INTRODUCTION: Cancer is a serious health problem showing significant impact on human health care system, even with advancements in diagnosis, prevention and therapy cancer still effecting millions of people worldwide.

Cancer is diseases characterized by uncontrolled proliferation of cells leading to the growth of abnormal tissue; on worldwide basis cancer represents the single largest cause of death in both men and women.

Cancer is one of the most dreaded diseases of the 20th century and spreading further continuously with increasing incidence in 21st century.

It is still affecting millions of patients worldwide and reducing their quality of life.
Cancer is a group of more than 100 different diseases, characterized by uncontrolled cellular growth, local tissue invasion, and distant metastases. The aim of current research has been based on the identification of natural compounds that can be used in prevention or treatment of cancer. An ideal anticancer agent should be both cell and tissue specific i.e, it should kill or incapacitate cancer cells without causing much damage to normal host cells. Unfortunately currently available cancer chemotherapeutic agents insidiously affect the host cells, bone marrow, epithelial tissue, reticulo-endothelial system and gonads.

The World cancer report documents that cancer rates are set to increase at an alarming rate globally. Cancer rates could increase by 50% new cases for the year 2020. Plants are the rich source of medicines from ages. They produce bioactive molecules which can used to ameliorate various types of disorders. Over the last few decades there has been increased interest by Pharmaceutical industries to discover the new drugs from the ethno botanicals to provide new and alternative drugs to synthetic drugs for treatment of dreadful diseases.

Potent anticancer drugs like taxol, vinblastine, vincristine, camptothecin derivatives, topotecan and irinotecan, etoposide and podophyllotoxin derived from plant sources are in efficient clinical use. Hence, the natural products now have been contemplated of exceptional value in the development of effective anticancer agents with minimum host cell toxicity.

In India, from ancient times number of plant extracts are used against various diseases treatments in different systems of medicine such as Siddha, Ayurveda and Unani. Only a few plants have been scientifically explored regarding their medicinal uses.

There were good number of plant products such as flavonoids, terpenes, alkaloids and glycosides etc. are used as remedies to treat various diseases and disorders. Because of their distinct pharmacological qualities including cytotoxic and cancer chemo preventive effects, it is inspired many scientists to take up independent investigations on a number of new medicinal plants.

*Mimosa rubicaulis lam* (Mimosaceae) is commonly known as *Mimosa himalayan; korinda, putta korinda* in Telugu. A large straggling deciduous shrub, branches ribbed, densely hairy; prickles present on nodes or inter nodes, straight or hooked, 4 mm or less long. Traditionally, it is used in treatment of leucoderma, leprosy, chronic diarrhoea, rheumatism, treatment of snake bite, fungal infections and used for cuts & wounds (good wound healing property).

Amarnath and Chowdary (2010) isolated and reported anti-bacterial activity of *Mimosa rubicaulis lam* stem, root extracts. Amir Muhammad khan (2012) isolated and reported that *Mimosa rubicaulis lam* collected from Margalla Hills, Pakistan, consists of flavonoids such as Luteolin (2.3%) and quercetin (65.38%). Quercetin is a flavonoid widely distributed in nature it is important chemical constituent of *Mimosa rubicaulis lam* stem, Quercetin has antiviral property, quercetin may be effective in the treatment of fibromyalgia because of its potential anti-inflammatory or mast cell inhibitory property and it has Monoamine-Oxidase inhibitor activity. But no systematic pharmacological work was done on this plant anticancer activity.

As the stem of *Mimosa rubicaulis lam* consists of quercetin this further suggests that MEMR stem might have anti-cancer property. The present study was carried out to evaluate the anti tumor activity of methanol stem extract of *Mimosa rubicaulis lam* against Ehrlich Ascites Carcinoma in Swiss Albino mice and also to evaluate cytotoxic effect of MEMR on EAC, MCF-7, MDA-MB 435S cell lines (Cytotoxic effect of MEMR on cancer cell lines was evaluated by XTT assay).

**MATERIAL AND METHODS:**

**Cell lines and culture:** Human cancer cell lines used in this study were procured from National Centre for Cell Science, Pune, EAC cells were obtained from Amala Cancer Research Center, Thrissur, Kerala, India. All cells were grown in Minimal essential medium (MEM, GIBCO) supplemented with 4.5 g/L glucose, antibiotics (Benzylic Penicillin – 50units/mL, Streptomycin -50 µg/ml and Amphotericin –B -50 µg/ml), 200mM L-glutamine and 5% fetal bovine serum (FBS) (growth medium) at 37°C in 5% CO₂ incubator.
Plant collection and extraction: The plant *Mimosa rubicaulis lam* was collected in the month of June 2013 from in and around Tirupathi, Chittoor district, Andhrapradesh, India and authenticated by Dr. K. Madhav Chetty, Assistant professor, Department of Botany, Sri Venkateswara University, Tirupati. The plant was shade dried, stems of (500g) were coarsely powdered passed through sieve no.40 and then extracted by using 400ml of methanol (100%v/v) as solvent using soxhlet extraction apparatus. Coarse powdered plant material was subjected to continuous hot percolation for 9h at 64.7 – 65°C. The solvent was distilled in reduced pressure and resulting semisolids mass was vacuum dried using rotary flash evaporator to yield thick semisolids mass. The percentage yield was found to be 11.08% w/w. Preliminary qualitative analysis of the methanol extract showed presence of alkaloids, flavonoids, and tannins.  

Animals: The study was carried out after obtaining permission from Institutional Animal Ethics Committee (no.TBLSTPRJ0242013) and CPCSEA regulations were adhered during the study. Female Swiss albino mice (22-29g, 7-9 weeks old) were selected for this study. The animals were maintained under standard environmental conditions and acclimatized to the experimental room having temperature 23 ±2°C, controlled humidity conditions, 12h light-dark cycle. Animals were caged in poly acrylic cages (38×23×10cm) with maximum of four animals per cage. The mice were fed with standard food pellets and water *ad libitum*.

Acute toxicity studies: The oral acute toxicity study of the extract was carried out in Swiss albino mice using up and down procedure as per OECD, 2001. Animals should be fasted prior to dosing (food but not water should be withheld for 3-4 hours). Following the period of fasting, the animals were weighed and the test substance was administered. After the substance has been administered, food was withheld for a further 1-2 hours in mice. Mice received methanol extract at various doses (ranges from 500-4000mg/kg) orally by gavage. They were observed for toxic symptoms continuously for the first 4h after dosing. Finally the number of survivors was noticed after 24h. In the toxicity study no mortality occurred within 24h under the tested doses of MEMR. A period of at least 24 hours was allowed between the dosing of each animal. All animals were observed for 14 days.

Tumor Cells: The Ehrlich ascites tumor cell is a spontaneous murine mammary adenocarcinoma (Ehrlich and Apolant, 1905) adapted to ascites form (Loewenthal and Jahn, 1932) and carried in outbred mice by serial intraperitoneal passage.

EAC cells were obtained from Amala Cancer Research Center, Thrissur, Kerala, India. The EAC cells were maintained in the ascitic form *in vivo* by sequential passages in Swiss albino mice, by means of intraperitoneal transplantation of $2\times10^6$ cells/mice after every 10 days.

Transplantation of Tumor: After inoculation of EAC cells in the animals they were maintained under standard environmental conditions and fed with standard food pellets and water *ad libitum*. Ascitic fluid was drawn out from EAC bearing mice of 10-12 days old after inoculation. The ascitic fluid from mice was withdrawn using an ≤ 20 gauge needle into sterile syringe. Tumor viability was determined by Trypan blue exclusion test and cells were counted by Haemocytometer. The ascitic fluid was suitably diluted in normal saline to get a concentration of $1\times10^7$ cells/ml of tumor cell suspension. From the stock suspension 0.2ml (i.e. $2.0\times10^6$ EAC cells/mice) was injected intraperitoneally (i.p.) to obtain ascitic tumor.

Treatment schedule: The animals were divided into six groups (n=10), total 60 Swiss albino mice used in the experiment were fed with food and water *ad libitum*. All the animals in each group except first group received 0.2ml of EAC cell suspension ($2.0\times10^6$ EAC cells/mice, i.p). This was taken as day ‘0’. The first group-I was served as normal control.

The second group-II animals served as EAC control. After 24h of tumor inoculation the group-III, group-IV and group-V animals received MEMR at the dose of 100, 200 and 400mg/kg body weight, orally for 13 consecutive days respectively. Group-VI animals received reference anti-cancer drug 5-fluouracil (500mg/10ml injection, purchased from Ankurpharmacy, Hyderabad, manufactured by; Biochem, batch no; SB7124026, Exp date; 06/14) at the dose 20mg/kg body weight, i.p for 13 consecutive days.
After 24h of the last dose and then 18h fasting, blood was collected from 5 animals of each group’s retro-orbital plexus for the estimation of haematological parameters and then sacrificed by cervical dislocation for the study of antitumor parameters such as to measure tumor volume, viable cells and nonviable cells. The rest of the five animals of each group were kept with food and water *ad libitum* to check mean survival time and percentage increase in life span (ILS) \(^{21}\).

**Determination of Tumor Volume:** The mice were dissected and the ascitic fluid was collected from the peritoneal cavity. The volume was measured by taking ascitic fluid in a graduated centrifuge tube.

**Estimation of viable and non-viable Tumor cell count:** The ascitic fluid was taken in a WBC pipette and diluted 100 times (1:100) using normal saline. 0.5 ml of cells suspension was added to 0.5 ml of a 0.4% w/v Trypan Blue Solution to a test tube mixed thoroughly allowed to stand for 5 minutes. (If there are too many cells, change the ratios by adding 0.3 ml of the saline solution and 0.2 ml of the cell suspension and mix thoroughly) Then a drop of diluted suspension was paled on Neubauer counting chamber and the cells were then stained with Trypan blue dye. This was not let to stand for too long because viable cells will also start to uptake trypan blue. Grid was focused using 10X objective lens. The cells that did not take up the dye were viable cells and those took the stain were non-viable cells were counted.

\[
\text{No. of cells/ml} = \frac{\text{Average no. of cells/square} \times 10^4}{\text{dilution factor}}.
\]

**Determination of Mean Survival Time and Percentage Increase in Life Span:** The effect of methanol extract of *M. rubicaulis lam* on mean survival time and percentage increase in life span was calculated on the basis of mortality of the experimental mice \(^{22}\).

Mean survival time (MST) = First death + last death \(\div 2\)

Percentage Increase in Life Span (%ILS) = 

\[
\frac{\text{MST of treated group} - \text{MST of control group} \times 100}{\text{MST of control group}}
\]

Time was denoted by number of days \(^*\).

**Estimation of Haematological Parameters:** Blood was withdrawn from the retro-orbital plexus of all groups and used for the estimation of haemoglobin (Hb), RBC, WBC, PCV. \(^{23, 24, 25}\)

**Statistical Analysis:** All values were expressed as Mean ± SEM. Statistical analysis was performed with one way analysis of variance (ANOVA) followed by Dunnet’s multiple comparison test of significance. \(P < 0.05\) was considered to be statistically significant when compared to EAC control group.

**In vitro XTT assay procedure:** In brief, the trypsinized cells from T-25 flask were seeded in each well of 96-well flat-bottomed tissue culture plate at a density of (EAC cells \(-2.32 \times 10^6\) cells/mL, MCF-7 2.48 \(\times 10^6\) cells/mL and MDA-MB 435S 2.16 \(\times 10^6\) cells/mL) cells/well in growth medium and cultured at 37°C in 5% CO\(_2\) to adhere. After 24hr incubation, the supernatant was discarded and the cells were pretreated with growth medium and were subsequently mixed with different concentrations of test compounds (6.25, 12.5, 25, 50, 100 & 200 µg/ml) in triplicates to achieve a final volume of 100 µl and then cultured for 48 hr. The compound was prepared as 1.0 mg/ml concentration stock solutions in DMSO. Culture medium and solvent are used as controls. Each well then received 50 µl of fresh XTT (0.9mg/ml in RPMI along with XTT activator reagent) followed by incubation for 2hr at 37°C. At the end of the incubation shacked the 96 micro well plate for 15sec. The Optical Density (OD) of the culture plate was read at a wavelength of 490 nm (reference absorbance at a wavelength of 630 nm) on an ELISA reader, Anthos 2020 spectrophotometer.

**Calculations:**

\[
\% \text{ cell survival (CS)}: 100 - \frac{(\text{At-Ab)} + (\text{Ac-Ab})}{100}
\]

Whereas, \(\text{At} = \) Absorbance of test, \(\text{Ab} = \) Absorbance of blank.

\(\text{Ac} = \) Absorbance of control.

\[
\% \text{ cell inhibition (CI)}: 100 - \% \text{ cell survival}
\]
DNA Laddering assay: A distinctive feature of apoptosis at the biochemical level is DNA fragmentation. EAC cells were exposed to plant extract in five different concentrations (500, 250, 125 and 62.5 μg/mL) for 24 hrs. Culture media was collected, add 1 ml of trypsin to cell monolayer on 100-mm dishes, scrape the cells, harvest cells (culture media and cell monolayer) by centrifugation (2,500 rpm, 5 min), and wash cell pellets with 1X PBS. 100 μl of lysis buffer (1% NP-40 in 20 mM EDTA, 50 mM Tris-HCl, pH 7.5) was added for 10 sec. (Preparation of lysis buffer (1 ml): 10% NP-40 100 μl + 200 mM EDTA 100 μl + 0.2 M Tris-HCl (pH 7.5) 250 μl + D.W. 550 μl).

Centrifugation (3,000 rpm, 5 min) and obtain supernatant. 10 μl of 10% SDS solution to pooled supernatant (final: 1% SDS), was added then treated with 10 μl of 50 mg/ml RNase A (final 5 μg/μl) and incubate for 2 h at 56°C. 10 μl of 25 mg/ml Proteinase K (final 2.5 μg/μl) was added and incubate for 2 h at 37°C. 1/2 vol. (65 μl) of 10 M ammonium acetate was added. 2.5 vol. (500 μl) of ice-cold ethanol was added and mix thoroughly, Stand for 1 h in –80°C freezer (“ethanol precipitation”).

Centrifuged for 20 min at 12,000 rpm, wash the white pellet with 200 μl 80% ice - cold ethanol and air-dry for 10 min at room temperature. The pellet was air-dried, resuspended in 20μl tris acetate EDTA buffer supplemented with 2μl of sample buffer (0.25% bromphenol blue, 30% glyceric acid), and electrophoretically separated on a 2% agarose gel containing 1 μg/ml ethidium bromide and visualized under ultraviolet transillumination.

RESULTS:

Acute toxicity studies: Animals were observed for toxic symptoms continuously for the first 4h after dosing. Finally the number of survivors after 24h was noticed, Changes in skin and fur, eyes, mucous membranes, respiration, motor activity, autonomic and central nervous system etc..., were observed.

In the acute toxicity study no mortality occurred within 24h under the tested doses of MEMR, even after 14 days no mortality occurred and there were no significant toxic effects below a dose of 4000 mg/kg of MEMR.

Effect on body weight and tumor volume: Methanol stem extract of the Mimosa rubicaulis lam significantly reduced the body weight and tumor volume of EAC bearing groups when compared with EAC control group. Body weight of EAC control group was found to be 38.10 ± 0.94 gm, where as it was 33.22 ± 0.69 gm, 30.10 ± 0.37 gm, 27.16 ± 0.50 gm and 26.24 ± 0.65 gm for the group treated with MEMR at the dose of 100, 200, 400 mg/kg body weight and 5-FU at the dose 20 mg/kg body weight respectively. Tumor volume of EAC control group was found to be 18.26 ± 0.48 ml where as it was 12.66 ± 0.23 ml, 11.22 ± 0.11 ml, 8.6 ± 0.24 ml and 3.18 ± 0.33 ml for the groups treated with MEMR at the dose of 100, 200, 400 mg/kg body weight and 5-FU at the dose 20 mg/kg body weight respectively.

Effect on survival time: The MST of EAC control group was found to be 17 ± 0.28 days and the effect of MEMR on MST of EAC bearing mice was 41.50 ± 0.57, 32.17 ± 0.88, 26.67 ± 0.88 and 20 ± 0.44 days for the group treated with MEMR at the dose of 100, 200, 400 mg/kg body weight and 5-FU at the dose 20 mg/kg body weight respectively. MEMR treated animals bearing EAC showed a significant increase in the MST when compared with EAC control group.

Effect on viable and non-viable tumor cell count: The viable cell count of the EAC control group was 7.08 ± 0.08 x10^7 cells/ml, where as it was 6.44 ± 0.10x10^7, 5.60 ± 0.07x10^7, 4.68 ± 0.08x10^7 and 2.13 ± 0.16x10^7 cells/ml for the group treated with MEMR at the dose of 100, 200, 400 mg/kg body weight and 5-FU at the dose 20 mg/kg body weight respectively. MEMR treated animals bearing EAC showed a significant increase in the viable cells count when compared with EAC control group.

The non-viable cell count of the EAC control group was 0.13 ± 0.016x10^7 cells/ml, where as it was 0.52 ± 0.017x10^7, 0.30 ± 0.019x10^7, 1.26 ± 0.067x10^7 and 0.84 ± 0.019 x10^7 cells/ml for the group treated with MEMR at the dose of 100, 200, 400 mg/kg body weight and 5-FU at the dose 20 mg/kg body weight respectively. MEMR treated animals bearing EAC showed a significant increase in the non-viable cell count when compared with EAC control group.
Effect of MEMR on haematological parameters:
Blood was withdrawn from each mice by retro orbital plexus and the haemoglobin content, RBC, WBC count and PCV were measured. Haemoglobin content, RBC count in the EAC control group was decreased as compared to the normal group. The animals that are treated with MEMR at the dose 100, 200, 400mg/kg body weight showed an increase in haemoglobin content and RBC count to more or less normal levels. WBC count was found to be increased in the EAC control group when compared with the normal group. Whereas MEMR reduced WBC count when compared with EAC control group.

XTT assay:

Effect of Tamoxifen and MEMR on EAC, MCF-7, MDA-MB 435S cell lines: Tamoxifen exhibited maximum cytotoxic effect of 99.3% on EAC, 95.5% on MCF-7, 99.4% on MDA-MB 435S cell lines at the concentration of 200µg/ml.
MEMR showed its maximum cytotoxic effect at 200µg/ml and it was found to be 78.3% on EAC, 79% on MCF-7, 83% on MDA-MB 435S cell lines.
MEMR showed significant cytotoxic effect on cancer cell lines. MEMR showed a concentration dependent cytotoxic effect on various cancer cell lines. Cell viability was assessed by optical density.

IC$_{50}$ of Tamoxifen on various cancer cell lines; on EAC IC$_{50}$ was found to be 22.42 µg/mL, on MCF-7 IC$_{50}$ was found to be 20.7 µg/mL, on MDA-MB 435S IC$_{50}$ was found to be 20.87 µg/mL.

IC$_{50}$ of MEMR on various cancer cell lines; on EAC IC$_{50}$ was found to be 72.326 µg/mL, on MCF-7 IC$_{50}$ was found to be 69.692µg/mL, on MDA-MB 435S IC$_{50}$ was found to be 80.565 µg/mL.

Apoptosis: Effect of MEMR in EAC cells to induce apoptosis was evaluated by DNA laddering assay. DNA laddering is a feature that can be observed when DNA fragments resulting from apoptotic DNA fragmentation are visualised after separation by gel electrophoresis. DNA laddering is a distinctive feature of DNA degraded by caspase-activated DNase (CAD), which is a key event during apoptosis. CAD cleaves genomic DNA at internucleosomal linker regions, resulting in DNA fragments that are multiples of 180–185 base-pairs in length.

EAC cells treated with MEMR for 12hr, fragments are Separated by agarose gel electrophoresis and subsequent visualization, by ethidium bromide staining, showed a characteristic "ladder" pattern(see figure.1). DNA laddering can be used as final state read-out method and has therefore become a reliable method to distinguish apoptosis from necrosis.

![DNA Laddering](image-url)
TABLE 1: EFFECT OF MEMR ON BODY WEIGHT, TUMOR VOLUME, MEAN SURVIVAL TIME, % INCREASE IN LIFE SPAN (%ILS) IN MICE BEARING EAC

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Body weight (gm)</th>
<th>Tumor volume (ml)</th>
<th>Mean survival time (days)</th>
<th>%ILS</th>
</tr>
</thead>
<tbody>
<tr>
<td>EAC control</td>
<td>38.10 ± 0.94</td>
<td>18.26 ± 0.48</td>
<td>17 ± 0.28</td>
<td>----</td>
</tr>
<tr>
<td>MEMR (100mg/kg)</td>
<td>33.22 ± 0.69**</td>
<td>12.66 ± 0.23***</td>
<td>20 ± 0.44*</td>
<td>22.52%*</td>
</tr>
<tr>
<td>MEMR (200mg/kg)</td>
<td>30.10 ± 0.37***</td>
<td>11.22 ± 0.11***</td>
<td>26.67 ± 0.88***</td>
<td>56.8%***</td>
</tr>
<tr>
<td>MEMR (400mg/kg)</td>
<td>27.16 ± 0.50***</td>
<td>8.6 ± 0.24***</td>
<td>32.17 ± 0.88***</td>
<td>89.23%***</td>
</tr>
<tr>
<td>5-FU (20mg/kg, IP)</td>
<td>26.24 ± 0.65***</td>
<td>3.18 ± 0.33***</td>
<td>41.50 ± 0.57***</td>
<td>144.1%***</td>
</tr>
</tbody>
</table>

Values are expressed as mean±SEM (n=5), *P<0.0001 MEMR treated group compared with EAC control group, **p<0.001 MEMR treated group compared with EAC control group, ***p<0.0001 MEMR treated group compared with EAC control.

TABLE 2: EFFECT OF MEMR ON Viable Tumor Cell and Non-Viable Tumor Cell Count in EAC Bearing Mice

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Viable cells (x10^6 cells/ml)</th>
<th>Non-viable cells (x10^6 cells/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>EAC control</td>
<td>7.08 ± 0.08</td>
<td>0.13 ± 0.016</td>
</tr>
<tr>
<td>MEMR (100mg/kg)</td>
<td>6.44 ± 0.10*</td>
<td>0.30 ± 0.019*</td>
</tr>
<tr>
<td>MEMR (200mg/kg)</td>
<td>5.60 ± 0.07**</td>
<td>0.52 ± 0.017**</td>
</tr>
<tr>
<td>MEMR (400mg/kg)</td>
<td>4.68 ± 0.08**</td>
<td>0.84 ± 0.019**</td>
</tr>
<tr>
<td>5-FU (20mg/kg, IP)</td>
<td>2.13 ± 0.16**</td>
<td>1.26 ± 0.067**</td>
</tr>
</tbody>
</table>

Values are expressed as mean±SEM (n=5), *P<0.01 MEMR treated group compared with EAC control group, **p<0.001 MEMR treated group compared with EAC control group.

TABLE 3: EFFECT OF MEMR ON HAEematological Parameters in EAC Bearing Mice

<table>
<thead>
<tr>
<th>Treatment</th>
<th>WBC (x10^3/µl)</th>
<th>RBC (x10^6/µl)</th>
<th>Haemoglobin (g/dl)</th>
<th>PCV %</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal</td>
<td>14.47 ± 0.3</td>
<td>9.09 ± 0.28</td>
<td>17.21 ± 0.12</td>
<td>56.46 ± 0.69</td>
</tr>
<tr>
<td>EAC control</td>
<td>64.26 ± 2.12</td>
<td>3.66 ± 0.28</td>
<td>5.21 ± 0.25</td>
<td>18.96 ± 1.12</td>
</tr>
<tr>
<td>MEMR (100mg/kg)</td>
<td>37.90 ± 0.86*</td>
<td>6.06 ± 0.05*</td>
<td>11.34 ± 0.40*</td>
<td>35.72 ± 0.84*</td>
</tr>
<tr>
<td>MEMR (200mg/kg)</td>
<td>27.18 ± 0.11*</td>
<td>8.83 ± 0.09*</td>
<td>13.92 ± 0.22*</td>
<td>46.98 ± 0.33*</td>
</tr>
<tr>
<td>MEMR (400mg/kg)</td>
<td>24.6 ± 0.87*</td>
<td>7.12 ± 0.06*</td>
<td>13.16 ± 0.22*</td>
<td>40.90 ± 1.04*</td>
</tr>
<tr>
<td>5-FU (20mg/kg, IP)</td>
<td>12.8 ± 0.11*</td>
<td>9.32 ± 0.07*</td>
<td>16.38 ± 0.14*</td>
<td>50.98 ± 0.8</td>
</tr>
</tbody>
</table>

Values are expressed as mean±SEM (n=5), *P<0.001 MEMR treated group compared with EAC control group.

XTT Assay:

TABLE 4: DOSE RESPONSE OF MEMR ON EAC CELLS

<table>
<thead>
<tr>
<th>Conc. (µg/ml)</th>
<th>Tamoxifen</th>
<th>MEMR</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>OD at 490 nm</td>
<td>% CS</td>
</tr>
<tr>
<td>6.25</td>
<td>0.583 ± 0.001</td>
<td>88.4</td>
</tr>
<tr>
<td>12.5</td>
<td>0.462 ± 0.002</td>
<td>68.7</td>
</tr>
<tr>
<td>25</td>
<td>0.345 ± 0.001</td>
<td>49.7</td>
</tr>
<tr>
<td>50</td>
<td>0.200 ± 0.013</td>
<td>26.1</td>
</tr>
<tr>
<td>100</td>
<td>0.116 ± 0.002</td>
<td>12.4</td>
</tr>
<tr>
<td>200</td>
<td>0.044 ± 0.001</td>
<td>0.7</td>
</tr>
</tbody>
</table>

Blank: 0.040; Control: 0.654. Values are expressed as mean ± SEM of three determinations.

TABLE 5: DOSE RESPONSE OF MEMR ON MCF-7 CELLS

<table>
<thead>
<tr>
<th>Conc. (µg/ml)</th>
<th>Tamoxifen</th>
<th>MEMR</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>OD at 490 nm</td>
<td>% CS</td>
</tr>
<tr>
<td>6.25</td>
<td>0.475 ± 0.012</td>
<td>82.4</td>
</tr>
<tr>
<td>12.5</td>
<td>0.402 ± 0.001</td>
<td>68.6</td>
</tr>
<tr>
<td>25</td>
<td>0.252 ± 0.001</td>
<td>40.2</td>
</tr>
<tr>
<td>50</td>
<td>0.143 ± 0.003</td>
<td>19.5</td>
</tr>
<tr>
<td>100</td>
<td>0.092 ± 0.015</td>
<td>9.8</td>
</tr>
<tr>
<td>200</td>
<td>0.064 ± 0.002</td>
<td>4.5</td>
</tr>
</tbody>
</table>

Blank: 0.040; Control: 0.568. Values are expressed as mean ± SEM of three determinations.
TABLE: 6: DOSE RESPONSE OF MEMR ON MDA-MB 435S.

<table>
<thead>
<tr>
<th>Conc. (ug/ml)</th>
<th>Tamoxifen</th>
<th>MEMR</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>OD at 490nm</td>
<td>% CS</td>
</tr>
<tr>
<td>6.25</td>
<td>0.500 ± 0.001</td>
<td>91.6</td>
</tr>
<tr>
<td>12.5</td>
<td>0.392 ± 0.012</td>
<td>70.1</td>
</tr>
<tr>
<td>25</td>
<td>0.241 ± 0.002</td>
<td>40</td>
</tr>
<tr>
<td>50</td>
<td>0.133 ± 0.001</td>
<td>18.5</td>
</tr>
<tr>
<td>100</td>
<td>0.095 ± 0.001</td>
<td>11</td>
</tr>
<tr>
<td>200</td>
<td>0.043 ± 0.005</td>
<td>0.6</td>
</tr>
</tbody>
</table>

Blank: 0.040; Control: 0.542. Values are expressed as mean ± SEM of three determinations.

TABLE 7: IC$_{50}$

<table>
<thead>
<tr>
<th></th>
<th>EAC</th>
<th>MCF-7</th>
<th>MDA -MB 435S</th>
</tr>
</thead>
<tbody>
<tr>
<td>IC$_{50}$ of TAMOXIFEN</td>
<td>22.42 µg/ml</td>
<td>20.7 µg/ml</td>
<td>20.87 µg/ml</td>
</tr>
<tr>
<td>IC$_{50}$ of MEMR</td>
<td>72.326 µg/ml</td>
<td>69.692 µg/ml</td>
<td>80.565 µg/ml</td>
</tr>
</tbody>
</table>

DISCUSSION: The Ehrlich ascites tumor cell is a spontaneous murine mammary adenocarcinoma (Ehrlich and Apolant, 1905) adapted to ascites form (Loewenthal and Jahn, 1932) and carried in outbred mice by serial intraperitoneal (i.p.) passage. It is a cancer of epithelial tissue that has glandular origin, glandular characteristics or both. It is a rapidly growing carcinoma with very aggressive in cell proliferation and is able to grown almost all strains of mice. In ascetic form it has been used as a transplantable tumor model to investigate the antitumor effect of various substances. Ascites (from Greek: askites) is a gastroenterological term for an accumulation of fluid in the peritoneal cavity.

In EAC tumor bearing hosts, a drastic increase in ascitic fluid volume was observed. The present study was carried out to evaluate the anti tumor effect of methanol extract of *Mimosa rubicaulis* lam (MEMR) against mice bearing EAC tumor. EAC tumor bearing mice which are treated with MEMR at the doses of 100, 200 and 400 mg/kg body weight. Showed significant decrease in tumor volume, tumor cell count, packed cell volume and brought back the haematological parameters to more or less normal levels.

Ascites fluid was the direct nutritional source for tumor cells and a rapid increase in ascites fluid with tumor growth would be a means to meet the nutritional requirement of tumor cells 26. EAC tumor bearing control group showed drastic increase in ascitic fluid volume. The animals which are treated with MEMR showed decrease in viable tumor cell count and increase in the percentage of trypan blue positive stained dead cells (non-viable tumor cell count), decrease in tumor volume and decrease in body weight were observed when compared with EAC control group. No visible sign of toxicity and changes in vital functions were observed in any of treated animals.

The reliable criterion for judging the value of any anticancer drug is the prolongation of life span of the tumor bearing animal 27. MEMR increased mean survival time and exhibited significant effect on percentage of increase in life span by decreasing the ascites fluid volume and viable tumor cell count. It may be assumed that MEMR showed anti tumor activity by decreasing the nutritional fluid volume and arresting the tumor growth. Based on the results it can be concluded that MEMR increased the survival time and delayed tumor growth in mice bearing EAC tumor.

Myelosuppression and anemia (reduced haemoglobin level) have been frequently observed in ascites carcinoma 28, 29. Anemia encountered in ascites carcinoma was mainly due to iron deficiency, either by haemolytic or myelopathic conditions which finally lead to reduced RBC number 30.

In this study, elevated WBC count, reduced haemoglobin and RBC count were observed in EAC control mice, and the oral administration of MEMR restored haemoglobin content and maintained more or less to normal values of RBC and WBC, thus supporting its haematopoietic protecting activity without inducing myelotoxicity, which is a common side effect of cancer chemotherapy. The XTT assay is a colorimetric assay for measuring the activity of cellular enzymes that reduce the tetrazolium dye.
This assay utilizes a colour reaction as a measure of viable cells. This assay was dependent on the cellular reduction of tetrazolium salt to a blue coloured formazan by the mitochondrial dehydrogenase of viable cells/metabolically active cells. The intensity of blue coloured formazan produced was directly proportional to the cell viability. MEMR showed cytotoxic effect on cancer cells such as EAC (Mice mammary gland cancer), MCF-7 (Human mammary gland cancer), MDA-MB435S (Human metastatic mammary gland cancer) in dose dependent manner, this cytotoxic effect was studied by XTT assay. The Optical Density (OD) of the culture plate was read at a wavelength of 490 nm (reference absorbance at a wavelength of 630 nm) on an ELISA reader, Anthos 2020 spectropho-meter. Based on percentage of cell inhibition results of XTT assay it can be concluded that MEMR showed cytotoxic effect on cancer cell lines.

Apoptosis is an important homeostatic mechanism that balances cell division and cell death for maintaining the appropriate cell number in the body. Disturbances of apoptosis and induction of apoptosis in cancer cells was one of the strategies for anticancer drug development. There are several crucial cellular and molecular biological features involving apoptosis, cell shrinkage, and disorganization of chromatin and activation of Caspase etc. Among this activation of the family of caspases was known as a crucial mechanism for induction of death signals in apoptosis. Caspases can be divided into initiator and effector caspases.

Initiator caspases (e.g. caspase-1, -2, -8, -9 and -10) containing a long prodomain involve in early stages of the proteolytic cascade, whatever effector caspases (e.g. caspase-3, -6, and -7) containing a short prodomain act downstream in cleavage of specific intracellular substrates (e.g. poly-ADP-ribose polymerase, focal adhesion kinase) resulting programmed cell death.

Separation of the fragments by agarose gel electrophoresis and subsequent visualization, by ethidium bromide staining, resulted in a characteristic “ladder” pattern. Based on this ladder pattern observed in DNA laddering assay it can be concluded that MEMR induced apoptosis in EAC cells. This apoptosis may be induced because of either cell shrinkage or disorganization of chromatin or activation of caspase or other mechanism, further investigation was needed to find out the specific mechanism which is responsible for induceing apoptosis in cancer cells by MEMR.

Preliminary phytochemical study indicated the presence of flavonoids, tannins, triterpenes, and carbohydrates in methanol extract. Flavonoids such as Quercetin, kaemoferol and their glycosides have been shown to possess antimutagenic and antimalignant effect. Furthermore, Flavonoids have a chemo preventive role in cancer through their effect on signal transduction in cell proliferation and angiogenesis. The cytotoxicity and anticancer activity was may be due to presence of flavonoids and triterpenes. In the earlier studies, it was found that Mimosa rubicaulis lam extracts of root and stem showed anti-microbial activity. This preliminary study was carried out to evaluate anticancer activity of Mimosa rubicaulis lam. Based on invitro results it can be concluded that the life span of EAC tumor bearing mice was increased which is a reliable criteria for judging the value of anti-cancer drug. Based on invitro results it can be concluded that MEMR exhibited cytotoxic effect on cancer cells and based DNA laddering assay it can be concluded that MEMR induced apoptosis in EAC cells. It is valuable for further investigation including elucidation of active components of MEMR responsible for anti tumor activity.

**ABBREVIATIONS:**

- °C = Degree Celsius
- μM = Micro Molar
- mM = Milli Molar
- mg/kg = Milligram per kilogram
- MEMR = methanol extract of *Mimosa rubicaulis lam*
- 5-FU = 5-fluorouracil
- EAC = Ehrlich Ascites Carcinoma
- LD<sub>50</sub> = lethal dose
- MST = Mean survival time
- RBC = Red blood cells
WBC = white blood cells
PCV = packed cell volume
%ILS = percentage of increase in life span
CAD = caspase-activated DNase

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