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Original Article

IN VITRO CYTOTOXIC ACTIVITY OF RUTIN AND PIPERINE AND THEIR SYNERGISTIC EFFECT ON HEP G2 AND HEP 3B CELL LINES

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ABSTRACT

The objective of the present study was to evaluate the cytotoxic activities of Rutin and Piperine and their synergistic effect on Hep G2 and Hep 3B cell lines. Cytotoxic activity of Rutin, Piperine and the combination of both was confirmed by the consideration of IC_{50} by MTT assay. The present study showed that combination of Rutin and piperine showed enhanced cytotoxic activity on tested Hepatocellular carcinoma cell lines (Hep G2 and Hep 3B) with IC_{50} values 30 µg/ml and 20 µg/ml respectively, compared with individual cytotoxic effect of Rutin and Piperine. Therefore the present study revealed that all samples, i.e. Rutin and Piperine and combination of both rutin and piperine showed potent cytotoxic and anti proliferation effect however the combination of Rutin and Piperine showed enhanced cytotoxic effect compared with individuals.

Introduction:

Natural resources,for example plants, microbe's, vertebrates and invertebrates are an indispensable source of bioactive compounds.[1] Hepatocellular carcinoma (HCC) also called malignant hepatoma, which is the most common primary liver cancer, accounts as the third leading cause of deaths worldwide after lung and stomach cancer.[2]A large number of drugs have been developed in medicinal practice from natural phytocontituents. At most 60 % of anticancer drugs are of natural origin, such as plant (Vincristine) and microorganisms (Doxorubicin, mitomycine).[3] Flavonoids are the polyphenolic compounds act as the major nutritional constituents of plant based food. Rutin is a common dietary flavonoid with a wide range of pharmacological activities which is present in many of plants such as citrus fruits, apple, tea, onion and red wine.[4-6]An alkaloid, piperine is used widely as an excellent remedy for the treatment of respiratory tract infection, sleeping problem, menstrual pain, chronic gut related pain and arthritic conditions.

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Beside these other reported useful effects as diuretic, central nervous system depression and alleviation of Anxiety. [7-8] Hep G2, & Hep3B, which are easy to handle and retain many of the morphological characteristics of liver parenchymal cells and certain several enzymes responsible for activation of various xenobiotics. MTT assay is a sensitive, Quantitative and trustworthy colorimetric assay that measures viability, proliferation and activation of the cells. The assay is based on conversion of the yellow water soluble substrate 3-(4, 5-dimethylthiazole-2-yl) 2, 5-diphenyl tetrazolium bromide (MTT) into a dark blue formazan product, which is insoluble in water due to the capacity of mitochondrial dehydrogenase enzymes in living cells.[9-10]

Viable cell are able to reduce the yellow MTT under tetrazolium ring cleavage to a water insoluble purple blue complexation, which precipitate in the cellular cytosol and can be dissolved after cell lysis, whereas cells being dead by a toxic damage, cannot transform MTT, this formation production is proportionate to the viable cell number and inversely proportional to the amount of cytotoxicity. The reaction is arbitrated by dehydrogenase enzyme associated with the endoplasmatic reticulum and the mitochondria.[11] However, many chemotherapeutic drugs are presently placed in a difficulty of reduced

therapeutic effect due to the problem of drug resistance. Chemotherapeutic drugs also possess toxicity to normal cells, which in turn causes the unpleasant side effects to the patients. For these reasons, research and development of new classes of anticancer agents, which exhibit efficient and selective toxicity in tumour cells is enticing increased attention.[12]

Materials & Methods:

Rutin, Piperine& MTT were procured from Sigma Aldrich Pvt. Ltd (Bangalore). HepG 2 and Hep 3B cell lines were purchased from National Centre for Cell Science (NCSS), Pune. All other chemicals purchased from S.D fine chemicals Ltd. Mumbai. All reagents were of analytical grade and all were subjected for screening for their genuinity, prior to use.

Methods:

Preparation of cell culture and stock drug:[13]

The cell lines used for the study were Hep G2 and Hep 3B were maintained in 96 wells micro titter plate containing Minimal essential medium (MEM), supplemented with 10 % heat inactivated fetal calf serum (FCS) containing 5 % of mixture of gentamicin (10 µg/ml), Penicillin (100 units/ml) and streptomycin (100 µg/ml) in presence of 5 % CO_2 at 37^0C for 48-72 hours. In brief, 10 mg of Rutin, Piperine and combination (1:1) were prepared in 1 ml Dimethyl sulfoxide (DMSO) as a stock, from this stock 10, 20, 25, 30, 50 µl were transferred into the wells containing 100 µl of medium.

MTT Assay:

The anticancer activity of sample on Hep G2 and Hep 3B cells were determined by (3-(4,5-dimethylthiazole-2-yl)2,5-diphenyl tetrazolium bromide (MTT) assay was used to assess the cytotoxicity[11]. *In vitro* growth inhibition effect of test compound was assessed by colorimetric determination of conversion of MTT into formazan blue by living cells. Cells (1 x 10^5/wells) were plated in 0.2 ml of medium/well in 96 well plates. For MTT assay supernatant was removed and fresh MEM solution was added and wells were treated with different concentration of samples (10, 20, 25, 30, 50 µl/ml) from the stock (10 mg/ml) were added to respective wells containing 100 µl of MEM medium, so that the final concentrations were 10, 20, 25, 30 & 50 µg/ml. incubated the plates for 48 hours at 37^0Cin a humidified atmosphere of 5% CO_2 .

After incubation,the MTT stock was added to each well (20 µl, 5mg/ml in sterile PBS). Then the plates were further incubated 4 hours, for cytotoxicity. Later the supernatant carefully aspirated and the precipitated crystals of Formazan blue were solubilised by adding 1 ml DMSO and left for 45 sec. presence of viable cells was visualized by the development of purple colour due to formation of formazan crystals. Concentration of Formazan was determined on a microplate reader (ELISA plus) at 570 nm. The Cell viability was calculated using DMSO treated cells as the 100 % viable control. The results represents the mean of five readings

$$Cell\ viability\ (\%) = \frac{Mean\ OD\ of\ test}{Mean\ OD\ of\ Control} \times 100$$

Results:

The cytotoxic activity of Rutin and Piperine and combinational effect of Rutin and Piperine against Hep G2 and Hep3B were determined by the MTT assay. The results of cytotoxic assay on Hep G2 & Hep 3B are presented in Table.1 & 2 respectively. Rutin, Piperine & Combinational sample of Rutin and Piperine were compared, which was described in the above tables, It suggested that the combined effect of Rutin and Piperine showed considerable additive effect, when compare with individual samples of Rutin and Piperine.

Discussion:

However, with the advancement of scientific technology and sophisticated instrumentation, we have now enormous chemical molecules having direct action on cellular and molecular level have been proved to be an effective on oncogenic cells without affecting adjacent cells and organs, with the outcome of the usage of natural and synthetic drugs in the treatment area of cancer. In this present research study individual Rutin and Piperine showed a potent cytotoxic effect on Hep G2 and Hep 3B cell lines. Then, on combination of Rutin and Piperine shows increased cytotoxic effect even when it is used in minimal quantity (20µg/ml) as per our study. Numerous studies demonstrated that piperine has shown best chemotherapeutic effect against breast and colon cancer cell lines[14] and rutin also proven as a best therapeutic agent against different cancer like Nuroblastoma, breast cancer cell lines.[15] however in this study piperine and rutin showed potent chemotherapeutic effect on HCC lines. And a combination of Rutin along with piperine showed an increased cytotoxic effect, this is may be bio enhancing activity of piperine.

S.No.	Sample	Concentration(µg/ml)	Results as observed	IC50
1.	Rutin	10 µg	No lysis	50 µg
2.		20µg	No lysis	
3.		25 µg	No lysis	
4.		30 µg	No lysis	
5.		50 µg	50% lysis	
6.	Piperine	10 µg	No lysis	50 µg
7.		20 µg	No lysis	
8.		25 µg	No lysis	
9.		30 µg	Partial lysis	
10.		50 µg	50% lysis	
12.	Combination of Rutin & Piperine	10 µg	No lysis	30 µg
13.		20 µg	No lysis	
14.		25 µg	No lysis	
15.		30 µg	50% lysis	
16.		50 µg	50% lysis	
17.	control		No lysis	

Table 1: IC50 values of Rutin and Piperine and their combination on Hep G2 Cell lines

S.No.	Sample	Concentration (µg/ml)	Results as observed	IC50 (µg)
1.	Rutin	10	No lysis	50 µg
2.		20	No lysis	
3.		25	25% lysis	
4.		30	25% lysis	
5.		50	50% lysis	
	Piperine			25 µg
6		10	25% lysis	
7		20	25% lysis	
8		25	50% lysis	
9		30	100% lysis	
10		50	100% lysis	
11	Combination of Rutin & Piperine	10	25% lysis	20 µg
12		20	50% lysis	
13		25	50% lysis	
14		30	50% lysis	
15		50	100% lysis	
			No lysis	
	control		No lysis	

Table 2. IC50 values of Rutin and Piperine and their combination on Hep 3B Cell lines

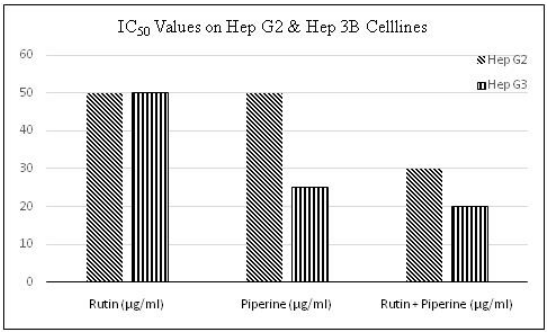
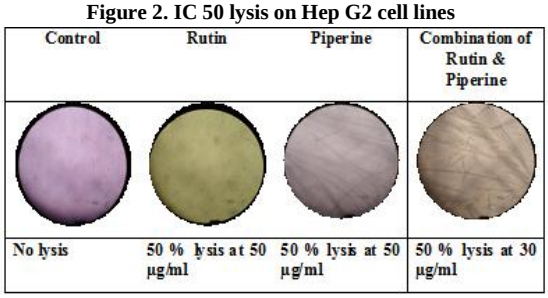


Figure 1. IC50 Values on Hep G2 & Hep 3B Celllines

Figure 2. IC 50 lysis on Hep G2 cell lines



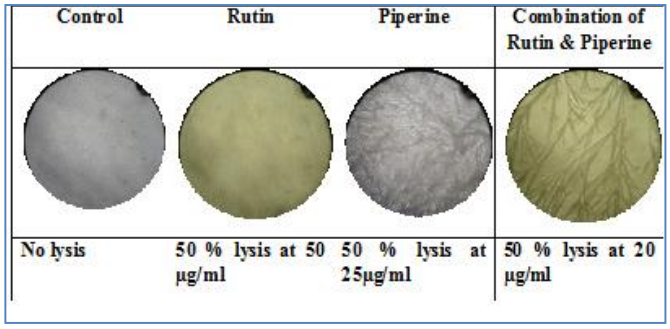


Figure 3. IC 50 lysis on Hep 3B cell lines

Conclusion:

In conclusion, the pure phytoconstituents of rutin and Piperine can significantly inhibit cell viability and induce apoptosis in Hep G2 and Hep 3B cells and also showed potent synergistic activity in combinational treatment of rutin and piperine in very low concentrations (< 20µg/ml) at which 50 % of cancer cell death occurred.

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Conflict of interest:

Authors declared no conflict of interest

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