ISSN: 0976-3031

Available Online at http://www.recentscientific.com

CODEN: IJRSFP (USA)

International Journal of Recent Scientific Research
Vol. 9, Issue, 3(J), pp. 25328-25330, March, 2018

International Journal of Recent Scientific Research

DOI: 10.24327/IJRSR

Research Article

ANTICANCER ACTIVITY OF L-ASPARAGINASE FROM *ASPERGILLUS ORYZAE* AGAINST HEP G2 AND HELA CELL LINES

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DOI: http://dx.doi.org/10.24327/ijrsr.2018.0903.1838

ARTICLE INFO

Article History:

Received 14th December, 2017
Received in revised form 20th January, 2018
Accepted 4th February, 2018
Published online 28th March, 2018

Key Words:

L-asparaginase, *Aspergillus oryzae,* anticancer activity, MTT assay.

ABSTRACT

Cancer is a leading cause of death worldwide. In recently cancer caused 8.2 million deaths, and cancers of the lungs, liver, colon, stomach, and breast are main types. L-asparaginase belongs to the group of hydrolytic enzymes that is responsible for hydrolysis of L-asparagine into L-aspartic acid and ammonia. It had been used as an effective therapeutic agent against anticancer activity. In current study partially purified L-asparaginase enzyme from *Aspergillus oryzae* was tested *in vitro* anticancer activity by using MTT assay. To determine anticancer activity, different concentration of partially purified L-asparaginase was tested on Hep G2 and Hela cancer cell lines by 3-(4, 5-dimethylthiazol-2-yl)-2, 5-diphenyltetrazolium bromide (MTT) assay. The enzyme showed a significant antiproliferative activity and a dose dependent effect was observed. Minimum viability of 2.60% was shown by enzyme at concentration 0.1µg/ml and maximum viability (66%) was observed at 2µg/ml. From the result it is conclude that this L-asparaginase can be used for the development of new preparations for the therapy of tumors.

INTRODUCTION

Cancer is a leading cause of death worldwide. In 2012, cancer caused 8.2 million deaths, and cancers of the lungs, liver, colon, stomach, and breast are main types (Ferlay *et al.,* 2012). A hallmark of cancer is the rapid growth of abnormal cells that extend beyond their usual limits and invade adjoining parts of the body or spread to other organs, a process known as metastasis. Cancer treatment requires careful selection of one or more therapeutic modalities, such as surgery, radiotherapy, or chemotherapy. Despite progress in anticancer therapies, the chemotherapeutic drugs used in cancer treatment have the serious drawback of nonspecific toxicity. The incidence of cancer has been rising alarmingly for the last few decades. In India, cancers of oral cavity, oro-pharynx, oesophagus, stomach, rectum, colon and lung are commonly seen in men, whereas cancers of the cervix and breast commonly affect Indian women. In spite of technical advancements in the diagnosis and management, cancer still remains a major health care burden throughout the globe.

Many enzymes have been used as drugs like wise Lasparaginase (L-asparagine amidohydrolase) attracted much attention because of its anti carcinogenic potential. The important application of the L-asparaginase enzyme is in the treatment of acute lymphoblastic leukemia (mainly in children), Hodgkin disease, acute myelocytic leukemia, acute myelomonocytic leukemia, chronic lymphocytic leukemia, lymphosarcoma treatment, reticulosarcoma and melanosarcoma (Head and Behm, 1995). L-asparaginase belongs to an amidase group that hydrolyses the amide bond in L-asparagine to aspartic acid and ammonia. L-asparaginase is very essential amino acid for the growth of tumor cells whereas the growth of normal cell is independent of its requirement (Berenbaum *et al.,* 1970). It can be produced within the cell by an enzyme called Asparagine synthetase. Most of the normal tissue synthesizes L-asparagine in amounts for their metabolic needs but the tumour cells (especially Malignant and Carcinoma Cell) require external source of L-asparaginase for their growth and multiplication (Broome, 1963). In the presence of L-asparaginase, the tumor cells deprived of an important growth factor and they may failure to survive. Thus this enzyme can be used as a chemotherapeutic agent.

The development of L-asparagine as a therapeutic agent began in 1953 and today it is one of the most biotechnologically and biomedically important therapeutic enzymes accounting for about 40% of the total worldwide enzyme sales (Capizzi *et al.*,

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1970). L-asparagine is broadly distributed among the plants, animals and microorganisms.

Microbial enzymes have various application and used in various industries. L-asparaginase (L-asparagine amido hydrolase, E.C.3.5.1.1) has been widely used as therapeutic agent in the treatment of certain human cancers. It catalyses the conversion of L-asparagine to L-aspartate and ammonia and this catalytic reaction is irreversible under physiological conditions (Prakash *et al.,* 2007). The clinical action of this enzyme is ascribed to the reduction of L-asparagine, since tumour cells unable to synthesize this amino acid is selectively killed by L-asparagine deprivation (Prista and Kyriakidis, 2000). The present research work was carried out anticancer activity of L-asparaginase against Hep G2 and Hela cell lines.

MATERIAL AND METHODS

L-asparaginase enzyme

L-asparaginase enzyme produced by *Aspergillus oryzae* using orange peel as a substrate under submerged fermentation was used for the present study. The enzyme was partially purified and used for further study.

In vitro anticancer activity of L-asparaginase against human cervical Cancer cell (HeLa) and human liver cancer cell (HepG2)

Human cervical cancer cell (HeLa) and human liver cancer cell (HepG2) Cell lines were obtained from National centre for cell sciences Pune (NCCS). The cells were maintained in Minimal Essential Media supplemented with 10% FBS, penicillin (100 U/ml), amphotericin B 20µg and streptomycin (1mg/ml).

Antitumor assay

The cytotoxicity activity of samples on Hela cell and Hep G2 was determined by the MTT assay (Mosmann, 1983). Cells (1×10^5/well) were plated in 0.2 ml of medium/well in 96-well plates. Incubate at 5 % CO_2 incubator for 72 hours. Then, add various concentrations of the samples in 0.1% DMSO for 24hrs at 5 % CO_2 incubator. After removal of the sample solution and 20µl/well (5mg/ml) of 0.5% 3-(4,5-dimethyl-2-thiazolyl)-2,5-diphenyl-tetrazolium bromide (MTT) in phosphate- buffered saline solution was added. After 4hrs incubation, 1ml of DMSO was added. Viable cells were determined by the absorbance at 540nm. Measurements were performed and the concentration required for a 50% inhibition of viability (IC_{50}) was determined graphically. All experiments were performed in triplicate.

Statistical analysis

The results were expressed as mean ± standard deviation (S.D.). Chi square test was applied to the primary and secondary screening data. Statistical significance of MTT assay was analyzed by one way ANOVA.

RESULTS AND DISCUSSION

L-asparaginase is an important chemotherapeutic agent used to treat a variety of diseases of the lymphatic system, and lymphomas such as acute lymphoblastic leukemia (Husain *et al.,* 2016). L-asparaginase is the first enzyme with antitumor activity to be intensively studied in human beings. In our present study, result showed that MTT assay was used for the evaluation of *in vitro* anticancer activity of L-asparaginase against human cervical cancer cell (HeLa) and liver cancer cell (HepG2). L-asparaginase showed a good anticancer activity against HeLa and HepG2 with IC_{50} maximum cell viability of 66.0 IU/ml (Hela figure-1 and table-1) and 58.8 IU/ml (HepG2 figure-2 and table-2) respectively in comparison with control. The Viability of cell death was increased with increasing concentration of sample. Soniyamby Ambi Rani *et al.* (2012) reported that the L-asparaginase purified from *Aspergillus flavus* (KUFS20) have good activity against human cervical cancer cell (HeLa). And also evidence of the present work was reported by several researchers (Prista *et al.,* 2001; Maysa *et al.,* 2010; Elshafei *et al.,* 2012; Patro and Gupta, 2012; EL-sabbagh *et al.,* 2013; Shrivastava *et al.,* 2016) against human cervical cancer cell.

Table 1 Anticancer activity of *Aspergillus oryzae* producing L-Asparaginase against Hela cell line

S. No.	Concentration IU/ml	Absorbance 540nm	% cell Viability
1	0.10	0.72	66.0
2	0.25	0.57	49.5
3	0.50	0.35	30.4
4	1.00	0.18	15.6
5	1.50	0.08	7.82
6	2.00	0.03	2.60
7	Control cells	1.15	100

Values are expressed Mean ± Standard Deviation (M±SD); n = 6.

Table 2 Anticancer activity of *Aspergillus oryzae* producing L-Asparaginase against HepG2 cell line

S. No.	Concentration IU/ml	Absorbance 540nm	% cell Viability
1	0.1	0.63	58.8
2	0.25	0.49	45.7
3	0.5	0.25	23.3
4	1.0	0.08	7.4
5	1.5	0.03	2.8
6	2	0.00	0
8	Control cells	1.07	100

Values are expressed Mean ± Standard Deviation (M±SD); n = 6

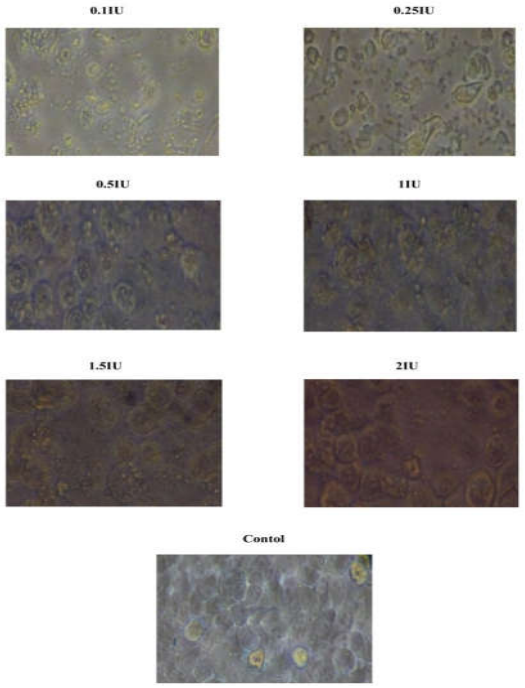
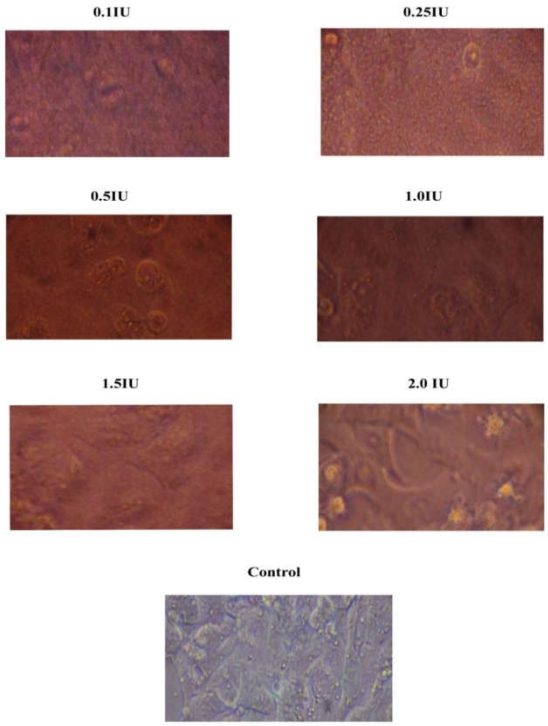


Figure 1 Anticancer activity of *Aspergillus oryzae* producing L-Asparaginase against Hela cell line



Figgure2 Anticancer activity of *Aspergillus oryzae* producing L-Asparaginase against Hep G2 cell line

CONCLUSION

The present study clearly indicated that the L-asparaginase purified from *Aspergillus oryzae* have a good activity against human cervical cancer cell (HeLa) and liver cancer (Hep G2). However, more detail investigation is required to characterize this microbial enzyme, which may be effectively used in the large scale production for commercial and pharmaceutical purpose in the future.

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How to cite this article:

Sudarkodi C and Sundar S.K.2018, Anticancer Activity of L-Asparaginase from Aspergillus Oryzae Against hep G2 And Hela Cell Lines. *Int J Recent Sci Res.* 9(3), pp. 25328-25330. DOI: http://dx.doi.org/10.24327/ijrsr.2018.0903.1838

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