DOI: 10.21276/IJIPSR.2017.05.12.619

International Journal of Innovative Pharmaceutical Sciences and Research
www.ijipsr.com

ASSESSMENT OF CYTOTOXICITY OF *GYROCARPUS ASIATICUS* CRUDE EXTRACT AGAINST HEPG2 CELL LINES BY INDUCING NUCLEAR MORPHOLOGY CHANGES & DNA FRAGMENTATION

[1]Vulasi Indrani, [1]P. Suvarnalatha Devi*

*Department of Applied Microbiology, Sri Padmavati Mahila Visvavidyalayam (Women's University), Tirupati- 517502, **INDIA***

Abstract

Plants have important substances with pharmacological effects and can be natural sources as new anticancer agents. Therefore in this study, Cytotoxicity of *Gyrocarpus asiaticus* were assessed by DNA fragmentation and nuclear morphology changes. MTT assay was used to assess the cytotoxicity of Gyrocarpus. The optical density (OD) of each well was measured at 560nm by using spectrophotometer. Results were generated from three independent experiments and each experiment was performed in triplicate. Cytotoxicity was expressed as the concentration of extract inhibiting cell growth by 50% (IC50 value**).** DAPI staining assay was performed to identify the nuclear morphological changes, where the cells were then treated at IC50 concentration of crude extract. Agarose gel electrophoresis was performed DNA fragmentation detection. The results of cytotoxic activity against HepG2 cancer cell lines shows IC50 of 250IC50 at 242.5 µg/ml indicates dose dependent manner. Further, nuclear morphology changes were carried by DAPI shows changes in morphology, apoptotic body formation. Apoptosis which was further confirmed through increasing nuclear DNA damage.The present study findings clearly indicated that *Gyrocarpus asiaticus* showed dose dependant cytotoxicity.

Keywords: HepG2 cell lines, MTT assay, DNA damage, DAPI, Apoptosis.

Corresponding Author:

P. Suvarnalatha Devi

Department of Applied Microbiology,

Sri Padmavati Mahila Visvavidyalayam

(Women's University),

*Tirupati- 517502, **INDIA***

***E-mail:** drsuvarnapallipati@gmail.com*

***Phone:** +91-8374820199*

DOI: 10.21276/IJIPSR.2017.05.12.619

INTRODUCTION

Cancer is a major clinical problem which is a socio economic challenge of the healthcare system. Cancer is a leading cause of death worldwide and cancer related deaths are projected to increase over 11 million people globally by 2030 (World Health Organization, 2010). Hepatocellular carcinoma (HCC) is the fifth most common cancer and the third most common cause of cancer-related death, in which the recovery of HCC is low and therapies used to treat are rarely beneficial [1,2]. Cancer therapy generally combines surgery, multi-therapeutic agents and ionizing radiation which induce cell cycle arrest and cell death by apoptotic, non-apoptotic mechanisms (necrosis, senescence, autophagy and mitotic catastrophe) [3,4]. Major issues involves in conventional anticancer chemotherapy are the occurrence of side effects induced by normal and cancer cells [5] leads to complications of fatigue, pain, diarrhea, nausea, vomiting, and hair loss, gradual resistance of cancer cells against treatment. To overcome these problems cancer research focused in drug discovery has become a major goal by ethnomedical approach from natural sources. Among natural sources, plants have played an important role as a source of effective anticancer agents [6,7,8] with biologically active compounds. Biological compounds found in plant-derived dietary components exhibit anticarcinogenic activity. India referred to as the medicinal garden of the world, in which many plants have been used in traditional treatment of various malignancies from centuries. Studies have evaluated the biological activities of various phytochemicals produced from plants exert their anti-cancer activity through different mechanisms, including altered carcinogen metabolism, induction of DNA repair systems, immune activation, suppression of cell cycle progression and induction of apoptosis leads to cancer prevention [9,10]. Compared with the conventional cancer chemotherapy, the mixture of phytochemicals extracted from medicinal plants may have synergistic effect that targets several pathways responsible for cancer pathogenesis. This increases the therapeutic efficacy along with minimal side effects. In the present study, in vitro anticancer activity of *Gyrocarpus asiaticus* crude extrct has been investigated against HepG2 cell lines by morphology changes and DNA fragmentation induction.

MATERIALS & METHODS

Determination of *In-vitro* Assay of Anticancer Activity

Cell lines and culture

Human hepato cellular carcinoma cell lines (HepG2) that is liver cancer cell lines were procured from national centre for cell sciences (NCCS), Pune. Stock cells were cultured in dulbecco's

DOI: 10.21276/IJIPSR.2017.05.12.619

modified eagle's medium (DMEM) supplemented with 10% inactivated fetal bovine serum (FBS), penicillin, streptomycin in a humidified atmosphere with 70-80% confluency. After receiving cells, culture was trypsinized and grown in fresh media of t-culture flasks. The t- culture flask was then marked with the seeding date, cell line, and the passage number. This cell suspension was then transferred to a new t-culture flask and allowed for incubation in 5% CO_2 at 37°C.

Preparation of Test Solutions for cytotoxicity studies

Powdered leaves were extracted with methanol in soxhlet. Extracts were separately dissolved in 1% DMSO and volume was made with DMEM supplemented with 2% inactivated FBS to obtain a stock solution of 1 mg/ml concentration and sterilized by filtration which are used to carry out cytotoxic studies [11].

Cytotoxicity assay (MTT Assay)

The 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT) colorimetric assay was used to screen the cytotoxic activity of plant extract. The HepG2 cells were grown overnight on 96-well flat bottom cell culture plates, and were then exposed to different concentrations include 0.78, 1.56, 3.12, 6.25, 12.50, 25, 50, 100, 200, 300, 400, 500 and 1000 µg / ml of plant extract for 24hours. DMSO is used as negative control. 10µl MTT reagent prepared in 5.0 mg/ml phosphate buffered saline (PBS) was added to each well and incubated for 3 hours at 37°C. Finally, the medium with MTT solution was removed, and 200µl of DMSO were added to each well and further incubated for 20 minutes. The optical density (OD) of each well was measured at 560nm by using spectrophotometer. Results were generated from three independent experiments and each experiment was performed in triplicate. The cell viability ratio was calculated by the following formula,

Inhibitory ratio (%) = (OD control – OD treated) / OD control × 100.

Cytotoxicity was expressed as the concentration of extract inhibiting cell growth by 50% (IC50 value**)** [12].

Detection of Apoptosis by DAPI, DNA fragmentation assay

Detection of nuclear morphological changes using DAPI fluorescent dye staining assay

A DAPI staining assay was performed to identify the nuclear morphological changes. The cells were seeded in 6-well plates in the medium and incubated for 24hours. The cells were then treated at IC**50** concentration of crude extract of *Gyrocarpus asiaticus* for 24hours. Untreated cells were

DOI: 10.21276/IJIPSR.2017.05.12.619

used as controls which contains only the complete medium and cells. After treatment, the cells were washed with 1X PBS once and harvested using centrifugation at 4000 rpm at 4 °C for 5 minutes. The treated cells were fixed at −20°C for 10minutes with 50µl of methanol and water (1:1). The 100µl (1 µg/ml) of DAPI dye was added to the frozen cells and the mixture kept at 37°C for 30minutes for staining protected from light. Excess DAPI was then removed with the supernatant by centrifugation at 4000 rpm at 4°C for 5minutes and then observed under 40× magnification using fluorescent microscope [13,14,15].

DNA fragmentation detection using DNA laddering assay

DNA extraction: HepG2 Cells were seeded in t-25 flask containing in DMEM medium and incubated at 37°C in 5% CO_2. The treated and control cell samples were collected in to centrifuge tubes, washed with PBS and resuspended in EDTA buffer with pH 8.0 (10mM Tris, 1mM EDTA). Then they were incubated in ice for 30minutes with lysis buffer (pH 8.0) containing 20mM EDTA, 50mM Tris, 1% Sodium Dodecyl Sulfate (SDS).After incubation cells were treated with the IC50 doses of plant extract along with a control (untreated cells).Centrifuge cells at 12000 rpm at 4°C for 10 minutes and discard supernatant. To the pellet add 0.5 ml volume of ice- cold 1M NaCl, 0.7ml of ice-cold isopropanol and vortex vigorously. Allow precipitation to proceed overnight at - 20°C. After, precipitation, recover DNA by centrifugation at 2700 rpm and the pellet was washed with 70% alcohol and dissolved in TE buffer. The solution was then incubated at 37°C for 30minutes with ribonuclease A (RNase A). To the pellet containing DNA add 70% alcohol and finally dissolved in TE buffer.

Agarose gel electrophoresis: For casting 1% Agarose gel add 0. 8gm of agarose in 80ml of diluted 1X TBE buffer with the addition of 0.5mg/ml of ethidium bromide. The agarose mixture was poured into an electrophoresis chamber, and a gel comb was inserted to create wells for samples. Once solidified, the gel was transferred into a gel buffer tank. 5 μL of DNA 100 bp ladder used as marker, samples were carefully loaded into the wells, the electrophoresis was run at a constant ~50 volts until the dye front has reached 1-2 cm from the bottom of the gel. The gel was then examined through UV illumination for the detection of DNA fragments [16].

RESULTS & DISCUSSION

Evaluation of Anticancer activity of *Gyrocarpus asiaticus* crude extract

The IC50 value was graphically obtained by plotting the percentage of growth inhibition against different concentrations of the crude extract of *Gyrocarpus asiaticus* shown in Figure 1. A

DOI: 10.21276/IJIPSR.2017.05.12.619

decrease in the viable cells were observed with the increase in the concentration of the extract. There was a dose depended increase in the cytotoxic activity. The crude extract at low concentration (0.78µg/mL) showed 99.62476548 % cell viability and at high concentration (500µg/mL) 17.3650198 % cell viability. The extract shows IC50 value 242.50 µg/mL which corresponds to cytotoxicity of approximately 250 µg/ml concentration.

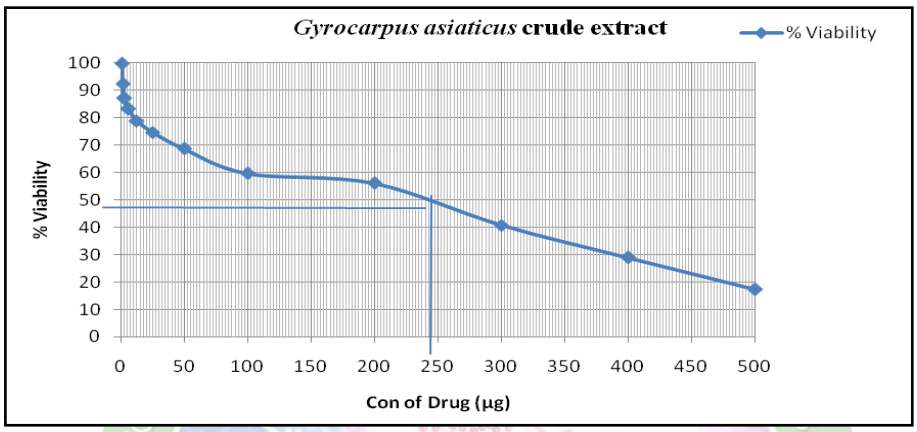
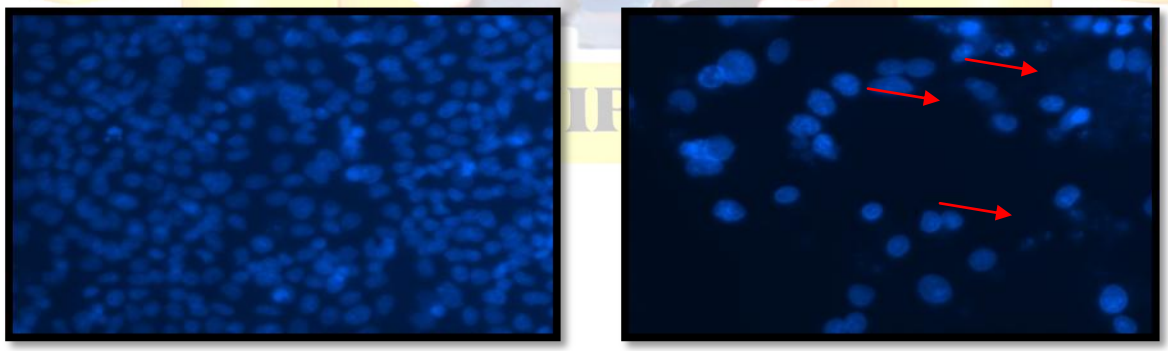


Fig.1: Cytotoxic activity of methanolic *Gyrocarpus asiaiticus* crude extract on HepG2 cell lines treated with different concentrations

DAPI fluorescent dye staining assay

Cells were stained with DAPI fluorescent dye is to assess apoptosis after treatment of cell lines with plant extract with IC50 concentration of *Gyrocarpus asiaticus* crude extract (250 µg/ml) observed under microscope. Plant extract were observed to cause alterations of nuclear morphology by cell shrinkage, membrane blebbing, and apoptotic bodies shown in Figure 2.



(a) (b)

Fig. 2 : Nuclear morphological change of HepG2 cells stained with DAPI fluorescent dye at 24 hours. a. Untreated HepG2 cells b. HepG2 cells treated with 250 µg/ml crude extract. Arrows indicate apoptotic nuclei by DNA condensation, apoptotic bodies.

DOI: 10.21276/IJIPSR.2017.05.12.619

DNA fragmentation

Plant extracts damage the genomic DNA shown in figure 3 indicating a clear smear which indicates extensive DNA fragmentation, whereas the control do not show smear of DNA.

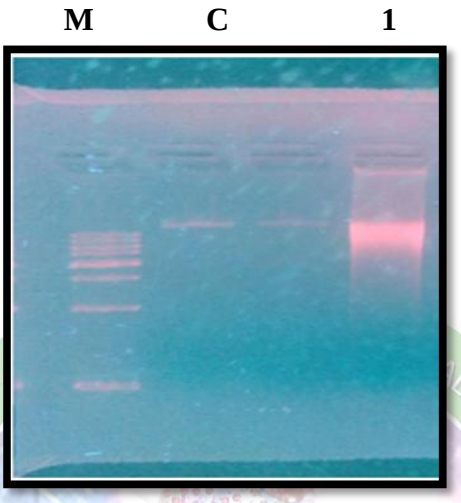


Fig.3: DNA fragmentation of *Gyrocarpus asiaiticus* crude extracts

M: DNAmarker ladder
C: Control
1: cell lines treated with *Alphonsea sclerocarpa* crude extract

Cancer treatment methods include surgery, chemotherapy and radiotherapy have serious side effects and complications of fatigue, pain, diarrhea, nausea, vomiting, and hair loss, gradual resistance of cancer cells against treatment. A great effort has been given towards the research on complementary and alternative medicine that deals with cancer treatment. Plant based formulations play an important role in cancer treatment. In the present study, *Gyrocarpus asiaticus* crude extract cytotoxicity was measured by which MTT assay, which is an a colorimetric approach used to determine cell growth and cell cytotoxicity, It measures cell membrane integrity by determining mitochondrial activity through enzymatic reaction of MTT to formazan.The amount of formazan crystals produced by MTT is directly proportional to the number of viable cells.% of viability was decreased as the concentration increases. Fragmentation induction is the hall mark of apoptosis. *Gyrocarpus asiaiticus* induces cyttoxicity by inducing apoptosis in HepG2 cell lines.

CONCLUSION

Results from the present study show a significant decrease in viability of HepG2 cell lines by dose dependent manner. Extract treated cells shows nuclear morphology changes and induces

DOI: 10.21276/IJIPSR.2017.05.12.619

DNA fragmentation. The DNA fragmentation is the separation or breaking of DNA strands into pieces which is a biochemical hallmark of apoptosis which is a direct or indirect outcome of cell death. The present study concludes that plant acting as an efficient anticancer source**.**

REFERENCES

1. Sheu JC. Molecular mechanism of hepatocarcinogenesis. Journal of Gastroenterology and Hepatology. 1997;12 : 309-13.
2. Thomas MB, Zhu AX. Hepatocellular carcinoma: the need for progress. Journal of Clinical Oncology. 2005;23:2892-2899.
3. Okada H, Mak TW. Pathways of apoptotic and non-apoptotic death in tumour cells. Nature reviews Cancer. 2004;4:592–603.
4. Brown JM, Attardi LD. The role of apoptosis in cancer development and treatment response. Nature reviews Cancer. 2005;5:231–237.
5. Gurung RL, Lim SN, Khaw AK, Soon JFF, Shenoy K . Thymoquinone induces telomere Shortening, DNA damage and apoptosis in human glioblastoma Cells. PLoS ONE. 2010; 5: e12124.
6. Butler MS. The role of natural product chemistry in drug discovery. Journal of Natural Products. 2004; 67: 2141–2153.
7. Li JWH, Vederas JC.Drug discovery and natural products: end of an era or an endless Frontier. Science. 2009; 325: 161–165.
8. Paterson I, Anderson EA.The renaissance of natural products as drug candidates. Science. 2005 ; 310: 451–453.
9. Sharif T, Alhosin M, Auger C, Minker C, Kim JH, Etienne-Selloum N, Bories P, Gronemeyer H, Lobstein A, Bronner C. Aronia melanocarpa juice induces a redox-sensitive p73-related caspase 3-dependent apoptosis in human leukemia cells. PLoS One. 2012 ; 7(3):e32526.
10. Ali R, Mirza Z, Ashraf GM, Kamal MA, Ansari SA, Damanhouri GA, Abuzenadah AM, Chaudhary AG, Sheikh IA: New anticancer agents: recent developments in tumor therapy. Anticancer. 2012: 32:2999–3005.
11. Muruganantham N, Solomon S, Senthamilselvi MM . Anti-cancer Activity of *Cucumis sativus* (Cucumber) flowers against human liver cancer. International journal of Pharmaceutucal and clinical research. 2016; 8(1): 39-41.

DOI: 10.21276/IJIPSR.2017.05.12.619

12. Ali S, Akra SN. Bio diesel production from Anabaena variabilis cyanobacterium. Indian journal of geo marine sciences. 2017; 46(2):385-390.

13. Pocasap P, Weerapreeyakul N, Barusrux S. Cancer preventive effect of thai rat-tailed radish (*Raphanus sativus* L. var. caudatus Alef). Journal of Functional Foods. 2014; 5(3):1372–1381.

14. Atale N, Gupta S, Yadav UCS, Rani V. Cell-death assessment by fluorescent and nonfluorescent cytosolic and nuclear staining techniques. Journal of Microscopy. 2014; 255(1):7–19.

15. Devika V, Mohandas S. Apoptotic induction of crude extract of Foeniculum vulgare extracts on cervical cancer cell lines. International Journal Of Current microbiology and Applied Sciences. 2014; 3(3): 657-661.

16. Mohd ZM, Zuraiza MZ, Fathilah AR. Apoptosis-inducing effect of three medicinal plants on oral cancer cells KB and ORL-48. The Scientific World Journal. 2014 : 1- 8.