IJISET - International Journal of Innovative Science, Engineering & Technology, Vol. 4 Issue 6, June 2017
ISSN (Online) 2348 – 7968 | Impact Factor (2016) – 5.264
www.ijiset.com

Anti Cancer Activity of *Moringa Oleifera* (Flowers) Against Human Liver Cancer

A. Rajeshkanna[1*], M. M. Senthamilselvi[2], D. Prabhakaran[3], S. Solomon[4], N. Muruganantham[5]

[1]Department of Chemistry, Ananda College, Devakottai, Tamil Nadu, India.

[2]Regional Joint Director of Collegiate Education, Tiruchirapalli , Tamil Nadu, India.

[3]Chettinad Cement Corporation Ltd., Ariyalur, Tamil Nadu, India.

[4]Department of Chemistry, Mother Terasa College of Engineering and Technology, Pudukottai, Tamil Nadu, India.

[5]Department of Chemistry, Roever Engineering College, Perambalur, Tamil Nadu, India.

ABSTRACT

Cancer is a public health problem all over the world. Medicinal herbs have been on the forefront whenever we talk about anticancer remedies, Herbal medicines have a vital role in the prevention and treatment of cancer. Large number of plants and their isolated constituents have been shown to potential anticancer activity. Our present investigation is focused on the anticancer activity of the compound isolated from the ethyl acetate fraction of flowers of *Moringa Oleifera* against human liver cancer HePG2 cell line by MTT assay using in-vitro method. The CTC_{50} value of the sample was 245.54 µg/ml against liver cancer HePG2 cell lines. Significant results were observed there by explaining the use of this plant in the traditional system of medicine.

Keywords: MTT assay, anticancer activity, *Moringa Oleifera*, Liver cancer HePG2

1. INTRODUCTION

Medicinal plants have various effects on living systems which include sedative, analgesic, antipyretic, cardioprotective, antibacterial, antiviral and antiprotozoal [1] . The specific constituents which impact medicinal values on the plants can be derived from whole or parts of the plant such as stems, leaves, fruits, flowers, seeds and roots [2]. The growing public interest and awareness in herbal medicine have led the pharmaceutical industry and biomedical researchers to give more attention on medicinal plants [3]. Cancer is one of the ailments which cannot be completely subdued by chemotherapy. The chemotherapeutic agents though effective against various types of tumor, they are not totally free from side effects [4]. Cancer is the leading cause of mortality worldwide. According to the cancer reports published by the World Health Organization (WHO) and

the World Cancer Research Fund, the incidence of cancer is still increasing especially due to diet, environment and carcinogenic virus infections [5-6]. *Moringa oleifera Lam.,* a member of the Moringaceae family also known as Drumstick or Horseradish-tree, is a Indian medicinal plant and has been reported as the significant sources of vitamins (A, B, C, E, riboflavin, nicotinic acid, folic acid, pyridoxine, ascorbic acid, beta carotene), iron, calcium and alpha tocopherol. Due to the importance of *M. oleifera* in traditional medicine, many investigations have previously reported on pharmacological properties such as antifertility, anti-inflammatory, antispasmodic, and diuretic activities [7-10].

2. MATERIALS AND METHODS

2.1 Collection of Flowers

Fresh flowers of *Moringa Oleifera* were collected from S. Pudur, Sivagangai (Dt), Tamil Nadu, India, during the month of January and identified by Dr.S.John Britto, Director, The rapinat Herbarium and Centre for Molecular Systematics (Authentication No. AR003 dated: 05/04/2017). St.Joseph's College (Campus), Trichy, Tamil Nadu, India.

2.2 Extraction and fractionation

Fresh flower (3 kgs) of *Moringa Oleifera* were extracted with 90% ethanol (5x500ml). The combined alcoholic extract was concentrated in vacuo and the aqueous extract was successively fractionated with petroleum ether (60-80^{0}C) (6x250ml), Peroxide free diethyl ether (4x250ml) and ethyl acetate (8x250ml). Petroleum ether fraction and diethyl ether fraction did not yield any isolable material. Ethyl acetate fraction on concentration yielded a dry powder which was dissolved in DMSO to get various concentrations and were used for further study.

3. MTT ASSAY METHOD

3.1 MTT-Assay-Chemicals

3-(4,5–dimethyl thiazol–2–yl)–5–diphenyl tetrazolium bromide (MTT), Fetal Bovine serum (FBS), Phosphate Buffered Saline (PBS), Dulbecco's Modified Eagle's Medium (DMEM) and Trypsin were obtained from Sigma Aldrich Co, St Louis, USA. EDTA, Glucose and antibiotics from Hi-Media Laboratories Ltd., Mumbai. Dimethyl Sulfoxide (DMSO) and propanol from E.Merck Ltd., Mumbai, India.

3.2 Cell Lines and Culture Medium

HePG2 (Liver cancer cell line) cell cultures were procured from National Centre for Cell Sciences (NCCS), Pune, India. Stock cells were cultured in Dulbecco's modified Eagle's medium (DMEM). Medium was supplemented with 10% inactivated Fetal Bovine Serum (FBS), penicillin (100 IU/ml), streptomycin (100 µg/ml) and amphotericin B (5 µg/ml) in an humidified atmosphere of 5% CO_2 at 37^0C until confluent. The cells were dissociated with TPVG solution (0.2% trypsin, 0.02% EDTA, 0.05% glucose in PBS). The stock cultures were grown in 25 cm^2 culture flasks and all experiments were carried out in 96 microtitre plates (Tarsons India Pvt. Ltd., Kolkata, India).

3.3 Preparation of Test Solutions

For cytotoxicity studies, each weighed test drugs were separately dissolved in distilled DMSO and volume was made up with DMEM supplemented with 2% inactivated FBS to obtain a stock solution of 1 mg/ml concentration and sterilized by filtration. Serially two fold dilutions were made from this for carrying out cytotoxic studies.

3.4 Determination of Cell Viability by MTT Assays

The monolayer cell culture was trypsinized and the cell count was adjusted to 1.0 x 10^5 cells/ml using medium containing 10% FBS and were used for the determination of cell viability by MTT assays as described by Francis and Rita (1986) respectively. The absorbance was measured using a microplate reader at a wavelength of 540 nm. The percentage growth inhibition was calculated by using the following formula and concentration of test drug needed to inhibit cell growth by 50% (CTC_{50}) values is generated from the dose-response curves for each cell line.

$$\% \text{ Growth inhibition} = 100 - \frac{\text{Mean OD of individual test group}}{\text{Mean OD of control group}} \times 100$$

4. RESULTS AND DISCUSSION

The different concentration of the compound isolated from the ethyl acetate fraction of *Moringa Oleifera* flowers were subjected for MTT assay and results are presented in table.1. The photographs (Fig. 1 to Fig. 5) show the effect of the compound on the human liver cancer HePG2 cell line. The sample concentrations of 1000µg/ml, 500 µg/ml, 250µg/ml, 125µg/ml and 62.5µg/ml show 72.34 µg/ml, 67.85 µg/ml,

52.37µg/ml, 48.69 µg/ml, 34.18 µg/ml CTC_{50} value against the human liver cancer HePG2 cell line respectively.

HePG2 cell line figures:

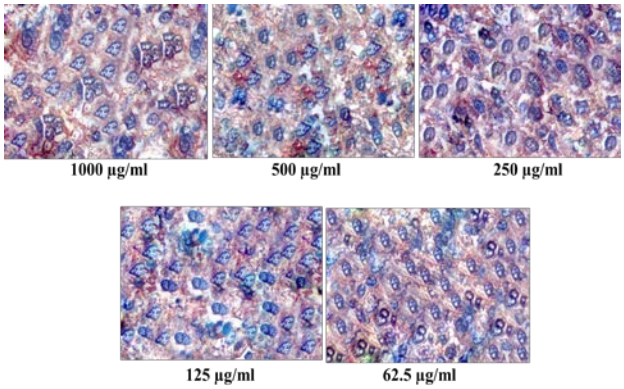
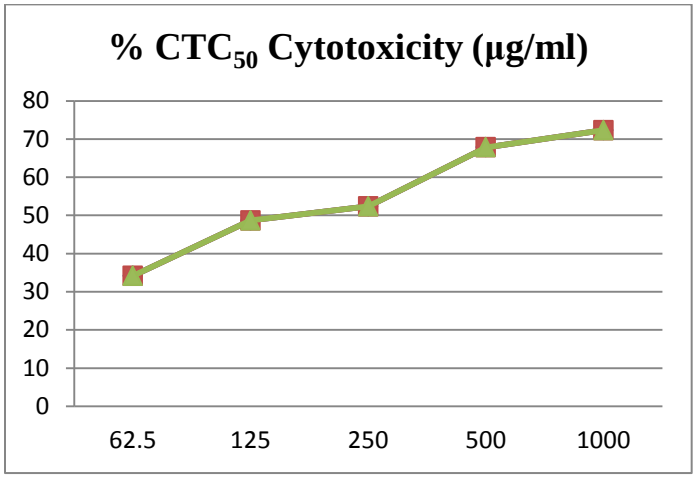


Fig: (1-5): Effect of the compound isolated from the ethyl acetate fraction of *Moringa Oleifera* flowers against human Liver cancer HePG2 Cell line in different concentrations

S. No	Concentration (µg/ml)	% CTC_{50} Cytotoxicity (µg/ml)	CTC_{50}
1	1000	72.34	245.54
2	500	67.85	
3	250	52.37	
4	125	48.69	
5	62.5	34.18	

Table.1: The CTC_{50} values of the compound isolated from the ethyl acetate fraction of *Moringa Oleifera* flowers against human Liver cancer HePG2 Cell line



Graphical representation of the CTC_{50} values of the compound isolated from the ethyl acetate fraction of *Moringa Oleifera* flowers against human Liver cancer HePG2 Cell line

5. CONCLUSION

The MTT assay of the compound isolated from the ethyl acetate fraction of flowers of *Moringa Oleifera* shows that all concentrations are having anticancer activity. So, it could be concluded that the compound has high anticancer potential.

6. REFERENCES

1. Olalaye, M.T., O.O. Adegboye and A.A. Akindahunsi, *Alchomea cordiforlia* extract protects Wister albino rats against acetaminophen-induced liver damage. Afr J. Biotchnol., 5(24), 2006, 2439-2445.

2. Attama, A.A., O.J. Okorooguand B.E. Onuigbo, Evaluation of the *in vitro* combined Antimicrobial activities of *Garcinia Kola*, Heckel and Honey. Bio Research, 7, 2009, 525-528.
3. Osinubi, A.A., O.G. Ajayi and A.E. Adesuyun, Evaluation of the anti-diabetic effect of aqueous leaf extract of *Tripinanthus butungil* in male Sprange Dawly rats. Medical Journal of Islamic World Academy of Science, 6(1), 2006, 41-47.
4. Christina AJ, Joseph DG, Packialakshmi M, Kothai R, Robert SJ, Chidambaranathan N *et al.* Anticarcinogenic activity of Withania somnifera Dunal against Dalton's ascetic lymphoma J Ethnopharmacol., 93:3, 2004, 59-361.
5. World Health Organization. World cancer report 2008, IARC,France. 2008.
6. World Cancer Research Fund / American Institute for Cancer Research. Food, physical activity, and the prevention of cancer: A global perspective, AICR, USA. 2007.
7. Fahey J.W, Moringa oleifera: a review of the medical evidence for its nutritional, therapeutic, and prophylactic properties. Part 1. Tree for Life Journal, 1, 2005, 5.
8. Shukla S, Mathur R, Prakash, A.O, Antifertility profile of the aqueous extract of Moringa oleifera roots. Journal of Ethnopharmacology 22, 1988, 51–62.
9. Dahot MU. Vitamin contents of the flowers and seeds of *Moringa oleifera*. Pak J Biochem, (1-2), 1988, 21- 24.
10. Caceres A, Saravia A, Rizzo S, Zabala L, De Leon E, Nave F, Pharmacologie properties of Moringa oleifera. 2: Screening for antispasmodic, anti-inflammatory and diuretic activity. Journal of Ethnopharmacology 36, 1992, 233–237.