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Studies on anticancer activity of ethanolic extract of Noni fruit (*Morinda citrifolia* L.)

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Keywords : *Morinda citrifolia,* Hep2 cells and HepG2 cells, MTT, SRB.

Abstract : Noni *(Morinda citrifolia* L.) is used in Indian system of medicine for treatment of a variety of diseases. This plant is enriched with flavanoids, anthroquinone and glycosides. The present work is to study the effect of ethanolic extract of *Morinda citrifolia* fruits (MCF-ET) on HepG2 (human liver cancer) cell culture and Hep2 (Human laryngeal epithelial carcinoma) cell culture respectively. Noni (*Morinda citrifolia* L.) fruits were collected from WNRF, Chennai were shade dried and extracted using ethanol to study its *in vitro* cytotoxicity activity against HepG2 (human liver cancer) cells and Hep2 (Human laryngeal epithelial carcinoma) cells using methods like MTT and SRB assay. Ethanolic extract of Noni fruits *Morinda citrifolia* L (MCF-ET) showed very potent cytotoxicity against HepG2 and Hep2 cells with CTC_{50} (cytotoxicity 50 %) values of 171 µg/ml and 181 µg/ml respectively. MCF-ET showed potent toxicity against two different human cancer cells from liver and laryngeal origin respectively. Hence this extract merits further investigation to screen its anti cancer activity using *in vitro* and *in vivo* models.

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Introduction

Noni (*Morinda citrifolia* L) is a versatile medicinal plant with a broad spectrum of pharmacological activities. *Morinda citrifolia* possesses hepatoprotective (Wang *et al.,* 2008a, b), anticancer (Akihisa *et al.,* 2008), immunomodulatory (Palu *et al.,* 2008), anti-inflammatory (Palu *et al.,* 2007), wound healing (Nayak *et al.,* 2007), antioxidant (Su *et al.,* 2005), anti-tubercular (Saludes *et al.,* 2002), wide spectrum of biological activities (Pawlus and Kinghorn., 2007) and anti-HIV (Umezawa *et al.,* 1992; Masakazu *et al.,* 2006; Bina *et al.,* 2007). Recently much attention was devoted for searching potential safe herbal medicines from natural products for the treatment of various diseases and M*orinda citrifolia* used for the treatment of a variety of diseases and safe herbal drug (West *et al.,*2006). The present work is to study the inhibitory activity of ethanolic extract of the fruit powder of M*orinda citrifolia* against Hep2 (Human laryngeal epithelial carcinoma) cells and HepG2 (Human liver cancer) cells.

Materials and Methods

Preparation of Extracts: The fruit powder of M*orinda citrifolia* is dried under shade and further powdered. The powder is extracted with ethanol for five days by cold maceration. It is then filtered to get the extracts evaporated to dryness under vacuum. The dried ethanolic extract (MCF-ET) is used for cytotoxicity studies in Hep2 (Human laryngeal epithelial carcinoma) cells and HepG2 (Human liver cancer) cells.

Preparation of suspensions

The ethanolic extract of Noni fruits (*Morinda citrifolia* L) was dissolved in DMSO and the volume was made up to 10ml with DMEM/MEM to obtain a stock solution of 1mg/ml concentration and stored at -20 °C prior to use. Further dilutions were made to obtain different concentrations ranging from 1000–62.5µg/ml with respective media and used for i*n vitro* investigations.

Cell lines and growth media

Hep2 (Human laryngeal epithelial carcinoma) cells and HepG2 (Human liver cancer) cells were cultured in MEM (minimum essential medium) and DMEM (Dulbecco's modified eagles medium) medium respectively. The medium also contains 10% fetal calf serum, penicillin (100 U) and streptomycin (100 µg).

In vitro cytotoxicity screening

The ability of the cells to survive a toxic insult is the basis of most cytotoxicity assays. The monolayer cell culture was trypsinized and the cell count was adjusted to $1.0x10^5$ cells/ml using medium containing 10% new born calf serum. To each well of the 96 well microtitre plate, 0.1ml of the diluted cell suspension (approximately 10,000 cells) was added. After 24 hours, when a partial monolayer was formed, the supernatant was flicked off, washed the monolayer once and 100ml of different drug concentrations was added to the cells in microtitre plates. The plates were then incubated at 37°C for 3 days in 5% CO_2 atmosphere, and microscopic examination was carried out and observations recorded every 24 hours. After 72 hours, the drug solutions in the wells were discarded and MTT (Francis D and Rita L., 1986.) and SRB (Philip *et al.,* 1990) assays performed.

Morphological observation by acridine orange staining

Staining cells with fluorescent dyes, such as acridine orange is used in evaluating the nuclear morphology of apoptotic cells. To confirm that apoptosis have been induced by *Morinda citrifolia* (MCF-ET) plant extract, HepG2 cells were analysed in the presence of acridine orange (AO). Acridine orange (AO) is a vital dye that will stain both live and dead cells (Javadev et al., 2004). Two different concentrations were chosen based on the IC_{50} values determined by MTT assay, which were 100 and 200 ìg/ml. As a control, HepG2 cells were cultured in complete media and stained with AO. Cells stained green

represent viable cells, whereas yellow staining represented early apoptotic cells, and reddish or orange staining represents late apoptotic cells. As shown in Figure 1 HepG2 cells treated with 100 and 200 ìg/ml of MCF-ET showed changes in cellular morphology, including chromatin condensation, membrane blebbing and fragmented nuclei. Our result clearly shows that the *Morinda citrifolia* (MCF-ET) induced apoptosis after 48 hours incubation at both the concentration of plant extract tested.

Results

Ethanolic extract of Noni fruits (*Morinda citrifolia* L) (MCF-ET) showed very potent cytotoxicity against HepG2 and Hep2 cells (Table 1&2) with CTC 50 (cytotoxicity 50 %) values of 171 µg/ml and 180 µg/ml respectively. MCF-ET showed potent toxicity against two different human cancer cells from liver and laryngeal origin respectively. Hence this extract merits further investigation to screen its anti cancer activity using *in vivo* models. It was evident from nuclear morphology studies that *Morinda citrifolia* (MCF-ET) showed nuclear morphology changes (Fig. 1) similar to that of apoptotic cell morphology in cancerous cell culture HepG2 (Human liver cancer cells). In normal cell culture tested, here was no such nuclear morphological change. This *in vitro* study has proved the selective toxicity *Morinda citrifolia* (MCF-ET) against cancer cells. Hence this work can be taken up for *in vivo* and pre clinical studies.

Table: 1 Determination of CTC $_{50}$ by using MTT and SRB assay in HepG2 (human liver cancer) cell cultures

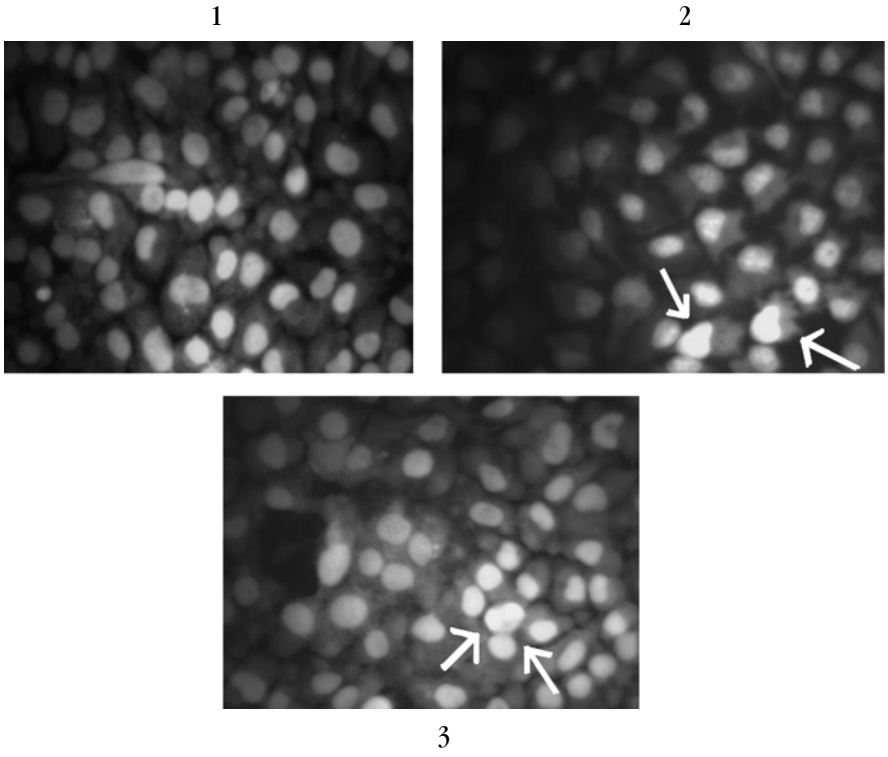
Extracts	CTC $_{50}$ in (µg/ml)	
	MTT	SRB
MCF-ET	163 ± 1.15	171.3 ± 1.66

Table: 2 Determination of CTC $_{50}$ by using MTT and SRB assay in Hep2 (Human laryngeal epithelial carcinoma) cell cultures

Extracts	CTC $_{50}$ in (µg/ml)	
	MTT	SRB
MCF-ET	180.5 ± 2.12	182.5 ± 2.11

Average of six determinations, values are mean $\pm$ SER

Figure 1 : Nuclear staining using acridine orange. 1) Normal HepG2 cells; 2) HepG2 cells + MCF-ET (100 ìg/ml) treated. 3) HepG2 cells + MCF-ET (200 ìg/ml) treated. Arrows indicate mem brane blebbing.



Discussion

The Polynesians utilized the whole Noni plant in various combinations for herbal remedies (Wang *et al.,* 2002, McClatchey W. 2002) like arthritis, diabetes, high blood pressure, muscle aches and pains, menstrual difficulties, headaches, heart disease, AIDS, cancers, gastric ulcers, sprains, mental depression, senility, poor digestion, atherosclerosis, blood vessel problems and drug addiction. Noni possessed wide spectrum of anticancer activity. From there studies, ethanolic extract of noni fruit was screened for anticancer activity. Ethanolic extract of noni fruit showed potent toxicity against two different human cancer cells from liver and laryngeal origin respectively. This extract merits further investigation to screen its anti-cancer activity using *in vivo* models and further studies regarding isolation of active constituents from ethanolic extract under way. Our result clearly shows that the *Morinda citrifolia* (MCF-ET) induced apoptosis after 48 hours incubation at both the concentration of plant extract tested.

References

Akihisa T, Matsumoto K, Tokuda H, Yasukawa K, Seino K.I, Nakamoto K, H. Kuninaga H, Suzuki T and Kimura Y.2008. Anti-Inflammatory and Potential Cancer Chemopreventive Constituents of the Fruits of *Morinda Citrifolia*, *J. Nat.Prod.*, 71(7): 1322-25.

Bina S. Siddiqui, Fouzia A. Sattar, Sabira Begum, Tahsin Gulzar, and Fayaz Ahmad. 2007. Chemical Constituents from the Stems of *Morinda citrifolia* Linn, *Arch Pharm Res.,* 30 (7): 793-798,

Francis D and Rita L., 1986. Rapid colorimetric assay for cell growth and survival: modifications to the tetrazolium dye procedure giving improved sensitivity and realiability. *Journal of Immunological Methods.,* 89, 271-277.

Jayadev R, Jagan MRP, Malisetty VS, Chinthapally VR: Diosgenin, a steroid saponin of Trigonella foenum graecum (Fenugreek), inhibits Azoxymethane-induced aberrant crypt foci formation in F344 rats and induces apoptosis in HT-29 human colon cancer cells. *Cancer Epidemiol Biomarkers and Prev.,* 2004, 13(8):1392-1398.

Masakazu Kamata, Raymond P. Wu, Dong Sung Anc, Jonathan P. Saxe, Robert Damoiseauxd, Michael E. Phelps, Jing Huang, and Irvin S.Y. Chena.2006. Cell-Based. Chemical Genetic Screen Identifies Damnacanthal as an Inhibitor of HIV-1 Vpr Induced Cell Death, *Biochem Biophys Res Commun*., 348(3): 1101–1106.

McClatchey W. 2002. From Polynesian healers to health food stores: changing perspectives of *Morinda citrifolia* (Rubiaceae). *Integr Cancer Ther.,* 1:110-20.

Nayak B.S, Isitor G.N, Maxwell N.A, Bhogadi V and Ramdath D. 2007. Wound-Healing Activity of *Morinda Citrifolia* Fruit Juice on Diabetes-Induced Rats. *J.Wound Care*., 16(2):83-7.

Palu A K,. Kim A.H, West B.J, Deng S, Jensen J and White L. 2007. Anti-Inflammatory and Potential Cancer Chemopreventive Constituents of the Fruits of *Morinda Citrifolia* (Noni), *J. Nat. Prod*., 70(5): 754-60.

Palu A K, Kim A.H, West B.J, Deng S, Jensen J and White L. 2008. The Effects of *Morinda Citrifolia* L. (Noni) on the Immune System, Its Molecular Mechanisms of Action, *J. Ethnopharmacol.,* 115(3): 502-7.

Pawlus A. D and Kinghorn D. 2007. A Review of the Ethnobotany, Chemistry, Biological Activity and Safety of the Botanical Dietary Supplement *Morinda citrifolia* (Noni). *J Pharm. Pharmacol*., 59(12): 1587-92.

Philip S., Rista S., Dominic S., Anne M., James., David V., Jonathan T.W., Heidi B., Susan K. and Michale R.B., 1990. New colorimetric cytotoxic assay for anti cancer drug screening. *Journal of National Cancer Instituite.,* 82, 1107-1112.

Saludes J.P, Garson M.J and Aguinaldo A.M. 2002. Antitubercular Constituents from the Hexane Fraction of *Morinda citrifolia* Linn. (Rubiaceae), *Phytother Res*., 16(7):683-8.

Su B.N, Pawlus A.D, Jung H.A, Keller W J, Mclaughlin J.L and Kinghorn A.D 2005. Chemical Constituents of the Fruits of *Morinda Citrifolia* (Noni) and their Antioxidant Activity, *J. Nat. Prod.,* 68(4): 592-8.

Umezawa K. 1992. Isolation of 1-methoxy-2-foremyl-3-hydroxyanthraquinone from *Morinda citrifolia* and neoplasm inhibitors containing the same. Japan Kokai Tokyo Koho JP 06 87, 736 (94-87, 736) Appl 1992; 92/264, 311 07.

Wang M.Y, Anderson G, Nowicki D and Jensen J. 2008a. Hepatic Protection by Noni Fruit Juice against CCl_4-Induced Chronic Liver Damage in Female SD Rats, *Plant Foods Hum. Nutr*., (3), 141-47.

Wang M.Y, Nowicki D, Anderson G, Jensen J and West B. 2008b. Liver Protective Effects of *Morinda Citrifolia* (Noni), *Plant Foods Hum. Nutr*., 63(2): 59-63.

Wang M.Y, West B.J, Jensen C.J, Nowicki D, Su C, Palu A.K, Anderson G . 2002. *Morinda citrifolia* (Noni): a literature review and recent advances in Noni research. *Acta Pharmacol Sin*., 23:1127-41.

West B.J, Jensen C.J and Westendorf J, 2006. Noni Juice is Not Hepatotoxic, World *J. Gastroenterol*., 12(22): 3616-20.