Rubia cordifolia Root Extract Induces Apoptosis in Cancer Cell Line

Sourabh Tiwari*, Ravi Upadhyaya, Ruchi Shroti, Sharad Trivedi Upadhyaya,
Department of Botany, Government PG College, Pipariya, Madhya Pradesh, India.

Article history:
Received 27th September 2012
Accepted 15th October 2012
Available online 11th December 2012

ABSTRACT

Thousands of herbal and traditional compounds are being screened worldwide to validate their use as anti-cancer drugs; Rubia cordifolia is one of them. R. cordifolia (Rubiaceae) is also known as, Manjishtha, Indian madder known to contain substantial amounts of anthraquinones, especially in the roots which is responsible for anti-tumor activity. The plant contains substantial amounts of anthraquinones, especially in the roots, which is responsible for its pharmacological activity. Cancer is a dreadful disease and any practical solution in combating this disease is of paramount importance to public health. An integrated approach is needed to manage cancer using the growing body of knowledge gained through scientific developments. In the present study, we investigated the influence of an ethanolic extract of root of R. cordifolia on the induction of apoptosis in HeLa human cervical cell line. Ethanol fraction of R. cordifolia extract exhibited potent inhibition of human cervical cancer cell line. R. cordifolia can be a source of potent pharmacophore for treatment of disease like cancer. Thus, cancer patients who already got crippled with this disease, who are further burdened by drug-induced toxic side effects, have now turned to seek help from the complementary and alternative medicine hoping for a better cure.

Key words: Apoptosis, Anthraquinone, Cervical cancer, Rubia cordifolia.

*Corresponding Author:
Ms. Sourabh Tiwari,
Near Hanuman Mandir,
Budni (District-Sehore), MP-466445
E. Mail: sourabhtiwari@gmail.com

How to cite this article:
Rubia cordifolia Root Extract Induces Apoptosis in Cancer Cell Line

Introduction:
Cancer is a disease characterized by unregulated proliferation of cells. Plants have been used as folk remedies and ethnobotanical literature has depicted the usage of plant extracts. There is an increasing need for search of new compounds with cytotoxicity activity at the treatment of cancer with the available anticancer drugs is often insufficient due to the problem cytotoxicity to the normal cells. For the last few decades, phytochemical examination has been making rapid progress and herbal products are becoming popular as sources of possible anticancer compounds (Patel et al, 2010).

The herb Rubia cordifolia is usually categorized as GRAS (generally recognized as safe) (Williamson 2002). R. cordifolia Linn. (Rubiaceae) is commonly known as Indian Madder Manjistha (Kirtikar and Basu 1980, Rao et al, 2006). It is a perennial, herbaceous climber. The roots are 4-8 cm long, reddish, cylindrical, flexuous, with a thin red bark. Stems often have a long, rough, grooved, woody base. The family Rubiaceae comprises about 450 genera and 6500 species and includes trees, shrubs and infrequently herbs. About 15 species occur in India. Some of these are Indian madder (R. cordifolia), Naga madder (R. Sikkimensis) & European madder (R. tinctorum) (Deshkar et al, 2008). It is distributed throughout the lower hills of Indian Himalayas in the North & the Western Ghats in the south and Japan, Indonesia, Ceylon, Malay, Peninsula, Java & tropical Africa in moist temperate & tropical forests, up to an altitude of 3500 meter grow well in light (sandy), medium (loamy) and heavy (clay) soils.

Plants belonging to this family are known to contain substantial amounts of anthraquinones, especially in the roots (Singh et al, 2005, Meena et al, 2010). It purifies the blood by removing toxins from the blood, dissolving obstructions in blood flow and removing stagnant blood. Furthermore, the Anthraquinones of the Rubiaceae family exhibit some interesting in vivo biological activities, such as antitumor (Adwankar and Chitnis 1982), anti-inflammatory (Kasture et al, 2001), urinary disorders (Itokawa et al, 1984), antistress antimicrobial (Singh et al, 2005), hepatoprotective (Rao et al, 2006), radio protective (Tripathi and Sing 2007), and anticancer (Son et al, 2008), antimicrobial, antifungal, hypotensive, analgesic, antimarial, antioxidant, antileukemic and mutagenic functions, immunomodulatory (Joharapurkar et al, 2003), anti-inflammatory (Antarkar et al, 1983).

The roots of this plant are of high medicinal value and are recognized as official (More et al, 2007). The major Phytoconstituents reported in this plant include free Alizarin & its glucosides, Purpurin, Xanthopurpurin, Munjistin, Ruberythric acid, Pseudo-purpurin, Physcion, Nordamnacanthal, Rubicoumaric acid, Mollugin, Furomollugin, various Anthraquione glycosides such as- 1-hydroxy-2-methyl anthraquione, 1,4-dihydroxy-6-methyl anthraquione etc., cyclic hexapeptides as RA-V, RA-VII, cyclic heptapeptides as RA-III, RA-IV etc. (Agrawal and Paridhavi 2007, Mukherjee 2002).

Materials and Methods:
Reagents

The culture petri-dishes were procured from BD Falcon (Rockville, MD, USA). Fetal calf serum was obtained from HyClone Labs (Logan, Utah, USA). Dulbecco’s Modified Eagle’s Medium (DMEM) growth medium was procured from Gibco/BRL Life Technologies, Inc. (NY, USA). Antibiotic-antimycotic solution was obtained from Hi-Media Labs (Mumbai, India). The cell growth supplements sodium pyruvate, nonessential amino acids and sodium bicarbonate were obtained from MP Biomedicals, Solon, USA. The activity of caspase-3 was determined by using PE Active Caspase-3 Apoptosis kit from BDTM Biosciences, San Diego, CA, USA.

Cell line & culture conditions

The human carcinoma cell line, HeLa was obtained from the National Centre for Cell Science (NCCS), Pune, India. The cells were seeded at 2 x 105 cells/60 mm culture dishes in DMEM supplemented with 10% fetal calf serum, 1.5 g/L sodium bicarbonate at 37°C in the humidified atmosphere of 5% CO2 in the air according to NCCS catalogue instructions. After optimum Confluency, the cells were treated with the experimental agent, free drugs and encapsulated drugs and harvested with trypsin-EDTA for use in the following experiments.

Plant material and extraction method

The R. cordifolia roots are authenticated by authors. The air dried roots of R. cordifolia reduced to a coarse powder and around 2 kg of dried powder was extracted separately with 95% v/v alcohol by a continuous hot percolation process using a Soxhlet apparatus. The ethanol extract was concentrated to a small volume and then evaporated to dryness. The percent yield of R. cordifolia root extract is 17.26%. The dry extracts were subjected to various chemical
tests to detect presence of different photochemical constituents.

Study design

Cells were treated with a fixed 0.005 µM concentrated of root extract of R. cordifolia at different sampling intervals (n=5) for dosage gradient of 1 to 100 µg following 6 hour of treatment.

Assessment of apoptotic index

The activity of active caspase-3 was measured by washing the cells with cold 1× PBS and then resuspending in BD cytofix/cytoperm solution at a concentration of 1×10^6 cells/ml followed by incubation for 20 minutes on ice. The cells were then harvested, washed, followed by incubation with antibodies for 30 minutes at room temperature. The cells were washed and analyzed by flow cytometry in FL2 channel.

Results and Discussion:

Fig 1: Caspase-3 activation in HeLa cell line.

Flow cytometric analysis for caspase-3 activation in HeLa cancer line following treatment with R. cordifolia root extract at different concentration: a FSC/SSC plot showing the population of cancer cell line; b control cells; c-g cells treated with 1, 5, 25, 50, 100 µg doses showing a dose dependent increase in activation of caspase-3 following a 6-h incubation period. Transparent zone represents untreated cells primarily negative for PE.

Fig 2: Graphical representations of apoptosis.

Graph shows a percentage of cells showing caspase-3 activation following treatment with R. cordifolia at 1, 5, 25, 50, and 100µg doses in recipient cells after a 6 h incubation period.

Discussion:

Active caspase-3, a marker for cells undergoing apoptosis, comprises a hetero-dimer of 17- and 112- kDa subunits, which in turn are derived from a 32- kDa pro-enzyme. Active caspase-3 proteolytically cleaves and activates other caspases and relevant targets in the cytoplasm. We measured the caspase-3 activity in R. cordifolia-treated cells. Maximum caspase-3 activity was observed at 100µg dose, i.e., 80.08%, while in time course study, the maximum percentage of cells showing significant activity was 64.33% at 24 h post-treatment. The rate of apoptosis was increased with increase in concentration of extract in HeLa cell line. Study results show that root extracts of R. cordifolia is promisingly cytotoxic against human cervical cancer.

Conclusion:

It is concluded that ethanolic extract of root of R. cordifolia possess significant anti-cancer activity against cancer cell line. This may be due to the presence of reported active phytoconstituents. So, this plant extracts may have clinical and therapeutic proposition in the most life threaten disease like cancer. The rate with which cancer is progressing, it seems to have an urgent and effective effort for making good health of humans.

Acknowledgment:

The authors are thankful to Mr. BP Singh, Faculty of Botany, for his technical support.
References:


