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RESEARCH ARTICLE

Lethality Assay of *Ficus religiosa* Leaves: An Indian Herb

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Abstract

For the treatment of cancerous cells in human body it needs to detect such medicine that kills the carcinoma cells without any adverse effects on human body. The anti-cancer drugs used previously exhibited relatively high toxicity not only to the tumour cells but also to the normal cells of the body part in which the cancer had developed. Currently assessment of pharmacological properties of plant has become one of the significant basic studies in biomedical sciences in recent years. In this context the present study was undertaken to evaluate the effect of *Ficus religiosa* plant leaves extract for cytotoxic study to calculate LD 50 value by using brine shrimp lethality (BSL) assay experimental method. The current research work was aimed to check the preliminary stage In-vitro cytotoxicity and anticancer activity of *Ficus religiosa* plant leaves with the help of Brine shrimp lethality bioassay experimental method. We concluded that if concentration of drug increases % Inhibition of cell line is also increases. The LD 50 value of the plant was found to be 61.06 µgm. It would be beneficial effects as anticancer use due to the easiness and low cost of *Ficus religiosa* leaves maintenance. This could accommodate the usage of this medicinal plant as anti- cancer. However, further study is needed to investigate the other anti-cancer mechanism of leaf extract.

Keywords: ADR, Anticancer, Brine Shrimp, Cytotoxicity, *Ficus religiosa*.

Introduction

In carcinogenic condition there is drastic increase in growth of cells and which is out of control (B. N. Meyer *et al.* 1982). The growth and multiplication can be occur in body and develops many cells and tissues (S. B. Chandrasekar *et al.* 2010). Generally, body cells form, developes and multiply (through a process called cell division) and shows formation of newer cells (Ayurvedic Pharmacopeia Of India, 2001). After some time new cells get old, damage and died and again forms newer cells (Prasad P *et al.* 2006). Sometimes abnormal or damaged cells grow and multiply instead of orderly process breaks down (Swami and Bisht 1996). These cells may form tumors, which are lumps of tissue (Bajracharya and Tuladhar (2011). Tumors can be cancerous or not cancerous (benign) (Quazi *et al.* 2017). Cancerous tumors spread into, or invade, nearby tissues and can travel to distant places in the body to form new tumors (a process called metastasis) (Carballo *et al.* 2002). Cancerous tumors may also be called malignant tumors (Saliba and Krzyz 1976). Many cancers form solid tumors, but cancers of the blood, such as leukemias, generally do not (Maity *et al.* 2017). Benign tumors do not spread into, or invade, nearby tissues (Shaikh *et al.* 2020). When removed, benign tumors usually don't grow back, whereas cancerous tumors sometimes do (Sahira Banu and Cathrine (2015). Benign tumors can sometimes be quite large, however (Kooti *et al.* 2017). Some can cause serious symptoms or be life threatening, such as benign tumors in the brain (Makhija *et al.* 2010).

Although, despite advances in surgical and radiation treatments, chemotherapy continues to be an important therapeutic option for different malignancies, especially for the primary, advanced and metastatic tumors (Enit *et al.* 2018). However, the efficacy of chemotherapy is substantially

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limited by the intrinsic and acquired resistance of cancer cells to anticancer drugs (Khandelwal and Sethi 2007). Resistance to chemotherapy is due to the variety of factors such as individual variations in patients (e.g. age, gender) and genetic differences in tumors (Choudhari *et al.* 2011). The most common reason of resistance to drugs is the change in the expression of one or more energy-dependent transporters, insensitivity to drug-induced apoptosis and induction of drug-detoxifying effect (Purnamasari *et al.* 2019).

Chemotherapy is used for the whole-body treatment of recurrent tumors using conventional anticancer drugs which lead to serious clinical side effects (Finney 1952). The side effects are mainly associated with the high dose, non-specific distribution severe toxicity to the normal cells, inadequate drug concentrations at tumours or cancerous cells, and thedevelopment of multidrug resistance (Niksic *et al.* 2021). Therefore, continuous research for improved anti- cancer therapies that can selectively target tumour cells with minimal side effects on normal tissues is going several approaches like immunotherapy, thermal therapy, phototherapy and gene therapy have been recently developed and tested for the suppression of such resistance (Nerdy *et al.* 2021).

Adverse Drug Reactions of Anticancer Drugs (Sung H ***et al.*** 2021**) –**

- Anemia, Appetite Loss, Bleeding and Bruising (Thrombocytopenia)
- Constipation, Delirium, Diarrhea, Edema (Swelling), Fatigue
- Fertility Issues in Boys and Men, Fertility Issues in Girls and Women
- Flu-Like Symptoms, Hair Loss (Alopecia), Infection and Neutropenia
- Lymphedema, Memory or Concentration Problems, Mouth and Throat Problems
- Nausea and Vomiting, Nerve Problems (Peripheral Neuropathy)
- Organ-Related Inflammation and Immunotherapy, Pain,
- Sexual Health Issues in Men, Sexual Health Issues in Women,
- Skin and Nail Changes, Sleep Problems, Urinary and Bladder Problems

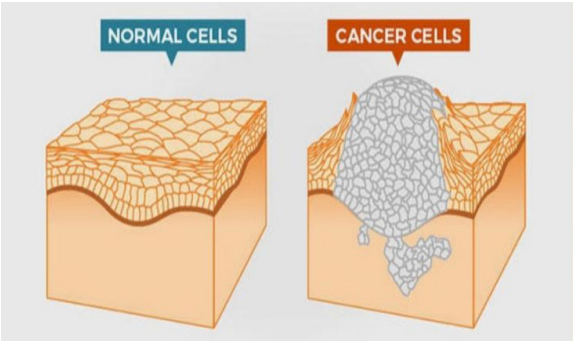

Figure 1: Cancer cells growth

Classification of Anticancer Drugs

Brine shrimp assay/test [24]

It has been proven that the brine shrimp lethality test (BSLT) has a good correlation with cytotoxic activity in some human solid tumors. Application of BSLT in cytotoxic assays has been described for several herbal plants. Although the BSLT is able to identify stronganticancer activity of tested compound, its main limitation is its sensitivity to distinguish between strong to moderate and weak anti-cancer potentials. Therefore, the BSLT represents a screening tool for potential cytotoxins, but a more sensitive distinction of anticancer activity requires testing on human cancer cells.

The brine shrimp (*Artemia salina*) lethality test (BSLT) method is often used to detect thep resence of compounds that have cytotoxicity in the process of active compounds isolation from natural materials by determination of the lethal concentration 50% (LC50) value. Based on the preliminary studies, the researchers are interested in using the brine shrimp lethality to measure the cytotoxicity of herbal plant anticancer potential.

Materials And Methods

Collection

All required *Ficus religiosa* plant leaves were collected from the local area. After collection of the required plant leaves for the study was washed with water for removing the dirt on the plant leaves surfaces. After this all leaves were kept for the drying.

Authentication

About 10 gm of *Ficus religiosa* plant leaves sample authenticated from the Botanist Dr. B. B. Dixit (Shamantak Enterprises). The authentication report/ certificate is attached in the following next page.

Drying

All collected Ficus religiosa leaves shade dried for the 7 days i.e. 1 week. In the processof the shade drying of the

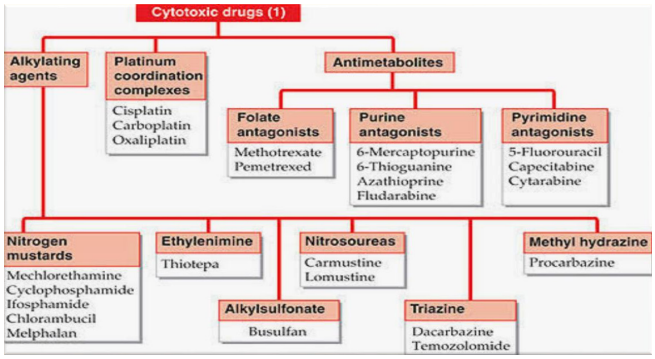

Figure 2: Classification of Anticancer Drug

Table 1: percent inhibition rate of ficusreligiosa extract on nauplii after 24 hours

Con. of extract	*Brine ShrimpTaken*	*Brine Shrimp Survived After 24Hr*		*Total Shrimp Survived*	*%Inhibition*
		T1	*T2*	*T1 + T2*	
10	10	9	8	17	15
20	10	8	7	15	25
30	10	7	6	13	35
40	10	6	6	12	40

leaves in that all moisture were present in the Ficus religiosa leaves were eliminated. After drying of the leaves converted into the coarse form for the extraction purpose.

Extraction - Decoction –This method is used for the extraction of the water soluble and heat s constituents from crude drug by boiling it in water for 20 - 40 minutes, cooling, straining and passing sufficient cold water through the drug to produce the required volume (Sahira Banu K., Dr. Cathrine L. (2015). The decoction method for the extraction of the Ficus religiosa plant leaves contain the boiling of coarse powder of the leaves with distilled water for 45 min. After cooling of liquid itfiltered and stored at cool and dry place.

- Water taken – 400 ml, Crude powder taken – 25 gm
- Ratio followed – 1 : 16, Heating time – 45 min
- Extract remained after boil – 205 ml, Store condition - 3 to 4 Degree Celsius

Brine Shrimp Lethality Assay

Brine shrimp lethality assay is an important tool for the preliminary cytotoxicity assay of plant extract and others based on the ability to kill a laboratory cultured larvae (nauplii). The nauplii were exposed to different concentrations of plant extract for 24 hours. The number of motile nauplii was calculated for the effectiveness of the extract. It is a simple,cost effective and requires small amount of test material. The larvae (nauplii; singular nauplius), about 22 mm long, are large enough to observe without high magnification andsmall enough for hatching in enormous amount without extensive workspace in a laboratory.

This assay was first proposed by Michael et al. in 1956. Subsequently, it was further developed by others. This lethality assay has been successively employed as a bioassay guide for active cytotoxic and antitumor agents in 1982 (Meyer et al., 1982).For the bioactive compound of either natural or synthetic origin, this is a rapid andcomprehensive test. It is also an inexpensive and simple test as no aseptic techniques are required. It easily utilizes a large number of organisms for statistical validation and requires no special equipment and relatively small amount of sample (2-20 mg or less) is necessary.

Materials Required

- Rectangular glass jar or glass bowl, Measuring cylinder (1000 mL)
- salt (27 g), Spatula, Brine shrimp eggs, Air pump & air stone with air controller
- Analytical balance, Pipette, Light source, Glass petriplates
- Test tubes, Magnifying glass, Test sample of plant extract

Protocol

Hatching brine shrimp

- Measure 3 liters of water using measuring cylinder and pour into the rectangular jar
- Weigh about 27 g of salt by an balance and add it into the jar containing water
- Mix the water with a spatula
- Place the tip of an airline from a air pump into the bottom of the jar maintaining properaeration
- Add about brine shrimp eggs at the top water level of the jar and mix with the water
- Switch on a light (60-100 Watt bulb) placed a few inches away from the jar
- After 20-24 hours, the nauplii will hatch
- Observe the eggs and nauplii
- Collect the nauplii after the next 24 hours
- Hatched nauplii must be separated from the empty egg. It can be done by turn off theair and switch off the lamp. The empty egg will float while the brine shrimp will concentrate in thewater column.
- Transfer 10 nauplii to a test tube using a pipette

Toxicity testing

- Expose the nauplii to different concentrations of the plant extract
- Count the number of survivors and calculate the percentage of death after 24 hours.

Result And Discussion

If concentration of drug increases from 10% to 40% Inhibition of cell line is also increases with probit value from

Table 2: Percent values in probit value

Con. of extract	*log C*	*%Inhibition*	*Probit value*
10	1	15	3.96
20	1.301029996	25	4.33
30	1.477121255	35	4.61
40	1.602059991	40	4.75

Table 3: Probit value table

%	0	1	2	3	4	5	6	7	8	9
0	-	2.67	2.95	3.12	3.25	3.36	3.45	3.52	3.59	3.66
10	3.72	3.77	3.82	3.87	3.92	3.96	4.01	4.05	4.08	4.12
20	4.16	4.19	4.23	4.26	4.25	4.33	4.36	4.39	4.42	4.45
30	4.48	4.50	4.53	4.56	4.59	4.61	4.64	4.67	4.69	4.72
40	4.75	4.77	4.80	4.82	4.85	4.87	4.90	4.92	4.95	4.97
50	5.00	5.03	5.05	5.08	5.10	5.13	5.15	5.18	5.20	5.23
60	5.25	5.28	5.31	5.33	5.36	5.39	5.41	5.44	5.47	5.50
70	5.52	5.55	5.58	5.61	5.64	5.67	5.71	5.74	5.77	5.81
80	5.84	5.88	5.92	5.95	5.99	6.04	6.08	6.13	6.18	6.23
90	6.28	6.34	6.41	6.48	6.55	6.64	6.75	6.88	7.05	7.33
--	0.0	0.1	0.2	0.3	0.4	0.5	0.6	0.7	0.8	0.9
99	7.33	7.37	7.41	7.46	7.51	7.58	7.65	7.75	7.88	8.09

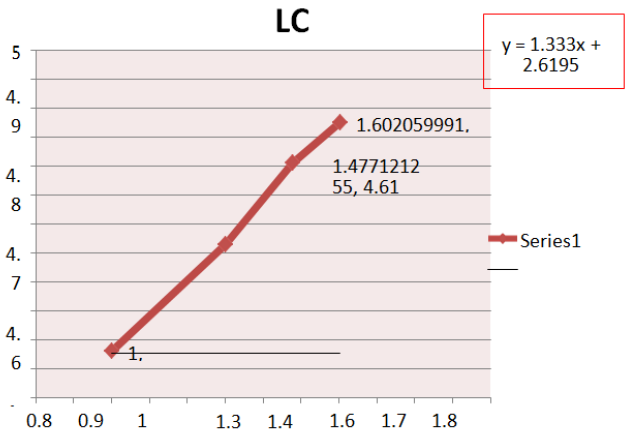

Figure 3: Scatter linear graph of observations in that probitvs log C and Showing the equation

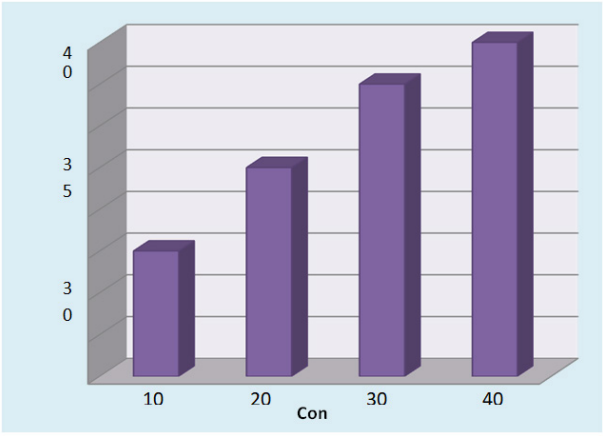

Figure 4: Statistical analysis of results - 3D bar graph of observations in that percent inhibition vs concentration

3.96 to 4.75 (2 and 3). Scatter linear graph of observations in that probits log C and Statistical analysis of results - 3D bar graph of observations are observed (Fig. 3 and 4).

Calculations

$y = 1.333x + 2.6195$ (i)

$R^2 = 0.997$

$y = mx + c$, $y = 5.0$ (for LD 50), ,$X = (y – c) / m$, $X = (5.0 – 2.6195) / 1.333$,

$X = 1.785821$, Anti Log X = LD 50, Anti Log X = 61.0690269

LD 50 = 61.06 µg

The lethal dose (LD50) Of *Ficus religiosa* plant leaves was found to be LD 50 = 61.06 µg by Lethality Assay.

The outcome of this research of lethality assay of *Ficus religiosa* leaves have provided insight of cytotoxic potential in nauplii of brine shrimp after 24 hrs observations are discussed for the herbal anticancer activity. The traditional regular medications of anticancer drugs causes the serious ADR on the human but herbal drugs not cause any serious ADR are also discussed. Future advanced study required to know API for cytotoxicity in the *Ficus religiosa* extract are also discussed in this experimental design of *Ficus religiosa* assay by brine shrimp method.

Conclusion

The current research work was aimed to check the preliminary stage In-vitro cytotoxicity and anticancer activity of *Ficus religiosa* plant leaves with the help of Brine shrimp lethality bioassay experimental method. We concluded that if concentration of drug increases % Inhibition of cell line is also increases. The LD 50 value of *Ficus religiosa*was found to be 61.06 µgm. It would be beneficial effects as anticancer use due to the easiness and low cost of *Ficus religiosa* leaves maintenance. This could accommodate the usage of this medicinal plant as anti-cancer. However, further study is

needed to investigate the other anti-cancer mechanism of leaf extract. Hence, the *Ficus religiosa* leaves may be useful in discovery for its anticancer potential, further investigation is required to understand the possible mechanism of API in leaves which responsible for its cytotoxicity.

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Disclosure of Conflict of Interest

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