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Anticancer activity of nano encapsulated formulation from the extracts of *Picrorhiza kurroa* against human cancer cell lines

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ABSTRACT

The following study is designed to determine the anticancer and cytotoxic potential of Nano encapsulated extract formulation from rhizome of *Picrorhiza kurroa* enriched with Apocynin, caffeic esters and cucurbitacins aglycone compounds, to produce any cytotoxic effect on mammalian cell lines. The test conducted using MTT method using human hepatocarcinoma cells (HepG2) and Madin Darby Canine Kidney (MDCK) cell lines as part of the in vitro preclinical characterization of compound. More than 100% increment in cell killing at a concentration of 100µg/ml recorded in both the cell lines, 52.5% cytotoxicity in HePG-2 cell line was recorded at 0.1µg/ml concentration, whereas 50.4% cytotoxicity assessed in MDCK cell line at 1µg/ml of Formulation concentration. Exhibited LC_{50} value of Formulation in HePG-2 and MDCK cell lines were recorded 1.2 µg/ml and 4.14µg/ml respectively. Cytotoxic effect against HePG-2 cancer cell line is considered as a predictive anticancer activity indicator also, where Doxorubicin is a standard anti-cancer agent which is a highly cytotoxic drug. MDCK cytotoxicity results support that formulation is less cytotoxic in normal cell lines, as MDCK is a Non-Cancerous cell line.

Keywords: Anticancer, HepG2, MDCK, Nano encapsulated, *Picrorhiza kurroa*

1. Introduction

Since last many years, plants have beneficial activity in different type of diseases producing in human beings. As per WHO calculate that about 80% of the world's inhabitants problem should treated by medicinal herbal drug for their primary health care [1-2]. Natural products remain an important source of new drugs, new drug leads and new chemical entities. The plant based drug discovery resulted mainly in the development of anticancer agents including plants (vincristine, vinblastine, etoposide, paclitaxel, camptothecin, topotecan and irinotecan), marine organisms (citarabine, aplidine and dolastatin 10) and micro-organisms (dactinomycin, bleomycin and doxorubicin) [4-5].

Till Current research on *Picrorhiza kurroa* has focused on its hepatoprotective, anticholestatic, antioxidant, and immune-modulating activity [6-7].

Kutkin is the active principal of *Picrorhiza kurroa* and is comprised of kutkoside and the Iridoid glycoside picrosides I, II, and III. Other identified active constituents are apocynin, drosin, and nine cucurbitacin glycosides [7-8]. Apocynin is a catechol that has been shown to inhibit neutrophil oxidative burst in addition to being a powerful anti-inflammatory agent [9]. while the curcubitacins have been shown to be highly cytotoxic and possess antitumor effects [10].

2. Materials and method

2.1 Test System Preparation

The cell lines propagated at 37 ± 1 °C in a gaseous environment of 5% ± 1% Carbon dioxide in 75 cm² tissue culture flasks containing Minimum Essential Medium (MEM)(Invitrogen), supplemented with 10% fetal bovine serum (Invitrogen) and penicillin(100 units) –streptomycin (100 µg) antibiotics (Invitrogen). The assay conducted using 96 well tissue culture flat bottom plates with optimized cell density of both the cell lines using cells per mL plating density from 70% confluent culture flask, properly labeled and incubated at a temperature of 37 ± 1°C in a 5% ± 1% CO_2 to obtain a confluent mono layer of cells prior to use

i) **Test Item:** Nano encapsulated formulation
ii) **Positive Control:** Doxorubicin (7 conc.).
iii) **Negative Control**: Minimum Essential Medium without test item, with 0.5% DMSO

2.2 MTT Assay

The tetrazolium salt 3-[4, 5-dimethylthiazol-2-yl]-2, 5-diphenyltetrazolium bromide (MTT) is used to determine cell viability in assays of cell proliferation and cytotoxicity [10-11]. MTT is reduced in metabolically active cells to yield an insoluble purple formazon product. Cells were harvested from maintenance cultures in the exponential phase and counted by a hemocytometer using trypan blue solution. The cell suspensions were dispensed in triplicate into 96-well culture plates at optimized concentrations for both the cell lines, after a 24-hr recovery period the Doxorubicin standard and Formulation were diluted and added. Seven dilutions of Doxorubicin and Formulation were tested (100, 10, 1, 0.1, 0.01, 0.001 and0.0001µg/ml) with an incubation period of 48 h. Doxorubicin was used as positive control and vehicle (MEM) as negative control. For median inhibition concentration (IC50) and/or lethal concentration (LC50) determination dose-response curves were conducted with a series of different concentrations of formulation [12-13]. To control wells, only culture medium was added. After an additional 48 h incubation period, the medium in each well was aspirated and replaced with MTT working solution. The cells were incubated at 37°C for 4 h and then the medium was aspirated and replaced with DMSO to dissolve the formazan crystals formed. The culture plates were shaken for 5 min and the absorbance of each well was read at 490 nm with 655 nm as the reference wavelength. The relative viability of the treated cells as compared to the control cells was expressed as the % cytoviability [14].

2.2.1 Procedure

100 µl of 5X104 cells/ml of HePG-2 cell line and 2X105 cells/ml of MDCK plated in 96 culture well plates and incubated for 24 hrs to reach at 70% confluency. Each well designated to indicate its contents and date of experiment**.** 1mg/ml stock prepared for formulation and Doxorubicin in DMSO. Compound diluted in plain MEM medium from the stock and prepared 2X working concentration to achieve final range of 10 log concentrations (0.0001µg-100µg). 100µl of drug added in triplicates from each concentration of Doxorubicin, Formulation and negative control in designated wells. Plate incubated for 48 hrs in 37 ± 1°C in a gaseous environment of 5% ± 1% Carbon dioxide, after drug addition. After 48 hrs medium replaced with 200 µl of fresh medium from the appropriate wells and 50 µl of 5 mg/ml of MTT dye added in each well. Plate covered with aluminum foil and incubated for 4.0 hrs at 37 ± 1°C incubator. Medium containing MTT removed from the plate and 200µl of DMSO added in appropriate Wells, to mix formazan crystals. Absorbance measured at 490nm on plate reader using a reference wavelength of 655nm.

2.2.2 Calculation

Percent Cytotoxicity calculated using below mentioned formula:

% Cytotoxicity = (sample abs/media control abs)*100. (Cell survival percentage was calculated based on standard curves. Percentage of cell survival in the negative control was assumed as 100 %.)

Mean and SD calculated for each blank, positive control, negative control and test sample. LC50 and Significance of cytotoxicity (P value) calculated using statistical software analysis.

3. Result and discussion

The in vitro screening for the cytotoxicity assessment of the formulation showed moderate cytotoxic activity against the hepatoma cancer cells (HePG2). More than 100% increment in cell killing at a concentration of 100µg/ml recorded in both the cell lines, 52.5% cytotoxicity in HePG-2 cell line was recorded at 0.1µg/ml concentration, whereas 50.4% cytotoxicity assessed in MDCK cell line at 1µg/ml of formulation concentration (Figure 1 and 2). Exhibited LC_{50} value of formulation in HePG-2 and MDCK cell lines were 1.2 µg/ml and 4.14µg/ml respectively (Table 1 and 2). In this study, it was observed that Formulation induces a concentration dependent inhibition of both the cell lines. The results obtained from the present study showed moderately cytotoxic activity on HePG-2 cell line. Cytotoxic effect against HePG-2 cancer cell line is considered as a predictive anticancer activity indicator also, where Doxorubicin is a standard anti-cancer agent which is a highly cytotoxic drug. MDCK cytotoxicity results support that Formulation is less cytotoxic in normal cell lines, as MDCK is a Non-Cancerous cell line.

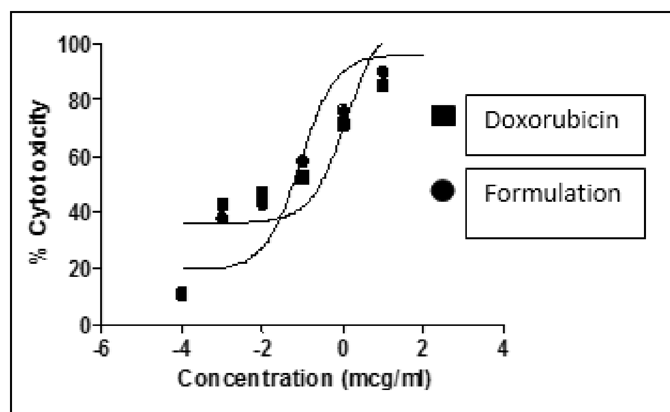

Fig 1: Cytotoxic effect of Nano Formulation on HEPG2 cell line

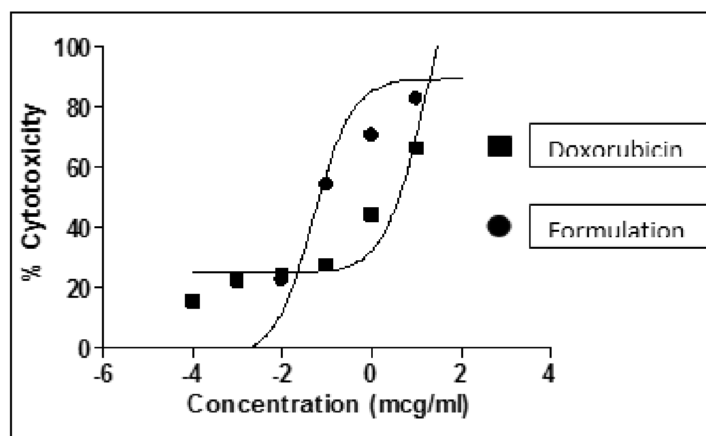

Fig 2: Cytotoxic effect of Nano Formulation on MDCK cell linen

Table 1: Effect of Nano Formulation on HePG-2 Cell Line

Conc. (microgram/ml)	Avg. OD	SD	% Cytotoxicity	LC_{50} (microgram/ml)	Significance (P value) P=<0.05	Summary
Doxorubicin						
100.00000	0.0784	0.0094	113.4		Yes	**
10.00000	0.1597	0.0146	90.0		Yes	***
1.00000	0.2070	0.0196	76.3		Yes	***
0.10000	0.2702	0.0377	58.1	**0.08747**	Yes	***
0.01000	0.3228	0.0204	43.0		Yes	***
0.00100	0.3400	0.0290	28.0		No	*
0.00010	0.5054	0.0656	0.0		No	*
Nano Formulation						
Conc. (microgram/ml)	**Avg. OD**	**SD**	**% Cytotoxicity**	**LC_{50} (microgram/ml)**	**Significance (P value) P=<0.05**	**Summary**
100.0000	0.0500	0.0025	120.4		Yes	**
10.0000	0.1786	0.0245	85.4		Yes	***
1.0000	0.2298	0.0073	71.4		Yes	***
0.1000	0.2992	0.0142	52.5	**1.212**	Yes	**
0.0100	0.3202	0.0196	26.8		No	*
0.0010	0.3345	0.0255	12.9		No	*
0.0001	0.4524	0.0441	10.8		No	*
Note: * Significance Summary calculated using Dunnett's Multiple Comparison Test.						

Table 2: Effect of Nano Formulation on MDCK Cell Line

Conc. (microgram/ml)	Avg. OD	SD	% Cytotoxicity	LC_{50} (microgram/ml)	Significance (P value) P=<0.05	Summary
Doxorubicin						
100.00000	0.0966	0.0092	109.5	**0.0516**	Yes	***
10.00000	0.1758	0.0119	82.8		Yes	***
1.00000	0.2121	0.0189	70.6		Yes	***
0.10000	0.2600	0.0424	54.4		Yes	**
0.01000	0.3543	0.0344	22.7		Yes	**
0.00100	0.4271	0.0676	0.0		No	*
0.00010	0.4511	0.0683	0.0		No	*
Nano Formulation						
Conc. (microgram/ml)	**Avg. OD**	**SD**	**% Cytotoxicity**	**LC_{50} (microgram/ml)**	**Significance (P value) P=<0.05**	**Summary**
100.0000	0.0619	0.0058	116.9	**4.14**	Yes	***
10.0000	0.2291	0.0381	70.0		Yes	***
1.0000	0.2994	0.0308	50.4		Yes	***
0.1000	0.351	0.0526	35.9		No	*
0.0100	0.4207	0.0674	16.3		No	*
0.0010	0.4281	0.0543	14.3		No	*
0.0001	0.4494	0.0347	8.3		No	*
Note: * Significance Summary calculated using Dunnett's Multiple Comparison Test.						

4. Conclusion

From the result it is concluded that more than 100% increment in cell killing at a concentration of 100µg/ml recorded in both the cell lines, 52.5% cytotoxicity in HePG-2 cell line was recorded at 0.1µg/ml concentration, whereas 50.4% cytotoxicity assessed in MDCK cell line at 1µg/ml of formulation concentration. Exhibited LC50 value of Formulation in HePG-2 and MDCK cell lines were recorded 1.2 µg/ml and 4.14µg/ml respectively. So, nano encapsulated extract formulation from rhizome of *Picrorhiza kurroa* could be a good candidate for more studies on other cancer cell lines and in vivo antitumor evaluation. It can be a new source for antitumor medicine.

5. Acknowledgements

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