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HEPATOPROTECTIVE ACTIVITY OF TRACHYSPERMUM AMMI (L.), TRIGONELLA FOENUM - GRAECUM L. AND PIPER NIGRUM L. ON HEPG2 CELL LINE

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ABSTRACT

The plant seed extract samples were evaluated for its In vitro hepato-protective activity against Ibuprofen induced toxicity in human HepG2 cell line, Firstly the plant seed extract samples were estimated for cytotoxicity with different concentrations from 1000µg/ml to 31.2µg/ml, which resulted to be moderate toxicity to hepatic cell line, and hence the non-toxic concentrations were taken for further studies. The percentage viability of cell line and the cytotoxicity activity of TA, TFG and PN against HEPG2 Cell line was carried out by using MTT assay method. In hepatoprotective study the test substances TA and TFG showed significant percentage protection against Ibuprofen induced toxicity than test substances PN.

KEYWORDS : *Trachyspermum ammi (L.), Trigonella foenum-graecum L. and Piper nigrum L., Hepatoprotective activity* Ibuprofen, HepG2 cell line.

1. INTRODUCTION

Ancient Ayurveda texts additionally taught important medical techniques, such as rhinoplasty, sutures and the extraction of foreign objects [1]. The crude aqueous extracts of medicinal plants are used to treat liver damage. Identification of energetic components of such plant extracts with acknowledged Hepatoprotective properties requires screening, isolation and characterization of large numbers of plant extracts obtained at some stage in fractionation and purification processes. Because of the cost and time involved, freshly isolated hepatocytes are no longer convenient for this large-scale screening of material separated from crude plant extracts and therefore an attempt was made to devise a reproducible microplate screening assay, based on protection of cells of the human-liver-derived HepG2 cell line [2].

The discovery of aspirin in 1946 followed by that of phenylbutazone was the beginning of the NSAIDs era. However, not until 1960 was indomethacin marketed. On the other hand, during the 1950s, Ibuprofen was the second drug (along with aspirin) approved to be sold as over the counter medication. The NSAIDs chemical classification recognizes four major groups of molecules: (1) carboxylic acids; (2) oxicams carboxamides; (3) sulphonanilides diaryl-substituted; and (4) pyrazole or furanone [3]. NSAIDs induced hepatotoxicity is associated with different patterns of clinical presentation, several mechanisms of liver damage and various pathological patterns. Ibuprofen has a recognized anti-inflammatory, analgesic and antipyretic property and is one of the most commonly NSAIDs used worldwide. Ibuprofen increases the risk of liver injury when administered to patients with chronic hepatitis C. An Ibuprofen associated increase of transaminases was recently reported as the causes of chronic hepatitis C, eventually confirmed by re-challenge [4].

The present study explains the *Trachyspermum ammi (L.)*, *Trigonella foenum-graecum L*. and *Piper nigrum L*. plant seed aqueous crude extracts used for the treatment of advanced stages of various hepatic diseases.

2. MATERIALS AND METHODS

2.1. Preparation of plant Extracts

The shade dried selected three plant seeds were grind as courase powder with help of electric mixer grinder. The seed powder stored in an air tight containers at 200 C for further studies. 50 g of each sample was extracted in a soxhlet apparatus at 700 C by using 500 ml of double distilled water as an aqueous solution extracted. Then the extractions of each sample were kept in a hot air oven at 800 C for 12 hrs. The paste like plant seed crude extracts were stored in a glass air tight labeled small vials and kept in a refrigerator at 40 C for further analysis.

2.2. Preparation of test solution

For studies, each weighed samples of plant crude extract were separately dissolved in DMSO and volume was made up with DMEM-HG supplemented with 2% inactivated FBS to obtain a stock solution of 10 mg/ml concentration and sterilized by filtration. Serial two fold dilutions were prepared from this for carrying out cytotoxic studies.

2.3. Cell line and Culture medium

Cell lines (Human hepatocellular carcinoma) were cultured in DMEM- HG media supplemented with 10% inactivated Fetal Bovine Serum (FBS), penicillin (100 IU/ml), streptomycin (µg/ml) and amphotericin B (5 µg/ml) in a humidified atmosphere of 5% CO2 at 370 C 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 cm2 culture flasks and all experiments was carried out in 96 microtitre plates.

2.4. Cytotoxicity studies

The cytotoxic analysis based on standard procedure of Francis D and Rita 1986 cell growth and survival modifications to the tetrazolium dye procedure giving improved sensitivity and reliability followed in cell line studies [5]. The monolayer cell culture was trypsinized and the cell count was adjusted to100, 000 cells/ml using DMEM-HG containing 10% FBS. To each well of the 96 well microtitre plate, 0.1 ml of the diluted cell suspension was added. After 24 h, when a partial monolayer was formed, the supernatant was flicked off, washed the monolayer once with medium and 100 l of different test concentrations of test drugs were added on to the partial monolayer in microtitre plates. The plates were then incubated at 37º C for 3 days in 5% CO2 atmosphere, and microscopic examination was carried out and observations were noted every 24 h interval. After 72 h, the drug solutions in the wells were discarded and 50 l of MTT in PBS was added to each well. The plates were gently shaken and incubated for 3 h at 37o C in 5% CO2 atmosphere. The supernatant was removed and 100 µl of propanol was added and the plates were gently shaken to solubilize the formed formazan. The absorbance was measured using a microplate reader at a wavelength of 540 nm. The percentage growth inhibition was calculated and concentration of test drug needed to inhibit cell growth by 50% (CTC50) values is generated from the dose-response curves for each cell line.

2.5. Determination of Hepatoprotective activities:

The monolayer cell culture was trypsinized and the cell count was adjusted to 1.0 x 105 cells/ml using DMEM medium containing 10% FBS. To each well of the 96 well microtitre plate, 0.1 ml of the diluted cell suspension (approximately 10,000 cells) was added. After 24 h, when a partial monolayer was formed, the supernatant was flicked off, washed the monolayer once with medium. 100 µl of DMEM with nontoxic concentration-50 % cell death (Ibuprofen amide) of

toxicant and 100 µl of different non-toxic test concentrations of test drugs were added. The plates were then incubated at 37o C for 24 h in 5% CO2 atmosphere. After 24 h, the cell supernatants were discarded and 50µl of MTT in PBS was added to each well. The plates were gently shaken and incubated for 3 h at 37ºC in 5% CO2 atmosphere. The supernatant was removed and 100 µl of propanol was added and the plates were gently shaken to solubilize the formed formazan. The absorbance was measured using a microplate reader at a wavelength of 540 nm. The percentage cell viability was determined, based on which the percentage protection offered by test and standard drugs was calculated over the Ibuprofen amide control. The induced toxicity in cell line procedure followed based on the Hepatoprotective effects of aqueous leaf extract and crude isolates of Murrayakoenigii against in vitro ethanol induced hepatotoxicity model. Experimental and toxicologic pathology official journal of the Gesellschaft fur ToxikologischePathologie [6].

3. RESULTS AND DISCUSSIONS

Table-1: Cytotoxic properties of seed extract samples drug against HepG2 cell line

S.NO.	Name of Test Sample	Ibuprofen Conc. (µg/ml)	% Cytotoxicity	CTC50 (µg/ml)
1.	TA	1000 500 250 125 62.5 31.2	38.23±0.37 26.29±0.23 17.74±0.38 10.54±0.14 5.30±0.19 2.95±0.39	>1000
2.	TFG	1000 500 250 125 62.5 31.2	50.56±0.09 36.31±0.23 32.00±0.86 26.12±0.28 13.54±0.57 7.74±0.28	980.46±2.94
3.	PN	1000 500 250 125 62.5 31.2	34.67±0.34 26.80±0.57 17.22±0.14 14.71±0.28 10.01±0.28 7.56±0.39	>1000

Table 2: Hepatoprotective activity of test substances in HepG2 against Ibuprofen induced toxicity.

Sl. No	Samples	Plant extracts Conc. (µg/ml)	% Protection over the control
1.	TA	300µg/ml 150µg/ml 75µg/ml	60.61±1.5 37.73±1.0 21.97±1.5
2.	TFG	100µg/ml 50µg/ml 25µg/ml	58.02±3.4 40.48±2.7 15.81±1.3
3.	PN	300µg/ml 150µg/ml 75µg/ml	36.21±3.1 13.44±3.5 10.69±0.3

Fig. 1: Cytotoxic effect of the TA, TFG and PN on HepG2 Cell line.

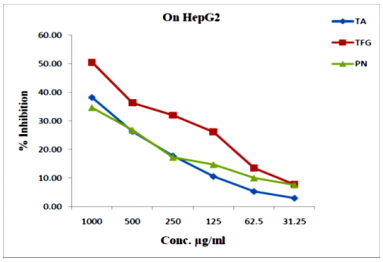
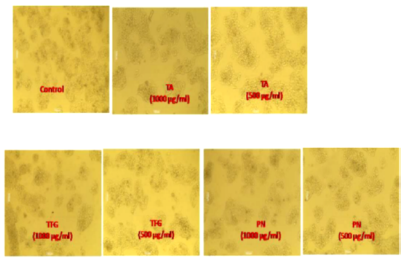


Fig.2. HepG2 cell line image treated with control and plant seed extracts of TA, TFG and PN



4. DISCUSSION

Test substances, tested for in vitro cytotoxicity studies against human Hepatoma cell line by MTT assay exposing the cells to different concentrations of test substance (1000 µg/ml to 31.2 µg/ml), and showed moderate toxicity. The test substances were evaluated in human hepatoma cell line. The test substance TA showed exhibited significant efficacy by offering 60.61±1.5 and 58.02±3.4 % protection against ibuprofen induced toxicity at 300 and 150 µg/ml, respectively. Similarly Extract TFG and PN showed exhibited Moderate efficacy by offering 58.02±3.4 and 36.21±3.1% protection against ibuprofen induced toxicity at higher concentration, respectively.

5. CONCLUSION

HepG2 cell line is an immortal cell line and it is derived from the human liver tissue. The human hepatic cells are epithelial in morphology. The HepG2 cells secretes a different major plasma proteins. They can grow successfully in large- scale cultivation systems. HepG2 cell lines are well respond to stimulation with human growth hormone [7]. HepG2 cells are an acceptable in vitro model system for the study of human hepatic cell line, nature and morphology of hepatocytes [8]. The proper culture conditions which leads the vigorous morphological and functional differentiation with controllable formation of apical and basolateral cell surface domains [9]. HepG2 cells are have their high degree of morphological and functional differentiation in vitro cell line research and this cell line model helps to study the intracellular trafficking and dynamics of membrane proteins, lipids in human hepatocytes [10]. HepG2 cells and their derivatives are also used as a model system for the study of liver metabolic functions and toxicity of xenobiotics [11]. The Hepg2 cell line researches are used to the detection of environmental and dietary, genotoxic, cytoprotective, anti- genotoxic, cytotoxic, hepatocarcinogenesis and drug targeting studies [12].

The various methods of liver metabolic pathways that have been studied appear to stimulate the performance of normal hepatocytes. Thus, these studies encourage a wider application of this HepG2 cell line to biological problems that relate specifically to the role of the liver [13]. Therefore, HepG2 cell line has been widely used in a variety of fields like liver metabolism development and hepatotoxicity [14]. The discovery and the application of natural drugs will play an important role in the future to treat human hepatic diseases. These three selected medicinal plant seed extracts have more potential and may have phytochemical compounds and naturally easily available source from our environment. The seed extracts of Trachyspermum ammi (L.), Trigonella foenum- graecum L. and Piper nigrum L. can be recommended as therapeutic drugs to treat the hepatic diseases.

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