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REGULAR ARTICLE

CYTOTOXIC, ANTICANCER STUDIES OF RUTIN, ASCORBIC ACID, MENINDIONE COMBINATION ON CELL LINES

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ABSTRACT

Rutin is a flavanoid with many biological activities including antiallergic, anti-inflammatory, anti-proliferative and ant carcinogenic properties. Rutin along with Vitamin C and K combination are used as neutraceuticals in the treatment of Cancer, Haemorrhoids, Vit K deficiency, varicose veins, Wound healing, Scurvy e. t. c. The present study evaluates the anticancer effect of Rutin, Ascorbic acid and Menindione (RAM) combination against two cell lines, one cancer and one normal cell line using Hep G2 (Human liver carcinoma cells) and L929 (fibroblast) cells by MTT assay. Morphological changes after exposure with RAM were studied under phase contrast microscope in a dose dependent manner. RAM shows an IC_{50} value of 47.99 µg/ml against Hep G2 and 23.612 against L929 respectively. This shows that the combination is more cytotoxic than promising anticancer activity which is contrary to the beneficial effects claimed by these formulations.

Keywords: MTT, L929, Hepg2, Rutin, Ascorbic acid, Menindione

INTRODUCTION

The term neutraceuticals are derived from two words" Nutrient" and pharmaceuticals. They differ from dietary supplements as they help in disease prevention and treatment [1,2]. Rutin is a flavanoid and is having many biological activities including antiallergic, anti-inflammatory, antiproliferative and anticarcinogenic properties. It is of natural origin and present in many fruits and vegetables [3]. Rutin along with Ascorbic acid (Vitamin C) and Menindione (Vitamin K) abbreviated as RAM, are used as neutraceuticals in the treatment of Cancer, Haemorrhoids, Vitamin K deficiency, Varicose veins, Wound healing, Scurvy e. t. c.

There is not much data available regarding the cytoprotective or potential anticancer benefits of this combination. The present study evaluates the anti cancer effect of this combination in HepG2 and LN29 Cell lines.

MATERIALS AND METHODS

In vitro cytotoxic and antiproliferative activity of RAM against cultured L929 and HepG2 cell

Cell lines

HepG2 (Liver hepato cellular carcinoma) and L929 (Murine Fibroblast cell) were procured from National centre for cell sciences (NCCS), Pune, India. The cells were maintained in Dulbecos modified Eagles medium (DMEM, Gibco, Invitrogen) which was supplemented with 10% FBS, L glutamine, sodium bicarbonate and antibiotic solution containing penicillin (100U/ml), Streptomycin (100U/ml) and amphotercin B (2.5 µg/ml) and kept at 37 °C in a humidified 5% CO_2 incubator (NBS Eppendorf, Germany) and 95% air. The relative humidity was maintained as 100%. The cells were sub cultured once they attained a confluency of 75-80%.

MTT assay [4, 5]

Cytotoxic and anti proliferative effect of RAM was determined against L929 and Hep G2 cell lines by MTT assay. The trypnized cells were suspended in 10% growth medium,100 microlitre cell suspension ($5x10^4$ cells/well) was seeded in 96 well tissue culture plate. It was then incubated at 37 ° C in a humidified 5% CO_2 incubator.1 mg of RAM (50:25:5) was mixed in 1 ml of DMEM and mixed using a cyclomixer. It was then sterilized using a Millipore filter of 0.22 µm. After 24 h the growth medium was removed, freshly prepared compound RAM in the ratio 50:25:5 in 5% DMEM were five times serially diluted by two fold dilution (100µg, 50µg, 25µg, 12.5µg, 6.25µg in 100 µl of 5% MEM) and each concentration of 100 µl were added in triplicates to the respective wells and incubated at 37°C in a humidified 5% CO_2 incubator for 24 h. The viability of cells was determined by MTT assay method

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Morphological observation [6]

Morphological observation was done by inverted phase contrast microscope to identify changes induced by the RAM to the cells like membrane shrinkage, presence of apoptic bodies, membrane blubbing, and presence of condensed nuclei e. t. c.

RESULTS

MTT assay

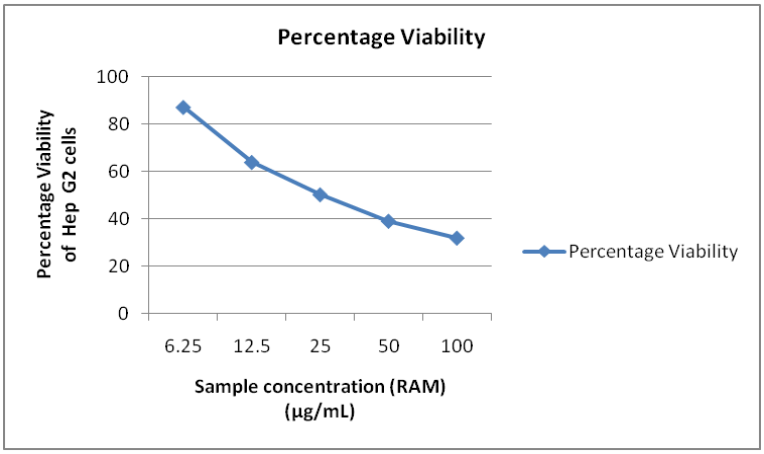

Fig. 1: Anticancer activity of RAM against Hep G2 cell lines

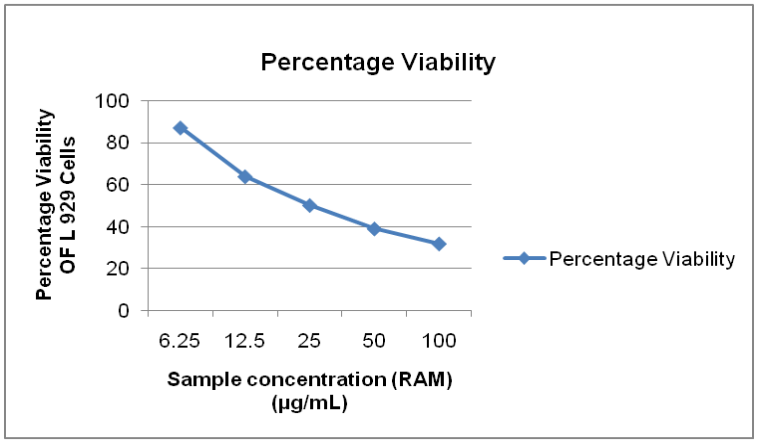

Fig. 2: Cytotoxic activity of RAM against L929 cell lines

Morphological observation

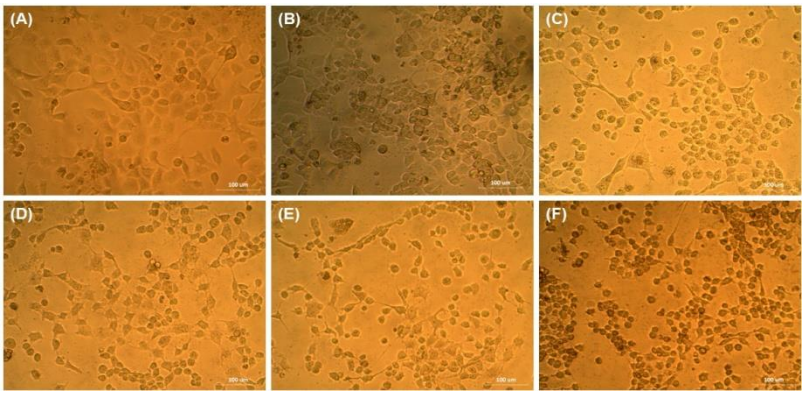

Fig. 3: Determination of *in vitro* anticancer effect of RAM on cultured HepG2 cells (Magnification 10X). (A) Untreated control cells (B) 6.25 µg/ml (C) 12.5 µg/ml (D) 25 µg/ml (E) 50 µg/ml (F) 100 µg/ml. Photomicrographs captured by Olympus CKX41 connected with Optika Pro5 CCD camera. Phase contrast analysis evidenced that the cells treated with the sample at different concentrations showing apoptotic bodies, blebbing, cell shrinkage and condensed nuclei

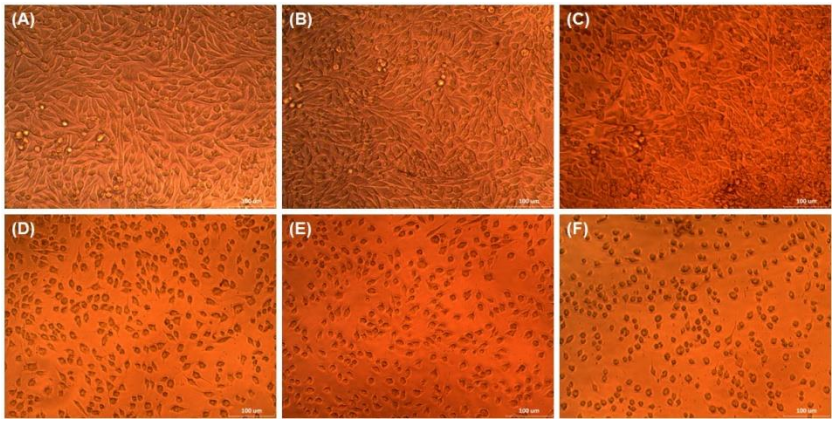

Fig. 4: Determination of *in vitro* cytotoxic effect of RAM on cultured L929 cells (Magnification 10X). (A) Untreated control cells (B) 6.25 µg/ml (C) 12.5 µg/ml (D) 25 µg/ml (E) 50 µg/ml (F) 100 µg/ml. Photomicrographs captured by Olympus CKX41 connected with Optika Pro5 CCD camera. Phase contrast analysis evidenced that the cells treated with the sample at different concentrations showing apoptotic bodies, cell shrinkage and condensed nuclei

DISCUSSION

Hepatic carcinoma accounts for 90% of liver cancers. It is one of the most common cancers in the world with high mortality rate [7]. Cytotoxic and anti proliferative studies using cell lines give relevant information about the anticancer potential of phytopharmaceuticals in this study we had selected RAM, a combination of Rutin which is flavanoid quarcetin and disaccharide rutinose along with Ascorbic acid (vitamin C) and Menindione (Vit k) which is available in the market as neutraceutical, claim to have many health benefits including anti cancer properties. The anti cancer property of rutin was reported in some literatures [8]. The requirement of anti cancer drug candidates is that they should kill the proliferarating cancer cells without affecting the normal cells. In the present study anticancer activity of RAM evaluated by MTT assay method against Hep G2 (Hepatic carcinoma cells) and L929 (Fibroblast) cell lines. Hep G2 are useful as a model system *in vitro* for human hepatic cancer cells. L929 is the representative of normal cell. The MIC values are found to be 47.99 against Hep G2 and 23.61 against L929. This indicates a certain degree of cytotoxicity against normal cell lines as per the guidelines set by national cancer institute of America [9]. Rutin exerts its anticancer effect by inducing apoptosis by arresting the G0/G1 phase in the cell cycle. This cell cycle arrest can be attributed by acting upon Bcl-2 family proteins which regulates all major types of cell death. The low MIC value of RAM towards L929 indicates that the tested drug is more cytotoxic towards normal cell lines. In this aspect the tested drug combination didn't show promising results against the claim. However, this study provides only a basic data and studies like genotoxicity etc can provide more insight in this regard [10].

CONCLUSION

The present study concluded that the tested combination of RAM was found to more cytotoxic towards normal cell lines L929 than Hepatic carcinoma cell line Hep G2.

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