EVALUATION OF ANTI-CANCER ACTIVITY OF DIKAMALIARTANE-A, A CYCLOARTANE ISOLATED FROM DIKAMALI, A GUM RESIN

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ABSTRACT

Cancer, one of the leading causes of death. Dikamalartiane-A, a cycloartane isolated from gum resin, Dikamali of Gardenia gummifera/Gardenia lucida was evaluated for in vitro and in vivo anti-cancer activity. The MTT (3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) assay was used for the evaluation of in vitro anti-cancer activity in HeLa(cervical cancer) and MCF-7(breast cancer) cell lines. The IC50 values of Dikamalartiane-A was 29.57 µg/mL & 29.99 µg/mL and standard Cisplatin were 1.43 µg/mL & 3.189 µg/mL respectively. The LD50 was found to be 500 mg/kg. Ehrlich Asctes Carcinoma bearing mice were used for screening of in vivo anti-cancer activity and different parameters like percentage increase in life span, body weight, mean survival time, tumor volume, viable cell count, packed cell volume and hematological parameters were estimated. The Dikamalartiane-A treated mice showed significant (p<0.001) anti-cancer activity as that of Cisplatin, standard drug. This shows the potential of Dikamalartiane-A to become a future drug candidate for cancer chemotherapy but it requires further attempts to reveal the exact mechanism of action.

Keywords: Dikamalartiane-A, Anti-Cancer activity, Cytotoxicity, MTT, EAC

INTRODUCTION

Gardenia gummifera is a shrub about 1.8 M height, flowers are non odorous a Calyx 1 cm. Long Corolla at first, white and later changes to yellow. Gardenia Lucida, a large glabrous or small tree reaching 6-7.5 M height young shoots, greyish green smooth resinous leaves 6.3-20 cm, flowers fragrant, the flowers open in the evening and changes color from white to yellow. It is known by various names in different parts of the India.

Dikamali is the gum resin obtained from the leaf buds of Gardenia gummifera/Gardenia lucida family Rubiaceae. The resin is secreted in the form of tears; it is transparent greenish yellow in color with a sharp pungent smell, it is marketed as cumbiresin. It is known by various names. The gum resin will be an exudating from Gardenia lucida/Gardenia gummifera. Gardenia lucida and Gardenia gummifera are geographically distributed in all districts of Tamil Nadu, Burma, Bangladesh, Konkan region, North Kanara and Malabar Coast.

Dikamali is claimed to have a number of medicinal properties which include antihelmintic, antispasmodic, carminative, diaphoretic, expectorant, potentiation of pentobarbitone induced sleep, Anti-epileptic, peripheral and central Analgesic, Cardiotonic, Antioxidant, and Antihyperlipidemic. It is also claimed to be useful in dyspepsia, flatulence for cleaning foul ulcers and wounds, and to keep off flies.

CAS name: (-(6β, 7β, 17R, 20R)-6,7,29 Trihydroxy- 23, 26-epoxy- 3α-secoesycocra-4(28), 23(24), 25(26)-trien-3-ok Acid).

Dikamalartiane-A for anti-cancer activity.

Extraction of plant materials

Lumps of dikamali resin were broken into small pieces and the leaf stalks were carefully removed. Powdered gum resin was extracted with benzene, the solvent was removed by evaporation and the extract was dried in desicators to yield benzene extract. The benzene extract was subjected to column chromatography. The column was eluted with benzene and benzene/ acetone mixtures. Benzene/ acetone 80:20 elution gave the 0.3% yield.

Acute toxicity studies

This was performed as per OECD guidelines 423. Four groups of 3 mice in each group were taken and Dikamalartiane-A at the doses of 5mg/kg, 50mg/kg, 300mg/kg, 2000mg/kg were administrated per oral and they were observed for next 48hrs for the morbidity and mortality response.

MATERIALS AND METHODS

Chemicals

Fetal bovine serum (FBS), Dulbecco’s modified eagle’s medium (DMEM), penicillin, amphotericin B and streptomycin were purchased from Himedia (Mumbai, India); 3-(4, 5-Dimethylthiazol-2-yi)-2,5-diphenyltetrazolium bromide (MTT) was purchased from Sigma Aldrich Company, USA. Cisplatin was procured from local market with trade name as Cytoplatin 50mg/50mL marketed by Cipla Pvt Ltd, Ahmedabad, India. Trypsin-EDTA, HiMedia, Mumbai, India.

Cell cultures

The cell cultures like HeLa (cervical), MCF-7 (breast) cancer cell lines were procured from National Centre for Cell sciences (NCCS), Pune, India. These cell lines were grown and maintained using suitable media (DMEM) and were grown in culture medium supplemented with 10% fetal bovine serum, 1% L-glutamine and 1% penicillin-streptomycin-amphotericin-B antibiotic solution. Cells were seeded in 25 cm² tissue culture flasks (Tarsore, Mumbai India).
at 250,000 cells/flask in a total volume of 9 mL. When confluent, all the cells were trypsinized and seeded in 96-well tissue culture plates (Tarsons, Mumbai, India).

**Cytotoxic activity invitro**

*In vitro* anticancer activity against HeLa and MCF-7 cancer cell lines was determined using 96-well tissue culture plates. The cell suspension of 1 x 10^4 cells/mL was prepared in complete growth medium. The drug solutions were serially diluted at concentrations of 3 µg/mL and 30 µg/mL with complete growth medium containing 50 µg/mL of Gentamycin to obtain working test drug solution of 300 ng/mL, 3 µg/mL, and 30 µg/mL concentrations (with <1% DMSO solution). The 100 µL of cell suspension was added to each well of the 96-well tissue culture plates. The cells were allowed to grow in a CO2 incubator (37 °C, 5% CO2, 90% relative humidity) for 24 hrs. The test drug solutions in complete growth medium (100 µL) were added after 24 hr incubation to the wells containing cell suspension. The optical density is directly correlated with cell quantity; it was read at 490 nm for optical density using ELISA reader and the anticancer activity against HeLa and MCF-7 cancer cell lines was calculated.

**Anti-Tumor parameters**

The antitumor parameters of the DK-1 Groups and standard drug Group were determined by the change in body weight, mean survival time (MST), percentage increase in life span (% ILS), Tumor volume, packed cell volume and viable tumor volume. The MST of each group containing five mice was identified by recording the mortality on a daily basis for 30 days, and the % ILS was calculated using the following equations:

\[
\text{MST}= (\text{day of the first death} + \text{day of the last death}) / 2
\]

\[
\text{ILS} = (\text{mean survival time of treated group}/\text{mean survival time of control group}) - 1 \times 100
\]

**RESULTS**

**Acute toxicity studies**

The acute toxicity study of Dikamaliartane-A was determined in mice and when a dose of 5 mg/kg, 50 mg/kg, 300 mg/kg, there were no death of mice were recorded and when 2000 mg/kg of Dikamaliartane-A was administered all three mice were died. From the schematic diagram data, the LD50 of Dikamaliartane-A was determined to be 500 mg/kg following OECD guidelines 423 and Therapeutic dose for animals was 1/10 of LD50 dose.

**Cytotoxic activity in vitro**

We have performed in vitro cytotoxicity tests for DK-1 using HeLa (cervical), MCF-7 (Breast) cancer cell lines by MTT assay method. The IC50 values were given in Table 1.

**Table 1: IC50 values of DKA and Cisplatin on HeLa and MCF-7 cell lines**

<table>
<thead>
<tr>
<th>S. No.</th>
<th>Compound</th>
<th>HeLa cells (µg/mL)</th>
<th>MCF-7 cells (µg/mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>DKA</td>
<td>29.57</td>
<td>29.99</td>
</tr>
<tr>
<td>2</td>
<td>Cisplatin</td>
<td>1.43</td>
<td>3.18</td>
</tr>
</tbody>
</table>

**Antitumor activity in vivo**

The effects of DK-1 in different doses (30 mg/kg and 50 mg/kg) on increase in body weight, mean survival time, % increased life span, tumor volume, packed cell volume and tumor cell count (viable cells) are shown in Table 2.

**Table 2: Antitumor activity of Dikamaliartane-A on EAC bearing mice**

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Tumor control</th>
<th>Cisplatin (50 mg/kg)</th>
<th>DK-A (30 mg/kg)</th>
<th>DK-A (50 mg/kg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Average increase in body weight (gm)</td>
<td>12.2 \pm 0.75</td>
<td>3.98 \pm 0.43 ***</td>
<td>10.41 \pm 0.39 **</td>
<td>7.70 \pm 0.49 ***</td>
</tr>
<tr>
<td>Mean survival time (days)</td>
<td>13.70 \pm 0.03</td>
<td>29.30 \pm 0.36 ***</td>
<td>16.40 \pm 0.67 **</td>
<td>22.10 \pm 1.03 ***</td>
</tr>
<tr>
<td>% Increase in Life span (%) (ILS)</td>
<td>---</td>
<td>113.86 ***</td>
<td>19.70*</td>
<td>61.31 ***</td>
</tr>
<tr>
<td>Tumor volume (mL)</td>
<td>12.10 \pm 0.89</td>
<td>3.01 \pm 0.35 ***</td>
<td>7.61 \pm 0.61 ***</td>
<td>4.50 \pm 0.39 ***</td>
</tr>
<tr>
<td>Packed cell volume (mL)</td>
<td>2.75 \pm 0.49</td>
<td>0.25 \pm 0.05 ***</td>
<td>1.25 \pm 0.23*</td>
<td>0.94 \pm 0.11 ***</td>
</tr>
<tr>
<td>Viable tumor cell count (x 10^6 cells/mL)</td>
<td>6.39 \pm 0.51</td>
<td>0.16 \pm 0.04 ***</td>
<td>4.05 \pm 0.35 ***</td>
<td>3.27 \pm 0.43 ***</td>
</tr>
</tbody>
</table>

All values are expressed as mean \pm SD (n=5). ***P < 0.001, **P < 0.01, *P < 0.05, compared to tumor control.
The DK-1 has significant activity (p<0.001) in both doses on body weight, the decrease in body weight in five EAC-bearing mice was observed. DK-1 in both doses, significantly increase in mean survival time, % increase in life span and decrease in tumor volume and packed cell volume, when compared with standard drug and tumor control values.

**Biochemical parameters of EAC bearing mice**

The remaining five mice from each group were selected for hematological parameters. This is done on the 14th day of starting the treatment. The blood was withdrawn from mice through retro-orbital plexus, the parameters were evaluated such as haemoglobin level, it was performed by Sahlis hemoglobinometer, erythrocytes and leucocytes counts was done by Neubauer’s slide and DLC was done on plain slide with blood smear. They were compared in the group EAC control with the standard drug Cisplatin and the groups treated with DK-1, as shown in Table 3, the biochemical and hematological parameters in the group treated with the compound DK-1 have been more or less near to the normal values.

**Table 3: Effect of Dikamaliartane-A on biochemical and hematological parameters in EAC bearing mice**

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Normal</th>
<th>Tumor control</th>
<th>Cisplatin (5mg/kg)</th>
<th>DK-A (30mg/kg)</th>
<th>DK-A (50mg/kg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hb(%)</td>
<td>13.10±0.862</td>
<td>5.750±0.655</td>
<td>11.50±1.025*</td>
<td>8.080±0.86*</td>
<td>9.30±1.033**</td>
</tr>
<tr>
<td>RBC (million mm³)</td>
<td>4.512±0.084</td>
<td>2.740±0.081</td>
<td>4.190±0.087***</td>
<td>3.020±0.066**</td>
<td>3.880±0.086**</td>
</tr>
<tr>
<td>WBC (10⁹cells/mm³)</td>
<td>7.160±0.120</td>
<td>20.60±0.545</td>
<td>9.160±0.231***</td>
<td>18.48±0.355*</td>
<td>13.92±0.476***</td>
</tr>
<tr>
<td>Lymphocytes(%)</td>
<td>69±1.31</td>
<td>23±0.567</td>
<td>63±0.782***</td>
<td>31±0.936**</td>
<td>49±0.759**</td>
</tr>
<tr>
<td>Neutrophils(%)</td>
<td>29±1.46</td>
<td>73±1.25</td>
<td>30±1.17***</td>
<td>54±0.85*</td>
<td>39±0.97***</td>
</tr>
<tr>
<td>Monocytes (%)</td>
<td>1±0.29</td>
<td>2±0.12</td>
<td>1.2±0.16***</td>
<td>2±0.36*</td>
<td>1.6±0.18**</td>
</tr>
</tbody>
</table>

All the values were expressed as mean ± SD (n=5). *p<0.05, ** p<0.01 and ***p<0.001, compared to Tumor Control.

**DISCUSSIONS**

The Anticancer activity of DK-1 was evaluated on HeLa (cervical) and MCF-7 (breast) cancer cell lines by using MTT assay method. MTT assay is the reliable assay to assess the in vitro cytotoxicity of the anticancer compounds. MTT is a water-soluble tetrazolium salt, which is converted to an insoluble purple formazan by cleavage of the tetrazolium ring by mitochondrial succinate dehydrogenase. The disruption of mitochondrial dehydrogenase system due to the mitochondrial dysfunction or apoptosis may reduce the color formazan production in the MTT assay. Here we carried out MTT assay on cell lines namely HeLa and MCF-7 cancer cell lines. The test drug DK-1 with IC₅₀ values 29.57µg/mL & 29.99 µg/mL showed anticancer activity against HeLa and MCF-7 cell lines respectively. They produce a dose dependent inhibition of growth of the cells. The IC₅₀ values of cisplatin on HeLa cell lines is 1.43µg/mL and MCF-7 is 3.189 µg/mL.

In vitro study was carried out by using liquid tumor model. Ehrlich tumor is a rapidly growing carcinoma with very aggressive behavior and is able to grow in almost all strains of mice. The Ehrlich ascitic tumor implantation induces a local inflammatory reaction, with increasing vascular permeability, which results in an intense edema formation, cellular migration and a progressive ascitic fluid formation. The ascitic fluid is essential for tumor growth, since it constitutes a direct nutritional requirement to tumor cells. The tumor cells directly draw the nutrition from ascitic fluid, it means that ascitic fluid continuously supply the nutritional requirement to tumor cells. The drug which reduces ascitic fluid volume might be a good anticancer agent. The reliable criterion for judging the antitumor activity of any molecule is the prolongation of life span of tumor inoculated mice. Usually in untreated mice, EAC inoculation causes 100% mortality within 18 days, and our present data support this fact. An enhancement of life span by 25% or more over that of control was considered as effective anticancer response. The DK-1 significantly decreased the ascitic fluid volume as compared to EAC control. These results could indicate an indirect local effect, which may involve macrophage activation and inhibition of vascular permeability. With the comparison of tumor control, the compound DK-1 significantly increases the mean survival time and percentage increase in life span. When compared with the tumor control, DK-1 treated groups significantly (p<0.001) decreased the tumor volume, packed cell volume and viable tumor cell count. The compound DK-1 increases the haemoglobin and red blood cell levels, when compared with the tumor control. The compound DK-1 decreases the white blood cell levels when compared with the tumor control. Treatment with the compound DK-1 brought back the differential leucocyte count more or less to normal levels. This indicates that the test drug possess protective action on hemopoietic system.

**CONCLUSIONS**

From the results it can be concluded that the anticancer activity is not selective to a particular cancer, rather non-selective in their type of action. This shows the compound DK-1 to become a future drug candidate for cancer chemotherapy but it requires further attempts to reveal the exact mechanism of action of this compound, so that, by structure activity relationships, a new potent analogue can be generated with the desired anticancer activity with good efficacy.

**REFERENCES**


