The different extracts of *Cardiospermum helicacabum* Linn. on hematological parameters in EAC induced mice and serum lipid levels in EAC induced mice

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**Abstract**

The present study deals with *Cardiospermum helicacabum* Linn ( Sapindecaceae) roots extracts screened for anticancer activity. The anticancer properties of plant extracts were analyzed using, auto toxicity studies, *in vivo* studies. Effect of different extracts of *Cardiospermum helicacabum* Linn on hematological parameters in EAC Induced mice and Effect of different extracts of *Cardiospermum helicacabum* Linn roots on various biochemical changes in EAC Induced liquid tumor model in mice. The plant possess activities like antimicrobial, antifungal, antiparasitic, antidiarrheal, anxiolytic, rubifiant, antipyretic and management of painful, arthritic inflammatory conditions. Further investigations are needed to assess its isolated mode of action on various activities.

**Keywords:** *Cardiospermum helicacabum* Linn, EAC, Induced, Extracts.

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1. Introduction
Cancer known medically as a malignant neoplasm, is a large group of different diseases, all involving unregulated cell growth. In cancer, cells divide and grow uncontrollably, forming malignant tumors, and invade nearby parts of the body. The cancer may also spread to more distant parts of the body through the lymphatic system or bloodstream.

Not all tumors are cancerous. Benign tumors do not grow uncontrollably, do not invade neighboring tissues, and do not spread throughout the body. Healthy cells control their own growth and will destroy themselves if they become unhealthy. Cell division is a complex process that is normally tightly regulated. Cancer occurs when problems in the genes of a cell prevent these controls from functioning properly.

These problems may come from damage to the gene or may be inherited, and can be caused by various sources inside or outside of the cell. Faults in two types of genes are especially important, oncogenes, which drive the growth of cancer cells, and tumor suppressor genes, which prevent cancer from developing. Determining what causes cancer is complex. Many things are known to increase the risk of cancer, including tobacco use, certain infections, radiation, lack of physical activity, poor diet and obesity, and environmental Pollutants [1]. These can directly damage genes or combine with existing genetic faults within cells to cause the disease [2]. Approximately five to ten percent of cancers are entirely hereditary. Cancer can be detected in a number of ways, including the presence of certain signs and symptoms, screening tests, or medical imaging. Once a possible cancer is detected it is diagnosed by microscopic examination of a tissue sample. Cancer is usually treated with chemotherapy, radiation therapy and surgery. The chances of surviving the disease vary greatly by the type and location of the cancer and the extent of disease at the start of treatment. While cancer can affect people of all ages, and a few types of cancer are more common in children, the risk of developing cancer generally increases with age. In 2007, cancer caused about 13% of all human deaths worldwide (7.9 million). Rates are rising as more people live to an old age and as mass lifestyle changes occur in the developing world [3].

Cancer is the name given to any illness resulting from one of our body’s own cells growing out of control. Cancer cells are also referred to as tumors or neoplasm. Uncontrolled growth, ability to invade local tissues and ability to spread or metastasize are the characteristics of a cancer cell [1]. Medicinal plants are valuable natural resources and regarded as potentially safe drugs and have been tested for biological, antimicrobial and hypoglycemic activity also play an important role in the modern medicine[4&5]. It is well known that even the most synthetic drugs have their from plant products [6].

Cardiospermum helicacabum Linn. (Sapinadaceae) is an herbaceous climber [7], commonly used in the treatment of rheumatism, lumbago earache, fever [8&9]. The plant Cardiospermum helicacabum Linn. (Sapinadaceae), is an annual or sometimes perennial climber. The plant is distributed in America, extending to Africa and Asia. It also occurs throughout the plains and in lower elevations (up to 1200m) of India, Bangladesh and Pakistan [10&11].

2. Materials and Methods:
Preparation of Plant Extracts:
The roots of the plant Cardiospermum helicacabum was shade dried and powdered. A weighed quantity of 2500g was taken for chemical investigation. The bark was dried in the shed and coarsely powdered. The powder was extracted with ethanol in a soxhlet apparatus for 72h. The ethanolic extract was evaporated in vacuo giving the residue (24%). The ethanolic extract obtained was suspended in distilled water in small amounts and was extracted successively and exhaustively with petroleum ether (60-80°C), benzene, chloroform and acetone in the order of increasing polarity. The left over fraction was considered as aqueous fraction. The extract and fractions were concentrated in a rotary evaporator at reduced pressure.

Method of estimation of Total White blood cell count
The Hemocytometer neubaur counting chamber was used for total WBC count. The blood was drawn up to 0.5 mark of WBC pipette and wiped off any excess blood from the tip. Then diluted with WBC diluting fluid (1% acetic acid, 1 ml. Glacial acetic acid was added to 99 ml. distilled water) up to the mark 11 and mixed thoroughly, first few drops were discarded and the fluid was placed in the counting chamber of Hemocytometer. One cover slip was placed over the chamber and counting was taken under the microscope at high power. Then the total count of WBC was calculated from the number of cells/mm³ of the areas of counting chamber.

Method of estimation of Differential count of White blood cells
Staining of blood films by Leishman’s method:
The blood film was prepared by spreading a large drop of blood in clean grease –free slide. The blood film was dried in air. The Leishman’s stain was added on the dried blood film and evenly distributed over the entire slide. After 2 min, double the amount of distilled water was carefully added and mixed with the stain by tilting the slide. The film was allowed to stain for 10 min. and the excess of stain was removed by washing with water for 2 min. dried the film in air and mounted with DPX. Preparation of Leishman’s stain: A Romanowsky type stain designed to differentiate Leukocytes. It was prepared by using Leishman’s powder (Methylene blue, Eosin) and methanol (Acetone free) 0.15 g. of Leishman’s stain powder was dissolved in few ml. of methanol and made the volume up to 100 ml. with buffer. Preparation of buffer: KH$_2$PO$_4$-50.4 ml.; Na$_2$HPO$_4$-49.6 ml. 

II Differential leukocytes count (DLC):
a. After the general examination of the blood film, the differential leukocytes were observed. Counted about 100 cells and taken an average percent of the cells. The following leukocyte count was observed.

b. The polymorpho nuclear Neutrophils: Showed a faintly pinkish tinted cytoplasm filled with nearly uniforms, fine granules, which took a pink color. The nucleus was divided irregularly into two to five lobes, which were connected by fine bands. It is a round cell with distinct membrane.

c. The polymorph nuclear Eosinophils: It was distinguished by compact coarse granules with eosin color, circular in shape, bilobed nucleus looked like spectacles.

d. Lymphocytes: With rounded, deeply stained nucleus and a clear basophilic cytoplasm.

Method of determination Hemoglobin Estimation:
N/10 HCl was placed up to the mark 20 in the graduated tube. 20 mm³ of blood (0.02ml) was added with the help of Hemoglobin meter pipette. The pipette was rinsed two to three times, mixed well and allowed to stand for 10 minutes, till the solution became dark brownish in color. The solution was diluted by distilled water, drop by drop, each time mixing the solution with stirring rod until it matched with the standard. The result was read from the scale on the graduated tube by observing graduation mark at the lower edge of the meniscus at the top of the liquid column. [Note: 2 % value was deducted when time taken was more than 10 minutes from the mixing of blood with N/10 HCl, for the hemoglobin determination.]

Method of estimation of Total RBC count:
The Hemocytometer neubaur counting chamber was used for total RBC count the blood was drawn up to 0.5 mark of RBC pipette and wiped off any excess blood from the tip. Then the pipette was filled to the 101 mark with Hayme’s red cell diluting fluid (0.5 g. mercuric chloride, 5 g. sodium sulfate and 2 g. sodium chloride, 0.5 g. sodium sulfate and 1 g. sodium chloride were dissolved in 200 ml. distilled water) and mixed thoroughly.

The first few drops diluted blood were blown out and few drops were allowed to place in the counting chamber of Hemocytometer. A cover slip was placed over the chamber so that run under the slip, filling the chamber. To count red blood cells, the counting chamber was placed under the microscope at high power. The cells were counted in the five fields. The average number of cells in each square was counted. The total number of cells/mm³ was calculated.

Statistical Analysis:
For *in vitro* assays linear regression analysis was used to calculate the IC₅₀ values. In case of *in vivo* studies the experimental results were expressed as mean ± SEM. Results were analyzed by the one- way ANOVA followed by Tukey-kramer post hoc multiple comparison test using Graph pad In Stat version 3.00. P value of <0.05 was considered as statistically significant.

**Procedures for estimation of Biochemical Parameters in tissue homogenates:**

**Glutathione (GSH)**

**Reagents**

a. 25% Trichloroacetic acid.

b. 0.3 M sodium phosphate solution-4.257 g Disodium hydrogen phosphate in 100 ml distilled water.

c. Phosphate buffer pH 8.

d. Solution A-0.6 g sodium dihydrogen phosphate in 100 ml distilled water.

e. Solution B –Disodium hydrogen phosphate in 100 ml distilled water.

f. Mixed solution of 5 ml solution A and 95 ml of Solution B.

g. DTNB-11.9 mg in 50 ml phosphate buffer (pH 8).

**Procedure:**
GSH Contents were measured by the method of Briefly, proteins were precipitated by 25% TCA, centrifuged and the supernatant was collected. 1 ml supernatant was mixed with 6 ml of 0.2 M sodium phosphate buffer pH 8.0 and 1 ml 0.06 m M DTNB and incubated for 10 minutes at room temperature. The absorbance of the samples was recorded against the blank at 412 nm in an UV-Visible Spectrophotometer and the GSH concentration was calculated from the standard curve.

**Glutathione-S- Tranferase (GST)**

**Reagents**
Phosphate buffer pH 6.5-1.7418 g potassium dihydrogen phosphate in 100 ml distilled water.

GSH (30 m M) –16 mg GS in 2 ml DDW.

CDNB (1-Chloro –2,4,Dinitro benzene)-62 mg CDNB in 10 ml ethanol.

**Procedure:**
Added 0.1 ml CDNB to 0.6 ml supernatant of Liver, kidney homogenates and 2.2 ml phosphate buffer pH 6.5. Incubated at 37°C for 5 min and added 0.1 ml 30 m M GSH. Absorbance was recorded at 340 nm at intervals 1,2,3,4,5 minutes. Blank was carried out in the same manner without the homogenate.

**Catalase**

**Reagents:** Hydrogen peroxides (30 m M) 340 micro liter of 30% hydrogen peroxide in 100 ml with phosphate buffer pH 7.0.

**Procedure:**
The absorbance at 240 nm of 0.9 ml of Hydrogen peroxide in phosphate buffer pH7.0 taken in a 1.5 ml quartz cuvette was read in a spectrophotometer. Blank absorbance should be 0.5. Then 100 µl of sample was added and the activity was measured by recording the difference in absorbance at 240nm in one minute, after the addition of sample. The average difference in absorbance in 30 sec was calculated. A unit of catalase is defined as the amount of protein that results in a decrease in absorbance of 0.05 in 30 sec.

**Lipid profile:**
On the 14th day Blood was drawn from the mouse through Tail vein and the lipid profile viz. Total Cholesterol and Triglycerides were estimated from blood serum using Auto analyzer equipment.
3. Results and Discussion

Hematological parameters:
In order to detect the influence of *Cardiospermum helicacabum* extracts on the hematological status of EAC bearing mice, a comparison was made among different groups of animals on the 14th day after inoculation. The hematological profile showed significant changes when compared with the normal animals (Table 1). The total WBC counts, proteins and PCV were found to increase with a reduction in the hemoglobin content of RBC. The differential count of WBC showed that the percentage of neutrophils increased (P<0.05) while that of leucocytes decreased (P<0.05). At the same time interval, treatment with different extracts of *Cardiospermum helicacabum* could change these altered parameters to near normal.

**Leucocytes count:** Tumor induction significantly (p<0.05) increased total number of WBC almost by 4 times. Camptothecin administration reversed this effect significantly (p<0.05).

### Table 1: Effect of different extracts of *Cardiospermum helicacabum* on Hematological parameters in EAC induced Mice

<table>
<thead>
<tr>
<th>Drug</th>
<th>Dose</th>
<th>W.B.C count (10cells/mm)</th>
<th>R.B.C Count (millions/mm)</th>
<th>Hb gm%</th>
<th>Lymphocytes</th>
<th>Neutrophils</th>
<th>Mono cytes</th>
<th>PCV</th>
<th>Protein</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal mice</td>
<td></td>
<td>8.62 ± 0.03</td>
<td>5.02 ± 0.29</td>
<td>16.23± 0.59</td>
<td>75 ± 0.92</td>
<td>28.5 ± 0.56</td>
<td>1</td>
<td>6.5 ± 0.2</td>
<td>8.58 ±0.66</td>
</tr>
<tr>
<td>Control</td>
<td></td>
<td>15.2 ± 0.35</td>
<td>3.03 ± 0.32</td>
<td>9.73 ± 0.49</td>
<td>33.5±1.56</td>
<td>64 ± 1.95</td>
<td>2</td>
<td>20.23 ± 0.35</td>
<td>13.25 ± 0.45</td>
</tr>
<tr>
<td>Standard (Camptothecin)</td>
<td>10</td>
<td>9.05 ± 0.36</td>
<td>4.35 ± 0.29</td>
<td>16.03± 0.26</td>
<td>70.52± 1.52</td>
<td>35 ± 0.79</td>
<td>1</td>
<td>8.62 ± 0.55</td>
<td>8.35 ± 0.06</td>
</tr>
<tr>
<td>Aqueous extract A</td>
<td>250</td>
<td>12.59 ± 0.35</td>
<td>3.5 ± 0.15</td>
<td>12.85±0.33</td>
<td>56.15± 2.5</td>
<td>45.3 ± 1.7</td>
<td>2</td>
<td>16.23 ± 0.57</td>
<td>9.62 ± 0.33</td>
</tr>
<tr>
<td>Aqueous extract B</td>
<td>500</td>
<td>12.90 ± 0.29</td>
<td>3.59 ± 0.23</td>
<td>12.99± 0.35</td>
<td>59.25± 2.23</td>
<td>43.17± 0.92</td>
<td>1</td>
<td>15.23 ± 0.43</td>
<td>9.25 ± 0.55</td>
</tr>
<tr>
<td>Alcoholic extract A</td>
<td>100</td>
<td>12.30 ± 0.20</td>
<td>3.72 ± 0.22</td>
<td>13.62± 0.21</td>
<td>59.92± 2.16</td>
<td>41.21± 0.98</td>
<td>2</td>
<td>12.25 ± 0.22</td>
<td>9.19 ± 0.66</td>
</tr>
<tr>
<td>Alcoholic extract B</td>
<td>200</td>
<td>11.05 ± 0.15</td>
<td>3.98 ± 0.25</td>
<td>13.98± 0.28</td>
<td>62.15± 1.45</td>
<td>39.92± 0.67</td>
<td>1</td>
<td>11.52 ± 0.35</td>
<td>9.05 ± 0.35</td>
</tr>
</tbody>
</table>

Each value represents Mean ± SEM of six observations; all the values are significant at 0.05 Level.

The administration of various extracts also reversed the tumor induced rise in total counts of WBC significantly. However, they were not efficacious as Camptothecin in reversing the tumor induced total counts. (Figure 1)

**Differential leukocyte counts**
On differential leukocyte counts, tumor induction caused a significant reduction in Lymphocytes count and a significant (p<0.05) increase in Neutrophil count. This was significantly (p<0.05) reversed by Camptothecin as well as by the administration of various extracts. (Figure 2&3)
et al,

restored these lipids to

SEM of data obtained from

mor bearing mice. Treatment with different extracts of

(Figure 6 & 7)

Effect of different treatments on Neutrophils

SEM of data obtained from

(SEM of data obtained from

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) Effect of different treatments on Neutrophils

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Effect of different treatments on R.B.C. count in

animals.

R.B.C. count:

Tumor induction caused significant decrease in RBC almost to the half of the normal animals. All the treatments including Camptothecin could significantly reverse this effect (Figure 5).

Hemoglobin content:

Tumor development in the animals caused significant anemia (decrease in hemoglobin content). While this was significantly (p<0.05) reversed by Camptothecin as well as by the extracts. (Figure 4).

Lipid profile:

The parameters in the serum such as total cholesterol, triglycerides levels were found to be significantly elevated in tumor bearing mice. Treatment with different extracts of *Cardiospermum helicacabum* restored these lipids to normal levels. (Figure 6 & 7)

All the values represent mean ± SEM of data obtained from 6 different animals.

Figure 2: Effect of different treatments on Neutrophils in tumor induced mice

Figure 3: Effect of different treatments on Lymphocytes in tumor induced mice

Figure 4: Effect of different treatments on Hemoglobin content in tumor induced mice

Figure 5: Effect of different treatments on R.B.C. count in tumor induced mice

Figure 6 & 7: Effect of different treatments on lipid profile (Total cholesterol & Triglycerides) in tumor-induced mice

All the values represent mean ± SEM of data obtained from 6 different animals.
Table 2: Effect of different extracts of *Cardiospermum helicacabum* roots on various biochemical changes in EAC induced Liquid tumor model in mice.

<table>
<thead>
<tr>
<th>Groups</th>
<th>Dose (mg/kg)</th>
<th>GSH (ng mg-1 protein)</th>
<th>Lipid per oxidation (%)</th>
<th>Catalase (µg mg-1 protein)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>-----</td>
<td>3.95±0.02</td>
<td>96.65±1.59</td>
<td>30.50±0.58</td>
</tr>
<tr>
<td>Standard (camptothecin)</td>
<td>10</td>
<td>5.02±0.19</td>
<td>57.85±1.59</td>
<td>41.05±0.37</td>
</tr>
<tr>
<td>Aqueous extract A</td>
<td>250</td>
<td>3.99±0.09</td>
<td>89.08±1.53</td>
<td>29.98±0.52</td>
</tr>
<tr>
<td>Aqueous extract B</td>
<td>500</td>
<td>4.05±0.23</td>
<td>75.65±2.35</td>
<td>38.35±0.66</td>
</tr>
<tr>
<td>Alcoholic extract A</td>
<td>100</td>
<td>4.14±0.34</td>
<td>86.85±1.85</td>
<td>31.52±0.57</td>
</tr>
<tr>
<td>Alcoholic extract B</td>
<td>200</td>
<td>4.56±0.19</td>
<td>68.28±1.52</td>
<td>39.66±0.69</td>
</tr>
</tbody>
</table>

Oxidative stress, Cancer: effect of *Cardiospermum helicacabum* Fractions

**Glutathione syntheses:**
In EAC induced tumor animals, Glutathione syntheses levels in Liver was significantly (P<0.05) lower compared to treated animals. In Camptothecin and extract treated animals these levels were recovered significantly (P<0.05). (Figure 8).

**Lipid per oxidation:** In EAC induced tumor animals, Lipid peroxide levels in Liver are significantly (P<0.05) increased compared to extract treated animals. In Camptothecin and extract treated animals these levels were recovered significantly (P<0.05). (Figure 9).

**Catalase:**
In EAC induced tumor animals, Catalase levels in Liver are significantly (P<0.05) lower compared to treated animals. In Camptothecin and extract treated animals these levels were recovered significantly (P<0.05). (Figure 10).

4. Conclusion
In the present research work anti Cancer activity of *Cardiospermum helicacabum* roots has been evaluated on mice. In vivo anti cancer activity of ethanolic and aqueous extracts of roots were evaluated by using Ehrlich ascites
Carcinoma (EAC) cell lines. Acute toxicity studies were also performed initially in order to ascertain the safety of root extracts. The results showed that the ethanolic root and aqueous extracts were having significant anti cancer activity.

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6. References