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In-Vitro Cytotoxicity Evaluation Of Makardhwaja Against Mcf-7 Human Cell Lines.

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Abstract: Herbo-mineral formulations are gaining negative propaganda in population. Hence, mankind can remain deficient from the bounty of effective and efficient *ayurvedic* formulation like *makardhawaja*. It is used as a rejuvenating & aphrodisiac *(rasayan and vajikaran)* drug. Modern medicine evolved a method of subjecting all assumptions to experimental verification and statistical validation. Therefore, current research had been undertaken with an aim to study cytotoxicity against selected MCF-7 human cell lines. *Makardhwaj* was prepared according to the reference of *rasachandanshu* which is a herbo-mineral formulation. Its cytotoxicity study was conducted at ACTREC. The solvent used for dissolution of the study drug was DMSO. Human cancer cell lines MCF-7 of breast were cryopreserved in liquid nitrogen and DMSO at -20 ^{0}C. Cell culture was performed in incubators at 37°C .The selected cell lines were inoculated in 96 well plates, in which study drug was tested at 4 dose levels i.e. 10, 20, 40, 80 µg/ml. Adriamycin was used as a positive control drug for comparative screening. According to SRB assay protocol Lc50values were calculated as declared by NCI. The study had revealed that, *makardhwaja* is not cytotoxic as the observed LC50 values were found > 80 in the study.

Introduction:

The *ayurvedic* classical formulary is rich & diverse and holds a very sound position. A good quality of medicine emphasises on a better results with minimum dose and without untoward effects. Due to issue of *parad* (Hg) & metals used in an *ayurvedic* medicine, *rasaushadhies* are giving negative propaganda in population. Hence, mankind can remain deficient from the bounty of effective and efficient herbo-mineral formulations. Demand for research to find out safe and efficient formulation like *makardhwaja* from Indian traditional medicine. This formulation is unique of its kind as its total 31 references.[1]are available since several centuries. It is a known rejuvenating *(rasayan)* & aphrodisiac *(vajikaran)* drug[2]. Review of previous researchers show ample of studies on the specific formulation which enables to state importance of this formulation. Amongst various classics, the study drug i.e.*makardhwaja* was prepared as per the reference of *rasachandanshu*[2]. The current research has been undertaken with an aim to study its in vitro cytotoxicity in MCF7 (breast) cell lines. This study may prove its contribution regarding safety application of *makardhwaja* in the field of research.

Research question: Does *makardhwaj* show cytotoxicity on MCF-7 cell lines

Keywords: *Makardhwaja*, Cytotoxicity, In Vitro study, MCF-7.

Material and Methods:

Material:

i) Study drug: *Makardhwaja*.

ii) Cell lines: MCF-7(breast human cell lines).

iii) Instruments: SRB colorimeter, 25 cm^2 tissue culture flasks, 15 ml centrifuge tubes, 96 well plate, incubators etc.

iv) Chemicals: DMSO, liquid nitrogen, SRB dye, 1% acetic acid, 10% TCA, humidified CO_2

Method of in vitro screening:

MCF-7 Cell lines were obtained from NCI, USA and NCCS, Pune respectively. Cell lines were cryopreserved in liquid nitrogen vapours. For cytotoxicity measurements, 100 µl cells/ well were seeded into 96-well microtiter plates so that every well receives $5X10^3$cells. The seeded plates were incubated at 37^0C in humidified CO_2 (5%) incubator for 24 hrs. DMSO was used as a solvent for the study drug i.e.*makardhwaja* in a proportion of 100mg/ml. All further dilutions of the drug were made with complete medium which is used for growing the cells. The study drug was tested at 4 dose levels at 10, 20, 40, 80 µg/ml. Adriamycin (Doxorubicin) was used as a positive control drug for comparative screening and appropriate dilutions of DMSO were used as a vehicle control. On addition of the drug, the plates were incubated further for 48 hours at 37^oC in humidified CO_2 (5%) incubator. After incubation, 50µL of 30% TCA was added to fix the cells to the bottom of the wells. After 60 minutes incubation at 4°C, plates was washed gently under tap water and air dried at room temperature. Then 100µL SRB reagent was added into each well and left for 15 minutes and the SRB dye was removed by washing the plates with tap water. 1% acetic acid was used to remove unbound SRB dye. After air drying, 0.1ml of 10mM unbuffered TRIS base was added and absorbance was measured at 540 nm. With the help of formulae, cytotoxicity of compound was estimated.[3,4].

Observations & Results:

A- Study drug.

V.C. - vehicle control.

ADR- Adriamycin positive control drug.

Table no.1: % of control growth of human breast cell line MCF-7

	Study drug concentrations in µg/ml															
	Experiment 1				Experiment 2				Experiment 3				Avg. values			
	10	20	40	80	10	20	40	80	10	20	40	80	10	20	40	80
A	89.1	86.2	83.9	80.9	97.9	94.2	85.3	74.9	83.6	82.9	83.4	69.5	90.2	87.8	84.2	75.1
V.C.	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
ADR	-28.9	-36.6	-55.2	-56.1	-22.6	-30.9	-52.7	-53.9	26.1	-27.5	-43.5	-44.6	-25.9	-31.0	-56.5	-51.5

Table no.2: Drug concentrations (µg/ml) calculated by formulae-

	MCF7		
	LC_{50}	TGI	GI_{50}
A	>80	>80	>80
ADR	60.1	22.0	<10

GI_{50} is Growth inhibition of 50 % (GI50) resulting in a 50% reduction in the net protein increase.
TGI Drug concentration resulting in total growth inhibition (TGI)
LC_{50} is concentration of drug resulting in a 50% reduction in the measured protein at the end of the drug treatment as compared to that at the beginning) indicating a net loss of 50% cells following treatment.

Discussion& conclusion:

The selected study drug *makardhwaja* was *kupipakva* as well as *khalvirasayan*. It was prepared according to *rasachandanshu (vajikaran)* in 72hrs of gradual heating. The proportion of Au:Hg:S was 1:8:16 i.e. *dwigunabalijarana.* Method adopted of *agnisanskar* for *jarana* was *bahirdhumpaddhati* and the type of *murcchana* was *sagandhasaagni.* In the final formulation the medicine collected at the neck of the bottle was mixed and triturated with *karpoora, jatiphala, lavanga, latakasturi and samudraphen* as per proportion mentioned in the classical text. As per analytical reports it was α-HgS, particle size was between 10-30µ and was having solubility partially in gastric buffer i.e. pH 1.2 buffer and partially in physiological buffer i.e. 7.4 which is seconded by previous research.[5] revealing the absorption of drug in gut. The study drug had shown the presence of 41ppm gold.

Makardhwaja was partially soluble in the solvent used for dissolution i.e. DMSO. In vitro confirmation of cytotoxicity was carried out on MCF-7 cell lines of breast. As per the pharmacokinetics of *makardhwaja* described by material medica[6], the study drug gets absorbed by gut and through the portal circulation it gets stored in the liver. According to the *shukravahastrotas* described by *aacharya sushruta*[7],*stana*(breast) and *vrishana*(testes) are the origin of *shukravahastrotas.* It is already proved by previous researches the action of *makardhwaja*is on *shukravahastrotas.* So, in the current study cell lines of breast were selected to rule out cytotoxicity of drug. Cell cultures can be used to screen for toxicity by estimation of the basal functions of the cells (i.e. both processes common to all types of cells) or by tests on specialized cell functions. Cytotoxicity tests mainly at the detection of the biological activity of the study drug can be carried out on many cell types (fibroblasts, HELA & hepatoma cells). A number of parameters including vital staining, cystolic enzyme to release, cell growth & cloning efficiency are used as end points to measure cytoxicity.

In the current study, to evaluate in vitro cytotoxicity of study drug i.e. *makardhwaja*, cancer cell lines of breast were selected. Cancer cells are normally highly specialized cells which have regressed to a much simpler, more primitive stage& which unlike the normal parent, divide continuously although inefficiently because a much higher proportion of CA cells are undergoing active division, they are most vulnerable than normal cells[8].

According to SRB assay protocol.[3,4], GI_{50}, TGI and LC_{50} values are calculated by the following formulae-

GI_{50}: Growth inhibition of 50 % calculated from [(Ti-Tz)/(C-Tz)] x 100 = 50, drug concentration resulting in a 50% reduction in the net protein increase.

TGI: Drug concentration resulting in total growth inhibition will be calculated from Ti =Tz.

LC_{50}: Concentration of drug resulting in a 50% reduction in the measured protein at the end of the drug treatment as compared to that at the beginning) indicating a net loss of 50% cells following treatment is calculated from [(Ti-Tz)/Tz] x 100 = -50.

The results indicate that the drug under test has failed to demonstrate any lethality (LC50) values >80 in both the cell lines). So, it can be concluded that *makardhwaja* is non cytotoxic drug against MCF-7 cell lines of breast.

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