Academic Sciences International Journal of Pharmacy and Pharmaceutical Sciences
ISSN- 0975-1491 Vol 4, Issue 4, 2012


Research Article

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

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*Received: 13 July 2012, Revised and Accepted: 29 Aug 2012*

**ABSTRACT**

Cancer, one of the leading causes of death. Dikamaliartane-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 $IC_{50}$ values of Dikamaliartane-A was 29.57 µg/mL & 29.99 µg/mL and standard Cisplatin were 1.43 µg/mL & 3.189 µg/mL respectively. The $LD_{50}$ (median lethal dose) was carried out in mice according to OECD Guidelines. The $LD_{50}$ was found to be 500 mg/kg. Ehrlich Ascites 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 Dikamaliartane-A treated mice shown significant ($p<0.001$) anti-cancer activity as that of Cisplatin, standard drug This shows the potential of Dikamaliartane-A to become a future drug candidate for cancer chemotherapy but it requires further attempts to reveal the exact mechanism of action.

**Keywords:** Dikamaliartane-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, grayish 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 [1]. 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 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 anthelmintic, 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 from wounds in veterinary practice [1,2,3]. A number of Flavanoids such as Gardenin A, B, C, D & E were isolated from Dikamali in the past [4, 5, 6, 7, 8]. Recently, a number of new cycloartanes Dikamaliartane - A, B, C, D, E & F and Gardenin E were reported from this source of which Dikamaliartane-A is the main cycloartane [9] which is coded as DK-1.

Dikamaliartane-A was isolated in our UCPSc, Pharmaceutical Chemistry lab, Kakatiya University and its structure was established (Fig. 1) [9].

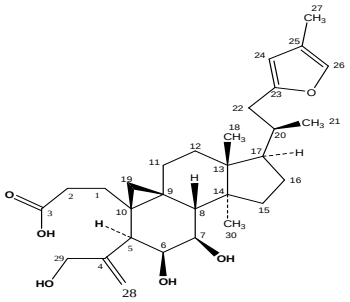

**Fig 1. Structure of Dikamaliartane-A**

**CAS name:** (=(6β, 7β, 17R, 20R)–6,7,29 Trihydroxy- 23, 26-epoxy-3,4- secocycloarta-4(28), 23(24), 25(26)-trien-3-oic Acid).

Cycloartanes isolated from the *Gardenia lucida/Gardenia gummifera* is found to possess Cytotoxic and anti-HIV activities [10, 11, 12, 13, 14]. This study was undertaken to screen the main cycloartane of Dikamali, Dikamaliartane-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 desiccators 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 Dikamaliartane-A at the doses of 5mg/kg, 50mg/kg, 300mg/kg, 2000mg/kg were administered per oral and they were was 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-yl)-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 $cm^2$ tissue culture flasks (Tarsons, 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 [15, 16] using 96-well tissue culture plates. The cell suspension of 1 x$10^5$ cells / mL was prepared in complete growth medium. The drug solutions were serially diluted at concentration of 3µg/mL and 30µg/mL with complete growth medium containing 50 mg/mL of Gentamycin to obtain working test drug solution of 300ng,1µg,3µg,10µg and 30µg/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 $CO_2$ incubator (37 $^0$C, 5% $CO_2$, 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. After 48 hrs of treatment with different concentrations of test drug solution, the cells were incubated with 20 µL of MTT (2.5 mg/ mL) for 2 hrs. After 24 hr medium was removed and 100 µL of <1%DMSO was added into each well to dissolve formazan crystals which is the metabolite of MTT. After thoroughly mixing, the plate was read at 490 nm for optical density using ELISA reader and the optical density is directly correlated with cell quantity [17].

$$\% \text{ Cytotoxicity} = 1 - \frac{\text{O.D. in sample}}{\text{O.D. in control well}} \text{ X } 100$$

**Antitumor activity *in vivo***

Adult female Swiss albino mice (Mahaveer Enterprises, Hyderabad, India) of 8 weeks old were selected for study with mean weights in the range of 20–25 grams and EAC mice was procured from CCMB Hyderabad housed in polypropylene cages in a room where the congenial temperature was 27±1°C and 12 hrs light and dark cycles were maintained. The animals were allowed to acclimatize to the animal house for 7 days and supplied with a standard pellet diet and water *ad libitum.* All procedures using animals were reviewed and approved by the Institutional Animal Ethical Committee of Kakatiya University, Warangal, India with approval number IAEC/03/UCPSc/KU/2010.

The animals were divided into five groups (*n* = 10). Group 1-Normal group, Group 2-Tumor control, Group 3-Standard Cisplatin drug, Group 4-DK-1 30mg/kg and Group 5-DK-1 50mg/kg body weight. The ascitic fluid from EAC bearing mice was withdrawn with sterile syringe and aseptically by intraperitoneal route. The normal group 1 was not inoculated with tumor cells, while four groups 2, 3, 4 & 5 were injected with Ehrlich Ascites Carcinoma (EAC) cells (0.2mL of 2×$10^6$ cells/mouse) intraperitoneally. This was taken as day 0 and the experiment was started after 24 hr. From the 1st day, Body weight of each animal was recorded and 100 µL/mouse per day of sterile saline was administered intraperitoneally to the negative control group (EAC-bearing mice). The test drug DK-1 at doses of 30mg/kg and 50mg/kg were administered each day to the treated groups and the standard drug Cisplatin at a dose of 5mg/kg was administered to each animal from the positive control group by intraperitoneal route. The pharmacological treatment lasted for Nine days. Fourteen days after the treatment, five mice from each group were sacrificed for the study of the antitumor parameters. The remaining five mice from each the groups were kept to check the Mean survival time of EAC tumor bearing hosts.

**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 of EAC bearing mice using the following equations [18]: MST= (day of the first death + day of the last death) / 2; ILS (%) = [(mean survival time of treated group/mean survival time of control group) −1] ×100.

**RESULTS**

**Acute toxicity studies**

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

That is equal to or less than 50 mg/kg

**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 $IC_{50}$ values were given in Table 1.

**Table 1: $IC_{50}$ values of DK-A and Cisplatin on HeLa and MCF-7 cell lines**

| S. No. | Compound | HeLa cells (µg/mL) | MCF-7 cells (µg/mL) |
|---|---|---|---|
| 1 | DK-A | 29.57 | 29.99 |
| 2 | Cisplatin | 1.43 | 3.189 |

**Antitumor activity *in vivo***

The effects of DK-1 in different doses (30mg/kg and 50mg/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: Anticancer activity of Dikamaliartane-A on EAC bearing mice**

| Parameter | Tumor control | Cisplatin(5mg/kg) | DK-A(30mg/kg) | DK-A(50mg/kg) |
|---|---|---|---|---|
| **Average increase in body weight (gm)** | 12.20±0.75 | 3.98±0.43*** | 10.41±0.39* | 7.70±0.49*** |
| **Mean survival time (days)** | 13.70±0.03 | 29.30±0.36*** | 16.40±0.67** | 22.10±1.03*** |
| **%Increase in Life span (%ILS)** | ------- | 113.86*** | 19.70* | 61.31*** |
| **Tumor volume (mL)** | 12.10±0.89 | 3.01±0.35*** | 7.61±0.61*** | 4.50±0.39*** |
| **Packed cell volume (mm)** | 2.75±0.49 | 0.25±0.05*** | 1.25±0.23* | 0.94±0.11*** |
| **Viable tumor cell count (x $10^7$ cells/mL)** | 6.39±0.51 | 0.16±0.04*** | 4.05±0.35*** | 3.27±0.43*** |

All values are expressed as mean±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 [19]**

The remaining five mice from each group were selected for hemotological parameters. This is done on the 14th day of starting of 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 Neubaur'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**

| Parameter | Normal | Tumor control | Cisplatin(5mg/kg) | DK-A (30mg/kg) | DK-A (50mg/kg) |
|---|---|---|---|---|---|
| Hb (%g) | 13.10 ± 0.862 | 5.750 ± 0.655 | 11.50 ± 1.025*** | 8.080 ± 0.86* | 9.30 ± 1.103** |
| RBC (million cells/$mm^3$) | 4.512 ± 0.084 | 2.740 ± 0.081 | 4.190 ± 0.087*** | 3.020 ± 0.066** | 3.880 ± 0.086** |
| WBC ($10^3$cells/$mm^3$) | 7.160 ± 0.120 | 20.60 ± 0.545 | 9.160 ± 0.231*** | 18.48 ± 0.355* | 13.92 ± 0.476*** |
| Lymphocytes(%) | 69± 1.31 | 23±0.567 | 63±0.782*** | 31±0.936** | 49±0.759** |
| Neutrophils(%) | 29±1.46 | 73±1.25 | 30±1.17*** | 54±0.85* | 39±0.97*** |
| Monocytes(%) | 1±0.29 | 2.4±0.12 | 1.2±0.16*** | 2±0.36* | 1.6±0.18** |

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 [20]. 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-7cancer cell lines. The test drug DK-1 with $IC_{50}$ 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 $IC_{50}$ values of cisplatin on HeLa cell lines is1.43µg/mL and MCF-7 is 3.189 µg/mL.

*In vivo* 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 [21]. 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 [22]. The ascitic fluid is essential for tumor growth, since it constitutes a direct nutritional source for tumor cells [23]. 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 [24]. 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 antitumor response [25, 26, 27]. 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 leukocyte count more or less to normal levels. This indicates that the test drug possess protective action on hemopoetic 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.

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