The anticancer activity of ethanolic extract of Cleome felina linn

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ABSTRACT

Background: In the last decades, there is again a growing popularity of medicinal plants and plant derived products. Cancer is a class of diseases in which a cell or a group of cells display uncontrolled growth, invasion and sometimes metastasis. The anticancer activities of certain cleome genera have been studied. The present work deals with the anti cancer activity of the ethanolic extract of Cleome felina Linn. (C.felina). Methods: The anticancer activity against HepG2 (Human hepatocellular liver carcinoma cell line) cells was analysed using 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltertrazolium bromide (MTT) assay. Data were collected from triplicate separate experiments and the percentage of C.felina extract induced cell growth inhibition was determined by comparing with DMSO treated control cells. Results: The maximum inhibition of cell growth was observed at the concentration of 200 µg/ml of C.felina extract. Conclusion: The plant extract has potent to decrease the viability of HepG2 cells in a dose – dependent manner.

KEYWORDS: Cleome felina, Anticancer activity, MTT assay, HepG2 cells

1. INTRODUCTION

Cleomaceae are a small family of flowering plants in the order Brassicales, comprising about 300 species in 10 genera. These genera were previously included in the family Capparaceae, but were raised to a distinct family when DNA evidence suggested that the genera included in it are more closely related to Brassicaceae than they are to Capparaceae. The APG II system allows for Cleomaceae to be included in Brassicaceae1,2. The medicinal properties of number of Cleome species have been studied; Analgesic, anti-inflammatory and antipyretic activities of Cleome rutidosperma3. Leaf Paste of Cleome viscosa L. has wound healing property4. Whole plant Juice of Cleome viscosa and Cleome ciliata has been used to treat ear infection, pain and deafness5,6.

Species like Cleome viscosa, Cleome gynandra and Cleome chelidonii have many medicinal applications, often in rubifacient and counter irritant preparations. They are also used for rheumatism and even headache Some species like C. gynandra are eaten as vegetable in extreme conditions. Oil of Cleome seeds has insect repellent properties so used against tick. Seeds of Cleome chelidonii are used as condiment [Flora of India vol 2]. Bala et al.7 studied anticancer activity of methanolic extract of Cleome gynandra in swiss albino mice and showed that plant has potent dose dependent anticancer activity. Cleome arabica leaf extract has anticancer properties in human cancer cells8. Venu Gopal et al (2012) have studied the antitumour activity of Cleome viscosa9. The present work aims at the anticancer of the C.felina. The medicinal plant – C. felina Linn is chosen for the present study as they are medicinally very important. C. felina Linn is widely distributed in Deccan districts of Madras Presidency. It is an annual erect herb, 30-60 cm high, much branched, leaves 3-foliolate, leaflets 10-25 mm. long, obovate, obtuse, equaling or shorter than the pedioles. Flowers axilliary, solitary on long pedicels, 12-18 mm. long, purple or pink. Stamens about 30; filiform. Capsule 8 times long as broad, compressed, linear anlong, acute at both ends, striate, glabrous, seeds large, glabrous, tubercled10.

2. MATERIALS AND METHODS

2.1. The Collection of plant Material
The leaves of C.felina were collected from Kolli Hills during December 2010. The shade dried plant material was extracted with 80% Ethanol. The extract was concentrated and subjected to anti-cancer activity.

2.2. Cell culture:
The HepG2 is a perpetual cell line (Human hepatocellular liver carcinoma cell line) obtained from the National Center for Cell Science (Pune, India) and grown in Dulbecco’s Modified Medium (DMEM) supplemented with 10% fetal bovine serum (FBS), penicillin
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(100U/ml)/streptomycin (100µg/ml), 2 mM glutamine and 1 mM sodium pyruvate. HepG2 cells were cultured as adherent monolayers and maintained at 37°C in a humidified atmosphere of 5% CO₂ and 95% air in 100% relative humidity. HepG2 cells were harvested after brief trypsinization. All cell cultures reagents were purchased from Himedia, Mumbai, India.

2.3. Cell growth inhibition study using the MTT assay:

Cell growth inhibition of C.felina crude extracts were analysed using the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-terrazolium bromide (MTT) assay. Briefly, HepG2 cells were seeded in 96 well plates at a density of 6x10⁴ cells per well. After treatment with 25, 50, 100, 150, 200µg/ml of C.felina plant crude extracts for 48 hours, 20µl MTT (5 mg/ml) was added. Four hours later, 100 µl DMSO was added to each well to dissolve the resulting formazan crystals. Absorbance was read at 490 nm using an enzyme-linked immunosorbent assay reader (SpectraMax; Molecular Devices, Sunnyvale, CA).

Data were collected from triplicate separate experiments and the percentage of C. felina plant crude extracts induced cell growth inhibition was determined by comparison to DMSO-treated control cells. For statistical analysis, data were analyzed using analysis of variance. Fisher’s least significant difference (LSD) at the 5% level was calculated using the statistical package for social science (Version 12.0 for Windows, SPSS Inc.)

3. RESULTS:

3.1 Treatment of C.felina crude extracts inhibits the growth of HepG2 cells

HepG2 cells were treated with different concentrations of (25, 50, 100, 150 and 200µg/ml plant crude extracts and their growth was monitored. C. felina plant crude extracts decreased the viability of HepG2 cells in a dose-dependent manner. The maximum inhibition of cell growth was observed at the concentration of 200µg/ml (45%) with IC50) of C. felina crude extract.

The decreased growth rates of HepG2 cells growth rates of HepG2 cells of about 4%, 8%, 16%, 23% and 45% observed at the C.felina plant crude extract concentration of 25, 50, 100 150 and 200µg/ml, respectively. All the concentrations used in the experiment decreased the cell viability significantly (P<0.05) in a concentration-dependent manner (Table 1).

Table 1. Effect of C.felina on HepG2 cells death One-way ANOVA

<table>
<thead>
<tr>
<th>Concentration (µg/ml)</th>
<th>Effect of C.felina on HepG2 cells death</th>
</tr>
</thead>
<tbody>
<tr>
<td>25</td>
<td>4±0.6 e</td>
</tr>
<tr>
<td>50</td>
<td>8±1.8 d</td>
</tr>
<tr>
<td>100</td>
<td>16±0.8 c</td>
</tr>
<tr>
<td>150</td>
<td>23±0.8 b</td>
</tr>
<tr>
<td>200</td>
<td>45±0.6 a</td>
</tr>
<tr>
<td>Control</td>
<td>100</td>
</tr>
</tbody>
</table>

Means followed by the same letter do not differ significantly using LSD (P=0.05)

3.2. C. felina crude extracts induces apoptosis of HepG2 cells

Treatments with C.felina crude extracts (200µg/ml) effectively decreased the total number of HepG2 cells and were accompanied by cell shrinkage, condensed nuclei, blebbing and shape changes (Fig 1). When the cells were stained with trypan blue, which can discriminate between apoptosis and necrosis, condensed nuclei, blebbing, indicators of typical apoptosis manifestation were observed in plant crude extracts treated cells.

C. felina crude extract treated HepG2 cells exhibited more than 45 increase in apoptosis and Investigation of the staining pattern indicated that the predominant cause of cell death in HepG2 was due to apoptosis. Based on the one-way ANOVA analysis compared the means of C.felina crude extracts induces apoptosis of HepG2 cells significantly (P<0.05) in a concentration-dependent manner.

3.3. Apoptotic effect of plant crude extracts on normal cells

Apoptotic effect of plant crude extracts on normal cells were studied to test whether C.felina extract plant extract induced similar cell death in normal cells. After 30 minutes of incubation with of C.felina extract (200µg/ml) resulted only 16.5% cell death (Fig.2).
Fig. 2. Cytotoxicity and apoptotic activity of *C. felina* crude extract against HepG2 (A) Controls cells; (B) *C. felina* treated HepG2 cells showing cytotoxicity and apoptotic activity

4. DISCUSSION
Large scale screening of plant crude extracts on cell culture is an important initial step to determine their potential efficacy in clinical application. Several reports have shown that plant crude extracts replicate at tumour sites under hypoxic conditions and stimulate the host immune response and gene expression, leading to the inhibition of tumour growth.

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REFERENCES

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