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A COMPARATIVE EVALUATION OF ANTI-CANCER EFFECT OF *PANCHGAVYA* AND *PANCHGAVYAGHRITA* BY LEWIS LUNG CARCINOMA CELL LINE IN VITRO STUDY

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ABSTRACT

The study is conducted to explore utilization of *Panchgavya* and *Panchgavyaghrita* involved in possible therapeutic application of cow product which is used traditionally for various conditions. In the present study cytotoxicity assay method was carried out to identify the effect of *Panchgavya* and *Panchgavyaghrita* as potent anticancer agent in treatment of lung cancer induced by LLC cell line. The test was conducted using MTT dyeand Lewis Lung Carcinoma cell line for in-vitro characterization of cow product, comparison was done against Doxorubicin as standard cytotoxic drug. Inhibition of cancer cell growth (50%)was found at 7.75 mg/ml of *Panchgavya* and 6.78 mg/ml of *Panchgavyaghrita*, for doxorubicin it was found at 1.087×10^{-10} mg/ml.

KEYWORD: *Panchgavya*, *Panchgavyaghrita*, Lewis Lung Carcinoma, MTT assay, Anticancer activity.

INTRODUCTION

Cow products has a unique place in Ayurveda. It is describe in 'Sushruta Samhita' and 'Ashtanga Sangraha' as the most effective substance/secretion of animal origin with innumerable therapeutic values. It has been recognized as life or '*Amrita'* (beverages of immortality), the nector of the God. Cowpathy is the unique method to treat diseases

traditionally practiced by *Govaidyak*. Now a day the whole world is shifted to natural remedy. *Panchagavya* and *Panchagavyaghrita* are mixtures used in traditional Hindu rituals, prepared by mixing of five ingredients - three direct constituents are cow dung, urine, and milk; other two derived products are curd and ghee.

From the long period, cow products have been used as a medicine. In Veda, cow urine is considered as nectar. *Panchgavya* products have been found to be beneficial in curing several human ailment and enhance the body's immunity and resistance to fight the infections. This kind of alternative treatment has been reported to be beneficial even for dreaded diseases like cancer, AIDS and diabetes. Cow urine along with the antibiotics can also prevent the development of resistance in microorganisms against the antibiotics. Cow urine distillate fraction, popularly known as '*ark'*, has been identified as a bio-enhancer of the activities of commonly used antibiotics, antifungal and anticancer drugs.

Cowpathy is used in tuberculosis and cancer, thus opening a new era in medical science. Milk contain lactoferrin, B12, B1, lipid, vitamin, minerals and bioactive compound. Lactoferrin (Lf) is a multifunctional protein and an essential element of innate immunity.Cow ghee contains many vital nutrients which help in making the body healthy and immune to diseases. In addition, cow ghee contains calcium, protein, lactose, fats, CLA, omega 3, estrogen etc. Anti-cancer activity of ghee is due to CLA (conjugated linoleic acid).Curd is nourishing food and valuable source of protein, essential vitamins and minerals. It improves body's immune system and protects body from infections. The dung is rich in disinfectant properties. It kills all the germs and bacteria and heals wounds. Cow urine enhances the immune competence and improves general health of an individual; prevent the free radicals formation and act as anti-aging factor; reduces apoptosis in lymphocytes and helps them to survive; and efficiently repairs the damaged DNA, thus is effective for the cancer therapy. Its main contents are water, urea, minerals, salt, hormones, and enzyme. All above contents are used in the treatment of cancer. Cow urine has capacity to fight cancer cell, its anticancer effect is due to uric acid antioxidant property.

Lewis lung carcinoma is a cell line established from the lung of a **C57BL mouse** bearing a tumor resulting from an implantation of primary Lewis lung carcinoma. The present study is carried out to prove the anti-cancer effect of Panchgavya and Panchgavyaghrita on Lewis Lung Carcinoma.

MATERIAL AND METHODS

PREPARATION OF PANCHGAVYA AND PANCHGAVYA GHRITA

Collection and preparation of Panchgavya and Panchgavya Ghrita

Panchgavya and Panchgavyaghrita were collected from Shree RMD Ayurved College and Hospital, KesarsuriGyanviharSankul, Wadhaldhara, Valsad.

Preparation of Panchgavya

In the early morning fresh cow product i.e. Milk, Dung and urine was collected and indirect product Ghee and Curd were also collected freshly in equal proportion. After that Cow Urine and dung was thoroughly mixed and filtered eight time by using fine cotton cloth followed by mixing of milk and curd in filtrate and again filtered it by using cotton cloth.

In the end the filtered was boiled until removal of water and ghee was added in the end to prepared panchgavyaghrita.

Prepare the stock

Calcein-AM dye in DMSO at a concentration of 1mg/ml was prepared. Similarly prepared (Propidium iodide) PI stock in sterile water at a concentration of 10mg/ml.

CELL VIABILITY STUDY

The stock of Calcein-AM dye in DMSO at a concentration of 1mg/ml was prepared, (Propidium iodide) PI stock in sterile water at a concentration of 10mg/ml was also prepared.

NOTE: These dyes are highly light sensitive and should be stored in smaller aliquots frozen in -20`C till use; PI is a suspected mutagen and hence should be handled with great care

Adherent cell cultured on sterile glass coverslip as either confluent or sub confluent monolayer (for e.g., fibroblasts are typically grown on the coverslip for 2-3 days until acceptable cell densities are obtain). The cell cultured inside 35 mm disposable Petri-dishes or other suitable containers, non-adherent cell was used.

NOTE: if inverted fluorescence microscope is used, then the cells can be grown in the culture plates and observed directly in to the microscope. If normal (upright) fluorescence microscope is available, cells can be grow on coverslips, loaded with dye and carefully mounted on the glass slides, and observed under the microscope.

The medium from the cells was removed and washed twice with PBS buffer to prevent interfere of serum traces in the medium with the loading of the dyes. Fresh buffer and Calcein-AM dye were added so the final concentration of the dye was obtained i.e. 1µg/ml.

After that incubated at 3 ˚C in dark for 15 minutes.followed by adding of PI dye so the final concentration was 10µg/ml. Again incubated in dark at 3 ˚C for a further 5 minute duration.

The dye containing buffer was removed and fresh buffer was added in it. After that it was Observed under the microscope. FITC filter was used for viewing the calcein stained live cells and Rhodamine filter was used for PI stained dead cells.

The number of calcein positive cells and PI positive cells along with the total number of cells in each field of view were counted. The cell viability was expressed as (the number of calcein positive cells/ total number of cells) x 100

MTT Assay (CYTOTOXICITY ASSAY)

1. Cell was plated at the concentration of 1×10^6 cells/plate in the wells of column 2 to column 11 suspended in a 150 µl of medium/well.
2. 150 µl of growth medium to the eight wells in columns 1 and 12 was added.
3. The plate has been incubated at 37^oC in CO_2 incubator for 24 hour, such that the cells are in the exponential phase of growth at the time of drug addition.
4. After that Prepared serial dilutions of the cytotoxic drug in growth medium to give eight different concentrations 10, 1, 0.1, 0.01 and 0.001 mg/ml. The set of concentrations has been chosen in such a way that the highest concentration kills most of the cells and the lowest kills none of the cells.
5. The medium was removed from the wells in columns 2 to 11. The cells in the wells in column 2 and column 11 has been feed with fresh growth medium containing 5% FCS.
6. The cytotoxic drug (doxorubicin) containing growth medium to the cells in the wells of column 3 to 10 was added.
7. The plate was incubated at 37^oC in CO_2 incubator for a defined exposure period.

8. At the end of the drug exposure period, the medium was removed from all the wells and the cells was fed with 100 µl of fresh medium containing 0.5 mg/ml MTT.
9. The plates were wrapped in aluminum foil and incubated for 3 hour at 37°C and left for 8 hours.
10. After that the medium and MTT were removed from the wells and the remaining MTT-formazan crystals was dissolved by adding 100 µl of DMSO to all of the wells in columns 1 to 11.
11. Absorbance was recorded at 5

Analysis of cytotoxic Assay

I. Graph of the absorbance was plotted on y-axis, considering cells without drug as 100% against the concentration of drug on x-axis.
II. The LC50 was Calculated (the drug concentration that is required to reduce the absorbance to half that of the control).

RESULT

The different concentrations of 10, 1, 0.1, 0.01 and 0.001 mg/ml of Panchgavya, Panchgavyagharita and standard drug Doxorubicin were evaluated for the in vitro anti-cancer activity by LLC(lewis lung carcinoma) cell line culture and LC50 value. LC50 value was calculated, that was defined as the concentration of Panchgavya and Panchgavyaghrita required to inhibit 50% of Anti-cancer activity.

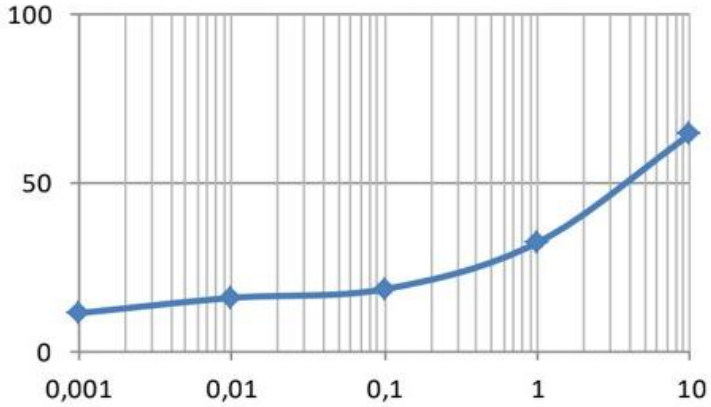
Concentration(mg/ml)	Panchgavya	Panchgavya Ghrita
10	61.85	64.34
1	15.33	32.15
0.1	12.54	18.33
0.01	10.2	15.74
0.001	1.26	11.2
LC50 Value mg/ml	7.75	6.78
Doxorubicin LC50	200nM(1.087×10^{-10} mg/ml)	

Comparison of LC_{50} values of *Panchgavya, Panchgavya Ghrita* and standard drug Doxorubicin

The concentration of *Panchgavya, Panchgavyaghrita* required to inhibit 50% of anticancer activity was found to be 10 mg/ml compare to standard with 50 % inhibition of 200nM.

***Panchgavya* and *Panchgavyaghrita* graph**

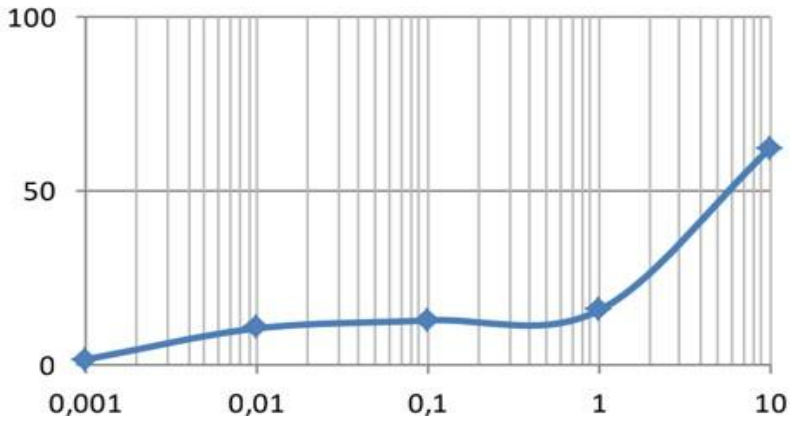
Panchgavyaghrita



X axis: concentration mg/ml **Y axis:** percentage inhibition

Figure 1: Graph of Panchgavya Ghrita LC50 Value.

Panchgavya



X axis: concentration mg/ml **Y axis:** percentage inhibition

Figure: 2 Graph of Panchgavya LC50 Value

DISCUSSION

Cancer is a generic term for a large group of diseases characterized by the growth of abnormal cells beyond their usual boundaries that can then invade adjoining parts of the body and/or spread to other organs. Other common terms used are malignant tumors and neoplasms. Lung cancer is a malignant lung tumor with various histological variants that arise from different cell type such as bronchial, epithelium, bronchioles, alveoli or bronchial mucous glands. Lung cancer is divided into two categories: small cell lung cancer and non–small cell lung cancer (NSCLC). Symptoms of lung cancer include anorexia, acute or chronic respiratory acidosis, wheezing, shortness of breath and pleural effusion. Smoking,

environmental exposure, clinical history, family history are causes of lung cancer. smoking is the major risk factor for lung cancer. For the treatment of lung cancer, different treatment options were discovered but they have unavoidable side effect/adverse drug reactions. Ayurveda is a branch which has comparatively less side effect observed. In present study we intended to identify effect of Panchgavya and PanchgavyaGhrita in treatment of lung cancer induced by LLC cell line. MTT [(3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetra sodium bromide)] was a pale yellow substrate that is cleaved by living cells to yield a dark blue Formozan product. This process requires active mitochondria and even freshly dead cells do not cleave significant amount of MTT. Thus the amount of MTT cleaved was directly proportional to the number of viable cells present, which is quantified by colorimetric methods. In present *in vitro* study it is reported that both Panchgavya and PanchgavyaGhrita have effectiveness in treatment of lung cancer. As the concentration of drug increased the % of inhibition was also increased and vice -a versa. LC_{50} value of PanchgavyaGhrita (6.78) is lower than the Panchgavya (7.75)which indicates that panchgavyaghrita is better in treatment of lung cancer.

CONCLUSION

Anticancer effect of panchgavya and Panchgavyaghrita cow product was already proven practically for the hepatic and breast cancer. For the first time in research we had used lewis lung cancer cell line model to explain the anti-cancer effect of panchgavya and panchgavyaghrita. In comparison between Panchgavya and Panchgavyaghrita LC_{50} value of PanchgavyaGhrita (6.78) is lower than the Panchgavya (7.75) so the result clearly indicated that the panchgavyaghrita showed better effect compare to Panchgavya. Which provide new era for research in the field of treatment for cancer.

Conflict of interest –Nil.