

Evaluation of In Vitro Anti-Cancer Activity of *Kukkutanakhi Guggula* on Liver, Prostrate, Ovary and Renal Cancer

Research Article

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Abstract

The medicines prepared by using exudates of *Commiphora mukul* (Stocks) Hook. are described in Ayurveda under *Guggula Kalpana* which are among such valuable dosage forms. According to retrospective literary review, the combination of *Kukkutnaki* (*Aspidium cicutarium* Sw.) and purified *Guggula* (*Commiphora mukul* (Stocks) Hook.) was first mentioned in the book *Chikitsa pradeep* named as *Kukkutnaki Guggula*. Since last 3 decades, it was documented as an herbal drug which is used for cysts, goiter, tumors, tonsillitis, abscess, *mansvaha strotas ailments*, which are burning issues worldwide. Due to its observed clinical efficacy in *Arbuda* (~Cancer), the current *in-vitro* anticancer study was conducted with an aim to check its anticancer effect on human hepatoma cell line-HEPG2 of Liver; PC-3 and DU145 cancer cell lines of Prostate; Ovar-3, A2780, SK-OV-3, PA-1 cancer cell lines of Ovary and ACHN renal cancer cell line of Kidney. The current in vitro study was conducted at ACTREC, Kharghar, Navi Mumbai. The selected cancer cell lines were procured from ATCC, USA and NCCS Pune. The Sulforhodamine B (SRB) Assay protocol was followed to observe the activity of the study drug. The positive control was Adriamycin in the study. The growth curve graphs were plotted and LC50, GI50, TGI values were calculated. *Kukkutnaki Guggula* was found safe for oral administration, non-toxic at cellular level (LC50 values were > 160) and have moderate activity on HEPG2, Ovar-3, DU145, ACHN cancer cell lines and had shown negligible activity on A2780, SK-OV-3, PA-1 and PC-3 cell lines. This work provides scope to study its effect on targeted cancers, specific in vivo scientific studies and human clinical trials for further researchers.

Key Words: *Kukkutnaki, Guggula, Cancer, SRB assay, In-vitro study, Cell lines.*

Introduction

The different kind of natural secondary metabolites produce by the Plant Kingdom which are being inspected for their anticancer activities leading to the development of new clinical drugs.(1) In human development and well-being the rise of Cancer worldwide is a major impediment in current era. By 2030, the burden of cancer will increase to 22 million new cases each year.(2) There is need of research & scientific documentation on newly derived and traditionally used herbal compounds and due to limitation of modern medicine & therapies, ayurvedic medicine is a ray of hope. Folklore and tribal sources

were utilized by Ayurvedic *Acharyas* while documenting *Materia medica* of Ayurveda.(3,4)

Kukkutnaki is an herb grown around the monsoon and rainy seasons and found along the foot hills of *Sahyadri* region. (5,6) It is botanically identified as *Aspidium cicutarium* Sw. of family *Dryopteridaceae*.(7,8) Initially it is mentioned in the text "*Gharguti Aushadhe*" and it is also called as *Kombadnakhi, Bichava, Nirvishi and Waghchavdi*. (9,10) The rhizomes of this herb being used as a folklore remedy for the treatment of *Shotha, Shula, Arbuda* etc. successfully.(11) *Guggula kalpana* i.e medicines prepared by using exudates of *Commiphora mukul* has its own importance in the arena of ayurvedic therapeutics.(12) *Kukkutnaki Guggula* was selected as a study drug according to retrospective literary review from various texts and scientific magazines and it is indicated in cysts, tumors, tonsillitis, abscess, elephantiasis etc.(13) It was first mentioned in the text "*Chikitsa pradeep*" for various *Mansavaha strotas ailments*.(14) However in the text "*Guggula kalpana*",

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its method preparation and proportion of the ingredients is mentioned.(15)

The study drug was formulated by using authenticated *Kukkutnakhi* and purified *Guggul* in the same proportion for oral administration as per text *Guggula Kalpana*.(16) Current in-vitro anticancer study was conducted by following SRB assay with an aim to check anticancer effect of *Kukkutnaki Guggula* on human hepatoma cell line-HEPG2 of Liver; PC-3 and DU145 cancer cell lines of Prostate; Ovcar-3, A2780, SK-OV-3, PA-1 cancer cell lines of Ovary and ACHN renal cancer cell line of Kidney.

Materials and Methods

Material

The selected cancer cell lines i.e human hepatoma cell line-HEPG2 of Liver; PC-3 and DU145 cancer cell lines of Prostate; Ovcar-3, A2780, SK-OV-3, PA-1 cancer cell lines of Ovary and ACHN renal cancer cell line of Kidney were procured from American Type Culture Collection (ATCC),USA and NCCS Pune. 96 well plate, 15 ml centrifuge tubes, SRB Calorimeter, 25 cm² tissue culture flasks, Millipore disposable filters, Liquid nitrogen container, CO₂ incubator, Laminar flow hood, Haemocytometer, Biological Microscope, Drug dispensing machine, Electronic balance, Multichannel automated pipette, Elisa reader were used for in vitro study. The chemicals like SRB dye, Ethanol, TCA liquid nitrogen, DMSO, RPMI medium, DMEM were used in the current study.

Method

The current in vitro study was conducted at ACTREC (Advanced Centre for Treatment, Research and Education in Cancer), Kharghar, Navi Mumbai. The Sulforhodamine B (SRB) Assay protocol was followed to observe the activity of the study drug and Adriamycin (Doxorubicin) was used as positive control.

In-Vitro Study

The selected cancer cell lines were checked for quality control and its cryopreservation was done in Liquid nitrogen vapors and DMSO(5-10%) in a liquid nitrogen container. 25 cm² tissue culture flasks containing RPMI 1640 medium i.e 10% Fetal Bovine Serum and 2nM L-glutamine were used for cell lines grown and its single cell suspension was made. Tissue culture flask was then kept in a CO₂ incubator at 37.5°C for 24-48 hrs for the purpose of cell division. Cells were counted with the help of haemocytometer and cell count is adjusted according to the titration readings (approximately 1x 10⁵cells/ml). This Cell culture was done under all aseptic condition inside the laminar flow hood to avoid bacterial contamination. Then inoculation

of prepared cell suspension(90µl) was done into 96-well Micro- Titer plates so that every well receives 5X10³ cells. Then the plates were incubated at 37°C, 95% air, 5% CO₂, and relative humidity(100%) for 24h prior to addition of experimental drugs. Study drug was solubilized in DMSO i.e. Dimethyl sulfoxide at 100 mg/ml and diluted to 1 mg/ml using water and stored frozen prior to use.(17)

By Drug dispensing machine aliquots of 10µl of the study drug dilution were added to in 96 well plates which previously containing of Cell suspension (90µl) in triplicate. DMSO (10 ml of 10% v/v) was added into each negative-control well and 10 ml Adriamycin was added into each positive-control well. Drug was tested at 4 dose levels (40,80,120,160µg/ml). Each experiment was repeated thrice. Then Cell fixation was done by 25 µl cold 50% (wt/vol) TCA(Trichloroacetic acid) and plates incubation was done at for 1 h at 4 °C. Then plates was stained by SRB dye (50 µl) of 0.04% (wt/vol) SRB solution to each well and left for 15minutes. Then washing of the plates with tap water was done to remove SRB dye. The unbound dye was washed by 1% acetic acid and plates were dried. Then protein-bound dye was solubilized after 30 min in Tris base solution. Then absorbance was read on the Elisa-Plate Reader at the wavelength of 540nm with reference to 690nm. Graphs were plotted and results was given in terms of GI50, TGI, LC50 values. Then optical density of control cells was compared to that of drug treated cells and growth inhibition calculated as values in percentage. Using the six absorbance measurements [time zero(Tz), control growth(C) and the test growth in the presence of drug at the four concentration levels (Ti)], the percentage growth was calculated at each of the drug concentration levels. On a plate by plate basis percentage growth was calculated for test wells relative to control wells. Percent growth was expressed as the ratio of average absorbance of the test well to the average absorbance of control wells. It was calculated as $[(Ti-Tz)/ C-Tz] \times 100$ for concentration for which $Ti \geq Tz$ (Ti-Tz) positive or zero $[(Ti-Tz)/ Tz] \times 100$ for concentration for which $Ti < Tz$. (Ti-Tz) negative. Growth inhibition of 50% (GI50) was calculated from $[(Ti-Tz)/ C-Tz] \times 100 = 50$, which was the drug concentration resulting in a 50% reduction in the net protein increase (as measured by SRB staining) in control cells during the drug incubation. The drug concentration resulting in total growth inhibition(TGI) was calculated from $Ti = Tz$. The LC50 (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 cells following treatment was calculated from $[(Ti-Tz)/ Tz] \times 100 = -50$. (18,19)

Observations and Results

The result of anticancer study on selected cancer cell lines are as follows:

Table no 1: % Control growth of human hepatoma cell line HEPG2:

Human Hepatoma Cell Line HEPG2																
% Control Growth																
Drug Concentrations (µg/ml)																
	Experiment 1				Experiment 2				Experiment 3				Average Values			
	40	80	120	160	40	80	120	160	40	80	120	160	40	80	120	160
KG	100.0	100.0	96.1	76.2	100.0	100.0	100.0	87.6	100.0	100.0	100.0	81.1	100.0	100.0	98.7	81.6
ADR	-35.2	-48.0	-54.4	-57.0	-42.4	-47.7	-60.7	-61.4	-45.3	-49.4	-63.1	-65.1	-40.9	-48.4	-59.4	-61.2

Table no 2: % Control growth of human prostate cell line- DU-145

Human Prostate Cancer Cell Line DU145																
% Control Growth																
Drug Concentrations (µg/ml)																
	Experiment 1				Experiment 2				Experiment 3				Average Values			
	40	80	120	160	40	80	120	160	40	80	120	160	40	80	120	160
KG	100.0	95.5	82.0	78.0	100.0	98.0	84.7	80.4	100.0	96.2	95.8	86.0	100.0	96.6	87.5	81.5
ADR	10.6	9.8	8.5	2.7	9.5	6.6	4.0	1.4	10.2	9.3	6.1	5.9	10.1	8.6	6.2	3.3

Table no 3: % Control growth of human prostate cell line PC3

Human Prostate Cancer Cell Line PC3																
% Control Growth																
Drug Concentrations (µg/ml)																
	Experiment 1				Experiment 2				Experiment 3				Average Values			
	10	20	40	80	10	20	40	80	10	20	40	80	10	20	40	80
KG	100.0	100.0	98.8	98.7	100.0	100.0	99.0	93.2	100.0	96.3	94.9	90.8	100.0	98.8	97.6	95.3
ADR	-69.1	-67.5	-76.3	-78.3	-67.5	-73.7	-73.9	-77.3	-68.7	-71.6	-74.4	-78.6	-68.5	-70.9	-74.8	-78.1

Table no 4: % Control growth of human ovarian cell line PA-1

Human Ovarian Cancer Cell Line PA-1																
% Control Growth																
Drug Concentrations (µg/ml)																
	Experiment 1				Experiment 2				Experiment 3				Average Values			
	40	80	120	160	40	80	120	160	40	80	120	160	40	80	120	160
KG	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	95.6	100.0	100.0	100.0	98.5
ADR	2.3	-7.3	-33.6	-33.7	-26.7	-32.8	-47.5	-51.8	-21.0	-33.9	-35.3	-43.8	-15.1	-24.7	-38.8	-43.1

Table no 5: % Control growth of human Ovarian cell line OVCAR-3

Human Ovarian Cancer Cell Line Ovarcar-3																
% Control Growth																
Drug Concentrations (µg/ml)																
	Experiment 1				Experiment 2				Experiment 3				Average Values			
	40	80	120	160	40	80	120	160	40	80	120	160	40	80	120	160
KG	100.0	100.0	80.9	47.1	100.0	100.0	100.0	100.0	100.0	100.0	100.0	99.1	100.0	100.0	93.6	82.1
ADR	-17.6	-41.6	-48.8	-50.2	-36.0	-44.0	-46.2	-48.1	-39.5	-46.0	-47.3	-47.8	-31.0	-43.9	-47.4	-48.7

Table no 6 : % Control growth of human ovarian cell line A2780

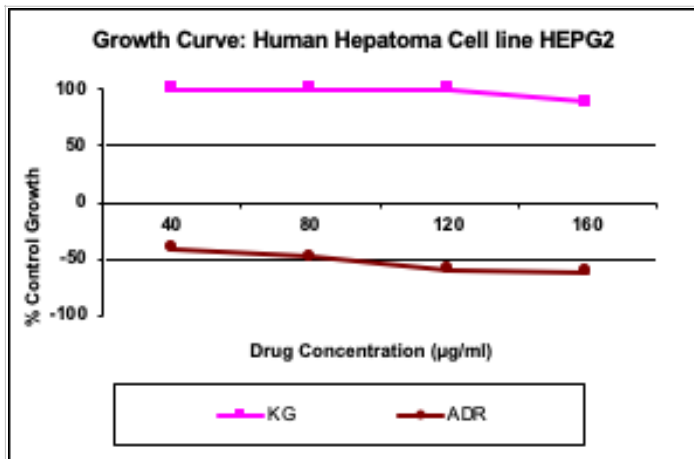
Human Ovarian Cancer Cell Line A2780																
% Control Growth																
Drug Concentrations (µg/ml)																
	Experiment 1				Experiment 2				Experiment 3				Average Values			
	40	80	120	160	40	80	120	160	40	80	120	160	40	80	120	160
KG	100.0	100.0	100.0	100.0	100.0	99.5	97.1	97.0	100.0	100.0	93.5	93.0	100.0	99.8	96.9	96.7
ADR	-34.0	-35.7	-41.2	-48.0	-29.3	-31.2	-42.4	-50.9	-31.9	-33.3	-47.6	-54.2	-31.7	-33.4	-43.8	-51.0

Table no 7: % Control growth of human ovarian cell line SK-OV-3

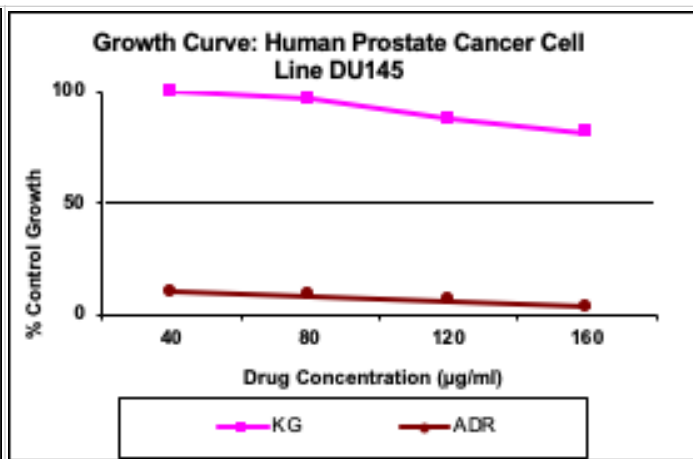
Human Ovarian Cancer Cell Line SK-OV-3																
% Control Growth																
Drug Concentrations (µg/ml)																
	Experiment 1				Experiment 2				Experiment 3				Average Values			
	40	80	120	160	40	80	120	160	40	80	120	160	40	80	120	160
KG	100.0	100.0	100.0	100.0	100.0	100.0	100.0	98.1	100.0	100.0	100.0	100.0	100.0	100.0	100.0	99.4
ADR	-23.0	-31.2	-33.9	-47.0	-31.7	-36.8	-43.5	-54.2	-36.8	-37.5	-40.4	-46.5	-30.5	-35.2	-39.2	-49.2

Table no 8 : % Control growth of human renal cell line ACHN

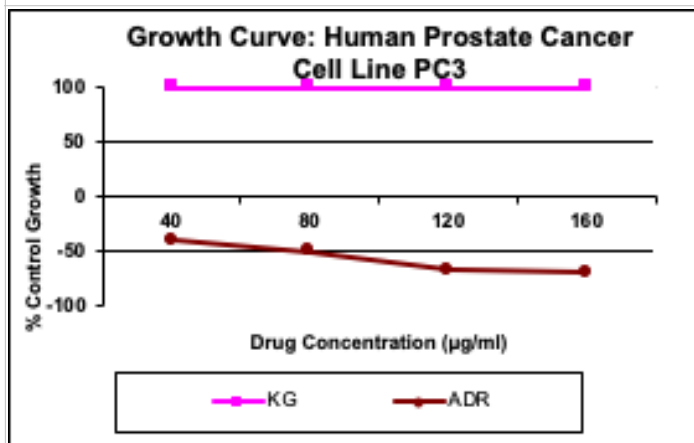
Human Renal Cancer Cell Line ACHN																
% Control Growth																
Drug Concentrations (µg/ml)																
	Experiment 1				Experiment 2				Experiment 3				Average Values			
	40	80	120	160	40	80	120	160	40	80	120	160	40	80	120	160
KG	83.7	77.2	75.9	66.2	87.2	81.7	79.1	67.5	82.0	78.5	64.7	64.3	84.3	79.1	73.2	66.0
ADR	-20.0	-21.8	-23.9	-24.4	-21.6	-22.9	-23.5	-26.2	-24.6	-26.2	-27.3	-31.9	-22.1	-23.6	-24.9	-27.5



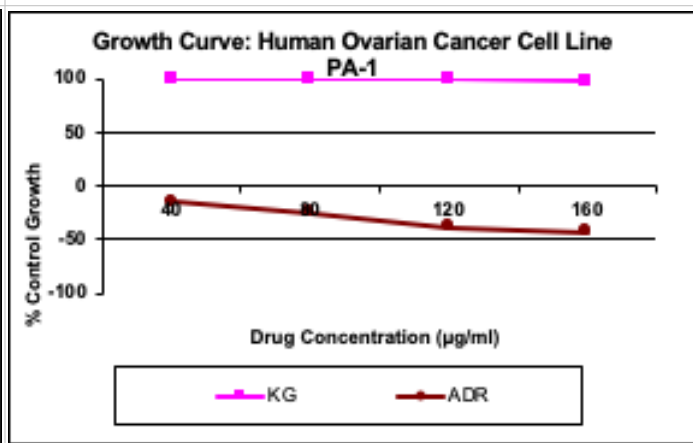
Graph no. 1: Growth curve of human hepatoma cell line HEPG2



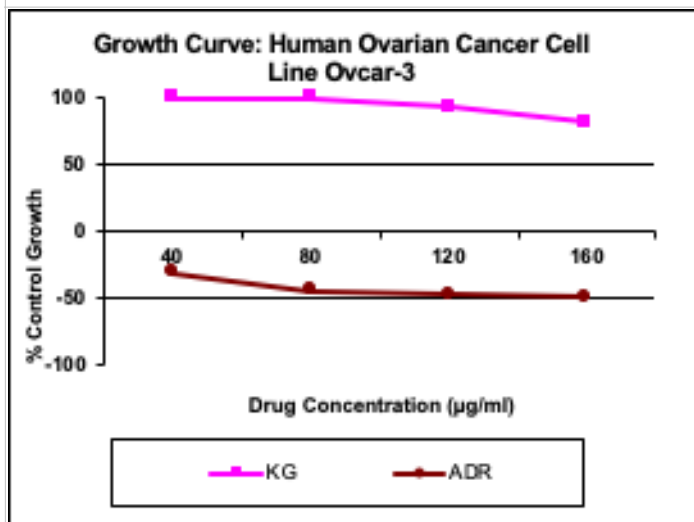
Graph no. 2: Growth curve of human prostate cell line DU145



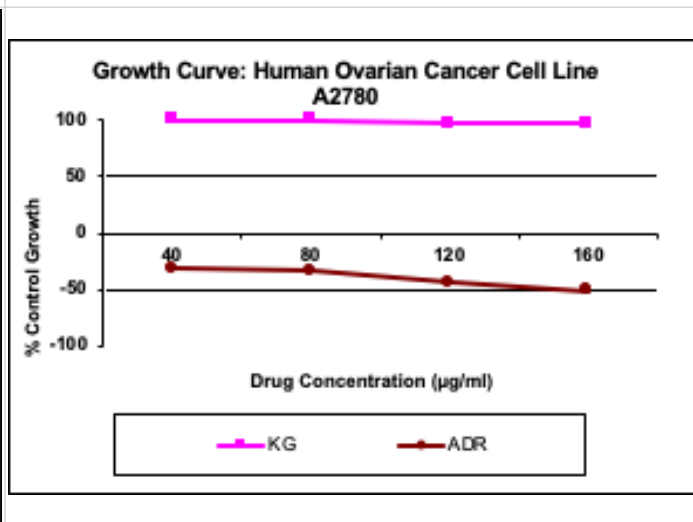
Graph no. 3: Growth curve of human prostate cell line PC3:



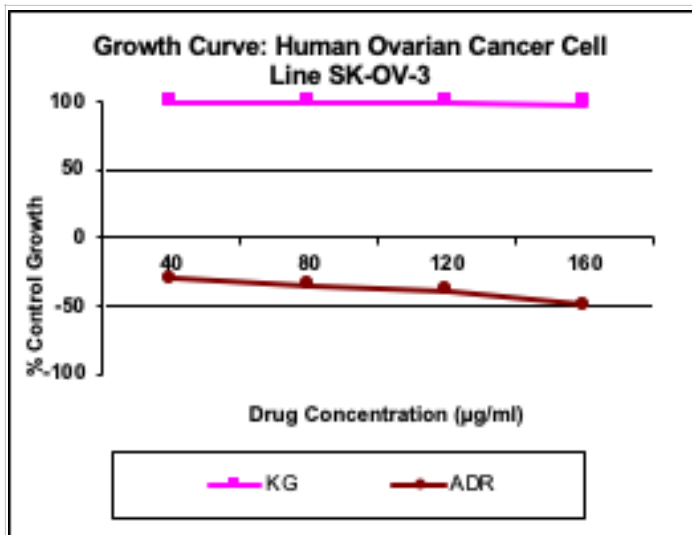
Graph no. 4: Growth curve of human ovarian cell line PA-1:



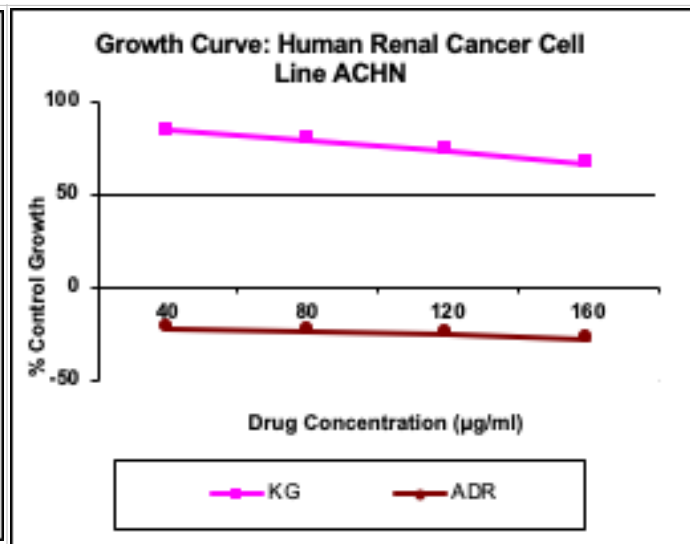
Graph no.5: Growth curve of human ovarian cell line OVCAR-3:



Graph no. 6: Growth curve of human ovarian cell line A2780:



Graph no. 7: Growth curve of human ovarian cell line SK-OV-3:



Graph no. 8: Growth curve of human renal cell line ACHN

KG: Kukkutnakhi guggul , ADR: Adriamycin

Discussion

In-vitro mechanism based screening of herbal medicine is mandatory in the preliminary phases of plant drug research before taking them to *in-vivo* study to evaluate their efficacy.(19) *Kukkutnakhi guggula* was selected for anticancer activity evaluation as it practices in tribal masses and also by senior *Vaidyas* in clinical practice on *Galaganda*(enlargement of glands of neck), *Granthi*(Cyst) and *Arbuda* (Tumor) etc. This proprietary drug prepared in teaching pharmacy by using authenticated *Kukkutnakhi* and *Guggula* in the same proportion for oral administration. In organoleptic analysis the colour of the product was found dark brown, *Rasa*(taste) was *Tikta*(Bitter) , *Katu* (Bitter), *Kashaya*(astringent) and odour was specific representing smell similar to *Guggula*. This is due to presence of *Guggula* as a major ingredients which contain few volatile phyto-constitutes having its specific odor. *Triphala kwath*(decoction) was used for purification of crude *Guggula*, as it is having proved anticancer activity. It has *Tridoshas shaman* and *Lekhana*(Scraping) properties which get embedded to purified *Guggula* and enhance the effect and remove the undesired effect of *Guggula*.

For anticancer screening of study drug, SRB (Sulphorhodamine B) assay was followed and each experiment was carried out at 4 dose levels (40, 80, 120, 160 µg/ml) in a triplicate. SRB binds to the basic amino acids of the cellular proteins as it is bright pink anionic protein staining dye and based on the measurement of cellular protein content it is mainly used for cell density determination.(19) Values obtained from optical density were estimated and average of observed values of each plate was mentioned in table and labeled as experiment 1, 2, 3 respectively. Graphs were plotted on the basis of average value obtained from each experiment which denotes the effect of study drug and control drug on selected cancer cell lines. Dye concentration was quantified by measuring absorbance

at 564 nm and the amount of remaining cells in treated samples was compared to untreated (control) and % inhibition of growth was determined. As per table and Graph no. 1, 2, 5 and 8 study drug had shown results in a range of 66 to 82 value of percentage control growth and inclination of line in graph. As the drug concentration increases which indicates moderate activity at maximum drug concentration i.e. 160µg/ml. As per table and Graph no. 3, 4, 6 and 7 study drug had shown results in range of 95-100 value of percentage control growth and very slight or no inclination in a graph at maximum concentration level i.e.160 µg/ml. This indicates the negligible activity, even if the concentration of drug is increased. The LC50, TGI and GI50 values of study drug were greater than 160µg/ml as compared to positive control group. As LC50 values of study drug is found to be greater than 160µg/ml which indicate that *Kukkutnakhi Guggula* is safe for oral administration and non-toxic at cellular level.

There are many advanced *in-vitro* screening techniques are available like SRB, MTT, Clonogenic assay, Fluorescent assays & dye exclusion test but SRB assay is particularly useful for qualitative analysis as it is highly sensitive and give better linearity with cell number than other assay.(20,21) In cell based studies it is widely used to investigate cytotoxicity and is also high cost-effective screenings method. It was utilize to determine the selective activity of the potential anticancer drugs.(22,23) There are certain limitations of SRB assay like less sensitive with non-adherent cells, several wash steps involved, but fixation required, and if drug is insoluble in solvent then it is difficult to perform assay.(24,25,26) This preclinical study had shown encouraging result in the parameter of growth inhibition i.e. GI50 on particular cell lines which shows the efficacy of the drug. But there was no activity seen on some cell lines which suggest that inactivity of the study drug on cancer cell lines of particular organ.

Conclusion

On the basis of retrospective literary review, the proprietary herbal formulation *Kukkutanakhi Guggula* is evaluated for its anticancer effect in 8 selected cancer cell lines. As per SRB assay protocol, *Kukkutanakhi Guggula* had shown moderate activity on HEPG2, ACHN, Ovar-3 and DU145 human cancer cell lines of Liver, Renal, Ovary and Prostate organs respectively and had shown no activity on A2780, SK-OV-3, PA-1 and PC-3 cell lines. *Kukkutanakhi Guggula* was found safe for oral administration, non-toxic at cellular level (LC50 values were > 160). This work provides scope to study its effect on targeted cancers, specific in-vivo scientific studies and human clinical trials for further researchers.

Conflict of interest- Nil

Acknowledgement- Nil

References

- Greenwell M, Rahman PK. Medicinal plants: their use in anticancer treatment. International journal of pharmaceutical sciences and research. 2015 Oct 1;6(10):4103.
- Ferlay J, Soerjomataram I, Dikshit R, Eser S, Mathers C, Rebelo M, Parkin DM, Forman D, Bray F. Cancer incidence and mortality worldwide: sources, methods and major patterns in GLOBOCAN 2012. International journal of cancer. 2015 Mar 1;136(5):E359-86.
- Acharya YT. Sushruta Samhita of Acharya Sushruta. reprint edi. Varanasi; Chaukhambha Orientalia publishers; 2009. 159p
- Tripathi RD. Charaka Samhita of Agnivesha; (editor), reprint edi. Varanasi; Chaukhambha Orientalia publishers; 2007. 358p
- Ghoghari AM, Bagul MS, Anandjiwala S, Chauhan MG, Rajani M. Free radical scavenging activity of *Aspidium cicutarium* rhizome. Journal of Natural Remedies. 2006 Jun 1;6(2):131-4.
- Hardik M, Nishteswar K, Patel BR, Harisha CR. Detailed Pharmacognostical Evaluation On Rhizome Of *Tectaria Coadunata* (Wall. Ex Hook & Grev.) C. Chr.-A FOLKLORE HERB. Global Journal of Research on Medicinal Plants & Indigenous Medicine. 2013 Aug 1;2(8):582.
- Dubal KN, Ghorpade PN, Kale MV. Studies on bioactive compounds of *Tectaria coadunata* (Wall. Ex Hook. & Grev.) C. Chr. Asian J Pharm Clin Res. 2013;6(2):186-7.
- Kulkarni M, Tambe R, Bhise K. Preliminary phytochemical screening and HPTLC studies of extracts of dried rhizomes of *Aspidium cicutarium*. Journal of Pharmacognosy and Phytochemistry. 2013 Sep 1;2(3):50-4.
- Sathe AP. *Gharguti Aushadhe*. 1st ed. Girgaon Mumbai; Ayurved Bhavan; 1922. 65p
- Desai VG. *Aushadhi Sangrah*. 2nd ed. Kothrud Pune; Rajesh Prakashan; 1975. 281p
- Pade SS. *Vanoushadhi Gunadarsh*. 2nd ed. Ghatkpoar Mumbai; Raghuvanshi prakashan; 1982. 306p
- Khan MB, Sathe N, Chavan R. Commiphora mukul Engl.–“Divya”: A Review. Ayurlog: National Journal of Research in Ayurved Science-2015.;3(2):1-2.
- Gordiya RH. Ayurved patrika-Katemulee or Kombadnakhi. Nashik; Ayurveda Sewa Sangha; 1967. 115p,
- Gokhale BV. *Chikitsa pradeep*. 1st ed. Sadashiv Peth Pune; Vaidyamitra Prakashan; 1989. 74p.
- Prabhu N. *Guggul kalpana*. 1st ed. Dombivali Mumbai; Karyavaha Maharashtra Ayurved Sammelan; 1999. 88p
- Khan Mujahid B., Sathe Ninad, Deshmukh Ashwini, Rathi Bharat. Pharmaceutical study of *Kukkutanakhi Guggul* and its modified dosage form as tablet. Joinsysmed; 2017, vol 5(1), pp-15-20.
- Gaidhani SN, Singh A, Kumari S, Lavekar GS, Juvekar AS, Sen S, Padhi MM. Evaluation of some plant extracts for standardization and anticancer activity. Indian Journal of Traditional Knowledge; October 2013; Vol. 12 (4), pp. 682-687
- Skehan P, Storeng R, Scudiero D, Monks A, McMahon J, Vistica D, Warren JT, Bokesch H, Kenney S, Boyd MR. New colorimetric cytotoxicity assay for anticancer-drug screening. JNCI: Journal of the National Cancer Institute. 1990 Jul 4;82(13):1107-12.
- Vichai V, Kirtikara K. Sulforhodamine B colorimetric assay for cytotoxicity screening. Nature protocols. 2006 Aug;1(3):1112-6..
- Lovitt CJ, Shelper TB, Avery VM. Advanced cell culture techniques for cancer drug discovery. Biology. 2014 Jun;3(2):345-67
- Chanda S, Nagani K. In vitro and in vivo methods for anticancer activity evaluation and some Indian medicinal plants possessing anticancer properties: an overview. Journal of pharmacognosy and phytochemistry. 2013 Jul 1;2(2).
- Patil LE, Kulkarni KI, Khanvilkar VI, Kadam V. In vitro evaluation of herb–drug interactions: a review. Int J Pharm Pharm Sci. 2014;6:9-12.
- Itharat A, Houghton PJ, Eno-Amooquaye E, Burke PJ, Sampson JH, Raman A. In vitro cytotoxic activity of Thai medicinal plants used traditionally to treat cancer. Journal of ethnopharmacology. 2004 Jan 1;90(1):33-8.
- Houghton P, Fang R, Techatanawat I, Steventon G, Hylands PJ, Lee CC. The sulphorhodamine (SRB) assay and other approaches to testing plant extracts and derived compounds for activities related to reputed anticancer activity. Methods. 2007 Aug 1;42(4):377-87.
- Chavan R, Khan M, Sathe N, Mankar NA. A Review: SRB Assay for Screening Anticancer Activity of Herbal drugs (in-Vitro). International Ayurvedic Medical Journal. 2016;4(2):66-70.
- Keepers YP, Pizao PE, Peters GJ, van Ark-Otte J, Winograd B, Pinedo HM. Comparison of the sulforhodamine B protein and tetrazolium (MTT) assays for in vitro chemosensitivity testing. European Journal of Cancer and Clinical Oncology. 1991 Jul 1;27(7):897-900.
