

Plate: 2.9 Insertion of Capillary Tube Withdrawal of Blood
Plate: 2.10 Completion of Blood collection
Plate: 2.11: Serum analyzer  Plate 2.12: BUN analyser

Plate: 2.13 Easy Lyte Analyser