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Original article

Investigating the pharmacological potential of *Iris kashmiriana* in limiting growth of epithelial tumors

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ABSTRACT

Background: *Iris kashmiriana*, a medicinal plant growing under Kashmir conditions, has been found very useful for pulmonary asthma, cancer, inflammation, liver and uterine diseases in traditional medicine. The medicinal importance of the plant prompted isolation of a variety of pharmacologically active compounds including quinones, triterpenoids, flavonoids, isoflavonoids and stilbene glycosides.
Objective: To evaluate the antiproliferative and antioxidant potential of methanolic extract of rhizomes of *I. kashmiriana*.
Material & methods: The effect of *I. kashmiriana* was evaluated against human epithelial cancer cell lines A549 and Caco-2 for their possible effect on cell proliferation. Free radical scavenging activity was tested by DPPH assay against known antioxidant Vitamin C. The extract was also analyzed for active components by RP-HPLC.
Results: The extract showed potent cytotoxic effect on both epithelial cell lines at all the tested concentrations with significant effect at 400 μg/ml ($IC_{50\ (A549)} = 128.7$ μg/ml; $IC_{50\ (Caco\text{-}2)} = 237.76$ μg/ml) as evaluated by MTT assay. The effect was, however, less pronounced on mouse fibroblast cell line NIH-3T3 ($IC_{50\ (NIH\text{-}3T3)} = 1182.92$ μg/ml), indicative of possible cell specific activity against epithelial cancers. Furthermore the free radical scavenging activity as verified by DPPH assay revealed that the methanolic extract of *I. kashmiriana* has strong antioxidant potential. HPLC analysis showed respectable amount of *Irigenin* and *Tectorigenin* present in the extract.
Conclusion: The results demonstrate pharmaceutical potential of *I. kashmiriana* for treatment of epithelial cancers and other inflammatory diseases.

1. Introduction

Plants are the main source of active principles for the treatment of diseases. The WHO reported that despite the availability of synthetic drugs, the big percentage of world's population relies on plant based therapies to cover the needs of primary health care.[1] Medicinal plants have got a priority position in the drug discovery and many modern day medicines have taken leads from the traditional use of medicinal plants. Despite major advances in molecular modeling, the medicinal plants remain an important source of new drugs and new drug leads.[2,3] Hence, for the treatment of disease states wherein drug therapy is a rational approach, plant materials represent legitimate starting materials for the discovery of new agents. In case of human cancers, many plant derived compounds have been approved for clinical use as anticancer drugs in the United States such as vinblastine, vincristine, vinorelbine, etoposide, teniposide, paclitaxel, docetaxel, topotecan, and irinotecan.[4–6]

Oxidative stress is defined as an imbalance between production of free radicals and reactive metabolites, so-called oxidants or reactive oxygen species (ROS), and their elimination by protective mechanisms, referred to as antioxidants. Free radicals, produced as a result of normal biochemical reactions in the body, play important role in the human body and become harmful only when they are produced in high amounts. Free radicals are implicated in wide spectrum of diseases such as cancer, atherosclerosis, aging,

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immunosuppression, inflammation, ischemic heart disease, diabetes, hair loss, and neurodegenerative disorders like Alzheimer's disease and Parkinson's disease.[7–9] Oxidative stress and cancer are highly interlinked. Cancer is a leading cause of death and may result from chronic injury to the epithelium by oxidants and other carcinogens. Under a sustained environmental stress, ROS over production may cause significant damage to cell structure and functions and may induce somatic mutations and neoplastic transformation.[10,11] Indeed, cancer initiation and progression has been linked to oxidative stress by increasing DNA mutations or inducing DNA damage, genome instability, and cell proliferation.[12] The human body possesses innate defense mechanisms to counter free radicals in the form of enzymes such as superoxide dismutase, catalase, and glutathione peroxidase. However, these endogenous systems are often insufficient for complete scavenging of ROS. Vitamin C, vitamin E, selenium, β-carotene, lycopene, lutein and other carotenoids have been used as supplementary antioxidants. Plant secondary metabolites such as flavonoids and terpenoids also play an important role in the defense against free radicals.[13–15]

The *Iris* plant belonging to family *Iridaceae* is worldwide in distribution, known for their ornamental relevance and medicinal value. The species of the genus *Iris* are very useful for pulmonary asthma, cancer, inflammation, liver and uterus diseases.[15] The intensive phytochemical investigations of various iris species have resulted in the isolation of a variety of compounds including quinones, triterpenoids, flavonoids, isoflavonoids and stilbene glycosides.[16] Flavonoids and isoflavonoids are important plant secondary metabolites with structural diversity and are consumed by human as dietary constituents.[17] The isoflavone rich dietary consumption is reported to reduce risk of cancer particularly breast and prostate cancer.[18,19] The role of isoflavones in cancer[18,20] osteoporosis, cardiovascular diseases and menopausal symptoms in addition to their antioxidant[21] antimicrobial[22] anti-inflammatory and estrogenic activities[18,23] is well documented. The current study investigated the effect of methanolic extract of *Iris kashmiriana*, a local medicinal plant against human epithelial cell lines to evaluate its anticancer potential.

2. Materials & methods

2.1. Plant material

I. kashmiriana was collected in June 2011 from *Naranag* area of Jammu and Kashmir. The voucher specimen has been kept in the herbarium of the Indian Institute of Integrative Medicine (CSIR) Srinagar with voucher specimen (No. I001/2010-I005/2010).

2.2. Extraction

The whole plant was dried under shade. The air dried plant was chopped and cut into small pieces and then grinded to the powdered form. The powdered material of the plant was soaked in methanol for 48 h. The extract was separated by filtration and evaporated to dryness on rotary evaporator under reduced pressure, which afforded 98 g of MeoH extract. The methanol extract was kept at −20 °C until use.

2.3. Phytochemical screening

The phytochemical screening was done using various standardized chemical tests:

2.3.1. Test for flavonoids

5–10 drops of dilute HCl followed by a piece of Zn or Mg were added to the test tube containing 0.5 ml of methanolic plant extract. The solution was boiled for few minutes. Presence of flavonoids is confirmed from the change in color to pink or reddish pink.

2.3.2. Test for phenols (ferric chloride test)

Small quantity of alcohol or aqueous extract is dissolved in 2 ml of distilled water. Few drops of 10% aqueous ferric chloride solution are added to the extract. The blue or green color indicates the presence of phenols.

2.3.3. Test for saponins

The extract is mixed with some drops of sodium bicarbonate and shaken vigorously. Heavy comb-like froth formation confirms the presence of saponins.

2.3.4. Test for glycosides

Small quantity of extract was added to 1 ml NaOH solution; yellow color indicates the presence of glycosides.

2.3.5. Test for tannins (ferric chloride test)

To 1–2 ml of extract add few drops of 5% aqueous ferric chloride solution, a bluish black color is produced, which disappears on addition of dilute sulfuric acid solution and formation of yellowish brown precipitate indicates the presence of tannins.

2.4. Cell culture

Cell lines A549 (human lung adenocarcinoma), Caco-2 (human colon adenocarcinoma) and NIH-3T3 (mouse non-neoplastic fibroblast) were kindly provided by Hybridoma Lab., National Institute of Immunology, New Delhi. Cells were maintained in Dulbecco's minimal essential medium (DMEM) or RPMI supplemented with 10% fetal bovine serum, 100U/ml penicillin and 100 μg/ml streptomycin at 37 °C in 5% CO_2. The medium was changed every two days or until the cells became confluent and then used for the experimentation.

2.4.1. Proliferation assay

MTT assay was used as a standard test for determining the effect of methanolic extracts of *I. kashmiriana* on cell proliferation. MTT assay is simple and reliable technique, which measures cell viability and is widely used for the screening of anticancer agents. The methanolic crude extract of *I. kashmiriana* was dissolved in DMSO (dimethyl sulphoxide), filter sterilized and then further diluted to attain required concentrations. Cell suspension containing 2×10^4 cells per well was seeded into a 96 well microtiter plate. After 24 h of seeding, cells were treated either with extract (at different concentration) or DMSO alone. The DMSO served as solvent control. Each concentration was tested in triplicate. The cells were incubated at 37 °C in a humidified incubator with 5% Co_2 for 24 or 48 h. MTT solution was added to the cells at 0.1 mg/ml concentration followed by incubation for 4 h at 37 °C in dark. The supernatant was removed and an equal volume of DMSO was added to dissolve the formazan crystals. The absorbance was measured at 565 nm (EPOCH Microplate Reader, Bio-Tek Instruments, USA).

Percentage proliferation inhibition was calculated as:

$$\frac{(\text{O.D Control} - \text{O.D Test})}{(\text{O.D Control})} \times 100.$$

IC_{50} values were calculated after 48 h of treatment.

2.4.2. DPPH assay

In this assay, free radical scavenging activity of crude methanolic extract of *I. kashmiriana* was determined from the bleaching of