ORIGINAL RESEARCH PAPER

Life Sciences

IN VITRO ANTICANCER ACTIVITY OF CARDIOSPERMUM HALICACABUM (LINN) AGAINST HEPATOCELLULAR CARCINOMA CELL LINE (HEP-G2).

KEY WORDS:
Cardiospermum halicacabum, Sapindaceae, Hep-G2, anticancer, cytotoxic

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ABSTRACT

Cardiospermum halicacabum is important medicinal plant which belongs to Sapindaceae family.In present study, ethanolic leaf extract of Cardiospermum halicacabum was examined for its anticancer activity against Hepatocellular carcinoma cell line (Hep-G2) using MTT assay. The leaf extract shows significant cytotoxic effect with 90% of inhibition when compared to standard drug.

INTRODUCTION

Cancer is considered as one of themost important health issue in both developed and developing countries(Shivkalim et.al.2020) Hepaticellular carcinoma of liver is third top reason for death in case of cancer patients (Rajesh and Sivakumari, 2020). It has proven that plant compounds have some active constituents which can help for prevention and cure of disease(Mohaddesi and Dudhrejiya, 2018) Plants are source of bioactive compounds, of which some may causes apoptosis in cancer cells under in vitro conditions.(Aishwarya et. al. 2014)

Cardiospermum halicacabum belongs to Sapindaceae family commonly known as balloon vine. It possess several medicinal properties such as antibacterial,anti diarrheal, antioxidant, anticancer and also used in treatment of rheumatism, nervous diseases,stiffness of limbs and snakebite.(Syed et. al. 2013, suresh et. al. 2013, Suresh, M. 2015). In present study attempts have been made to investigate the cytotoxic effect of ethanolic leaf extract of *Cardiospermum halicacabum* on hepatocellular carcinoma (Hep-G2) cell line.

MATERIALS AND METHODS

Plant sample

Healthy leaves of *Cardiospermum halicacabum* were collected from village Honali, District Latur, Maharashtra. The leaves and stems were cleaned, air dried in shade, coarsely powdered and stored in airtight container for further use.

Preparation of plant extract

Thirty gm of powder was extracted using ethanol as a solvent. The extraction was done by using soxhlet apparatus.The temperature was maintained 10^0-20^0 below boiling point of solvent. The time was fixed to 6hrs for each extraction. The solvent was then kept for evaporation at room temperature and concentrated to one fourth of its original volume and stored at 4^0C.

Screening for Anticancer activity

Materials

Hepatocellular Carcinoma cell line (Hep-G2) and DMEM with high glucose (Cat No-11965-092), FBS (Gibco, Invitrogen, Cat No -10270106), Antibiotic – Antimycotic 100X solution (Thermo fisher Scientific-Cat No-15240062) were used for the experiment.

MTT assay

The anticancer effect of crude extract was investigated against the Hep-G2 cancer cell lines using MTT assay described by VanMeerloo et. al., 2011,

Cells were incubated at a concentration of 1 × 104 cells/ml in culture medium for 24 h at 37°C and 5% CO2. Cells were seeded at a concentration (100µl/ml) 104 cells/well in 100µl/ml culture medium and 20, 40, 60, 80, 100mg of Samples into micro plates respectively (tissue culture grade, and 96 wells). Control wells were incubated with DMSO (0.2% in PBS) and cell line. All samples were incubated in triplicate. Controls were maintained to determine the control cell survival and the percentage of live cells after culture. Cell cultures were incubated for 24 h at 37°C and 5% CO2 in CO2 incubator (Thermo scientific BB150). After incubation, the medium was completely removed and Added 20µl/ml of MTT reagent (5mg/min PBS). After addition of MTT, cells incubated for 4 hours at 37oC in CO2incubator. Observed the wells for formazan crystal formation under microscope. The yellowish MTT was reduced to dark colored formazan by viable cells only. After removing the medium completely. Added 200□lof DMSO (kept for 10 min) and incubate at 370C (wrapped with aluminum foil). Triplicate samples were analyzed by measuring the absorbance of each sample by a micro plate reader (Benesphera E21) at a wavelength of 550 nm.

Percent cell inhibition was calculated as:[AT - AC / AC] × 100, where, AT and AC represented Absorbance of the treated and control samples respective.

RESULT AND DISCUSSION

The anticancer activity of ethanolic leaf extract was tested on Hep-G2 cell line in comparison with standard drug 5 fluro-uracil at concentration of 20 µg/ml, 40 µg/ml, 60 µg/ml, 80 µg/ml and 100 µg/ml. With increase in concentration the level of Cytotoxicity is also increases. Up-to 85.56% inhibition, after the addition of 100 µg/ml standard drug was reported. Whereas the leaf extract showed 52.04, 69.38, 86.73, 86.73 and 90.81 % inhibition at 20, 40, 60, 80 and 100µg/ml respectively in Hep-G2cell line.

Table 1 shows the effect of extract on Hep-G2 cell line and Figure 1 depicts the graphical representation of inhibition of the cell growth of cancer cell lines, treated with 20, 40, 60, 80 and 100µg extract/ml respectively. The Images of tested cancer cell lines have been presented in Figure 2.

Table 1 Effect of Ethanolic leaf extract of Cardiospermum halicacabum on Hep-G2 cell line.

Sample	Concentration (µg/ml)	%inhibition
Standerd	20	80.76
	40	81.79
	60	82.9
	80	84.97
	100	85.56
Sample	20	52.04
	40	69.38
	60	86.73

	80	86.73
	100	90.81

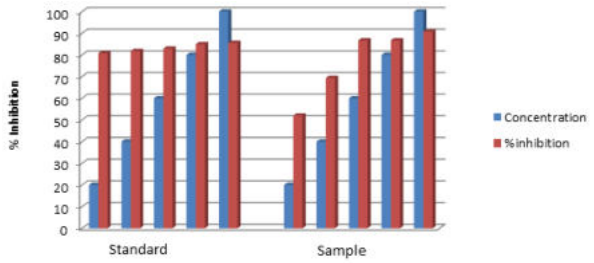
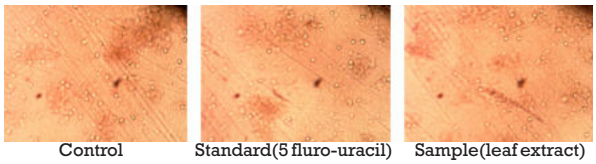


Fig.1 Graphical representation of Effect of ethanolic leaf extract of Cardiospermum halicacabum on proliferation of Hep-G2 cell line.



Control Standard(5 fluro-uracil) Sample(leaf extract)

Fig.2. Images of Hep-G2 Hepatocellular Carcinoma cell line under microscope after addition of test sample

MTT [3-(4,5-dimethylthiazol-2-yl)-2,5 diphenyltetrazolium bromide] assay is a colorimetric assay to evaluate cell viability. The Yellow coloured MTT is get reduced to insoluble, dark purple coloured formazan by mitochondrial succinate dehydrogenase enzyme. (Berridge, et. al., 2005; Suresh M. et. al. 2015).

In the present study, MTT assay was performed to check the anticancer activity of the ethanolic extracts of leaves of Cardiospermum halicacabum against Hepatocellular carcinoma cell line (Hep-G2). At a concentration of 100 µg/mL standard shows 85.56% inhibition while the extract shows remarkable inhibition i.e 90.81% as compared to standard drug.

According to Vaishnavi Sivakali and lakshmi Thangavelu (2020) Cardiospermum halicacabum extract shows cytotoxic effect against oral cancer cell line SCC25 . similarly S. Arunkumar et. al.2013 reported anticancer effect of aerial parts of *Cardiospermum halicacabum* extract.

CONCLUSION

The result of this study thus indicated that the *Cardiospermum halicacabum* leaf extract showed potential anticancer activity by causing 90% inhibition in the proliferation of Hepatocellular carcinoma cell(Hep-G2) cell line. Therefore it can be used in cancer management.

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