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Original Research Article

In vitro anticancer screening of herbal syrup formulation of turmeric extract

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ABSTRACT

In continuation of previous research, the anticancer screening of formulation of Herbal syrup of Turmeric extract was performed. The formulation of herbal syrup was performed as per reported literature method. The anticancer cancer screening was performed on two Human cancer cell lines. These cell lines were Human Lung Cancer cell line A549 and Human Breast Cancer Cell line MCF7. The formulation was found to be devoid of anticancer properties.

1. Introduction

Cancer is a complex disease that results from the uncontrolled growth and division of abnormal cells. It can occur in any part of the body and spread to other parts of the body, making it difficult to treat. The impact of cancer on society is significant both in terms of human suffering and financial costs.[1]

1.1. Here are some ways cancer affects society

1.1.1. Human suffering

Cancer can cause physical pain, mental suffering and emotional trauma to patients and their families. It can affect all aspects of a person's life, from work to relationships and daily activities. The impact of cancer on a patient's quality of life can be devastating.

1.1.2. Mortality

Cancer is one of the leading causes of death worldwide. In 2020, it caused an estimated 9.9 million deaths worldwide. Certain cancers, such as pancreatic and lung cancer, have particularly high death rates.

1.1.3. Financial costs

Cancer treatment can be expensive, and the financial burden of cancer is significant. The costs of cancer treatment include not only the cost of treatment, but also the loss of productivity and quality of life. In 2020, the global economic cost of cancer was estimated at $1.16 trillion.

1.1.4. Health system

The high incidence and prevalence of cancer places a significant burden on health systems. Cancer patients require special care and the resources needed to treat cancer can be limited. The demand for cancer treatment is expected to continue to grow as the population ages and the incidence of cancer increases.[2]

1.1.5. Research and development

The search for new and better cancer treatments is an ongoing process. Research and development in the field requires significant resources and investments. Although advances in cancer treatment have been made in recent

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years, much remains to be done to improve outcomes for cancer patients.[3]

2. Turmeric[4]

1. Curcumin is the active compound in turmeric that has been studied for its potential anti-cancer effects.
2. Curcumin has anti-inflammatory, antioxidant and immunomodulatory effects that may contribute to its anti-cancer effects.
3. The ability of curcumin to inhibit the growth and reproduction of cancer cells, to induce apoptosis and angiogenesis (formation of new blood vessels necessary for tumors) has been studied.[5]

2.1. Overview of curcumin and its mechanism of action.[6]

Curcumin is a natural compound found in turmeric, extracted from the root of the turmeric plant (Curcuma longa). It has been extensively studied for its potential health benefits, including anti-cancer activity. Here is an overview of curcumin and its mechanism of action:

1. Curcumin is a polyphenolic compound with powerful antioxidant and anti-inflammatory properties. It has been shown to regulate several important signaling pathways involved in cancer development and progression, including the NF-κB pathway, the PI3K/Akt pathway, and the MAPK pathway.
2. Curcumin has been shown to induce apoptosis (programmed cell death) in many types of cancer cells, including breast, prostate, lung, colon and leukemia cells. This effect is thought to be mediated by up-regulation of pro-apoptotic proteins and down-regulation of anti-apoptotic proteins.[7,8]
3. Curcumin has also been shown to inhibit cancer cell growth and invasion by regulating cell cycle progression and regulating the expression of matrix metalloproteinase (MMP), which is involved involved in the breakdown of extracellular matrix proteins.[9]
4. In addition to its direct effect on cancer cells, curcumin has been shown to have immunomodulatory effects, including activation of natural killer cells and suppression of regulatory T cells, which may contribute to part of its anticancer activity.[10]
5. Curcumin has also been shown to inhibit angiogenesis, the process by which new blood vessels form to provide nutrients to cancer cells. This effect is thought to be mediated by downregulation of vascular endothelial growth factor (VEGF), a key regulator of angiogenesis.[11] Overall, curcumin's mechanism of action is complex and involves the regulation of multiple signaling pathways and cellular processes involved in cancer development and progression. Its powerful antioxidant and anti-inflammatory properties, combined with its effects on apoptosis, cell proliferation, invasion, angiogenesis, and immune function, make it become a promising candidate for the development of new anti-cancer therapies. However, more research is needed to fully understand its potential and optimize its use in cancer treatment.[12]

2.2. Herbal syrup[13–16]

Medicated herbal syrup was prepared as per by reported by Nerkar et al. Herbal syrup is prepared by herbal extract decoction with appropriate herbal excipients such as flavored sugar syrup, preservatives such as regenerated alcohol, flavoring, and herbal additives. To increase the shelf life of the prepared formulation it was mixed with sugar helps to build the viscosity and as natural preservative. Herbal syrup contains extracts of medicinal plants. Several herbal and medicinal syrups have been prepared and evaluated as per reported literature methods.

3. Curcumin structure

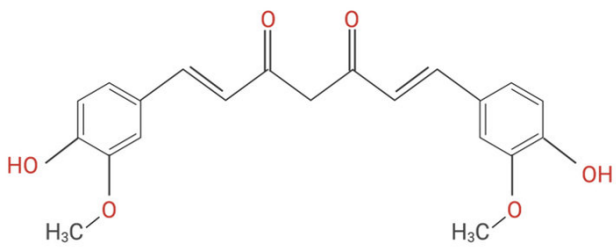

Fig. 1:

4. Material and Methods

4.1. Formulation of medicated herbal syrup of turmeric extract

This was prepared as reported by Nerkar et al. Further the syrup was dried in petri dishes and 0.5g dried syrup was dissolved in 5 ml of water to give a concentration on 100mg/ml. Further anticancer cytotoxicity assay was performed on 2 Cell lines viz Human Lung Cancer Cell line A549 and Human Breast cancer Cell Line MCF7. The turmeric extract was coded as RNT.

4.2. In vitro anticancer activity against cancer cell lines (anticancer cytotoxicity assay)

4.2.1. SRB assay

The assay relies on the ability of SRB to bind to protein components of cells that have been fixed to tissue-culture plates by trichloroacetic acid (TCA). The cell lines were

Table 1: In vitroanticancer activity of herbal formulation of turmeric extract against a549 human lung cancer cell line

Code	Human Lung Cancer Cell Line A549															
	% 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
RNT	100.0	94.6	89.5	75.0	91.4	87.1	86.7	72.2	99.6	97.1	91.7	73.3	97.0	92.9	89.3	73.5

Table 2: In vitro anticancer activity of herbal formulation of turmeric extract against mc7 hunan breast cancer cell line

Human Breast Cancer Cell Line MCF7																
	% 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
RNT	100.0	100.0	100.0	92.3	93.4	87.3	77.6	70.6	90.7	86.0	84.1	74.5	94.7	91.1	87.3	79.1

Table 3: Parameters for anticancer in vitro anticancerscreening LC50, TGI, GI50 of RNT against MCF7 Cell line

	Drug concentrations (μg/ml) calculated from graph		
MCF7	**LC50**	**TGI**	**GI50**
RNT	>80	>80	>80
ADR	3.7	3.2	0.2

Table 4: Parameters for anticancer in vitro screening LC50, TGI, GI50 of RNT againstA549 Cell Line

	Drug concentrations (μg/ml) calculated from graph		
A549	**LC50**	**TGI**	**GI50**
RNT	>80	>80	>80
ADR*	3.7	3.2	0.2

*Adriamycin

grown in RPMI 1640 medium containing 10% fetal bovine serum and 2 mM L-glutamine. For present screening experiment, cells were inoculated into 96 well microtiter plates in 100 μL at plating densities. After cell inoculation, the microtiter plates were incubated at 37°C, 5% CO2, 95% air and 100% relative humidity for 24 h prior to addition of experimental drugs. Experimental drugs were initially solubilized in dimethyl sulfoxide at 100mg/ml and diluted to 1mg/ml using water and stored frozen prior to use. At the time of drug addition, an aliquot of frozen concentrate (1mg/ml) was thawed and diluted to 100μg/ml, 200μg/ml, 400μg/ml and 800μg/ml with complete medium containing test article. Aliquots of 10μl of these different drug dilutions were added to the appropriate microtiter wells already containing 90μl of medium, resulting in the required final drug concentrations i.e.10μg/ml, 20μg/ml, 40μg/ml, 80μg/ml. After compound addition, plates were incubated at standard conditions for 48 hours and assay was terminated by the addition of cold TCA. Cells were fixed in situ by the gentle addition of 50μl of cold 30% (w/v) TCA (final concentration, 10% TCA) and incubated for 60 minutes at 4°C. The supernatant was discarded; the plates were washed five times with tap water and air dried. Sulforhodamine B (SRB) solution (50μl) at 0.4% (w/v) in 1% acetic acid was added to each of the wells, and plates were incubated for 20 minutes at room temperature. After staining, unbound dye was recovered and the residual dye was removed by washing five times with 1% acetic acid. The plates were air dried. Bound stain was subsequently eluted with 10mM trizma base, and the absorbance was read on an plate reader at a wavelength of 540nm with 690nm reference wavelength. Percent growth was calculated on a plate-by-plate basis 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 the control wells x 100. Using the six absorbance measurements [time zero (Tz), control growth (C), and 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. Percentage growth inhibition was calculated as: [Ti/C] x 100% Percentage growth inhibition, total growth inhibition TGI) and LC50 was calculated. GI50 value of $\leq$10 μg/ml is considered to demonstrate activity in case of pure compounds. For extracts, GI50 value $\leq$20 μg/ml is considered to demonstrate activity. Above three parameters were calculated only when the level of activity was observed. The values were expressed as greater or less than maximum or minimum concentration tested when the

effect was not reached or exceeded. $

5. Result and Discussion

From results as mentioned in Table 1, 2, 3 and 4. It is found that the Herbal Formulation of Turmeric is devoid of anticancer activity at concentrations of 10μg/ml, 20μg/ml, 40μg/ml and 80μg/ml as compared to parameters of LC50, TGI and GI50 and standard drug Adriamycin used for the assay.

6. Conclusion

The herbal formulation of syrup of turmeric extract is devoid of anticancer activity against Human A549 and Human MCF-7 Cell Line.

7. Source of Funding

None.

8. Conflict of Interest

None.

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