Original Article

# Pharmacological Evaluation of *Caesalpinia pulcherrima* Leaf Extract for Anticancer Activity against Ehrlich Ascites Carcinoma Bearing Mice

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## ABSTRACT

**Objectives:** The aim of the study is to assess the anticancer activity of *Caesalpinia pulcherrima* leaf extract against Erlich Ascites Carcinoma (EAC) in mice. **Materials and Methods:** BALB/c mice with Erlich Ascites Carcinoma were used for the present study. Ethanolic extract of *C. pulcherrima* (EECP) was administered p.o. in three different doses i.e. 200, 400 and 800 mg/kg body weight, after 24 hr of tumor inoculation in mice for 14 days. Effect of EECP on haematological parameters, liver enzymes marker, biochemical parameters/ antioxidants was evaluated after 14 days treatment. **Results:** Effect of EECP on Haematological parameters were evaluated and Hb, Lymphocytes, RBC and Monocytes showed significant increase in their count when compared to Disease. WBC and neutrophil count was decreased in EECP group compared to disease. Effect of EECP on Serum parameters were evaluated and showed significant decrease in ALP, TC, AST, ALT and TG compared to disease. TP was significantly increased in EECP groups compared to DISEASE group. Effect of EECP on Antioxidant activity showed significant increase in CAT, SOD, GSH and GPx counts compared to Disease. LPO level was significantly reduced in all the treated groups compared to Disease group. **Conclusion:** Ethanolic extract of *C. pulcherrima* leaf exhibited significant antioxidant and anticancer activity against Erlich Ascites Carcinoma.

**Key words:** Anticancer, Antioxidant, *Caesalpinia pulcherrima*, EAC, Malignancy.

## INTRODUCTION

Cancer is a disease in which a group of cells shows uncontrolled development, intrusion that encroaches upon and wreck adjoining tissue and spread to different areas in the body by means of lymph or blood.[1,2] Experimental tumors have great importance for the purposes of modeling and Ehrlich ascites carcinoma (EAC) is one of the commonest. EAC is referred to as an undifferentiated carcinoma and is originally hyper diploid, has high transplantable capability, shorter life span, rapid proliferation, no-regression, 100% malignancy and also does not have tumor specific transplantation antigen.[3] The chemotherapeutic agents provide a temporary improvement of signs and symptoms of cancers and most of the chemotherapeutic agents are given in combination with the radiation therapy and surgery, due to which the cost of treatment is high.[4] Along with it, patient also has to undergo the treatment for a longer duration. Thus to reduces such problems, in the last few years, novel chemo preventive agents of natural origin have been targeted with herbal plants being a key interest due to high content of bioactive compounds.

Traditional medicines have been reported to constitute multiple secondary metabolites and are useful in the management of multiple pathogenesis including both infectious and

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non-infectious pathogenesis.[5-12] Herbal plants which have antioxidant properties could add a beneficial effect in the management of multiple pathogenesis including cancer as they possess broad spectrum of biological activities.[13] Further, the cytotoxic effects of multiple plant-based agents have been reported as cytotoxic agents demonstrated via *in silico* and *in-vitro* approach.[14,15]

*Caesalpinia pulcherrima* is one known plant to be rich in flavonoids which possesses various pharmacological properties including antibacterial, antifungal, antiviral, anticancer and anti-inflammatory effects.[16] *Caesalpinia pulcherrima* indicated high particular cytotoxic properties against malignant growth cells (MCF-7).[17] However, no studies are available on antitumor and antioxidant activity of the of *Caesalpinia pulcherrima* leaf extract extract against Erlich Ascites Carcinoma (EAC) in mice. Hence, present study was considered to determine the antioxidant and antitumor activity of ethanolic leaf extract of *Caesalpinia pulcherrima* against ehrlich ascites carcinoma bearing mice.

## MATERIALS AND METHODS

### Plant material collection and extraction

Leaves of *C. pulcherrima* were collected from the College of Horticulture Arabhavi, Belagavi. The plant material were subjected to shade drying and identified by Dr. Harsha Hegde, Scientist D, RMRC (ICMR) Belagavi Voucher no. RMRC-1401. Leaves of *C. pulcherrima* were dried in room temperature, powdered and stored in a sealed compartment at room temperature. Herbal constituents was separated using 95% ethanol by Soxhlet extraction technique. The concentrate was concentrated to dryness utilizing Rotary evaporator.[5] Percentage yield extract was determined utilizing the formula:

$$\% \text{ yield} = \frac{\text{Weight of extract}}{\text{Crude weight}} \times 100$$

Dried extract was stored in sealed glass container for further evaluation.

### *In vitro* Antioxidant Activity

### DPPH radical scavenging assay[18]

The Stock solution was prepared by taking 1mg/ml of plant extract and 1mg/ml of Ascorbic acid. The stock solution was prepared with 50, 100, 200, 400 and 800µg/ ml of plant extract and Ascorbic acid. The solvent used was methanol.

Preparation of 0.1mM DPPH solution was done by dissolving 5.85mg of DPPH in 150ml of methanol.

Procedure: 0.4 ml of plant extract (50-800 µg/ ml) and the standard drug was mixed with 3.6 ml methanol solution of DPPH (0.1 mM). A Same quantity of methanol. (0.4 ml) was used as a blank (control) with DPPH soln (3.6ml). The above mixture was vortexed for 1 min. After incubation, the change in absorbance of each sample against methanol as blank was noted using plate reader at 517 nm. Percentage DPPH inhibition was calculated using the formula:

$$\text{DPPH inhibition } (\%) = \frac{\text{A Control} - \text{A Sample}}{\text{A Control}} \times 100$$

The results were reported as $IC_{50}$ value.

### Nitrous oxide (NO) radical scavenging activity[19]

Stock solution was prepared by taking 10mg/ml of plant extract and 1mg/ml of Ascorbic acid. This stock solution was prepared with different concentrations of 100, 200, 400, 800, 1000µg/ mL of plant extract and Ascorbic acid.

Preparation of Griess reagent: 25ml of 1% sulphanilamide was prepared using 2.5% phosphoric acid mixed with 25ml of 0.1% naphthyl ethylene diamine di hydro chloride using 2.5% phosphoric acid.

Preparation of sodium nitroprusside (10 mM): 0.2979 g of sodium nitroprusside was added in 100ml of pH 7.4 phosphate buffer.

To each 1ml different concentration of extract and standard drug (100-1000µg/ mL) added 0.5mL of 10mM sodium nitroprusside in phosphate buffered saline. To this solution, same vol. of Griess reagent was added. Blank was prepared without using plant extract and standard drug. The solution was administered to ninety six well plate and absorbance was taken at 546nm. The % inhibition of plant ext. and the std drug was found out using the formula:

$$\text{Nitric oxide scavenged } (\%) = \frac{\text{A Control} - \text{A Sample}}{\text{A Control}} \times 100$$

The results were reported as $IC_{50}$ value.

### Tumor Volume and Tumor Weight

TV and TW were determined by collecting ascetic fluid and measuring weight before and after sacrificing. Difference in the weight will give tumor weight and measurement of ascitic fluid from peritoneal cavity will give tumor volume.[20]

### Determination of cell viability by trypan blue exclusion method

50µl of cell culture/suspension was spotted in a cryo-vial. 0.4% of trypan blue dye was added to the suspension to get 1-2 dilution (ex: 50µl of cells - 50µl of trypan

blue) and was mixed. This cell culture was incubated for 3 minutes at room temp. Cells were counted using Haemocytometer by placing 10-20µl of cell suspension to each side of haemocytometer counter covered with cover slip. The cells were counted by placing the Haemocytometer on the stage of microscope.[21] The cells were focused and counted and % of viable cells was calculated using formula.

$$\%\ \text{cell viability} = 1 - \frac{\text{number of blue cells}}{\text{number of total cells}} \times 100$$

### Experimental animals

Female BALB/c mice (20 - 30g) were procured from *in vivo* Biosciences, Bengaluru. Animals were kept under standard husbandry conditions with temperature 22-28°C, relative humidity 65±10% and 12 hr light/dark cycle respectively. The research study was carried out obeying Institution Animal Ethics Committee (IAEC Reg. 221/Po/Re/S/2000/CPCSEA) Belagavi.

### Induction of erlich ascites carcinoma

Ehrlich Ascites Carcinoma (EAC) cell line was secured from NCCS Pune, India. The ascites bearing mice was taken after 15 days of tumor induction. The ascitic fluid was withdrawn using sterile/purified syringe and 21 gauge needle. Sufficient quantity was examined for microbial contamination. The ascetic fluid was diluted in normal saline to get concentration. of 106 cells / ml of tumor suspension. This was injected intraperitoneally to new healthy mice. All data were collected from the experimental animal model and seven animals were placed in each group. Animals from group 2 to 6 were transplanted intraperitoneally with EAC (0.1 ml) 1 × 106 cells/ ml. All groups were inoculated with EAC cells excluding Normal control. On zero day the weight of mice was noted. A day of incubation was left for proliferation of cells and group III was administered with standard drug Doxorubicin (i.p.) and for groups IV, V and VI received 200 mg/ kg, 400 mg/ kg and 800 mg/ kg of extracts of *Caesalpinia pulcherrima* (p.o.) and this treatment was continued up to 14 days.

### The animals were divided into following groups

Group I: Normal

Group II: Disease control (EAC control (injected i.p. with sterile physiological saline 0.9% w/v of NaCl);

Group III: Standard control (EAC+Doxorubicin (1.2mg/kg i.p. Administration)

Group IV: Treatment I (EAC bearing EECP treated 200 mg/kg -p.o. Administration)

Group V: Treatment II (EAC bearing EECP treated 400 mg/kg -p.o. Administration)

Group VI: Treatment III (EAC bearing EECP treated 800 mg/kg -p.o. Administration)

Evaluation of anticancer properties of plant extracts on physical parameters. The weight of each animal was recorded once in three days (0, 3, 6, 9, 12, 15$^{th}$ days). All the animals were scarified on the 15$^{th}$ day. Tumor volume and Tumor weight were determined by collecting ascetic fluid and taking weight before and after sacrificing the mice. Difference in the weight will give Tumor weight and measurement of ascitic fluid from peritoneal cavity will give tumor volume.

### Hematological Profile and Serum Biochemical Parameters Estimation

At the end of the experiment, blood was collected retro-orbitally and centrifuged at 3000 rpm for 10 mins to separate serum for the estimation of LPO, SOD, CAT, GSH and GPx.

### Statistical analysis

All values are expressed as Mean±SEM. ANOVA followed by *post hoc* test as required. The min value of $p < 0.05$ was reflected as significant.

## RESULTS

### Effect on tumor volume and tumor weight

Results showed that there was reduction in the tumor weight and tumor volume in standard Doxorubicin group and in all 3 Test groups. Tumor Volume and Tumor Weight of Disease control group was found to be 3.95 ± 0.94ml and 4.02 ± 0.64g respectively and it was significantly reduced to 3.08 ± 0.25ml and 2.98 ± 0.31g (200mg/kg), 2.18 ± 0.32ml and 2.18 ± 0.49g (400mg/kg), 1.33 ± 0.29ml and 1.53 ± 0.37 (800mg/kg) in ethanolic extract *C. pulcherrima* treated group where as in the Standard Doxorubicin Group it was found to be 0.86 ± 0.44ml and 1.08 ± 0.38g. When compared to the Standard Doxorubicin Group, 200mg/kg (3.08 ± 0.25 and 2.98 ± 0.31) and 400mg/kg (2.98 ± 0.32 and 2.18 ± 0.49) groups were showed significant increase ($p<0.001$) in Tumor Volume and Tumor Weight. When compared with 200mg/kg group, 400mg/kg and 800mg/kg groups showed significant reduction in TV and TW. When compared with 400mg/kg group, 800mg/kg (1.33 ±

0.29 and 1.53 ± 0.37) displayed significant ($p<0.001$) decrease in TV (Table 1).

## Effect on body weight

Towards the end of the 3rd day every animal gained body wt. compared to 0th day. Body wt. of Normal group was found to be 23.8 ± 0.43g and it was significantly increased in Disease control (24.25 ± 1.14g), when compared to treated 800mg/kg group (26.13 ± 1.05g), Doxorubicin group (22.23 ± 0.60g), Body weight in the diseased group was significantly reduced. At the end of 6th day, body weight of Normal was found to be 24.95 ± 0.62g and it was significantly increased in Disease control (28.43 ± 1.36g). When evaluated against treated groups such as 200mg/kg (26.90± 0.74g), 400mg/kg (25.96 ± 0.73g), 800mg/kg (26.83 ± 0.92g) and Doxorubicin (23.46 ± 0.50g), body weight of Disease Control was significantly reduced. On 9th day, the body weight of Normal was found to be 25.96 ± 0.73g and it was significantly increased, in disease control group was found to be 31.38 ± 1.35g. When compared to treated 200mg/kg (28.4 ± 1.36g), 400mg/kg (27.15 ± 0.61g) and 800mg/kg (27.25 ± 0.84g) groups, Body weight of disease control was significantly reduced. On 12th day, the body wt of Normal was (26.8 ± 0.65g) and it was significantly increased in disease control group (34.2 ± 0.88g). In comparison with treated groups such as 200mg/kg (28.08 ± 1.33g), 400mg/kg (26.71 ± 0.67g), 800mg/kg (25.4 ± 0.68g) and Doxorubicin (23.28 ± 0.49g), disease control showed significant decrease in bodyweight. At the end of 14th day, the body weight of Normal was 27.55 ±0.83g and it was significantly increased in Disease Control (38.05 ± 0.81g). Judged against treated group 200mg/kg (27.8 ± 1.12g), 400mg/kg (26.01 ± 0.58), 800mg/kg (24.8 ± 1.11g) and Doxorubicin (22.9 ± 0.66), Disease control group showed significant increase in the body weight (Table 2).

## Hematological parameters

When compared against Normal (13.55 ± 0.47), Disease (7.61 ± 1.15) group exhibited significant reduction in Hb count, as compared to all 3 EECP Treatment groups such as 200mg/kg (9.39 ± 1.17), 400mg/kg (10.14 ± 0.8) and 800mg/kg (11.05 ± 0.38), Disease control group showed significant decrease in Hb levels, whereas Doxorubicin (11.97 ± 1.34) group also exhibited significant increase in Hb count comparing with Disease group. Compared to Doxorubicin, 200mg/kg and 400mg/kg groups exhibited significant reduction in Hb levels (Table 3).

Relative to Normal control (8.09 ± 0.47), Disease control (20.8 ± 2.8) displayed significant rise in WBC count. On comparing with treated groups like Doxorubicin (11.5 ± 1.26), 400mg/kg (14.34 ± 1.77) and 800mg/kg (12.03 ± 1.33) groups, Disease control displayed significant ($p<0.001$) increase in WBC level. With reference to Doxorubicin, Treated group 400mg/kg exhibited significant ($p<0.001$) elevation in WBC levels. Measured in opposite to 200mg/kg group (18.76 ± 1.75), 400mg/kg and 800mg/kg groups displayed significant decrease in WBC levels (Table 3).

Relative to Normal (75.33 ± 5.21), Disease control (32.9 ± 4.53) displayed significant reduction in lymphocyte count. In comparison to treated groups like Doxorubicin (70.41 ± 8.28), 400 mg/kg (56.9 ± 5.35) and 800mg/ kg (68.22 ± 5.04), Disease group showed significant reduction

**Table 1: Effect of EECP leaf on Tumor volume and Tumor weight.**

| Group | Tumor Volume (ml) | Tumor Weight (g) |
|---|---|---|
| Normal | - | - |
| Disease | 3.95±0.94*** | 4.02±0.64*** |
| EAC + Doxorubicin | 0.86±0.44### | 1.06±0.38### |
| EECP 200mg/kg | 3.08±0.25### | 2.98±0.31## |
| EECP 400 mg/kg | 2.18±0.32@@@### | 2.18±0.49###@ |
| EECP 800 mg/kg | 1.33±0.29^^^ @@@### | 1.53±0.37@@@### |

All values are expressed as Mean±SEM
***$p<0.001$ compared to normal, ##$p<0.01$, ###$p<0.001$ compared to disease, @$p<0.05$, @@@$p<0.001$ compared to 200 mg/kg, ^^^$p<0.001$ compared to 400 mg/kg

**Table 2: Effect of EECP leaf on Body weight**

| Groups | Body weight (gm) | | | | | |
|---|---|---|---|---|---|---|
| | 0th day | III day | VI day | IX day | XII day | XIV day |
| Disease | 23.45±1.13 | 24.25±1.14 | 28.43±1.36 | 31.38±1.35 | 34.2±0.88 | 38.05±0.81 |
| Normal | 23.03±0.48 | 23.8±0.43 | 24.95±0.62*** | 25.96±0.73*** | 26.8±0.65*** | 27.55±0.83*** |
| EAC + Doxorubicin | 23.01±0.5 | 22.23±0.60*** | 23.46±0.50*** | 23.71±0.49*** | 23.28±0.49*** | 22.9±0.66*** |
| EECP 200mg/kg | 23.2±0.5 | 24.5±0.66 | 26.9±0.74* | 28.4±1.36*** | 28.08±1.33*** | 27.8±1.12*** |
| EECP 400 mg/kg | 23.4±0.44 | 24.58±0.54 | 25.96±0.73*** | 27.15±0.61*** | 26.71±0.67*** | 26.01±0.58*** |
| EECP 800 mg/kg | 23.53±0.69 | 26.13±1.05*** | 26.83±0.92** | 27.25±0.84*** | 25.4±0.68*** | 24.8±1.11*** |

All Values are exressed as Mean ± SEM where $n=6$. *$p<0.05$, **$p<0.01$ and **$p<0.001$compared to Disease

in lymphocyte count. With reference to Doxorubicin group, 200 mg/ kg and 400 mg/kg groups displayed significant decrease in lymphocyte count. Evaluated against 200mg/kg group (38.16 ± 4.63), 400mg/kg and 400mg/kg group exhibited significant ($p$<0.001) elevation in lymphocyte count (Table 3).

As opposed to Normal control (22.68 ± 3.54), Disease control (72.62 ± 4.01) exhibited significant ($p$<0.001) rise in Neutrophils count. Judged against treated groups such as Doxorubicin (18.59 ± 2.9), 3 EECP test groups 200, 400 and 800mg/kg groups, Disease Control displayed significant increase in Neutrophils count. In contrast to Doxorubicin group, leaf extracts indicated significant enhancement in Neutrophils count. By comparing with 200mg/kg group (56.2 ± 3.98), 400mg/kg (35.12 ± 3.64) and 800mg/kg group (27.35 ± 3.42) displayed significant ($p$<0.001) decrease in Neutrophils count. In relation to 400mg/kg group, 800mg/kg group showed significant ($p$<0.01) decrease in Neutrophil count (Table 3).

In relation to Normal control (9.24 ± 1.05), Disease control (1.12 ± 0.31) showed significant decline in RBC count. Related to treated groups such as Doxorubicin (10.2 ± 0.8) and 800mg/kg (6.71 ± 1.02), Disease showed significant decrease in RBC count. With reference to Doxorubicin, 200mg/kg (6.71 ± 1.02) and 400mg/kg (7.84 ± 0.8) groups exhibited significant decrease in RBC count. As opposed to 200mg/kg, 800mg/kg group showed significant increase in RBC count (Table 3).

With reference to Normal (3.15 ± 0.37), Disease (1.12 ± 0.3) showed significant decrease in Monocytes count. In relation to treated groups such as Doxorubicin (2.54 ± 0.4), 400mg/kg (2.29 ± 0.38) and 800mg/kg (2.5 ± 0.25), DISEASE group displayed significant reduction in Monocytes count. In accordance with Doxorubicin, 200mg/kg group (1.67 ± 0.53) showed significant ($p$<0.01) decrease in Monocytes count. On comparing with 200mg/kg group, 800mg/kg group displayed significant ($p$<0.01) increase in Monocytes count (Table 3).

## Serum estimation

In comparison with Normal control (27.68 ± 4.21), Disease control (101.1 ± 7.08) showed significant ($p$<0.001) rise in ALP count. With reference to treated groups such as Doxorubicin (45.03 ± 5.63), 200mg/kg (78.9 ± 4.01), 400mg/kg (63.63 ± 3.74) and 800mg/kg (50.75 ± 4.35), Disease control exhibited significant ($p$<0.001) increase in ALP count. In contrast to Doxorubicin, 200mg/kg and 400mg/kg was showing significant enhancement in ALP count. In contrast to 200mg/kg, 400mg/kg and 800mg/kg group was showing significant ($p$<0.001) decrease in ALP count. Dealing with 400mg/kg, 800mg/kg group displayed significant ($p$<0.01) reduced in ALP count (Table 4).

**Table 3: Effect of EECP leaf on Hematological variables.**

| Group | Hematological Parameters | | | | | |
|---|---|---|---|---|---|---|
| | Hb | WBC | Lymphocytes | Neutrophils | RBC | Monocytes |
| Normal | 13.55±0.47 | 8.09±0.47 | 75.33±5.21 | 22.68±3.54 | 9.24±1.05 | 3.15±0.37 |
| Diseasecontrol(EAC) | 7.61±1.15*** | 20.8±2.8*** | 32.9±4.53*** | 72.62±4.01*** | 6.38±0.76*** | 1.12±0.31*** |
| EAC + Doxorubicin | 11.97±1.34### | 11.5±1.26### | 70.41±8.28### | 18.59±2.9### | 10.2±0.8### | 2.54±0.4### |
| 200mg/kg | 9.39±1.17# | 18.76±1.75 | 38.16±4.63 | 56.2±3.98### | 6.71±1.02 | 1.67±0.53 |
| 400 mg/kg | 10.14±0.8## | 14.34±1.77###@@ | 56.9±5.35###@@@ | 35.12±3.64###@@@ | 7.84±0.8 | 2.29±0.38### |
| 800 mg/kg | 11.05±0.38### | 12.03±1.33###@@@ | 68.22±5.04###@@@ | 27.35±3.42###@@@^^ | 9.11±0.7###@@@ | 2.5±0.25###@@@ |

All values are expressed as Mean±SEM

***$p$<0.001 compared to normal, ###$p$<0.001 compared to disease, @@$p$<0.01, @@@$p$<0.001 compared to 200 mg/kg, ^^$p$<0.01 compared to 400 mg/kg

**Table 4: Effect of EECP leaf on Serum Estimation Parameters.**

| Group | Serum Estimation Markers | | | | | |
|---|---|---|---|---|---|---|
| | ALP | TP | TC | AST | ALT | TG |
| Normal | 27.68±4.21 | 6.87±0.80 | 142.34±13.29 | 40.45±5.8 | 34.4±3.3 | 66.25±5.31 |
| Disease (EAC) | 101.1±7.08*** | 4.66±0.79*** | 180.77±7.11*** | 82.9±5.49*** | 79.26±4.5*** | 154.18±7.23*** |
| EAC + Doxorubicin | 45.03±5.63### | 6.27±0.72## | 151.003±3.76### | 55.65±2.84### | 43.18±5.18### | 85.18±4.48### |
| 200mg/kg | 78.9±4.01### | 4.93±0.81 | 160.18±7.21### | 70.78±2.53### | 64.21±4.04### | 125.9±5.5### |
| 400 mg/kg | 63.63±3.74 ###@@@ | 5.43±0.39 | 155.85±2.85### | 61.1±4.38###@@ | 53.03±4.6###@@ | 111.16±7.98###@ |
| 800 mg/kg | 50.75±4.33### ^^ @@@ | 6.03±0.24# | 150.65±2.89### | 58.8±4.29###@@@ | 48.58±4.98###@@@ | 95.25±4.94### ^^^ @@@ |

All values are expressed as Mean±SEM

***$p$<0.001 compared to normal, #$p$<0.05,##$p$<0.01, ###$p$<0.001 compared to disease, @$p$<0.05, @@$p$<0.01, @@@$p$<0.001 compared to 200 mg/kg, ^^$p$<0.01, ^^^$p$<0.001 compared to 400 mg/kg

In accordance with Normal (6.87 ± 0.80), Disease control (4.66 ± 0.79) displayed significant ($p<0.001$) reduction in TP level. In opposite treated groups such as Doxorubicin (6.27 ± 0.72) and 800mg/kg (6.03 ± 0.24), DISEASE displayed significant ($p<0.01$ and $p<0.05$) decrease in TP count. As regards to Doxorubicin, 200mg/kg group (4.93 ± 0.81) showed significant ($p<0.05$) decreased in TP count (Table 4).

Measured against Normal control (142.34 ± 13.29), Disease control (180.77 ± 7.11) observed significant ($p<0.001$) upgrade in TC count. Judged against treated groups such as doxorubicin (151.003 ± 3.76), 200mg/kg (160.18 ± 7.21), 400mg/kg (155.85 ± 2.85) and 800mg/kg (150.65 ± 2.89), Disease control resulted significant ($p<0.001$) decrease in TC count (Table 4).

In contradiction of normal (40.45 ± 5.8), disease control (82.9 ± 5.49) showed significant increase in AST count. In accordance with treated groups such as doxorubicin (55.65 ± 2.84), 200mg/kg (70.78 ± 2.53), 400mg/kg (61.1 ± 4.38) and 800mg/kg (58.8 ± 4.29), Disease control exhibited significant ($p<0.001$) increase in AST count. Contrary to Doxorubicin, 200mg/kg group was showing significance ($p<0.001$) elevation in AST count. Relative to 200mg/kg group, 400mg/kg and 800mg/kg group showed significant decrease in AST level (Table 4).

On compared to normal (34.4 ± 3.3), Disease control (79.26 ± 4.5) resulted significant ($p<0.001$) rise in ALT count. Measured against treated groups such as doxorubicin (43.18 ± 5.18), 200mg/kg(64.21 ± 4.04), 400mg/kg(53.03 ± 4.6) and 800mg/kg(48.58 ± 4.98), DISEASE resulted significant ($p<0.001$) increase in ALT count. Relative to doxorubicin, 200mg/kg and 400mg/kg groups were showing significant increase in ALT level. Evaluated against 200mg/kg, 400mg/kg and 800mg/kg was showing significant decrease in ALT level (Table 4).

On comparing with normal (66.25 ± 5.31), Disease (154.18 ± 7.23) group displayed significant ($p<0.001$) elevation in TG count. In contrast to treated groups such as doxorubicin (85.18 ± 4.48), three drug extracts 200, 400 and 800mg/kg, DISEASE displayed significant increase in TG count. Evaluated against Disease, 200mg/kg(125.9 ± 5.5) and 400mg/kg(111.16 ± 7.98) groups displayed significant increase in TG count. Compared against 200 mg/ kg group, 400 mg/kg and 800 mg/kg(95.25 ± 4.94) group showed significant reduction in TG level. As opposed to 400mg/kg, 800mg/kg group showed significant ($p<0.001$) reduction in TG level (Table 4).

## Effect on enzymatic/non-enzymatic antioxidant biomarkers

In accordance with normal control (26.13 ± 1.05), Disease control (9.39 ± 1.17) group was showing significant ($p<0.001$) decrease in CAT count. In contrast to treated groups such as doxorubicin (22.2 ± 0.6), 200mg/kg(18.59 ± 2.95), 400mg/kg(20.84 ± 2.85) and 800mg/kg(21.33 ± 2.87), disease results significant ($p<0.001$) increase in CAT count (Table 5).

In dealing with normal group (8.06 ± 1.28), Disease control (25.2 ± 1.99) showed significant ($p<0.001$) elevation in LPO count. In opposite to treated groups such as doxorubicin (10.92 ± 0.53), 200mg/kg (19.44 ± 1.31), 400mg/kg(15.07 ± 0.68) and 800mg/kg (10.02 ± 0.45), Disease control displayed significant ($p<0.001$) rise in LPO count. On comparing with Doxorubicin group, 200mg/kg and 400mg/kg groups were showing significant increase in LPO count. Judged against 200mg/kg group, 400mg/kg and 800mg/kg group showed significant ($p<0.001$) decrease in LPO count. When it is seen with 400mg/kg, 800mg/kg group exhibited significant ($p<0.05$) decrease in LPO count (Table 5).

Was against normal group (13.16 ± 3.25), Disease control (18.43 ± 3.13) showed significant ($p<0.001$) decrease in SOD count. Measured against treated groups such as Doxorubicin (32.03 ± 1.19), 400mg/kg(24.4 ± 3.54) and 800mg/kg(30.71 ± 2.19), DISEASE displayed significant increase in SOD count. Reference to DISEASE, 200mg/kg (22.6 ± 5.24) and 400mg/kg groups displayed significant ($p<0.001$, $p<0.01$) reduction in SOD count.

Table 5: Effect of EECP leaf on Antioxidants.

| Group | Antioxidants | | | | |
|---|---|---|---|---|---|
| | CAT | LPO | SOD | GSH | GPx |
| Normal | 26.13±1.05 | 8.06±1.28 | 39.16±3.25 | 2.26±0.3 | 7.02±0.89 |
| Disease control (EAC) | 9.39±1.17$^{***}$ | 25.2±1.99$^{***}$ | 18.43±3.13$^{***}$ | 1.55±0.27$^{**}$ | 3.11±0.43$^{***}$ |
| EAC + Doxorubicin | 22.2±0.6$^{###}$ | 10.92±0.53$^{###}$ | 32.03±1.9$^{###}$ | 2.02±0.43 | 6.26±0.72$^{###}$ |
| EAC 200mg/kg | 18.59±2.95$^{###}$ | 19.44±1.31$^{###}$ | 22.6±5.24 | 1.63±0.22$^{*}$ | 3.51±0.32 |
| EAC 400 mg/kg | 20.84±2.83$^{###}$ | 15.07±0.68$^{### @@@}$ | 24.4±3.54$^{#}$ | 1.74±0.27 | 4.68±0.28$^{### @}$ |
| EAC 800 mg/kg | 21.33±2.87$^{###}$ | 10.02±0.45$^{### \wedge\wedge\wedge @@@}$ | 30.71±2.19$^{### \wedge @@}$ | 1.9±0.27 | 5.48±0.48$^{###@@@}$ |

All Values are expressed as Mean ± SEM where $n$=6.

$^{***}p$<0.001compared to Normal, $^{#}p$<0.05, $^{###}p$<0.001 compared to DISEASE, $^{@}p$<0.05, $^{@@}p$<0.01 and $^{@@@}p$<0.001compared to 200mg/kg, ^$p$<0.05, ^^^$p$<0.001compared 400mg/kg.

In contradiction of 200mg/kg group, 800mg/kg group was showing significant ($p<0.01$) increase in SOD count. With reference to 400mg/kg, 800mg/kg group resulted significant ($p<0.05$) rise in SOD count. Glutathione on comparing with normal group (2.26 ± 0.3), Disease (1.55 ± 0.27) showed significant ($p<0.01$) reduction in GSH count (Table 5).

Glutathione Peroxidase was evaluated against to Normal Group (7.02 ± 0.89), Disease control (3.11 ± 0.43) was showing signifying (p<0.001) decrease in GPx count. In relation to treated groups such as Doxorubicin (6.26 ± 0.72), 400mg/kg (4.68 ± 0.28), 800mg/kg (5.48 ± 0.48), DISEASE group displayed significant decrease GPx count. In accordance with Doxorubicin group, 200mg/kg (3.51 ± 0.32) and 400mg/kg groups was showing significant ($p<0.001$) decrease in GPx count. Evaluated against 200mg/kg group, 400mg/kg and 800mg/kg group results significant rise in GPx count (Table 5).

### Histopathology study

Damaged hepatocellular architecture with neoplastic lesions and changed hepatocytes were observed to be developed with more than one nucleus and hyper chromatic in nature. Invasion of lymphocytes and stamped widening of central vein were found in DC group. Slight damaged hepatocellular architecture with neoplastic lesions was observed in 200mg/kg and 400mg/kg groups. Reversal of this damage observed in Doxorubicin and 800mg/kg groups (Figure 1).

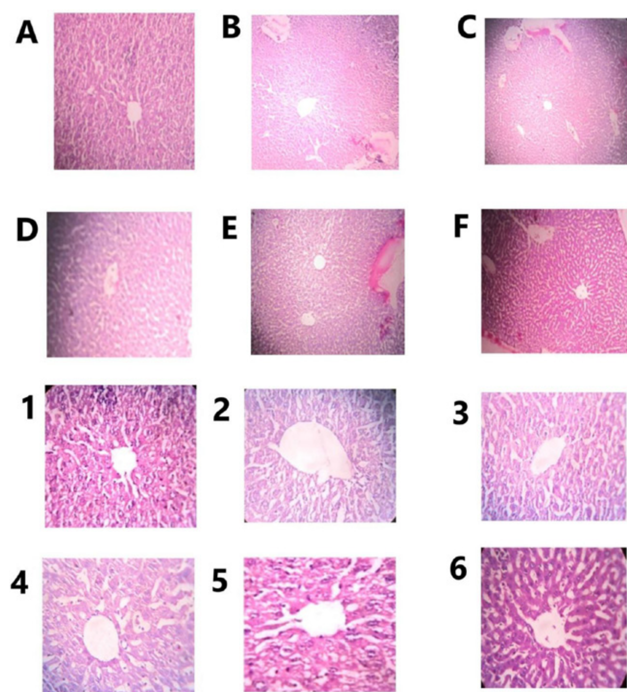


**Figure 1: Histopathology of Liver. A, B, C, D, E and F are 10X and 1, 2, 3, 4, 5 and 6 are 40X ; where A (1) is Normal group, B (2) is Disease control, C (3) is Doxorubicin, D (4) is 200mg/kg group, E (5) is 400mg/kg group and F (6) is 800mg/kg group.**

## DISCUSSION

The present research was carried out to assess the anti-oxidant and antitumor activity of ehthanolic extract of *C. pulcherrima* in EAC bearing mice. The EECP treated animals at the doses of 200 mg/ kg, 400 mg/ kg and 800 mg/kg significantly reduced TV and TW. Treated groups showed remarkable reduction in the VCC and major increase in the NVCC. Although Standard drug Doxorubicin showed a significant result compared to the other treated groups and was more effective than the *C. pulcherrima* doses. Phytochemical investigation displayed the existence of Alkaloids, glycosides, phenols and flavonoids. Basically alkaloids are used in the treatment of cancer (vincristine and vinblastine).[22] Cardiac Glycosides were also having the significant activity against cancer cell lines.[23] Similarly Phenols and flavonoids were also shown significant activity in cancer chemotherapy.[24]

Till today, in malignant growth, the problems due to chemotherapy that are being experienced are myelosuppression and anemia. Anemia occurred in tumor bearing mice is principally because of decrease in Red Blood Cells or Hb level and this might happen because of iron inadequacy or because of hemolytic or myelopathy conditions. Treatment with the drug EECP normalized the WBC, Red Blood Cells, Hb, Lymphocytes, Neutrophils and Monocyte count. This demonstrated the activity of drug on the hematological variables.[25]

Raised hepatic catalysts and bilirubin in serum lead to liver damage, liver metastases and bile duct hindrance.[26] ALP value observed to be expanded in different liver and bone disorders. Due to the spread of malignancy in different organ systems ALP level increase and the drugs used for treatment should reduce the ALP level to the normal. Similarly the levels of AST and ALT were also declined to the normal treatment groups.[27] Liver is the principle sources of serum protein. Protein intake is directly identified with the dimensions of total proteins (TP). Prior research reports propose that expended protein demonstrates liver dysfunction which impedes protein synthesis.[28] Total cholesterol (TC) level is more in the tumor bearing mice because cholesterol consumption leads to tumor growth. Hence the elevated cholesterol level reduced to normal level by treatment groups.[29]

Increased level of triglycerides (TG) indicates the liver damage and cell membrane disruption. The remarkable inversion of biochemical variables towards the normal

by treatment demonstrates a defensive impact on liver functions. In the ongoing investigation, biochemical inspection of EAC inoculated animals demonstrated noticeable changes, showing tumor growth. The standardization of these impacts seen in the serum cured with extracts supported the anti-tumor action of the EECP.

Due to the too much construction of free radicals cause oxidative strain that clues to the destruction of macromolecules such as fatty acids and can bring LPO *in vivo*. Rise in LPO content causes collapse of tissue.[30] Lipid peroxide shaped in essential site is to be exchanged through the flow and incite harm by spreading the progression of LPO.[31] In diseased condition, the end product of LPO i.e. Malondialdehyde found to be higher than in treated group organs. SOD and Catalase gives barrier against potential harming reactivity of Superoxide and hydrogen peroxide.[32] Tumor growths was accounted or reported because of blockade of SOD and Catalase.[33] SOD and Catalase were showed remarkable enhancement in the treated groups. The association of free radicals in tumor was reported.[34] Due to the excessive oxidative stress GSH level were decreased in Disease group but in the treatment group GSH levels enhanced towards the normal levels, which may be due to decreased proliferation of cells.

One of the important outcome of the present study could via the interaction of multiple bioactives with multiple targets associated to the cancer pathogenesis. Previously Khanal *et al*. reported the probable interaction of secondary metabolites from plant-based medicines to interact with multiple proteins involved in the disease state via the principle of "*multi-component-multi protein interaction*".[35-38] Since, the present study investigated the *Caesalpinia pulcherrima* as anti-cancer agent and is a herbal agent, the probable outcome of the could be via the interactions of multiple secondary metabolites with various proteins involved in cancer pathogenesis.

## CONCLUSION

The treatment of ethanol extract of *C. pulcherrima* in tumor bearing mice showed significant variations in hematological and biochemical variables near to normal level as related to Disease control mice. Ethanol extract of *C. pulcherrima* leaf exhibited significant antioxidant and anticancer activity, which may be the outcome of synergistic action of Chemical constituents present in the drug. Further research work on the clarification of its mechanism of action and separation of its dynamic components may exhibit potent anticancer property.

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## CONFLICT OF INTEREST

The authors declare no conflict of interest.

## ABBREVIATIONS

**Disease:** Disease Control; **GSH:** Glutathione; **SOD:** Superoxide dismutase; **LPO:** Lipid Hydroperoxide; **ALP:** Alkaline phosphatase.

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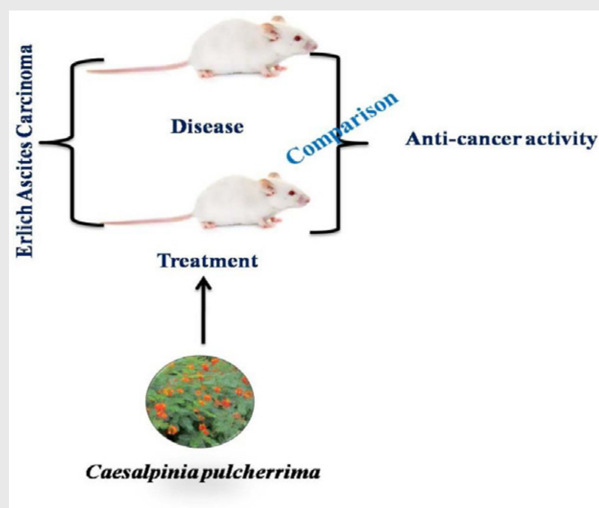
## PICTORIAL ABSTRACT




## SUMMARY

The Present study aim is to evaluate the anticancer activity of *Caesalpinia pulcherrima* leaf extract against Erlich Ascites Carcinoma (EAC) in mice. The ethanolic extract of *C. pulcherrima* (EECP) was administered p.o. at three different doses i.e. 200, 400 and 800 mg/kg body weight, after 24 hours of tumor inoculation in mice for 14 days. Effect of EECP on haematological parameters, liver enzymes marker, biochemical parameters/ antioxidants was evaluated after 14 days treatment. *Caesalpinia pulcherrima* leaf extract shows significant reduction of tumor size by Antioxidant and Anticancer activity of Phytoconstituents of the *Caesalpinia pulcherrima.*

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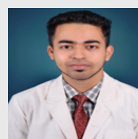

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