3. MATERIALS AND METHODS

3.1. PLANT MATERIALS

The leaves of *Girardinia heterophylla* and *Colocasia esculenta* were collected from Uttarakhand Himalayan region with the help of local plant collector. The plants were identified by Forest Research Institute of Dehradun, India. The plants were authenticated and a voucher specimen (No.Dis/2010-Bot./4-12(Herb.)) is maintained in the systematic botany discipline Botany Division, FRI, Dehradun.

3.2. PREPARATION OF THE EXTRACTS

The process of solvent extraction is involved in the preparation of most synthetic drugs or those produced by fermentation are after purified or separated by the mother liquor by liquid extraction. Constituents contained within plant or animal tissues are also extracted by means of solvents but a preliminary leaching process is usually necessary. Fixed oils
may be obtained in this way but are also removed by direct expression of the tissues and volatile oils are separated by steam distillation. With the advent of numerous synthetic drugs the demand for drugs of natural origin is diminishing. Some plant and animal products still remain important however including alkaloids, glycosides and other sugar derivatives spices, fixed and volatile oil, proteins and polypeptides.

3.2.1. Soxhlet

A small scale extraction apparatus consist of a flask, a soxhlet extractor and a reflux condenser. The raw materials (leaves of Girardinia heterophylla and Colocasia esculenta) is usually placed in a thimble made of filter paper and inserted into a wide central tube of the extractor. Solvent (alcohol) is placed in the flask and boiled, its vapour pass up the large right hand tube into the central space above the bark and hence to the condenser. The condensate then drops back onto the bark, through which it percolates, leaching solutes in the process. When sufficient of the solution has collected to raise its level to that of the top of the syphon tube, the whole of the collected percolate siphons over into the flask. The suction effect of the siphoning assists permeation of solvent through the drug. A limited amount of hot salient is thus made to percolate repeatedly through the raw material the solutes from which are transferred to the flask.

The principle of continuous hot extraction is sometimes used to extract a drug for the purpose of assay. It is the simple, which has the advantage that the hot, rising vapors, encircle the material to be extracted.

3.2.2. Distillation

Distillation is a method of separating substances which differ appreciably in their vapour pressures if is used in
pharmacy either to extract volatile active principles from vegetable drug or to separate volatile substances from their less volatile impurities. It also provides a method of recovering volatile solvents such as alcohol.

3.2.2.1. Simple Distillation

We have used the simple distillation method - the process of converting a liquid into its vapour, transferring the vapour to another place and recovering the liquid by condensing the vapour, usually by leading it in to contact with cold surface. The apparatus used consists of essentially three parts- the steal in which the volatile material is vaporized, the condenser in which the vapours are condensed; and the receiver in which the distillate is collected. Simple distillation can produce partial separation of components with different boiling point in a liquid mixture the more volatile components having obtained in increased concentration in the vapour. The process is generally used for the separation of liquid from non-volatile solids. Eg - preparation of distilled water and recovery of alcohol in the preparation of dry extracts.

Procedure

We have done the extraction by two methods

(1) Methanol
(2) Aqueous

Methanol extraction:

- The leaves of plants were taken and washed. Shaded and dried it properly in (tray drier) oven. at 50°C
- The leaves were crushed properly and first treated with petroleum ether in soxhlet assembly for remove the steroids.
- The assembly run till the drop became colourless. The raw material taken was 500 gm and volume of petroleum ether is about 2 liter.
- After soxhleting, distill off till all the liquid is condensed under reduced pressure.
- The raw material was treated with alcohol or (methanol) again for the alcoholic extraction. The extraction apparatus was run till the drop becomes colourless.

Aqueous extraction: The procedure is as same as alcoholic extraction.
3.3. ANTIMICROBIAL ACTIVITY

The antimicrobial activities of *Girardinia heterophylla* and *Colocasia esculenta* were assayed against six bacterial strains *Staphylococcus aureus* (MTCC-96), *Klebsiella pneumoniae* (MTCC-109), *Streptococcus mutans* (MTCC-890), *Pseudomonas fragi* (MTCC-2458), *Escherichia coli* (MTCC-483) and *Bacillus subtilis* (MTCC-121). Two fungal strain, *Aspergillus niger* (MTCC-281) and *Candida albicans* (MTCC-227) were also used to evaluate anti-fungal activity. Antibiotic assay medium no.1 (HiMedia) were used for bacterial culture and Sabouraud’s dextrose medium (HiMedia) were used for fungal culture. All the chemicals for the media formulation were procured from Himedia, India. The concentrations of bacterial suspensions were adjusted to $10^4$ CFU and fungal suspension to $10^3$ CFU.

3.4. ANTIMICROBIAL ASSAY

Antimicrobial activities of *Girardinia heterophylla* and *Colocasia esculenta* were determined by modified agar well diffusion method (Parekh *et al.* 2006) at four different concentrations, i.e., 100 mg/ml, 200 mg/ml, 300 mg/ml, 400 mg/ml using specified growth media for different test organisms (2% agar used for media solidification and agar was procured from Marc). The experiments were repeated for another 2 times and antimicrobial activity was determined by measuring the diameter of the zone of inhibitions after 18 h incubation for bacterial and 36 h incubation for fungal test organisms and mean values are determined (Table 1). Chloramphinicol (200 µg/ml) and Rifampicin (200 µg/ml), procured from Sigma, were used as positive controls for antibacterial and antifungal activities respectively.

3.5. PHYTOCHEMICAL ANALYSIS

3.5.1. Determination of total phenolic contents

The amount of total phenolics in extract was determined with Folin–Ciocalteu reagent according to the method of Singleton and Rossi (1965) with slight modification using gallic acid as a standard. 20 µl of sample was mixed with 1.58 ml of distilled water and added 100 µl Folin-Ciocalteu rea’gents. Mix well and incubated for 30 sec and 8 min at
room temperature. The absorbance was measured at 765 nm using a UV-Vis spectrophotometer.

3.5.2. Determination of flavonoids

Flavonols in *C. esculenta* extracts were expressed as quercetin equivalent. Quercetin (Sigma, Germany) was used to perform the calibration curve (standard solutions of 6.25, 12.5, 25.0, 50.0, 80.0 and 100.0 µg mL⁻¹ in 80 % ethanol (V/V)). Sample extracts (1g plant material in 25 ml extract) were all evaporated to dryness and re-dissolved in 80 % ethanol to be ready for the analytical test. Taken 50 µl sample and added 950 µl of phosphate buffer followed by 3000 µl follin’s reagent and 4000 µl sodium carbonate. After incubation at room temperature for 45 minutes, the absorbance of the reaction mixture was measured at 765 nm (Chang et al. 2002)

3.6. EXPERIMENTAL ANIMALS

Permission from the institutional ethical committee for laboratory use of animals was duly obtained. The experiments were performed on Wistar albino mice (3-4 months) obtained from Indian Veterinary Research Institute, Bareilly. The animals were kept in the experimental lab animal house at our Institute. The mice were housed in clean polypropylene cages and feed *ad libitum* with the commercially available feed and water. The litter was changed after every 5 days.

3.6.1. Labelling of Animals

The mice were labeled with picric acid solution containing small amount of dilute HCl. Each cage contained six mice. A total of 24 animals were equally divided into 6 groups (N = 6 in each subgroup). The details of the groups are given below:

*Table 2.1: Allocation of animals according to treatment groups*
3.6.2. Treatment Schedule

Acute Toxicity Studies

Acute oral toxicity (AOT) of alcohol was determined using Wistar albino mice. The animals were administered with single dose of the AEGH along with 2% alcohol and observed for mortality up to 90 days period.

<table>
<thead>
<tr>
<th>GROUP</th>
<th>TYPE</th>
<th>TREATMENT</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>Normal</td>
<td>No Treatment</td>
</tr>
<tr>
<td>II</td>
<td>Alcohol</td>
<td>Alcohol (160 mg /100 gm body wt)</td>
</tr>
<tr>
<td>III</td>
<td>Positive</td>
<td>Alcohol + Silymarin (10 mg /100 gm body wt)</td>
</tr>
<tr>
<td>IV</td>
<td>AEGH</td>
<td>Alcohol + Aqueous plant extract (16mg/100 gm body wt)</td>
</tr>
<tr>
<td>V</td>
<td>AECE</td>
<td>Alcohol + Aqueous plant extract (16mg/100gm body wt)</td>
</tr>
</tbody>
</table>

3.6.3. Dissection of the Animal

The animals were sacrificed by mild ether anesthesia. Take anesthetized mice fixed on dissection tray by ventral side. Lay down the mice by pinning it in the dissecting tray ventro-dorsally and pinning it in the limbs. With the help of a fresh sterile syringe withdraw blood from the heart while the heart is pumping. Store the blood in sterile plain vials and label them. Cut and remove the liver and kidney then washed two or three times in saline. Liver and kidney were weighed and then stored in cold phosphate buffer (50mM pH 7).

3.6.4. Homogenization of Tissue

The small pieces of organs were transformed to homogenizing tube to prepare homogenate in 50mM phosphate buffer saline in cold conditions. The homogenate was centrifuged at 3000rpm for 10 minute at 4°C then collected the supernatant for further...
tests. The blood samples were allowed to clot at room temperature for 45 min. The serum was separated by centrifugation at 2500 rpm at 30°C for 15 min and was stored at 4°C.

3.7. BIOCHEMICAL TEST IN TISSUE HOMOGENATE

3.7.1. Lipid Peroxidation

3.7.1.1. Principle
Assay of lipid per oxidation was done by Esterbauer and Cheeseman (1993) method. Malonaldehyde (MDA) is most abundant individual aldehyde from lipid per oxidation and its determination by thiobarbituric acid (TBA) is most common method of estimation of lipid per oxidation.

3.7.1.2. Methodology
Lipid per oxidation was determined by estimating the level of malonaldehyde MDA by the Easterbauer method (1993). Homogenized samples were mixed one by one thoroughly with an equal volume of 50% w/v tri chloroacetic acid (TCA) and kept in refrigerator. After 30 min tubes were centrifuged at 8000 rpm for 10 min. Supernatant was taken and mixed with equal volume of 0.87% Thiobarbituric acid, vortex and immediately placed in boiling water bath for 15 min. followed by rapid cooling. O.D. was taken at 535nm. MDA was expressed as nmoles MDA/ml plateletes suspension and were calculated based on extinction co-efficient of 153,000 for MDA-TBA adduct. It has been estimated that 99.7% of the absorbance at 535nm which is found in std.TBA assay resulted from MDA and only 0.3% or less due to all other aldehydes. Each sample was analyzed in triplicate and their mean was taken further analysis.

3.7.2. Superoxide Dismutase

3.7.2.1. Principle
An indirect assay method Mishra and Fridovich (1972) was used for the estimation of SOD activity. Auto oxidation of epinephrine in solution at alkaline pH values produces O´. Once formed participates in the oxidation of further molecules of epinephrine in a chain reaction to give rise to adrenochrome exhausts an absorption maximum at 480 nm. The addition of SOD greatly slows down the rate of oxidation of epinephrine because of the simultaneous and rapid utilization of O´ by the enzyme. This retardation in the production of adrenochrome from epinephrine in the presence of SOD provides a very convenient
method for the assay of the enzyme. Here epinephrine act as both as source of $O^-$ as well as the detector molecule. The rate of oxidation of epinephrine to adrenochrome is measured by the charge in absorbance at 480 nm (Mishra and Fridovich 1972).

3.7.2.2. Methodology

A standard curve between epinephrine concentration and absorbance at 480 nm was plotted to determine the concentration to be used in assay system at $25^0C$. The activity of SOD was measured according to Mishra and Fridovich. For control 3.0 ml, contain 1.0 ml tris buffer-(0.2m)PH-10.2,0.8 ml of 0.15m KCl, water is added to make final volume to 3.0 ml than added 0.2 ml of 0.025M epinephrine to start the reaction. Change in absorbance was measured at 480 nm and $25^0C$ at 15 seconds intervals for 60 seconds.

Unit of SOD activity

The unit of enzyme activity is defined as the amount of enzyme required to inhibit the rate of autoxidation of 5µmoles of epinephrine by 50% under experimental conditions. Percentage inhibition in sample is compared with blank and control.

3.7.3. Catalase

3.7.3.1. Principle

The absorption maximum of $H_2O_2$ observed at 240 nm. $H_2O_2$ decomposes to water and oxygen by the catalytic action of catalase on decomposition. The absorbance at 240 nm decreases with time and the rate of decreasing is used as measures of enzyme activity.

$$2H_2O_2 \rightarrow 2H_2O + O_2$$

3.7.3.2. Methodology

The Catalase activity was measured by the method of Beers and Sizers (1952). The spectrophotometer was adjusted to 100% transmittance at 240nm with a cuvette containing 0.3ml of phosphate buffer, 2.5ml H$_2$O and 0.1ml of H$_2$O$_2$.The sample was diluted with water to make up final volume equal to 3ml.The matching experimental cuvette contained 3.0ml of buffer having approximately 40 moles $H_2O_2$ at $25^0C$ and the sample. The absorbance was recorded at the interval of 15 sec for 1 min. The unit of enzyme activity was calculated based on molar extinction co-efficient of 0.04 cm$^2$/µmole of decomposed H$_2$O$_2$ at 240 nm.

Unit of catalase activity
One unit of catalase has been defined as the amount of enzyme required for the decomposition of one micromole of hydrogen peroxide in one minute under the experimental conditions. Specific activity has been expressed as unit/mg protein. The enzyme activity was followed by measuring initial velocities and within the time frame.

3.7.4. Glutathione

3.7.4.1. Principle
The procedure was followed initially as describe by Ellman (1959). Reduced glutathione (GSH) interacts with 5-5’-dithiobis (2-nitrobenzoic acid) (DTNB) to form the colored product 2-nitro-5-thiobenzoic acid, which is measured at 412 nm, and oxidized glutathione (GSSG). GSSG is then reduced by glutathione reductase to form reduced glutathione GSH, which is again measured by the preceding method.

3.7.4.2. Methodology
To measure the reduced GSH level, the tissue homogenate (in 0.1M phosphate buffer pH 7.4) was taken. The procedure was followed initially as describe by Ellman (1959). The homogenate was added with equal volume of 20% trichloro acetic acid (TCA containing 1mM EDTA) to precipitate the tissue proteins. The mixture was allowed to stand for 5 min. and then centrifuged at 2000 rpm for 10 min. The supernatant is taken and assay was done. After completion the reaction solutions were measured at wavelength 412 nm against blank. Absorbance values were compared with a standard curve generated from known GSH. The detail of assay procedure is given in appendix.

3.7.5. Protein Estimation
Protein was estimated by method describe by Lowry et. al. (1951) in liver tissue homogenate as well as in kidney homogenate.

3.7.5.1. Principle
The blue color developed in Lowry result of reaction of peptide bonds of protein with copper under alkaline conditions to reduction of molybdic acid by tyrosine and tryptophan residues of proteins, color shows maximum wavelength at 720nm.

3.7.5.2. Methodology
Tissue homogenate (0.1) was added and final volume was made up to 1ml with distilled water, 5ml reagent D (alkaline Copper Sulphate reagent) was added and it was incubated
for 10 min. After that 0.5ml folin’s reagent was added and again incubated for 30 min. at room temperature. O.D. was taken at wavelength 660 nm. The details of the assay procedure are given in appendix.

3.8. BIOCHEMICAL TEST IN BLOOD

3.8.1. Liver Function Test

3.8.1.1. Aspartate transaminase (AST)/SGOT
AST tests were performed by standard Autopeck kit methods.
(Appendix 2)

3.8.1.2. Alanine transaminase (ALT/SGPT)
ALT tests were performed by standard Autopeck kit methods.
(Appendix 3)

3.8.1.3. Alkaline Phosphatase (ALP)
ALP tests were performed by standard Autopeck kit methods.
(Appendix 4)

3.8.1.4. Bilirubin Total And Direct
Bilirubin tests were performed by standard Autopeck kit methods.
(Appendix 5)

3.8.2. Renal Function Test

3.8.2.1. Urea
Urea tests were performed by standard Autopeck kit methods.
(Appendix 6)

3.8.2.2. Creatinine
Creatinine tests were performed by standard Autopeck kit methods.
(Appendix 7)

3.9. HISTOPATHOLOGICAL EXAMINATIONS
Histopathological examination involved microscropic study of the tissues of various organs. This is done by preparing slides of thin section of the desired tissue.

3.10. HEMATOLOGICAL EXAMINATIONS

3.10.1. Total Leukocytes Count
Twenty µl of blood was diluted with 380 µl of WBC diluting fluid. The sample was kept at room temperature for 10 minutes and was then loaded in Neubaeur’s counting...
chamber. The counting chamber was kept in moist chamber for 10 minutes. The counting of cells was done from left to right and then from right to left and so on. The number of cells was counted using the following formula.

\[ \text{TLC} = \frac{\text{No. of the cells} \times \text{dilution factor}}{\text{area counted} \times \text{depth of fluid}} \]

- Dilution factor = 20
- Area = 4 sq. mm
- Depth of fluid = 0.1 mm
- No. of cells \times 20 / 4 \times 0.1

\[ \text{No. of cells} \times 50 / \text{cu mm} \]

### 3.10.2. Differential Leukocytes Count

**Making a thin blood smear:**

1) Take a clear, dry and grease free slide.
2) Put a small drop of blood near the edge of the slide.
3) Prepare a smear with the help of another glass slide.
4) Prepare a smear with the help of another glass slide.
5) Dry the blood smear at RT.

**Staining:**

1) Cover the smear with the staining solution (Leishman’s staining) by adding 10-15 drops on the smear and wait for 1-2 min.
2) Add equal volume of buffer solution.
3) Mix the solution by gentle blowing by a pipette.
4) Wait for 10-15 min.
5) Wash under tap water.
6) Air dry the smear at RT.
7) Observe the smear under microscope.

Plate 7.1 Microscopic examination
4. RESULTS

4.1. ANTIBACTERIAL ACTIVITY

The antibacterial and antifungal activities of *Girardinia heterophylla* and *Colocasia esculenta* gave different mean zone diameters of inhibition on the bacterial as well as fungal isolates tested (Table 3.1, 4.1). In this antimicrobial assay AECE showed maximum activity against *S. mutans* and *K. pneumoniae*. The zone diameters depict that the crude aqueous extract gave very promising results. In this study, the antimicrobial activity of the AECE was more effective in both the cases of bacteria than fungus. It may be assumed that further different solvent fractions and pure active ingredient will be more potent antimicrobial in comparison to the positive controls.

Plate 8.1

Plate 8.2 Against *K.pnemoniae*
Table 3.1: The results of *in vitro* preliminary activity of AECE in terms of zone of inhibition in millimeter around the agar wells.

<table>
<thead>
<tr>
<th>Test Organisms</th>
<th>Diameter of Zone of Inhibition (mm)</th>
<th>Concentration of AECE (mg / ml)</th>
<th>Chloramphenicol (200 µg / ml)</th>
<th>Rifampicin (200 µg / ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>100</td>
<td>200</td>
<td>300</td>
</tr>
<tr>
<td><strong>Bacterial strains</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>P. fragi</em> (MTCC 2458)</td>
<td>-</td>
<td>8.23±0.13</td>
<td>9.67±0.08</td>
<td>12.03±0.10</td>
</tr>
<tr>
<td><em>S. aureus</em> (MTCC 96)</td>
<td>-</td>
<td>3.83±0.16</td>
<td>4.67±0.12</td>
<td>8.50±0.09</td>
</tr>
<tr>
<td><em>S. mutans</em> (MTCC 890)</td>
<td>10.09±0.15</td>
<td>12.23±0.13</td>
<td>13.67±0.08</td>
<td>15.33±0.10</td>
</tr>
<tr>
<td><em>B. subtilis</em> (MTCC 121)</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>7.03±0.11</td>
</tr>
<tr>
<td><em>E. coli</em> (MTCC 483)</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>9.00±0.06</td>
</tr>
<tr>
<td><em>K. pneumoniae</em> (MTCC109)</td>
<td>-</td>
<td>-</td>
<td>10.53±0.21</td>
<td>13.00±0.06</td>
</tr>
<tr>
<td><strong>Fungal Strains</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>A. niger</em> (MTCC 281)</td>
<td>-</td>
<td>-</td>
<td>5.56±0.20</td>
<td>11.61±0.09</td>
</tr>
<tr>
<td><em>C. albicans</em> (MTCC 227)</td>
<td>-</td>
<td>5.03±0.011</td>
<td>10.07±0.07</td>
<td>12.76±0.13</td>
</tr>
</tbody>
</table>

Catch: “-” no measurable zone, values are mean ± S.E. of 6 replications of AECE, “XX” not taken as positive control; The pure antibiotic salts were used as positive controls while distilled water and blank media supernatants (for respective test organisms) were
used as negative control in comparison to the aqueous plant extract. Negative controls did not give any zone of inhibitions, so they are not given in table.

In this antimicrobial assay, Table 4.1 shows the zone of inhibition of *G. heterophylla* against *P. fragi*. At 24 hours contact period, aqueous extract of *G. heterophylla* exerted greater inhibitory activity against *P. fragi* with a zone of 10 mm in diameter. The zone of inhibition of aqueous extract of *G. heterophylla* on *S. aureus* at 400 mg/ml is 7.7 mm. The zone of inhibition of aqueous extract of *G. heterophylla* on *S. mutans* is maximum as compared to *B. subtilis, E. coli* and *K. pneumonia* i.e. 12 mm. The two fungal strains i.e. *A. niger* and *C. albicans* have moderate antimicrobial activity as compared to bacterial strains. Several investigations had reported that plants contain antimicrobial substances (El-Said *et. al.* 1971, Lewis 1980, Zaria *et. al.* 1975, Akinjobi *et. al.* 2004). The antimicrobial properties exhibited by the extracts may be associated with the presence of tannins, saponins, cardiac glycosides and alkaloids found in the plant extracts, a large number of flavonoids have been reported to possess antimicrobial properties (Bastista *et. al.* 1994, Tsuchiya *et. al.* 1996, Boris 1996, Olowusulu and Ibrahim 2006). The results of the antimicrobial tests indicate that the extract components from *G. heterophylla* with larger inhibition zones at the same microgram quantities as the antibiotics tested are generally more effective than those reference antimicrobial agents (Erturk *et. al.* 2006).
Table 4.1: The results of in vitro preliminary activity of AEGH in terms of zone of inhibition in millimeter around the agar wells.

<table>
<thead>
<tr>
<th>Test Organisms</th>
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<th>Rifampicin (200 µg / ml)</th>
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<tr>
<td><strong>Bacterial strains</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>P. fragi</em> (MTCC 2458)</td>
<td>-</td>
<td>5.13±0.13</td>
<td>8.27±0.07</td>
</tr>
<tr>
<td><em>S. aureus</em> (MTCC 96)</td>
<td>-</td>
<td>4.73±0.12</td>
<td>5.57±0.11</td>
</tr>
<tr>
<td><em>S. mutans</em> (MTCC 890)</td>
<td>9.08±0.14</td>
<td>11.20±0.13</td>
<td>10.57±0.07</td>
</tr>
<tr>
<td><em>B. subtilis</em> (MTCC 121)</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td><em>E. coli</em> (MTCC 483)</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td><em>K. pneumoniae</em> (MTCC 109)</td>
<td>-</td>
<td>-</td>
<td>10.40±0.21</td>
</tr>
<tr>
<td><strong>Fungal Strains</strong></td>
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<td></td>
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<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td><em>C. albicans</em> (MTCC 227)</td>
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<td>-</td>
<td>8.07±0.07</td>
</tr>
</tbody>
</table>

Catch: “-” no measurable zone, values are mean ± S.E.M of 3 replications of AEGH, “XX” not taken as positive control; The pure antibiotic salts were used as positive controls while distilled water and blank media supernatants (for respective test
organisms) were used as negative control in comparison to the aqueous plant extract. Negative controls did not give any zone of inhibitions, so they are not given in table.

4.2. PHYTOCHEMICAL ANALYSIS

4.2.1. Preliminary analysis

The result of the phytochemical screening of *G. heterophylla* and *C. esculenta* leaves aqueous extract are shown in table 5.1. The result revealed slight to moderate presence of alkaloids, tannins, phenolics, flavanoids, cardiac glycosides, deoxysugars and coumarin glycosides. The alkaloids are slightly present in *G. heterophylla* aqueous extract but moderately present in *C. esculenta* aqueous extract. Tannins are absent in both the extracts but phenolics are detected in case of *G. heterophylla* and slight presence in *C. esculenta*. Flavanoids were detected in *G. heterophylla* and a good presence in case of *C. esculenta*. Cardiac glycosides, deoxysugars and coumarin glycosides were not detected in the aqueous extracts of both the plants.
Table 5.1: Preliminary phytochemical tests for aqueous solvent extract of leaves of *G. heterophylla* and *C. esculenta*.

<table>
<thead>
<tr>
<th>TESTS</th>
<th>AEGH</th>
<th>AECE</th>
</tr>
</thead>
<tbody>
<tr>
<td>Alkaloids</td>
<td>+</td>
<td>++</td>
</tr>
<tr>
<td>Tannins</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Phenolics</td>
<td>+++</td>
<td>++</td>
</tr>
<tr>
<td>Flavanoids</td>
<td>++</td>
<td>+++</td>
</tr>
<tr>
<td>Cardiac glycosides</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Deoxysugars</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Coumarin glycosides</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

+ = Slight presence, ++ = Moderate presence, +++ = Good presence, - = Absence, AEGH = aqueous extract of *G. heterophylla* and AECE = aqueous extract of *C. esculenta*
4.2.2. Total phenolic estimation
A number of studies have focused on the biological activities of phenolic compounds, which are potential antioxidants and free radical scavengers (Marja et al. 1999; Sugihara et al. 1999). It is well known that flavonoids possess a wide range of antioxidant activities. These antioxidant properties are based on their phenolic structures.

The total phenolic content value of AEGH plant has been reported 8.66µg/gm fresh wt. of leaves where gallic acid used as a standard. Similarly the value of AECE has been reported 30µg/gm fresh wt. of leaves respectively.

4.2.3. Total flavanoid estimation
Total flavanoid content value of AEGH has been reported 113.5 µg/gm fresh wt. of leaves where quercetin used as a standard. Similarly the value of AECE has been reported 393.4µg/gm fresh wt. of leaves respectively.

4.3. EXAMINATION OF BODY WEIGHT

4.3.1. Weight of mice before sacrificed and liver and kidney wt. after sacrificed
The body weight measured on day first of study and every day till the end of the study is shown in Table 6.1. In normal group the mean wt. was 28 gm on day first and 32 gm on 30 days and 33 gm on 90 days. In case of alcohol the mean wt. was 28 gm on day first and 27 gm on 30 days and 25 gm on 90 days. The reduction in weight is the sign of toxicity as compared to normal and in positive one. In case of positive the weight found 20 gm on day first, 23 gm on 30 days and 24 gm on 90 days. In case of AEGH the weight found 22 gm on day first, 25 gm on 30 days and 27 gm on 90 days, similarly in AECE the weight found 22 gm on day first, 26 gm on 30 days and 28 gm on 90 days of study period.

After sacrificing mice, we had measured the liver and kidney wt. of all mice. The mean wt. of liver found 1.74 gm and kidney 1.21 gm in case of normal. In alcoholic case, the mean wt. of liver was of 0.96 gm and in kidney 0.59 gm. In positive one, the mean wt. of
liver was 1.62 gm and 1.10 gm of kidney. In case of AEGH, the wt. found 1.61 gm of liver and 1.18 gm of kidney and in AECE, the wt. found 1.52 gm of liver and 0.96 gm of kidney.

Table 6.1: Different groups having body wt. before sacrificed and liver and kidney wt. after sacrificed observed in mice.

<table>
<thead>
<tr>
<th>GROUP</th>
<th>Body wt. (gm)</th>
<th>Liver wt. (gm)</th>
<th>Kidney wt. (gm)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0 Day 30 Day 90 Day</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Normal</td>
<td>28 32 33</td>
<td>1.74 ± 0.06</td>
<td>1.21 ± 0.08</td>
</tr>
<tr>
<td>Alcohol</td>
<td>28 27 25</td>
<td>0.96 ± 0.07</td>
<td>0.59 ± 0.05</td>
</tr>
<tr>
<td>Positive</td>
<td>20 23 24</td>
<td>1.62 ± 0.09</td>
<td>1.10 ± 0.09</td>
</tr>
<tr>
<td>AEGH</td>
<td>22 25 27</td>
<td>1.61 ± 0.06</td>
<td>1.18 ± 0.08</td>
</tr>
<tr>
<td>AECE</td>
<td>22 26 28</td>
<td>1.52 ± 0.13</td>
<td>0.96 ± 0.09</td>
</tr>
</tbody>
</table>

Values are mean ± SE, (N=6). *P<0.05 compared with negative group. The values are significant according to results.
4.4. GENERAL CLINICAL OBSERVATION

General clinical observation were done for skin, fur, eyes, lacrymation, salivation, pupil size, posture, mobility, response to handling on day 1 and every week thereafter.

The parameters were scored as follows:

Table 7.1: General Clinical observations studied in case of mice.

<table>
<thead>
<tr>
<th></th>
<th>SCORES</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0</td>
</tr>
<tr>
<td>Skin</td>
<td>Healthy</td>
</tr>
<tr>
<td>Fur</td>
<td>Normal</td>
</tr>
<tr>
<td>Eyes</td>
<td>Normal</td>
</tr>
<tr>
<td>Lacrimation</td>
<td>Normal</td>
</tr>
<tr>
<td>Salivation</td>
<td>Normal</td>
</tr>
<tr>
<td>Pupil size</td>
<td>Normal</td>
</tr>
<tr>
<td>Posture</td>
<td>Normal</td>
</tr>
<tr>
<td>Mobility</td>
<td>Normal</td>
</tr>
<tr>
<td>Response to handling</td>
<td>Normal</td>
</tr>
</tbody>
</table>
4.5. BIOCHEMICAL TEST IN TISSUE HOMOGENATE

4.5.1. Liver

4.5.1.1. Lipid peroxidation
The level of MDA in liver was elevated in alcohol intoxicated mice (51.16 nmol/L). In mice treated with AEGH, the level was reduced to near normal (20.39 nmol/L). Similarly in case of AECE (18.18 nmol/L) the level was reduced. Silymarin restored the level of MDA to the normal.

4.5.1.2. Superoxide dismutase
The effect of AEGH on SOD activity in liver is shown in Table 8.1. SOD activity of the liver homogenate in alcohol treated group was examined to be lower than in normal group. SOD activity in AEGH was observed to be higher than in alcohol intoxicated group. SOD activity of AEGH was improved by 47.3 %, as compared to alcohol treated group. Similarly in case AECE (48.5%), the SOD level was markedly improved as shown in Table 9.1. Silymarin almost restored the 51.1% activity of SOD in alcohol treated groups.

4.5.1.3. Catalase
Catalase activity of liver homogenate in alcohol treated group was found to be strikingly lower than in normal group as seen in table 8.1. Catalase activity in AEGH is increased by 63.2 %, as compared to alcohol treated group. Similarly in case of AECE (64.0%), the Catalase activity is improved as compared to alcoholic treatment (Table 9.1). Silymarin significantly restored the enzyme activity almost to the normal level.

4.5.1.4. Glutathione
The effect of the aqueous extract of Girardinia heterophylla on glutathione content is shown in Table 8.1. GSH level of liver homogenate in alcohol treated group was found to be lower than in normal group but GSH level of AEGH was increased by 29.5 % as compared to the alcohol treated group whereas Silymarin was restoring the level back to
near normal. The AECE (25.0%), increased the levels as compared to toxicity (Table 9.1).

TABLE 8.1: Effect of AEGH on alcohol intoxication and antioxidant enzyme in liver.

<table>
<thead>
<tr>
<th>GROUP</th>
<th>MDA (nmol/L)</th>
<th>SOD (U/mgProtein)</th>
<th>CAT (U/mgProtein)</th>
<th>GSH (µg/mgProtein)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal</td>
<td>18.18 ± 1.78</td>
<td>1.70 ± 0.14</td>
<td>0.57 ± 0.04</td>
<td>141.27 ± 10.42</td>
</tr>
<tr>
<td>Alcohol</td>
<td>51.16 ± 2.80</td>
<td>0.88 ± 0.12</td>
<td>0.18 ± 0.08</td>
<td>83.46 ± 03.64</td>
</tr>
<tr>
<td>Positive</td>
<td>18.96 ± 2.11</td>
<td>1.80 ± 0.05</td>
<td>0.48 ± 0.03</td>
<td>112.81 ± 03.63</td>
</tr>
<tr>
<td>AEGH</td>
<td>20.39 ± 3.84</td>
<td>1.67 ± 0.06</td>
<td>0.49 ± 0.03</td>
<td>118.42 ± 02.76</td>
</tr>
</tbody>
</table>

Values are mean ± SE, (N=6).*P<0.05 compared with negative group.
TABLE 9.1: Effect of AECE on alcohol intoxication and antioxidant enzyme in liver.

<table>
<thead>
<tr>
<th>GROUP</th>
<th>MDA (nmol/L)</th>
<th>SOD (U/mgProtein)</th>
<th>CAT (U/mgProtein)</th>
<th>GSH (µg/mgProtein)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal</td>
<td>18.18 ± 1.78</td>
<td>1.70 ± 0.14</td>
<td>0.57 ± 0.04</td>
<td>141.27 ± 10.42</td>
</tr>
<tr>
<td>Alcohol</td>
<td>51.16 ± 2.80</td>
<td>0.88 ± 0.12</td>
<td>0.18 ± 0.08</td>
<td>83.46 ± 03.64</td>
</tr>
<tr>
<td>Positive</td>
<td>18.96 ± 2.11</td>
<td>1.80 ± 0.05</td>
<td>0.48 ± 0.03</td>
<td>112.81 ± 03.63</td>
</tr>
<tr>
<td>AECE</td>
<td>18.18 ± 2.26</td>
<td>1.71 ± 0.15</td>
<td>0.50 ± 0.04</td>
<td>111.30 ± 05.77</td>
</tr>
</tbody>
</table>

Values are mean ± SE, (N=6).*P<0.05 compared with negative group.
Nutraceutical evaluation of edible plants Colocasia esculenta and Girardinia heterophylla from high altitude

Antioxidant Profile of liver homogenates

Mean Values

(A)Graph
Nutraceutical evaluation of edible plants Colocasia esculenta and Girardinia heterophylla from high altitude

Figure 7.1: Effect of AEGH and AECE on (A) SOD & Catalase (B) MDA & GSH content of ethanol treated mice liver. Results are presented as the mean (n=6).
4.5.2. Kidney

4.5.2.1. Lipid peroxidation
The level of MDA in Liver was elevated in alcohol-intoxicated mice (33.50 nmol/L). In mice treated with AEGH, the level was reduced to near normal (19.80 nmol/L). Similarly in case of AECE (20.26 nmol/L), the level was reduced. Silymarin restored the level of MDA to the normal level.

4.5.2.2. Superoxide dismutase
The SOD activity of the kidney homogenate is shown in table 10.1. The SOD activity in the alcohol treated group was measured to be lower than in the normal group. SOD activity in AEGH was improved by 41.8 % whereas silymarin significantly increases the level as compared to alcohol group. Similarly in case of AECE (36.2%), the SOD level was markedly improved (Table 11.1).

4.5.2.3. Catalase
CAT activity of the kidney homogenate in the alcohol treated group was found to be strikingly lower than in normal group. CAT activity in AEGH was increased by 37.7 % as compared to alcohol treated groups. Similarly in case of AECE (36.3%), the Catalase level was markedly improved. Silymarin restored the enzyme activity almost to the normal level.

4.5.2.4. Glutathione
GSH level in normal group was measured to be higher (by 41.9 %) than in alcohol treated group as well as in silymarin treated groups by 26.8% as shown in table 10.1. GSH level of AEGH was increased by 31.6 %, as compared to the alcohol group. Similarly in case of AECE (28.0%), the GSH level was markedly improved (Table 11.1).
Table 10.1: Effect of AEGH on alcohol intoxication and antioxidant enzyme in kidney.

<table>
<thead>
<tr>
<th>GROUP</th>
<th>MDA (nmol/L)</th>
<th>SOD (U/mgProtein)</th>
<th>CAT (U/mgProtein)</th>
<th>GSH (µg/mgProtein)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal</td>
<td>16.11 ± 1.62</td>
<td>1.96 ± 0.13</td>
<td>0.43 ± 0.04</td>
<td>137.94 ± 9.00</td>
</tr>
<tr>
<td>Alcohol</td>
<td>33.50 ± 3.44</td>
<td>1.11 ± 0.08</td>
<td>0.28 ± 0.04</td>
<td>80.13 ± 2.28</td>
</tr>
<tr>
<td>Positive</td>
<td>20.90 ± 2.76</td>
<td>1.58 ± 0.17</td>
<td>0.47 ± 0.04</td>
<td>109.48 ± 3.84</td>
</tr>
<tr>
<td>AEGH</td>
<td>19.80 ± 1.64</td>
<td>1.91 ± 0.13</td>
<td>0.45 ± 0.05</td>
<td>117.25 ± 1.98</td>
</tr>
</tbody>
</table>

Values are mean ± SE, (N=6).*P<0.05 compared with negative group.
**Table 11.1:** Effect of AECE on alcohol intoxication and antioxidant enzyme in kidney.

<table>
<thead>
<tr>
<th>GROUP</th>
<th>MDA (nmol/L)</th>
<th>SOD (U/mgProtein)</th>
<th>CAT (U/mgProtein)</th>
<th>GSH (µg/mgProtein)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal</td>
<td>16.11 ± 1.62</td>
<td>1.96 ± 0.13</td>
<td>0.43 ± 0.04</td>
<td>137.94 ± 9.00</td>
</tr>
<tr>
<td>Alcohol</td>
<td>33.50 ± 3.44</td>
<td>1.11 ± 0.08</td>
<td>0.28 ± 0.04</td>
<td>80.13 ± 2.28</td>
</tr>
<tr>
<td>Positive</td>
<td>20.90 ± 2.76</td>
<td>1.58 ± 0.17</td>
<td>0.47 ± 0.04</td>
<td>109.48 ± 3.84</td>
</tr>
<tr>
<td>AECE</td>
<td>20.26 ± 2.67</td>
<td>1.74 ± 0.10</td>
<td>0.44 ± 0.03</td>
<td>111.30 ± 5.77</td>
</tr>
</tbody>
</table>

Values are mean ± SE, (N=6).*P<0.05 compared with negative group.
Nutraceutical evaluation of edible plants *Colocasia esculenta* and *Girardinia heterophylla* from high altitude

**Antioxidant Profile of kidney homogenates**

<table>
<thead>
<tr>
<th></th>
<th>AECE</th>
<th>AEGH</th>
<th>Positive</th>
<th>Negative</th>
<th>Normal</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Mean Values</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CAT</td>
<td>2.5</td>
<td>2.0</td>
<td>1.5</td>
<td>1.0</td>
<td>.5</td>
</tr>
<tr>
<td>SOD</td>
<td>1.0</td>
<td>1.0</td>
<td>0.5</td>
<td>0.0</td>
<td>0.0</td>
</tr>
</tbody>
</table>

(A)Graph
Nutraceutical evaluation of edible plants *Colocasia esculenta* and *Girardinia heterophylla* from high altitude

**Figure 8.1:** Effect of AEGH and AECE on (A) SOD & Catalase (B) MDA & GSH levels of ethanol intoxicated mice kidney. Results are presented as the mean (n=6).

(B) Graph
4.6. BIOCHEMICAL TEST IN BLOOD

4.6.1. Liver Function Tests

4.6.1.1. Aspartate transaminase (AST)/SGOT
The activity of AST is shown in table 12.1. There was elevation in the level of enzymes in the alcohol intoxicated mice (79.47 U/L). The AST activity was decreased in AEGH (45.05 U/L), treated mice, as compared to the alcohol treated group. Similarly in case of AECE (46.87 U/L), the AST activity was decreased as compared to the alcohol treated group (Table 13.1).

4.6.1.2. Alanine transaminase (ALT)/SGPT
The activity of ALT is shown in table 12.1. There was elevation in the level of enzymes in the alcohol intoxicated mice (75.17 U/L). The ALT activity was decreased in AEGH (45.32 U/L) as compared to the alcohol treated group. Similarly in case of AECE (50.00 U/L), the ALT activity was decreased as compared to the alcohol treated group (Table 13.1).

4.6.1.3. Alkaline Phosphatase
The level of alkaline phosphatase is shown in table 12.1. There was elevation in the level of the enzymes in the alcohol intoxicated mice (89.38 U/L). The level was decreased in AEGH (38.55 U/L), treated mice. Similarly in case of AECE (43.92 U/L), the ALP activity was decreased as compared to the alcohol treated group.

4.6.1.4. Bilirubin Total And Direct
The level of bilirubin is shown in table 12.1. There was elevation in the levels of the enzyme in the alcohol intoxicated mice (0.19 mg/dl). The level was decreased in AEGH (0.05 mg/dl), treated mice. Similarly in case of AECE (0.06 mg/dl), the bilirubin level was decreased as compared to the alcohol treated group (Table 13.1).
Table 12.1: Liver Function Test: Effect of AEGH on serum AST, ALT, ALP and Bilirubin levels on alcohol treated mice.

<table>
<thead>
<tr>
<th>GROUP</th>
<th>SGOT (U/L)</th>
<th>SGPT (U/L)</th>
<th>ALP (U/L)</th>
<th>BILIRUBIN (mg/dl)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal</td>
<td>38.47 ± 0.79</td>
<td>39.43 ± 0.45</td>
<td>33.87 ± 0.62</td>
<td>0.05 ± 0.006</td>
</tr>
<tr>
<td>Alcohol</td>
<td>79.47 ± 1.99</td>
<td>75.17 ± 3.60</td>
<td>89.38 ± 0.63</td>
<td>0.19 ± 0.008</td>
</tr>
<tr>
<td>Positive</td>
<td>44.88 ± 0.51</td>
<td>44.58 ± 0.61</td>
<td>40.23 ± 0.53</td>
<td>0.05 ± 0.006</td>
</tr>
<tr>
<td>AEGH</td>
<td>45.05 ± 1.05</td>
<td>45.32 ± 0.74</td>
<td>38.55 ± 0.55</td>
<td>0.05 ± 0.007</td>
</tr>
</tbody>
</table>

Values are mean ± SE, (N=6).*P<0.05 compared with negative group.
Table 13.1: Liver Function Test: Effect of AECE on serum AST, ALT, ALP and Bilirubin levels on alcohol treated mice.

<table>
<thead>
<tr>
<th>GROUP</th>
<th>SGOT (U/L)</th>
<th>SGPT (U/L)</th>
<th>ALP (U/L)</th>
<th>BILIRUBIN (mg/dl)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal</td>
<td>38.47 ± 0.79</td>
<td>39.43 ± 0.45</td>
<td>33.87 ± 0.62</td>
<td>0.05 ± 0.006</td>
</tr>
<tr>
<td>Alcohol</td>
<td>79.47 ± 1.99</td>
<td>75.17 ± 3.60</td>
<td>89.38 ± 0.63</td>
<td>0.19 ± 0.008</td>
</tr>
<tr>
<td>Positive</td>
<td>44.88 ± 0.51</td>
<td>44.58 ± 0.61</td>
<td>40.23 ± 0.53</td>
<td>0.05 ± 0.006</td>
</tr>
<tr>
<td>AECE</td>
<td>46.87 ± 1.29</td>
<td>50.00 ± 0.38</td>
<td>43.92 ± 0.64</td>
<td>0.06 ± 0.006</td>
</tr>
</tbody>
</table>

Values are mean ± SE, (N=6).*P<0.05 compared with negative group.
Nutraceutical evaluation of edible plants *Colocasia esculenta* and *Girardinia heterophylla* from high altitude

(A) Graph

Liver Function Profile

- SGOT
- SGPT
- ALP

Mean Values

- Normal
- Negative
- Positive
- AEGH
- AECE

(A) Graph
Figure 9.1: Effect of AEGH and AECE on (A) AST, ALT & ALP (B) Bilirubin levels of ethanol intoxicated mice. Results are presented as the mean (n=6).
4.6.2. Renal Function Test

4.6.2.1. Urea

The level of urea is shown in table 14.1. There was elevation in the level of urea in alcohol intoxicated mice (30.11 mg/dl). The level of urea was decreased in the AEGH (12.63 mg/dl), treated mice as compared to the alcohol -intoxicated mice. Similarly in case of AECE (14.98 mg/dl), the level of urea was decreased as compared to the alcohol treated group (Table 15.1).

4.6.2.2. Creatinine

The level of creatinine is shown in table 14.1. There was elevation in the level of creatinine in alcohol-intoxicated mice (6.26 mg/dl). The level of creatinine was decreased in the AEGH (2.44 mg/dl), treated mice as compared to the alcohol -intoxicated mice. Similarly in case of AECE (2.71 mg/dl), the level of creatinine was decreased as compared to the alcohol treated group (Table 15.1).
Table 14.1: Renal function Test: Effect of AEGH on serum Urea and Creatinine levels on alcohol intoxicated mice.

<table>
<thead>
<tr>
<th>GROUP</th>
<th>UREA (mg/dl)</th>
<th>CREATININE (mg/dl)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal</td>
<td>09.63 ± 0.17</td>
<td>2.13 ± 0.10</td>
</tr>
<tr>
<td>Alcohol</td>
<td>30.11 ± 0.54</td>
<td>6.26 ± 0.11</td>
</tr>
<tr>
<td>Positive</td>
<td>11.41 ± 0.33</td>
<td>2.40 ± 0.15</td>
</tr>
<tr>
<td>AEGH</td>
<td>12.63 ± 0.25</td>
<td>2.44 ± 0.10</td>
</tr>
</tbody>
</table>

Values are mean ± SE, (N=6). *P<0.05 compared with negative group.
Table 15.1: Renal function Test: Effect of AECE on serum Urea and Creatinine levels on alcohol intoxicated mice.

<table>
<thead>
<tr>
<th>GROUP</th>
<th>UREA (mg/dl)</th>
<th>CREATININE (mg/dl)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal</td>
<td>09.63 ± 0.17</td>
<td>2.13 ± 0.10</td>
</tr>
<tr>
<td>Alcohol</td>
<td>30.11 ± 0.54</td>
<td>6.26 ± 0.11</td>
</tr>
<tr>
<td>Positive</td>
<td>11.41 ± 0.33</td>
<td>2.40 ± 0.15</td>
</tr>
<tr>
<td>AECE</td>
<td>14.98 ± 0.30</td>
<td>2.71 ± 0.10</td>
</tr>
</tbody>
</table>

Values are mean ± SE, (N=6).*P<0.05 compared with negative group.
Figure 10.1: Effect of AEGH and AECE on UREA & CREATININE levels of ethanol intoxicated mice. Results are presented as the mean (n=6).
4.7. HISTOPATHOLOGICAL EXAMINATIONS

4.7.1. In Liver

Administration of ethanol produces a decrease in the hepatic content of glutathione (GSH), which is an important biomolecule that affords protection against chemically-induced cytotoxicity (Thakur 2002). The administration of ethanol enhances the alanine transaminase level (ALT), aspartate transaminase (AST), and gamma glutamyl transferase levels and also makes the reduced glutathione/oxidized glutathione ratio abnormal. Wang et. al. showed the protective effect of silymarin against ethanol-induced changes in these parameters (Wang et. al. 1996). Valenzuela et. al., showed the neutralization of the lipid peroxidation, using acute intoxication in rats as the experimental model, (Valenzuela et.al. 1985) while Valenzuela and Garrido showed reduction in the liver alterations, using chronic intoxication in rats as the model (Valenzuela and Garrido 1994).

Karimi et. al. (2011) study revealed that in animal experiments, silymarin and silybinin are indicated to have protective effects on rat or mouse liver against hepatotoxicity in acute ethanol intoxication, carbon tetrachloride, cisplatin, thioacetamide, thallium, D-galactosamine and acetaminophen (Wen et.al. 2009). Treatment with ethanolic extract (100 mg/kg bw) of silymarin seed most significantly declined the rats liver enzymes while using against carbon tetrachloride-induced (2 ml/kg bw) liver damage. Moreover, in oxidative experiment, ethyl acetate extract of silymarin showed the most enhancements in glutathione level and HDL/LDL (Saller et.al. 2007; Shaker et.al.2010). Pre-treatment of male mice with silymarin modulated the alteration of oxidative stress, cell cycle, cytoskeletal network, cell–cell adhesion, extra-cellular matrix, inflammation, apoptosis, cell-signaling and intermediary metabolism that was induced by pyrogallol. These effects leaded to the differential expression of 79 genes/transcripts (27 upregulated and 52 down-regulated) in comparison to the pyrogallol treated group. The results showed that, effects of silymarin could be due to its multiple functions as well as its antioxidant activity (Upadhyay et.al.2010). Findings in animal and human studies revealed the highest concentrations and therefore the more effects of silymarin in the liver (Morishima et.al.2010).
These are the pictures of different tissues slides which show the effect of toxic substances as compared to normal, positive and our different plant extracts. The histopathology of control section of liver tissue is normal. There is no necrosis where as ethanol treated groups show severe necrosis, hepatic damage and formation of cirrhosis. The positive control groups treated with silymarin which is a good hepatoprotective agent. Silymarin is obtained from *Silybum marianum* (milk thistle), an edible plant that has been used medicinally for centuries as a herbal medicine for the treatment of liver-related disorders. It is widely prescribed by herbalists and has almost no known side effects. The plant is native to the Mediterranean and grows throughout Europe and North America (Luper 1998; Pepping 1999) and shows almost less harmful effects on liver while aqueous extract of *Girardinia heterophylla* and *Colocasia esculenta* shows protective effects and cure alcoholic injury in liver.
Plate 9.1: Histological Section of Liver normal

(A)
Plate 9.2: Histological Section of Liver treated with ethanol
Plate 9.3: Histological Section of Liver treated with Silymarin
Plate 9.4: Histological Section of Liver treated with AEGH
(E)

**Plate 9.5:** Histological Section of Liver treated with AECE
4.7.2. In Kidney

Karimi et. al. (2011) study revealed that the effect of silymarin has been tested in alloxan-induced diabetes mellitus models in rats. Alloxan produces reactive oxygen species (H\textsubscript{2}O\textsubscript{2}, •O\textsubscript{2} and •OH) (39), which injure renal tissue (Sha et. al. 2007; Soto et. al. 2004). Silymarin was administrated 20 days after 9 weeks treatment with alloxan and it was effective on the renal tissue injuries. It has antioxidant effects via increase of gene expression of antioxidant enzymes and a number of the most important protection mechanisms against free radicals damage containing super-oxide dismutase, glutathione peroxidase, and catalase. Therefore, silymarin can be used as a drug for diabetic nephropathy therapy (Soto et. al. 2010). Oxidative stress (ROS) reduces glomerular filtration. Treatment with silymarin or vitamin E improved alteration in serum creatinine concentrations in the gentamicin-treated dogs (Varzi et. al. 2007).

These are the pictures of different tissues slides which show the effect of toxic substances as compared to normal, positive and our different plant extracts. The histopathology of control section of kidney tissue is normal, there is no necrosis. The ethanol treated groups show severe necrosis, hepatic damage and formation of cirrhosis. The positive control groups treated with silymarin which is a good hepatoprotective agent and shows almost less harmful effects on liver where as the group treated with aqueous extract of Girardinia heterophylla and Colocasia esculenta shows protective effects and cure alcoholic injury in kidney.
(B)

**Plate 10.2:** Histological Section of Kidney treated with ethanol
Plate 10.3: Histological Section of Kidney treated with Silymarin
Plate 10.4: Histological Section of Kidney treated with AEGH
Plate 10.5: Histological Section of Kidney treated with AECE
4.8. HEMATOLOGICAL EXAMINATIONS

These are the results of hematological studies in which we have calculated the total leukocyte count and differential leukocyte count. The hematological results interpreted that total leukocyte count in case of normal is 7800 cells/cumm as compared to alcohol where the total leukocyte count is 16200 cells/cumm. Similarly the value is 9400 cells/cumm in case of positive and 12300 cell/cumm and 14100 cell/cumm in AEGH and AECS respectively as shown in table 16.1.
Table 16.1: Different hematological parameters observed in mice.

<table>
<thead>
<tr>
<th>Group</th>
<th>Total Leukocyte Count (TLC)</th>
<th>Differential leukocyte Count (DLC)</th>
<th>Blood Picture</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal</td>
<td>7800 cells/cumm</td>
<td>14±02 84±05 02±01</td>
<td>Normal</td>
</tr>
<tr>
<td>Alcohol Treated</td>
<td>16200 cells/cumm</td>
<td>06±02 92±05 02±01</td>
<td>Anisopoikilocytosis</td>
</tr>
<tr>
<td>Silymarin Treated</td>
<td>9400 cells/cumm</td>
<td>11±02 88±05 01±01</td>
<td>Less damaged RBC’s</td>
</tr>
<tr>
<td>Alcohol + AEGH</td>
<td>12300 cells/cumm</td>
<td>06±02 93±05 01±01</td>
<td>Lymphocytosis</td>
</tr>
<tr>
<td>Alcohol + AECS</td>
<td>14100 cells/cumm</td>
<td>05±02 72±05 08±01</td>
<td>Lymphocytosis</td>
</tr>
</tbody>
</table>
Malik and Wickramasinghe (1986) study revealed that the haematological parameters in control mice and in mice exposed to ethanol vapour (20-38 mg/l of air) for 24 h in which the blood ethanol levels varied between 150 and 560 mg/dl. Many of the alcohol-treated mice showed locomotor depression and ataxia. It is evident from the table that several statistically significant haematological changes were induced by alcohol; these were leucopenia, neutrophil granulocytopenia, lymphopenia, monocytopenia, thrombocytopenia and a slight increase in the absolute reticulocyte count. The body weights and haematological findings in mice reared in an ethanol-containing atmosphere for 20-43 days, together with the findings in age matched control animals studied simultaneously. It can be seen that the only statistically significant change seen in the group of animals exposed chronically to ethanol vapour was thrombocytopenia and that only some of the alcohol-treated animals showed platelet counts below the 95% confidence range for control mice. It is noteworthy that the Hb and MCV were unaffected by chronic treatment with ethanol.