Chemico-Biological Interactions 179 (2009) 160–168

Contents lists available at ScienceDirect

Chemico-Biological Interactions

journal homepage: www.elsevier.com/locate/chembioint

Anticancer activity of an essential oil from *Cymbopogon flexuosus*

Parduman R. Sharma, Dilip M. Mondhe, Shanmugavel Muthiah, Harish C. Pal, Ashok K. Shahi, Ajit K. Saxena*, Ghulam N. Qazi

Indian Institute of Integrative Medicine, Canal Road, Jammu 180001, India

ARTICLE INFO

Article history:
Received 2 July 2008
Received in revised form 4 December 2008
Accepted 4 December 2008
Available online 11 December 2008

Keywords:
Cymbopogon flexuosus
Essential oil
Cytotoxicity
Tumor growth inhibition
Apoptosis

ABSTRACT

The essential oil from a lemon grass variety of *Cymbopogon flexuosus* was studied for its *in vitro* cytotoxicity against twelve human cancer cell lines. The *in vivo* anticancer activity of the oil was also studied using both solid and ascitic Ehrlich and Sarcoma-180 tumor models in mice. In addition, the morphological changes in tumor cells were studied to ascertain the mechanism of cell death. The *in vitro* cytotoxicity studies showed dose-dependent effects against various human cancer cell lines. The IC_{50} values of oil ranged from 4.2 to 79 μg/ml depending upon the cell line. In 502713 (colon) and IMR-32 (neuroblastoma) cell lines, the oil showed highest cytotoxicity with IC_{50} value of 4.2 and 4.7 μg/ml, respectively. Intraperitoneal administration of the oil significantly inhibited both ascitic and solid forms of Ehrlich and Sarcoma-180 tumors in a dose-dependent manner. The tumor growth inhibition at 200 mg/kg (i.p.) of the oil observed with both ascitic and solid tumor forms of Ehrlich Ascites carcinoma was 97.34 and 57.83 respectively. In case of Sarcoma-180, the growth inhibition at similar dose of oil was 94.07 and 36.97% in ascitic and solid forms respectively. Morphological studies of the oil treated HL-60 cells revealed loss of surface projections, chromatin condensation and apoptosis. The mitochondria showed apparent loss of cristae in the cells undergoing apoptosis. The morphological studies of Sarcoma-180 solid tumor cells from animals treated with the oil revealed condensation and fragmentation of nuclei typical of apoptosis. Morphological studies of ascites cells from animals treated with the oil too revealed the changes typical of apoptosis. Our results indicate that the oil has a promising anticancer activity and causes loss in tumor cell viability by activating the apoptotic process as identified by electron microscopy.

1. Introduction

Plants have been regarded as a potential source of cancer chemoprevention drug discovery and development [1,2]. Grasses are an important group of plants in the life of human beings and animals. Essential oil yielding grasses mostly belong to the family Poaceae of which *Cymbopogon* forms an important genus. The members of the genus afford essential oil, which has a wide variety of chemical constituents used in perfumery, pharmaceutical industries and yield isolates which are used in high grade perfumery. There are up to 60 species of *Cymbopogon* native to tropical and subtropical regions of Africa and Asia. The most significant species include *C. citratus* (DC) Stapf which gives West-Indian Lemon grass oil; *C. flexuosus* Stapf is the source of East Indian lemon grass oil; *C. nardus* (L) Watson is the source of Ceylon citronella oil; *C. winterianus* Jowilt is used to produce Java citronella oil [3]. The genus *Cymbopogon* is of great interest due to its commercially valuable essential oils and also in native medicines [4]. The essential oils from plants such as Guacatonga (*Casearia sylvestris* Sw.), Sweet fern (*Comptonia peregrina* L.), Guava (*Psidium guajava* L.), Sweet basil (*Ocimum basilicum* L.), *Tanacetum gracile* and lemon grass (*Cymbopogon flexuosus*) have been reported to be cytotoxic to human cancer cells [5–9]. Several Thai edible plants such as Lemongrass (*Cymbopogon citratus* D.C.), Holy Basil (*Ocimum sanctum*) and Sweet Basil (*O. basilicum*) possess antitumor activity in mice [10]. *C. flexuosus* oil is also thought to help with stress-related disorders, and has been shown to have antifungal and antimicrobial properties [11]. The chemical composition of *C. flexuosus* oil has been reported [9]. The various constituents (%) present in the oil from lemon grass variety of *C. flexuosus* such as geraniol (20.08), geranyl acetate (12.20), α-bisabolol (8.42) and isointermedeol (24.97) have been individually reported for their cancer cell cytotoxicity [12–14,9]. The limonene (3.5%) and borneol (1.90%) present in *C. flexuous* oil are known for their immunostimulatory activity, analgesic and anaesthetic activities respectively [15–16]. The outstanding feature of apoptosis is its remarkable stereotyped morphology showing condensation of nuclear heterochromatin, cell shrinkage and loss of positional organization of organelles in the cytoplasm [17]. A successful anticancer drug should kill or incapacitate cancer cells without causing excessive damage to normal cells. This ideal situation is achievable

* Corresponding author. Fax: +91 191 2569333/2569019/2569017.
E-mail address: aksaxena2003@yahoo.com (A.K. Saxena).

0009-2797/$ – see front matter © 2008 Elsevier Ireland Ltd. All rights reserved.
doi:10.1016/j.cbi.2008.12.004

by inducing apoptosis in cancer cells. Thus, modulating apoptosis may be useful in the management and therapy or prevention of cancer. Apoptotic induction has been a new target for innovative mechanism based drug discovery [18]. It is thus considered important to screen apoptotic inducers from plants, either in the form of crude extracts or as components isolated from them. Chemopreventive agents comprise a diverse group of compounds with different mechanisms of action, but their ultimate ability to induce apoptosis may represent a unifying concept for the mechanism of chemoprevention. To the best of our knowledge, the effect of *C. flexuosus* oil treatment on various other human cancer cell lines *in vitro* and on tumor development *in vivo* has hitherto not been reported. It was also of the interest to ascertain the effect of oil on morphology of tumor cells both *in vitro* and *in vivo* to have better insight into antineoplastic activity. The morphological analysis of tumor cells by light and electron microscopy constitutes an important and decisive tool to precisely identify the type of cell death and thus far, has been the gold standard for the most accurate detection of apoptosis [17,19]. The aim of the present study was to investigate the activity of *C. flexuosus* oil both in *vitro* using human cancer cell lines and *in vivo* using murine Ehrlich and Sarcoma-180 tumors and to ascertain the mechanism of cell death by light and electron microscopy.

2. Materials and methods

2.1. Chemicals

RPMI-1640, Minimum essential medium (MEM), trypsin, gentamycin, penicillin, 5-fluorouracil, Paclitaxel, Mitomycin-C, Sulforhodamine-B (SRB) and dimethyl sulphoxide (DMSO) were purchased from Sigma Chemical Co., U.S.A. The epoxy embedding kit and propylene oxide were procured from Fluka Chemie AG, Switzerland. Glutaraldehyde and osmium tetroxide were procured from Merck, Germany. All the other chemicals were of analytical grade and procured locally.

2.2. Isolation of essential oil of C. flexuosus (Nees ex Steud.) Wats [RRL, (J) CF HP]

C. flexuosus strain designated as RRL, (J) CF HP is a perennial thickly tufted grass with no signs of any disease and exhibits high survival under adverse conditions. The grass is grown in our Institute's farm. The oil (density 0.89) was isolated as reported [9].

2.3. Cell lines and cell cultures

The human cancer cell lines were obtained either from National Center for Cell Science, Pune, India or National Cancer Institute, Frederick, U.S.A. Colon (HT-29, HCT-15, SW-620, 502713), lung (A549, HOP-62, H-226), cervix (SiHa), oral (KB), prostate (DU-145) and promyelocytic leukemia (HL-60) cells were grown and maintained in RPMI-1640 medium, pH 7.4 whereas MEM was used for neuroblastoma (IMR-32) and liver (Hep-g-2). The media were supplemented with 10% fetal calf serum, glutamine (2 mM), penicillin (100 units/ml) and streptomycin (100 μg/ml). The cell cultures were grown in a carbon dioxide incubator (Heraeus, GmbH, Germany) at 37 °C with 90% humidity and 5% CO_2 as reported previously [20].

2.4. In vitro cytotoxicity against human cancer cell lines

The stock solution of the oil (10%) was prepared in DMSO and serially diluted with growth medium to obtain desired concentrations. The *in vitro* cytotoxicity of the oil, at concentrations ranging from 0.89 to 890 μg/ml, was determined by semi-automated assay [21] using sulforhodamine-B (SRB), as described earlier [20]. The untreated control cultures received only the vehicle (DMSO, <0.1%). The results are reported in terms of IC_{50} values.

2.5. Quantification of apoptosis by light microscopy

HL-60 cells were incubated with 22.2 and 44.5 μg/ml (0.025 and 0.05 μl/ml) of the oil for 12 h and apoptosis was assessed using light microscopy. The cells were spun onto glass slides. The air-dried smears were fixed for one minute in absolute methanol and stained with modified Giemsa stain. The percentage of cells undergoing apoptosis was determined after characterizing 200 cells per slide, using the standard morphological criteria such as condensation of the nuclear chromatin, vacuolization of the cytoplasm, fragmentation of the nucleus, and formation of apoptotic bodies [22].

2.6. In vivo anticancer activity

In vivo anti-tumoral activity of the oil was evaluated in murine models. Non-inbred Swiss albino mice and inbred BALB/c mice from an in-house colony were used in the present study. The animals were housed under standard husbandry conditions as per Guide for the care and use of laboratory animals (1996). Animals were provided pelleted mice feed (M/s Ashirwad Industries, Chandigarh, India) and autoclaved water *ad libitum*. The study and the number of animals used were approved by the Institutional Animal Ethics Committee, Indian Institute of Integrative Medicine, Jammu.

Ehrlich ascites carcinoma (EAC) cells and Sarcoma-180 (S-180) ascites cells were collected from the peritoneal cavity of the Swiss albino and BALB/c mice respectively harbouring 8–10 days old ascitic tumor. On day 0, the male animals weighing 18–23 g were selected and 1×10^7 EAC cells were injected (i.m.) in right thigh of the Swiss albino mice (43 nos.) and a similar number of S-180 cells in the right thigh of BALB/c mice (43 nos.). On the next day, animals in both experiments were randomized and divided into four test groups (7 animals each) and one control group (15 animals). In both the experiments, three test groups were treated with 100, 150 and 200 mg/kg, i.p. of oil, respectively from days 1 to 9. The fourth test group was treated with 5-fluorouracil (22 mg/kg, i.p.) and it served as positive control. The emulsion of oil was prepared in Tween-80 (0.5%, v/v) so that the required dose to be administered to each animal was contained in 0.2 ml. The control group was similarly administered normal saline (0.2 ml, i.p). On day 13, tumor weight was determined as per the standard procedure [23].

Ascitic tumors were produced by injecting EAC and S-180 ascites cells (10^7 cells/animal, i.p.) in Swiss albino and BALB/c mice, respectively. The group of animals and treatment with oil etc was the same as described for solid tumor experiments except the 5-fluorouracil was administered at the dose of 20 mg/kg (i.p.). On 12th day, animals were sacrificed and ascitic fluid was collected from peritoneal cavity of each mouse for evaluation of tumor growth [23].

2.7. Morphological analysis

2.7.1. Light, scanning and transmission electron microscopy of HL-60 cells

HL-60 cells were seeded in complete medium and treated with 22.2 and 44.5 μg/ml of the oil for 12 h and processed for light microscopy (LM) and transmission electron microscopy (TEM). The cells were sedimented at 1800 rpm for 10 min. Cell pellets were fixed instantly with 2.5% glutaraldehyde in 0.1 M PBS, (pH 7.2) at 4° C for 1 h, post-fixed with 1% OsO_4 in the same buffer, dehydrated with graded ethanol solutions and embedded in Epon 812 [24]. Semi-thin sections were stained with 1% aqueous toluidine blue at 40 °C and were observed with a Vanox light microscope (Olympus). To quantify apoptosis, semi-thin sections from three independent