

EVALUATION OF *IN VITRO* CYTOTOXIC AND ANTICANCER ACTIVITY OF HEPATOPROTECTIVE POLYHERBAL FORMULATION IN CELL LINE MODEL

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Received: 23 January 2019, Revised and Accepted: 22 March 2019

ABSTRACT

Objective: The use of natural products as anticancer agents has a long history that began with folklore medicine and through the years has been incorporated into traditional and allopathic medicine. Several drugs currently used are derived from medicinal plants. The main objective of this study is to investigate the cytotoxic potential of hepatoprotective polyherbal formulation in normal and cancer cell lines.

Methods: A 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide assay was utilized to screen the cytotoxic activity.

Results: The results revealed that the formulation does not induce much mortality in normal liver and kidney cell lines, and LC₅₀ value of liver cell lines was found 1716.355 µg/ml and kidney cell lines 2464.910 µg/ml. The *in vitro* anticancer activity was performed on liver, colon, and prostate cancer cell lines, and IC₅₀ values are found 2.077, 3.850, and 11.989 µg/ml, respectively, which show excellent anticancer activity.

Conclusion: Based on the results obtained, the hepatoprotective polyherbal formulation is safe for normal cells and cytotoxic for cancer cells. Further, identification and quantification of phytoconstituents responsible for the activity are in progress.

Keywords: Polyherbal formulation, Phytochemicals, Cytotoxicity, Anticancer activity.

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INTRODUCTION

In recent years, most of the world population is witnessing medicine going back to nature - a shift in global trend from synthetic to natural medicine. In the ancient days also, medicinal herbs are used to treat several diseases. Even today for health maintenance, the Indian traditional medical systems such as Ayurveda, Siddha, Unani, Homeopathy, Yoga, and Naturopathy are practiced and accepted by worldwide population. Nowadays, more research is going on phytomedicine, and several botanical preparations are evaluated for its potential therapeutic efficacy. Among those botanical drugs, anticancer and hepatoprotective agents are standing in front [1]. Oncology-related cases are increasing day by day due to environmental pollution, food, and lifestyle modifications. Currently, cancer is treated by chemotherapy, surgery, and radiation therapy. Most of the synthetic anticancer drugs are used for the treatment which show severe adverse effects [2]. Recently, most of the oncology-related researchers are trying to identify botanical preparations which show maximum anticancer effect with a minimum side effect. Semisynthetic or isolated active components from botanical origin such as *Catharanthus roseus*, *Taxus brevifolia*, *Podophyllum emodi*, and *Camptotheca acuminata* have been used in the treatment cancer [3]. The selected polyherbal formulation is made up of hydroalcoholic extract of different indigenous medicinal plants with specific ratio. The main objective of this study is to investigate cytotoxic potential and anticancer activity of a polyherbal ayurvedic formulation in cell line model.

METHODS

Chemicals and cell lines

All the required chemicals and reagents are analytical grade and procured from Loba Chemie Laboratory Reagents and HiMedia. The cell lines were obtained from the National Center for Cell Sciences,

Pune, India, and grown on Dulbecco's Modified Essential Medium supplemented with 10% (v/v) fetal calf serum, penicillin (100 U/ml), and streptomycin (100 µg/ml).

Preparation of hydroalcoholic extract

The formulation is made up of five herbal ingredients in different ratios. Phytochemical extraction was done by cold maceration method using double-distilled water and ethanol in the ratio of 7:3 and kept for 48 h by occasional shaking. The residue was filtered through double-lined muslin cloth and evaporated to dryness under reduced pressure using vacuum evaporator. The resulting greenish-black material (LVR05) was kept in an airtight container at 4°C.

Determination of *in vitro* cytotoxicity and anticancer activity

In vitro cytotoxicity and anticancer activity were assessed by 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay [4-9]. Two different experiments were carried out using the same procedure for assessing cytotoxicity and anticancer efficacy of test drug, and the percentage of cell viability and mortality was calculated using standard formula.

RESULTS AND DISCUSSION

In vitro cytotoxicity studies

The *in vitro* cytotoxicity studies give us a good idea about advantages and disadvantages of the model for screening and mechanistic analysis of potentially toxic compounds. A wide array of morphologic and biochemical markers are available for obtaining information at the cellular and molecular levels to detect chemical-induced disruption [10]. Among the methods, *in vitro* cell line models are quite easy to perform in laboratory conditions. Different concentrations of LVR05 were tested against normal liver and kidney cell lines, and the toxicity was assessed

by MTT assay (Tables 1 and 2) and calculated LC₅₀ using lethal dose probability line software (Figs. 1 to 10).

In vitro cytotoxicity data are useful for assessing the toxicity of particular material and also indicate the need for specific kinds

Table 1: Cytotoxic effect of different concentrations of LVR05 on normal cell lines (Chang liver cells)

S. No	Concentration (µg/ml)	Absorbance (O.D)	Cell mortality (%)	Cell viability (%)
1	500	0.89	29.92	70.07
2	250	0.95	25.19	74.80
3	125	1.02	19.68	80.31
4	62.5	1.10	13.38	86.61
5	31.25	1.18	7.08	92.91
6	15.63	1.21	4.72	95.27
7	7.81	1.25	1.57	98.42
9	Cell control	1.27	0	100

µg: Micrograms, O.D: Optical density, LVR05: Hepatoprotective polyherbal formulation

Table 2: Cytotoxic effect of different concentrations of LVR05 on normal kidney cell lines (vero cells)

S. No	Concentration (µg/ml)	Absorbance (O.D)	Cell mortality (%)	Cell viability (%)
1	500	0.80	31.04	68.96
2	250	0.95	18.11	81.89
3	125	1.02	12.07	87.93
4	62.5	1.05	9.49	90.51
5	31.25	1.10	5.18	94.82
6	15.63	1.12	4.45	95.55
7	7.81	1.14	1.73	98.27
9	Cell control	1.16	0	100

µg: Micrograms, O.D: Optical density, LVR05: Hepatoprotective polyherbal formulation

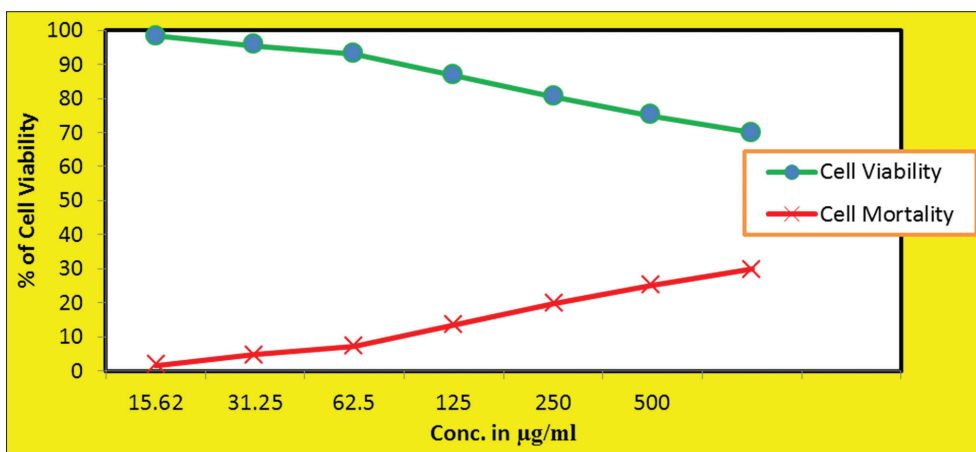
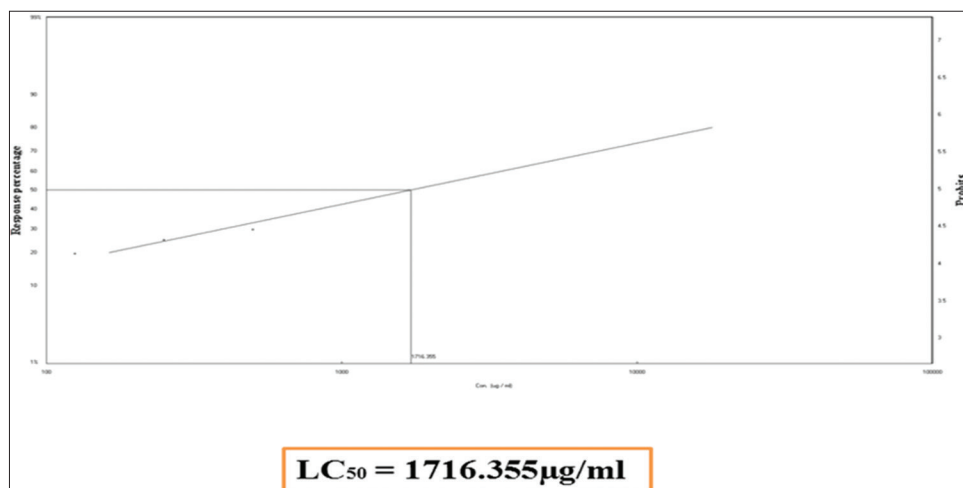


Fig. 1: Cytotoxic effect of LVR05 on normal liver cell lines percentage of viability and mortality



LC₅₀ = 1716.355µg/ml

Fig. 2: LC₅₀ value of LVR05 on normal liver cell lines was calculated by lethal dose probability line software

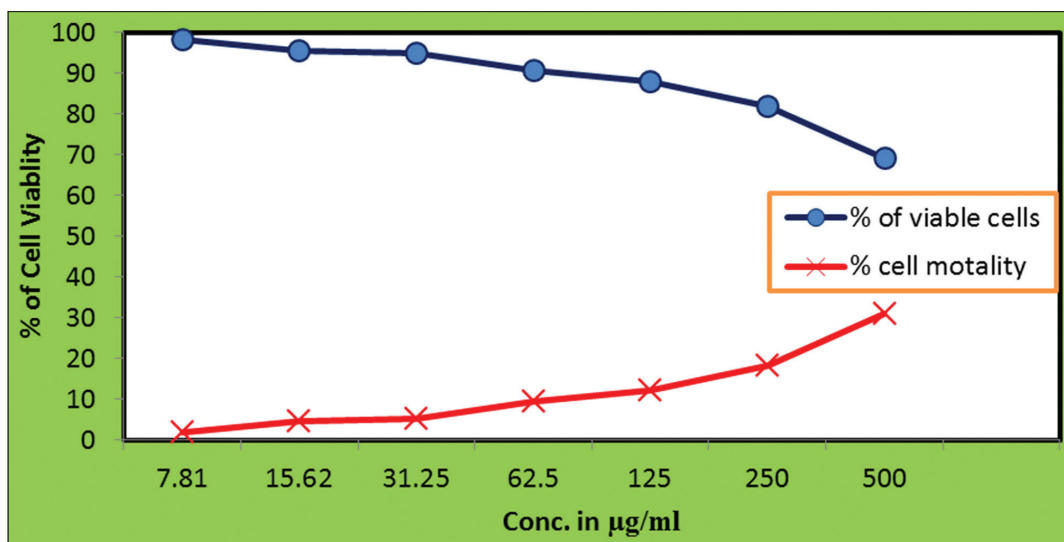


Fig. 3: Cytotoxic effect of LVR05 on normal kidney (vero) cell lines percentage of viability and mortality

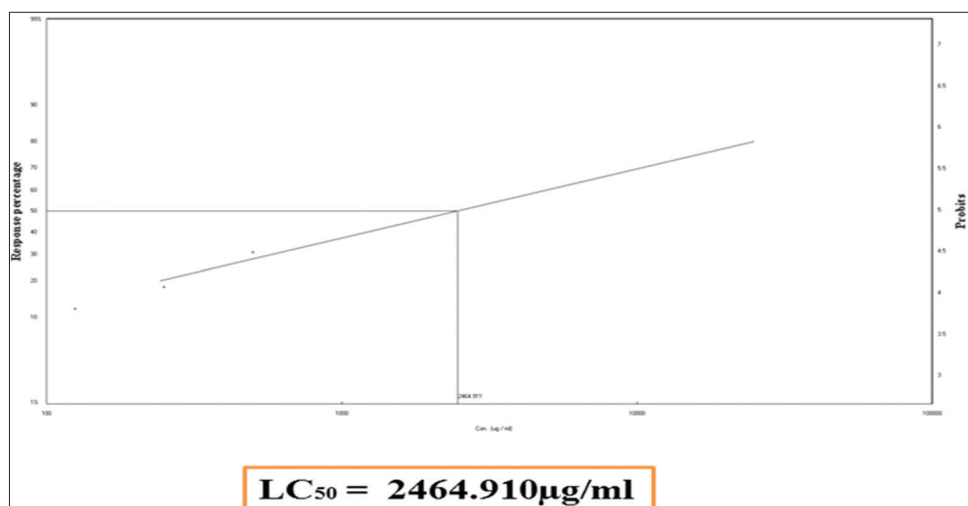


Fig. 4: LC₅₀ calculation by lethal dose probability line software

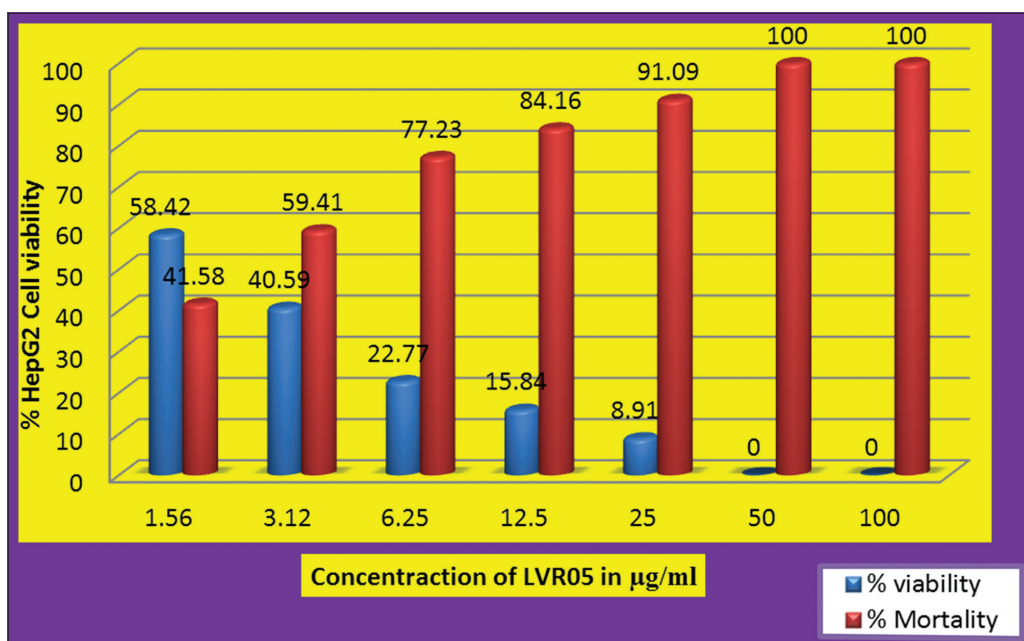


Fig. 5: Anticancer activity of different concentrations of LVR05 on liver cancer (HepG₂) cell lines

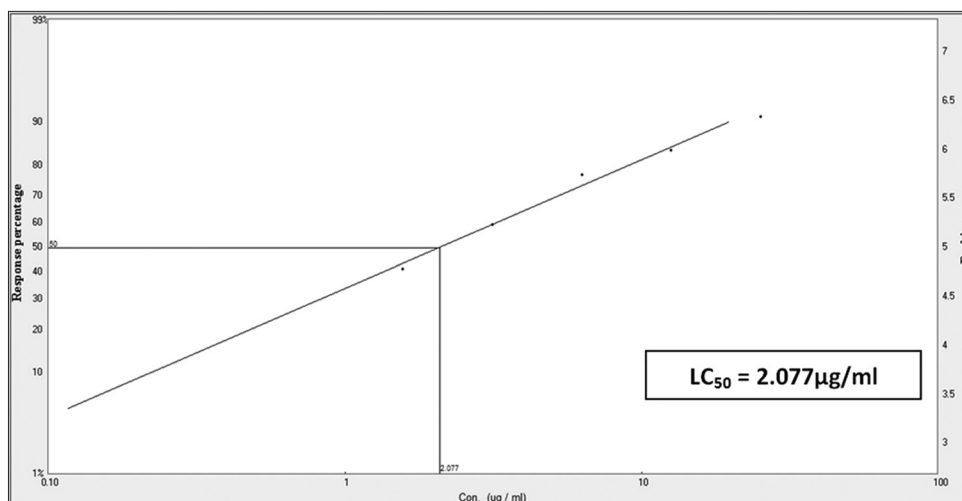


Fig. 6: LC₅₀ calculation by lethal dose probability line software

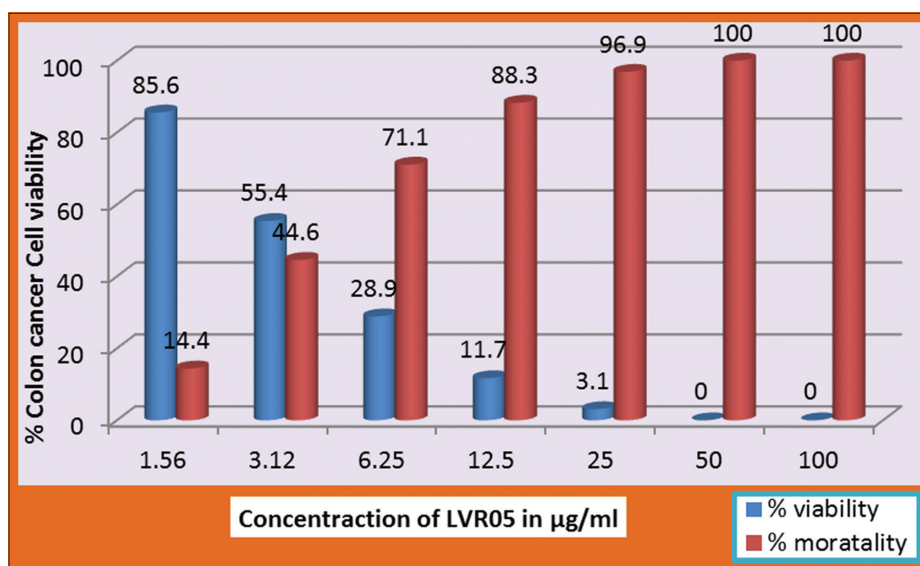


Fig. 7: Anticancer activity of different concentrations of LVR05 on colon cancer cell lines

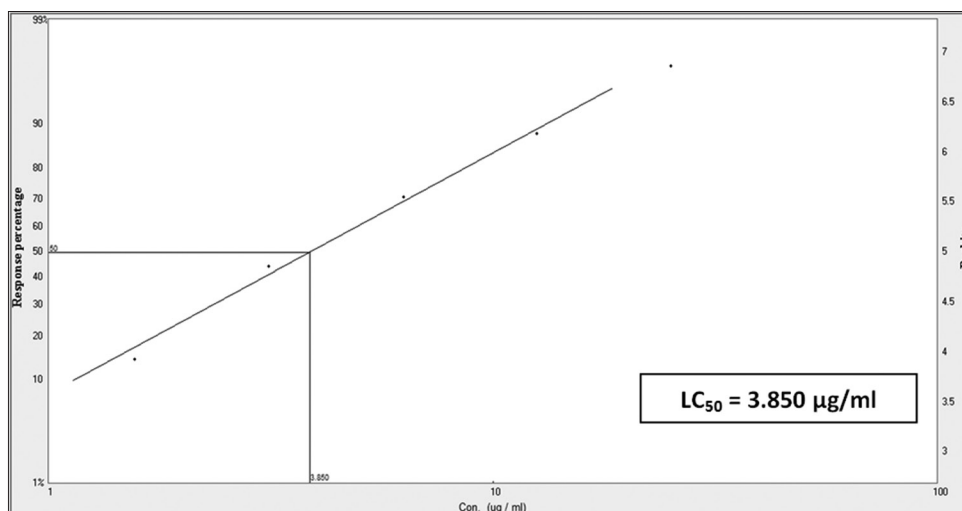


Fig. 8: LC₅₀ calculation by lethal dose probability line software

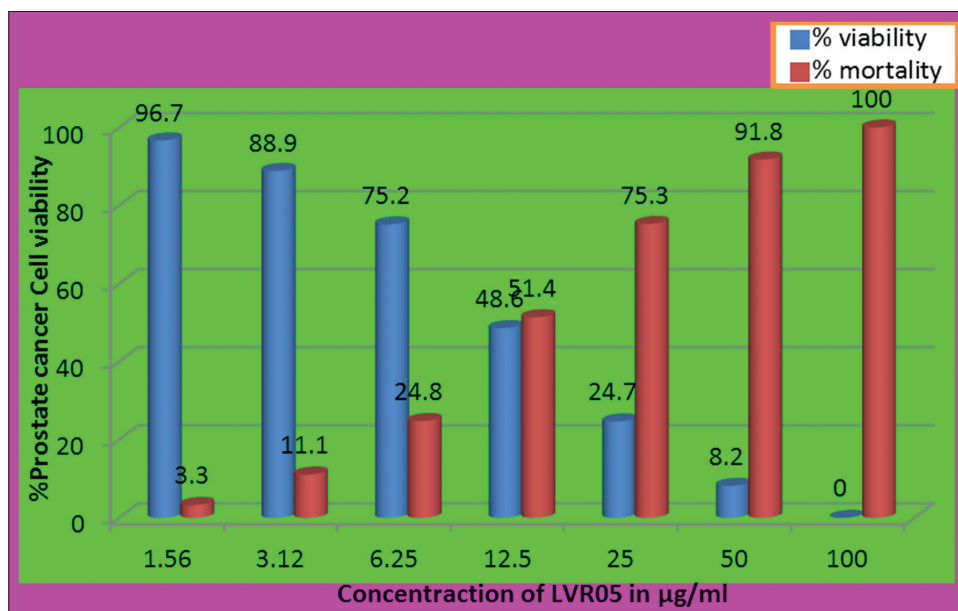


Fig. 9: Anticancer activity of different concentrations of LVR05 on prostate cancer cell lines

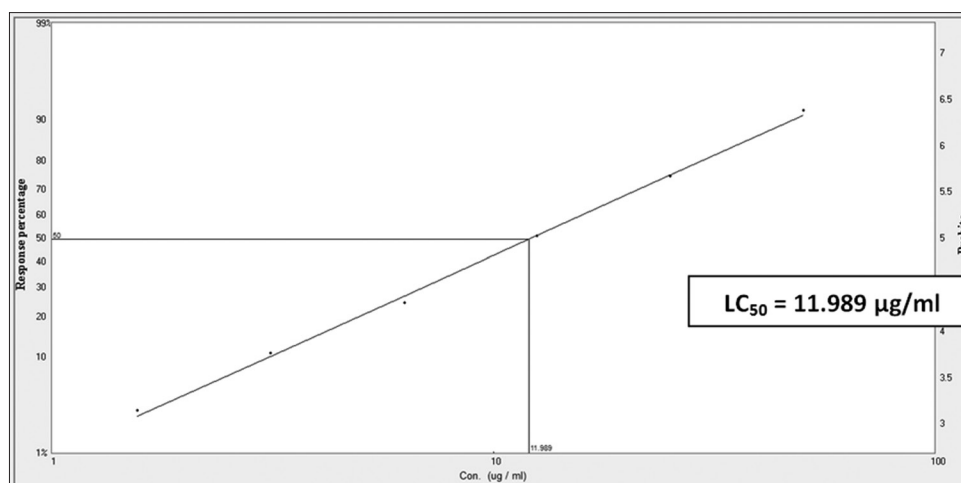


Fig. 10: LC₅₀ calculation by lethal dose probability line software

of additional toxicity tests that would be required. Any drug administered in to the system, undergoes metabolism in the liver. The liver is the primary organ which is exposed more to the drugs and toxicants. After the metabolism completed, it should eliminate through the kidney. During the elimination process, there are chances of causing damage to the kidney cells. Based on the results obtained from the cytotoxic activity, the polyherbal formulation does not induce any abnormality in normal liver and kidney cells which indicates its safety.

In vitro anticancer studies

Cellular screening in cancer research mainly consists of human tumor cell lines. It is the most suitable system in terms of management and reproducibility [11]. Currently, different concentrations of LVR05 were tested against liver, colon, and prostate cell lines and results found very effective.

Liver cancer or hepatocellular carcinoma has a low survival rate, and major risk factors include excessive alcohol intake, hepatitis, smoking, and high-grade fatty liver. Symptoms generally appear when cancer reaches an advanced stage. Treatment options for liver cancer include surgery and liver transplantation [12]. There is a

huge demand for safe and promising therapeutic agents which can reduce the incidence of liver cancer. Based on results obtained, the polyherbal formulation shows potent anticancer activity in dose-dependent manner. Even at low concentration (50 µg/ml), there was no cancerous cell viable which indicates the anticancer efficacy of the formulation.

Colon cancer is cancer of the large intestine (Colon), which is the final part of the digestive tract. Colon cancer starts with small, non-cancerous clumps of cells called adenomatous polyps. These polyps can become cancerous when continuous inflammation occurs in the colon. Till date, it is not clear what causes colon cancer and the cases of colon cancer are increasing [13,14]. The formulation is inhibiting the growth of colon cancer cells in dose dependent manner. At the concentration of 25µg/ml, cancer cell viability was 3.1% and mortality 96.9%. At the concentration of 50µg/ml there was no growth at all.

The incidence of prostate cancer is one of the most widespread cancer diagnoses in every part of the world. The pathogenesis of prostate cancer has remained a perplex issue [15]. In this study, the LVR05 inhibits prostate cancer cell growth 91.8% at the concentration of 50 µg/ml.

CONCLUSION

Based on the above results, it is concluded that the polyherbal formulation has not induced much toxicity in normal liver and kidney cell lines which indicate safe when taken internally. The hydroalcoholic extract possesses excellent anticancer activity on liver, colon, and prostate cancer cell lines. Further, identification and quantification of phytoconstituents responsible for the activity are in progress.

ACKNOWLEDGMENT

The author is thankful to the Director, Rumi Herbals Pvt. Ltd, Chennai, for providing test drug as a gift sample and necessary facilities to carry out the work.

AUTHORS' CONTRIBUTION

Research work was designed and executed by the corresponding author under the guidance of second, third, and fourth authors and technically supported by fifth author.

CONFLICTS OF INTEREST

All the authors declared no conflicts of interest. The major part of the work was handled by the corresponding author. Remaining authors are contributed in the required places.

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