An Elsevier Indexed Journal ISSN-2230-7346

Journal of Global Trends in Pharmaceutical Sciences

INDUCTION OF APOPTOSIS IN HUMAN LIVER CARCINOMA CELL LINE AND THE SAFETY OF LIVER BY *POLYCARPAEA CORYMBOSA*, LAM.

Sakthi Abirami. M*, Monika N
Institute of Pharmacology, Madras Medical College, Chennai – 600 003

Corresponding author E-mail: m36sakthiabirami@yahoo.co.in*

ARTICLE INFO

Key Words
HepG2, *Polycarpaea corymbosa*, agarose gel electrophoresis, Toxicity studies

ABSTRACT

The ethanol extract of *Polycarpaea corymbosa*, Lam. was investigated for induction of apoptosis by DNA fragmentation assay. The toxicity profiles towards normal liver cells were studied by MTT assay on Chang cell line. The selectivity of the drug was determined. The ethanol extract of *Polycarpaea corymbosa*, Lam. induces apoptosis in HepG2 which is indicated by DNA ladder formation and it doesn't produce significant toxicity towards normal liver cell line (*Chang*). The ethanol extract of *Polycarpaea corymbosa*, Lam. produces cell death by inducing apoptosis in HepG2 cells and it is safe towards normal liver cells.

INTRODUCTION

The term cancer refers to a disease of cells that show uncontrolled proliferation, dedifferentiation (anaplasia), invasiveness and the ability to metastasize (spread to distant parts of the body). The appearances of such abnormal gene sequences are called oncogenes. When such cells proliferate excessively, they form local tumours which can compress or invade nearby normal cellular structures. Small cores of cells within this tumour, which retain the ability to undergo repeated proliferation and to metastasise, are called tumour cells. These have colony forming capability and can readily survive in the multicellular environment of the host. The extent of metastasis and deterioration in metabolic processes, resulting from cancer leads to eventual death of the patient, unless it is eradicated with suitable treatment. A drug which is cytotoxic to cancer cells may cytotoxic to normal cells also. Hence the selectivity of an anticancer drug is very limited.[1] Hepatocellular carcinoma is the 5th most occurring neoplasm in the world. After diagnosis, the patient with liver carcinoma dies within one year. The treatment such as surgery and chemotherapy doesn't produce curative effects and it has many side effects. Therefore, search for highly efficient antitumor drugs was high. [2] The whole plant *Polycarpaea corymbosa*, Lam. [3] belong to the family *Caryophyllaceae* (Synonyms - *Achyranthus corymbosa, Polycarpaea nebulosa)* which is commonly known as Old man's cap. In Tamil, it is commonly known as nilaisedachi. It is an annual or perennial herb consist leaves which are opposite or

appear in whorls, linear up to 3.5cm long with a brittle at the tip. The Flowering Season of this plant is from August to September. Flowers are borne in compact heads at the end of stems. Sepals are silvery white, turning rich brown with age. Petals are small, pink to orange in colour. Fruit is a minute ellipsoid capsule. It is distributed in India, Burma, Ceylon, Western Peninsula and ascending the western Himalayas to 7000 feet. [3] It is used both internally and externally as remedy for venomous bites from reptiles and animals. Pounded leaves were used as poultice over boils and inflammatory swellings, used for animal bites and given along with molasses in form of a pill in jaundice. Roots were used for boils and liver complaints. [4] The previous study on this plant proved the inhibitory effect of *Polycarpaea corymbosa,* Lam. in various cell lines at different concentrations. [5] In that study, it proved that the drug has anti proliferative effect against hepatocellular carcinoma, still the curiosity of knowing whether it has apoptosis inducing ability, and its safety towards the normal liver cells remains. Hence we aimed to investigate the drug *Polycarpaea corymbosa,* Lam., its ability of inducing apoptosis which is determined using agarose gel electrophoresis and its safety towards normal liver cells was analysed using *Chang* cell line by MTT assay. This provides new opportunity for research with regard to their prevention and cure of human liver cancer in future.

Materials and methods:

Preparation of Extracts: The whole plant *Polycarpaea corymbosa,* Lam. was collected from Tirunelveli district, Tamilnadu, India and was authenticated by botanical survey of India. The whole plant *Polycarpaea corymbosa,* Lam. was collected, cleansed, shade dried for about 2 weeks. The dried plant was pulverized to a coarse powder by grinding in mixer and passed via sieve no. 40 and stored in an air tight container.

The extract was prepared by hot percolation method. The dried coarsely powdered plant material was extracted with ethanol using soxhlet apparatus at 60^0-70^0 for 18h, until the solvent become colourless in the siphon tube. The extracts were concentrated and the semisolid residue obtained was weighed and stored in desiccators till it is required for use. The yield value of the residue obtained was calculated. Further studies were conducted with the obtained residue from the above process.

Phytochemical analysis: The phytochemical analysis is used to determine the nature of phytoconstituents present in the plant were determined. The identification test for alkaloids, flavonoids, carbohydrates, glycosides, phytosterols, fixed oils and fats, saponins, phenolic compound and tannins, proteins, amino acids, gum and mucilage were performed in the ethanol extract[6].

Cell culture studies: HepG2 cells and Chang cells were purchased from the National Centre of Cell Sciences (NCCS), Pune. All the chemicals were obtained from HIMEDIA, Mumbai, India. The cells were maintained in DMEM supplemented with 10% Fetal bovine serum. Antibiotics added were penicillin (100units/ml) and 100 µg/mL of streptomycin in a humidified atmosphere of 50 µg/ml at 37℃.

DNA Fragmentation assay: The anti-proliferative effect of drug on HepG2 cell line was carried out by MTT assay and its IC_{50}value is 10µg. HepG2 cells were plated in 6 well plates and kept in CO_2 incubator to attain confluency. The IC_{50} concentration of sample was added into the well and incubated for 24 hrs. After this, cells were harvested using TPVG solution. The cells were centrifuged at 200xg at 4℃ for 10 minutes followed by addition of 0.5ml of TTE (Tris-TAPS-EDTA) solution to the pellet and it is vortexed vigorously. Then, again centrifuge the tubes at

20,000xg for 10 minutes at 4℃. Carefully, remove the supernatant and add 500µl of TTE solution into the pellet followed by addition of 500µl of ice-cold NaCl and vortex vigorously. Then, add 700µl of ice-cold isopropanol and vortex vigorously and allow it to precipitate by overnight at -20℃.After precipitation, recover DNA by pelleting for 10 min at 20,000x g at 4℃. Rinse the pellets by adding 500-700µl of ice-cold 70% ethanol. Centrifuge tubes at 20,000x g for 10 min at 4℃ and dissolve DNA by adding to each tube 20-50 µl of Tris- EDTA (TE) solution and place the tubes at 4℃. The samples of DNA were mixed with loading buffer by adding 10x loading buffer to a final concentration of 1X. The additi-on of loading buffer to samples allows to load in wells more easily and to monitor the run of samples. Run the electrophoresis in standard TE buffer after setting the voltage to the desired level. During electrophoresis it is possible to monitor the migration of samples by following the migration of bromophenol blue dye contained in the loading dye. The electrophoresis process was stopped when the dye reaches about 3 cm from the end of the gel. Place the gel on a UV Transilluminator and visualize the DNA ladder.

Toxicity studies: *Chang* Cells (1 x 10^{-4}) were plated in 24 well plates and incubated in 37^0 C with 5 % CO_2 condition. After the cell reaches the confluence, the various concentrations of the samples were added and incubated for 24 hrs. After incubation, the sample was removed from the well and washed with phosphate- buffered saline (pH 7.4) or DMEM without serum. 100µl/well (5mg/ml) of 0.5% 3-(4,5-dimethyl-2- thiazolyl)-2,5- diphenyl-tetrazolium bromide(MTT) was added and incubated for 4 hrs. After incubation, 1ml of DMSO was added in all the wells. The absorbance at 570nm was measured with UV- Spectrophotometer using DMSO as the blank. Measurements were performed and the concentration required for 50% inhibition (IC_{50}) was determined graphically. Graph is plotted using the percentage cell viability at y-axis and concentration of the sample in x-axis [7-10]. The selectivity index of the drug towards HepG2 cells were determined by comparing the effect of drug in normal liver cells.

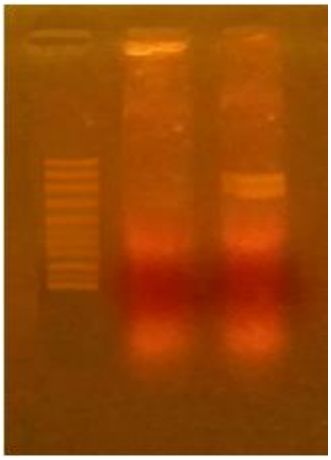
RESULTS AND DISCUSSION: The whole plant *Polycarpaea corymbosa*, Lam. was extracted with ethanol and it was rich in alkaloids, flavonoids, carbohydrates, glycosides, saponins, phenolic compound and tannins which were determined by identification tests.

Detection of *Polycarpaea corymbosa*, Lam. induced apoptosis of HepG2 cells by agarose gel electrophoresis: The biochemical hallmark of apoptosis is the fragmentation of the genomic DNA, an irreversible event that commits the cell to die.

Table no. 1: Effect of Ethanol extract of *Polycarpaea corymbosa* Lam. on *Chang* cell line

S.no	Concentration(µg)	Percentage Cell viability (%)
1	7.8	92.47
2	15.6	88.99
3	31.2	83.66
4	62.5	77.61
5	125	72.29
6	250	66.23
7	500	60.36
8	1000	54.12

Figure 1: DNA fragmentation on HepG2 cells



1 2 3

Lane 1: Marker; Lane 2: Control; Lane 3: Ethanol extract of *Polycarpaea corymbosa* Lam.

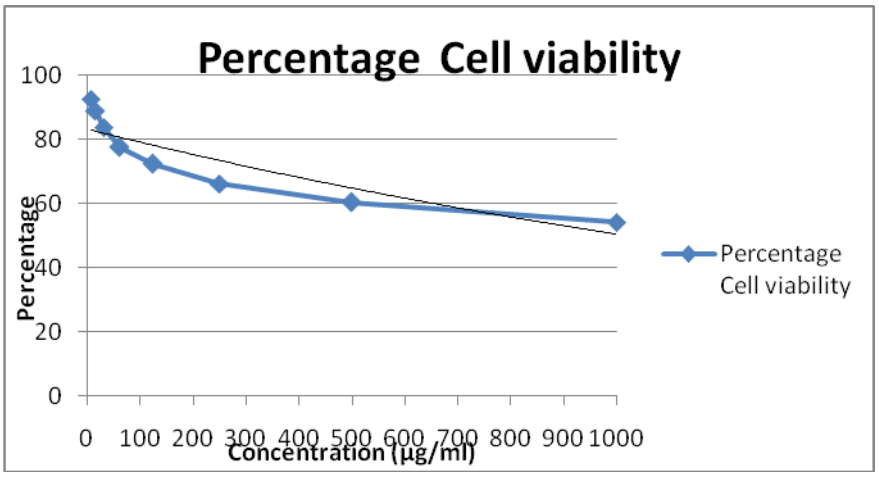


Fig. 2: Effect of Ethanol extract of *Polycarpaea corymbosa,* Lam. on Chang cell line

Apoptosis and cell mediated cytotoxicity are characterized by cleavage of the genomic DNA into discrete fragments prior to membrane disintegration. Because DNA cleavage is a hallmark for apoptosis, assays which measure prelytic DNA fragmentation are especially attractive for the determination of apoptotic cell death. The DNA fragments was assayed by using DNA "ladders" (with the 180 bp multiples as "rungs" of the ladder) derived from population of cells. DNA agarose gel electrophoresis showed that treatment of HepG2 cells with 10µg (IC_{50}) of *Polycarpaea corymbosa*, Lam. for about 24h resulted in typical DNA ladders, which indicates apoptosis was held. The DNA fragmentation or DNA ladder formation was compared with DNA marker, HepG2 untreated cells and extract treated HepG2 cells which were shown in the figure 1.

Toxicity Studies: The Effect of Ethanol extract of *Polycarpaea corymbosa*, Lam. on Chang cell line was represented in the above table 1 and it was graphically shown in the below figure 2. The dose dependent cell growth inhibition was observed. The ethanol extracts of the whole plant

Polycarpaea corymbosa, Lam. was safe at therapeutic concentration, and it possesses cytotoxic effect only at higher dose.

DISCUSSION:

The whole plant *Polycarpaea corymbosa,* Lam. was found to be enriched with phyto compounds such as flavonoids and polyphenols. They are potent bioactive compounds which possess anti-carcinogenic effects which interfere with initiation, development and progression of disease by the modulation of cellular proliferation, differentiation, apoptosis, angiogenesis and metastasis. The inhibitory effect of flavonoids was accompanied by an induction of apoptosis which was determined using DNA fragmentation assay. The result proves that *Polycarpaea corymbosa,* Lam. induces apoptosis at lower dose itself (Fig.no: 1) and the mechanism involved was, during DNA fragmentation, it activates endogenous Ca^{2+} and Mg^{2+} dependent nuclear endonuclease within the cell which cleaves DNA at sites located between nucleosomal units which generates mono and oligo- nucleosomal DNA fragments. These fragments reveal a distinctive ladder pattern consisting of multiples of an approximately 180bp subunit which indicates the sign of apoptosis. Even though it possess apoptotic activity on liver cancer cell, the safety towards normal liver cell is more vital. Because, nowadays more than therapeutic effect, side effects were more in most anti-cancer drug. Hence it was investigated in Chang cell line which reveals that 50% toxicity of *Polycarpaea corymbosa,* Lam. on normal liver cells was observed only at a dose of 1000µg (Table no. 1 & Fig.no. 2). The selectivity index was found to be 100 which prove that the ethanol extract of *Polycarpaea corymbosa,* Lam. was 100 times selective towards cancer cells and safe towards normal liver cells at therapeutic concentration. The chemo preventive effect of this plant was quite specific towards cancer cells than the normal cells.

CONCLUSION:

It concludes that the ethanol extract of *Polycarpaea corymbosa,* Lam. causes cell death by inducing apoptosis. The toxicity studies reveals that ethanol extract of *Polycarpaea corymbosa,* Lam. was safe and it did not produce any toxicity on normal liver cells at therapeutic dose and it is more selective towards cancer cells which may be due to the presence of phyto compounds such as polyphenols and flavonoids. In future, the mechanism by which *Polycarpaea corymbosa* Lam. induces apoptosis and the gene responsible for it is to be determined for further clinical use.

Conflict of interest: None

REFERENCES:

1. HL Sharma, KK Sharma; Principles of pharmacology; 3rd edition; Paras medical Publisher; 853-858

2. Trojan J, Zangos S, Schnitzbauer AA. Diagnostics and Treatment of Hepatocellular Carcinoma in 2016: Standards and Developments. Visc Med. 2016, 32(2):116–20.

3. Nadkarni K.M. Indian Materia Medica with Ayurvedic, Unani – Tibbi, Siddha, Allopathic, Homeopathic, Naturopathic & Home Remedies, Appendices & Indexes. Bombay Popular Prakashan Pvt. Ltd. 1976, 1(1), 997.

4. Chopra R.N, Nayar S.L and Chopra I.C. Glossary of Indian medicinal plants. CSIR, New Delhi, 1956, 1:199.

5. SakthiAbirami M, Muthusamy P; *Invitro* cytotoxic activity of various

extracts from whole plant of *Polycarpaea corymbosa* Lam.; Journal of global trends in pharmaceutical sciences, 2016, 7 (1), 2997-2999.

6. C.K.Kokate, A.P. Purohit, S.B. Gokhale; Pharmacognosy; Nirali Prakashan; 48th Edition, 2013, A.21-26

7. Gupta SK. Drug Screening Methods: Preclinical Evaluation of New Drugs. Jaypee Brothers, Medical Publishers Pvt. Limited, 2008, 209 - 217

8. Guide to sub culturing cell line monolayer. [Internet]. 2010. Available from: http://www.atcc.org
9. Wyllie AH, Kerr JF, Currie AR. Cell death: the significance of apoptosis. Int Rev Cytol. 1980, 68, 251–306.
10. Apoptosis and Cell proliferation, 2nd Edition; Boerhinger Mannheim, Biochemica, 1998, 70-72.