ORIGINAL ARTICLE

FUTURE JOURNAL OF PHARMACEUTICALS AND HEALTH SCIENCES

Published by Pharma Springs Publication Journal Home Page: https://pharmasprings.com/fjphs

Evaluation of *In-Vitro* Cytotoxic Potential of *Ficus benghalensis* Tender Prop Roots Extract

Badugu Kranti Kumar[*1], Kondoju Arun Kumar Chary[2], Kiran[2], Gajarlu Shivani[2], Abdul Rahaman S K[2], Boidi Mamatha[2]

[1]Department of Pharmacognosy, St. Mary's Group of Institutions, Deshmukhi (Village), Pochampally (Mandal), Yadadri Bhuvanagiri (Dist)-508284, Telangana, India
[2]St. Mary's Group of Institutions, Deshmukhi (Village), Pochampally (Mandal), Yadadri Bhuvanagiri (Dist)-508284, Telangana, India

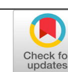
Article History:

Received on: 15 May 2022
Revised on: 03 Jun 2022
Accepted on: 04 Jun 2022

Keywords:

Colon Adenocarcinoma,
Colo 320 Cell Lines,
Tryphan Blue Assay,
MTT Assay

ABSTRACT



Cancerous cell lines might grow to develop a kind indispensible interconnect within stages through the structure–based drug to a collection of medical applicants such as cancer therapeutics. The current study is one such effort to screen for *in vitro* cytotoxic potential of methanolic prop root extract of *Ficus benghalensis* against human colon adenocarcinoma (*Colo 320*) cell cultures besides Tryphan turquoise assay as well as MTT microarrays following the 24 hours exposure. A present work demonstrated a reduction in the count of total viable cell by Tryphan blue assay. However, we have reported anti-proliferative potential of methanolic extract of *Ficus benghalensis* on *colo 320* cell cultures with just an Ic50 values like 87.03μg/milliliter. Extensive research needs to be employed in the near future to explore the anti cancer potential of various solvent extracts of different parts of the plant to evolve a molecule of interest.

*Corresponding Author

Name: Badugu Kranti Kumar
Phone: +91 9160394919
Email: baduga.kranthi19@gmail.com

eISSN: 2583-116X pISSN:

DOI: https://doi.org/10.26452/fjphs.v2i3.275

Production and Hosted by

Pharmasprings.com

© 2022 | All rights reserved.

INTRODUCTION

Colorectal cancer treatment decisions are made now since identifying the best choices available again for phase and site of carcinoma, and also the benefits and risks associated among each [1]. Colorectal cancer individuals with colorectal cancer, especially throughout early phases just might have to undertake a few type like operation to remove tumors and adjuvant chemotherapy or radiation might be needed. Carcinoma *in situ*, this surgical procedure has been performed by eliminating abnormal growth and could be achieved through polypectomy but rather municipal resection and through fluoroscope surgical excision of such a section of such colorectal and necessary is if tumour cell is much too huge just to be excluded through native colon resection. Confined phase [2]. In this surgical excision has to be performed to eliminate a carcinoma, together under a size like colorectal along both part of a cancer cell as well as efferent lymphatic single node. Regional stage, in this stage surgical excision of a section like colorectal comprising a cancer cell could be the very first diagnosis if a carcinoma tumour has not spread to nearby lymph nodes. If the carcinoma is probably going of about recur with it's character underneath the magnifying glass either because it is going to grow into some other connective tissue, radiotherapy, as well as chemotherapeutics, could be suggested as well [3]. If indeed the cancer that has spread of about regional lymph source node, clinical excision of a section like colorectal compris-

ing a cancer cell might be the first diagnosis, followed through chemotherapeutics. Chemo treatments predicted upon that opioid Fe_3O_4 (5-fu) were shown to enhance patient survival to stage ii as well as scenario 3 disorder, improved by reducing disorder re-occurrence. Radiation treatment might be suggested if a carcinoma does have risen into the adjoining connective tissue. Distanced phase at it phase, a cancer is spreading of about faraway organs or tissues, including the kidneys, lung tissue, peritoneal cavity (lining of a abdomen), but rather ovarian follicles [4]. An objective like surgical (segmental excision rather than deviating colostomy) inside this stage includes to alleviate as well as avoid blocking of a colorectal and also to inhibit side local health problems. When there are just a couple of metastatic disease to kidneys as well as lung tissue, operation to remove these, but also the colorectal cancer cell could be an opportunity. Rectal cancer except with faraway phase carcinoma, surgeries are needed to remove a testicular cancer is typically the primary diagnosis. Additional treatment options, such like chemo and radiation, often are involved already when surgical (neoadjuvant therapy) either after surgical procedure (adjuvant therapy) to scale back the danger anyway re-occurrence but also malignancy. A chemo drugs used throughout the diagnosis like testicular cancer were the same as these are used such as colorectal tumour [5].

MATERIALS AND METHODS

RPMI 1640 was gift sample from Difco, invitrogen corp, CanadaTryphan blue was found from CDH, New DelhiColor. Foetal Bovine Serum, Antibiotics, DMSO, Trypsin, EDTA, MTT was found from Zibco pharmaceuticals Pvt. Ltd. All abundant chemical as well as chemical agent utilized in this study are of analytical grade.

Plant Material Collection and Authentification

The tender prop roots were collected from *Ficus benghalensis* tree was collected from the same was authenticated by the Botanist at SV Govt. arts and science college, Tirupati and the specimen was preserved for reference [6].

Preparation of the Aerial Prop Root Powder for Extraction

The Aerial prop roots of *Ficus benghalensis* were shade dried and pulverized to get coarse powder followed by sieving (Sieve Number 44) to remove the unwanted waste material and to obtain the coarse powder [7].

Process of Extraction

About 100 g of the *Ficus benghalensis* crude root powder was subjected to extraction by using 600ml of methanol successfully by Continuous hot percolation method by using Soxhlet apparatus at a constant temperature of about 45- 55°C. The crude powder was extracted with the solvent for 4 consecutive days. After extraction, the extract was collected and removed of solvent by using rota vapour apparatus to get well-dried extract, weighed and the percentage yield was calculated from the weighed powder of the plant [8]. The percentage yield of the extract obtained was about 4.75%. The obtained extract was further used to evaluate for its anti-cancer activities by preparing concentrations of 10,25,50,75 and 100 ug/ml in double-distilled water.

Calculation of Percentage Yield

The percentage yield of the extract after successful completion of the extraction process has been estimated using the equation [9] and a proportion yield of a prop roots extract was found to be 4.75%.

$$Percentage \quad Yield \quad \% = \frac{weight\ of\ the\ dried\ extract\ sample}{weight\ of\ the\ original\ sample} \times 100$$

In-Vitro Anticancer Activity

Cell Lines

COLO 320 cell lines were obtained from Sugen Life Sciences Pvt. Ltd., Tirupati and cultured in RPMI 1640 medium (Difco, invitrogen corp, Canada).

Culture Method

A bottom moderate for this entire human cell seems to be constructed RPMI-1640 moderate. Of about create the entire medium containing adds additional ingredients of about the bottom moderate: fetus's invitrogen to such a desired concentration like 10%.

Sub Culturing

Culture conditions Temp 37°C

Volumes included in this guidelines are just for 75 cm two bottle; proportionate decrease as well as increase in financial like depersonalisation moderate as a civilization warships of all other sizes [10].

Shake bottle. Eliminate growth media to such desiccators.

Thoroughly sanitize a cell layer to 0.25% (w/v) Trypsin-0.53 micrometers EDTA solute to eliminate the whole trace amounts like retinol where it consists trypsin inhibitor.

Add 2.0 of about 3.0 ml like Trypsin-EDTA solute to bottle but also notice cellular underneath a reversed magnifying glass till the cell layer seems to be diffused (5 to 10 min). Of about eliminate large clumps need not stir up that whole cellular through whack-

ing as well as convulsing a bottle whereas awaiting a cellular complete disassociate. Cellular those that are complicated of about disassociate could be positioned there as 37°C of about expedite dispersal [11].

Add 6.0 to 8.0 millilitre like entire medium containing as well as aspirate cellular through softly pipette tips.

Eliminate pepsin EDTA solute, transmit suspension cell to a desiccators only with moderate as well as cellular through the step 1 but also rotates about as nearly for five to ten mins. Simply dismiss culture medium as well as resuspend cellular through clean liquid nutrient. Incorporate suitable aliquots like suspension cells of about fresh culture vessels.

Positioned civilization warships through new ventures about as 37°C. Sub -cultivation Ratio: The subcultivation ratio of 1:3 to 1:4 were suggested

Moderate Revival**:** Each 3 to 4 days

Freeze moderate: Complete growth medium supplemented with 5% (v/v) DMSO

Storage temp: Liquid nitrogen vapour phase

Cryopreservation

Freeze moderate: Entire liquid nutrient augmented with 5% (v/v) DMSO

Storage temp: Liquid nitrogen vapor phase

Culture observations: Temp: 37°Centigrade

Tryphan Blue Dye Exclusion Assay Method

Experimental Design

A constructed research consisted like 3 kinds namely: negative control, control, experiment, within negative control team a cell cultures have been cultured only with intermediate for a period of 24 hour shifts. Such a team has been constructed of about preclude the possibility of almost any growth inhibitory impact with certain molecules like intermediate [12].

A controlled study has been constructed of about govern out through the impact of any residue left as well as trace amounts like solutes whereby the extricate has been able to prepare upon that inhibition of growth like cell cultures. Here that the diluent appointed has been methanol and thus it's indeed decided to add just at accumulation like 0.1% (v/v) through deionised water [Table 1].

Through sample group various concentrations like experiment extricate i.e, 10, 25, 50, 75 as well as 100μg/ml have been cultured as for *colo 320* cell cultures for a period of 24 hour shifts. Above team has been used to analyse the influence through cultured cells effectiveness.

Principle

The Tryphan blue dye exclusion experiment has been used to specify the amount like viable cells are present inside a suspension cell. It's indeed founded on the supposition a certain cell lines acquire internal cell membrane surface which exclude definite colorants. Such like Tryphan turquoise, eosin, as well as propidium, so although dead tissue do not [13]. Through such an experiment, one suspension cell is just combined to stain and thereafter visual elements investigated to determine yet if cells are able that exclude stain. Inside the guidelines described here, one live cell has an evident cytosol even though an unsuitable organelle would have a dark blue cytosol.

Procedure

1. An equal amount like suspension cell becoming examined as effectiveness has been centrifuge tube just for five min and supernatant has been decanted. Dimensions of an equal amount is dependent upon an approximate number like cell lineages. An equal amount seems to be captured whereby it consisted one efficient amount of cells of about add up inside a haemocytometer while suspension through 1 ml PBS from thereafter solubilized by once again melding to 0.4% Tryphan turquoise(e.g., 5×10^5 cells/ml).

2. An organelle beads has been re suspended through 1 ml pbs as well as serum–free medium entire moderate. Serum peptides blemish as for Tryphan turquoise and therefore can generate inaccurate results. Thus the legal decisions has to be created through serum-free solution.

3. Combining 1 section of 0.4% Tryphan turquoise but also 1 part suspension cell (dilution of cells) cellular have been combined but also permitted to impregnate there as ambient temp for roughly three minutes, cells were collected inside of three to five min of blending with Tryphan turquoise, since prolonged fermentation durations might result in cell damage as well as lower viable cells qualifies. Combining has been conducted in the well of such a microtiter or even a tiny plastic tubular utilizing 10 to 20 μl each one of suspension cell but also Tryphan turquoise.

4. One fall of a Tryphan turquoise weird mix has been implemented through haemocytometer. The haemocytometer on a phase of such a stereoscopic magnifying but also cell lines have been decided to focus plainly.

Table 1: Experimental Design to Study the Effect of Methanolic Extract of Ficus Benghalensis Prop Roots on *Colo 320* Cell Line Viability by Tryphan Blue Assay

S.No.	Group	Treatment
1.	Negative control	*Colo 320* cell lines with medium (RPMI 1649)
2.	Control	*Colo 320* cell lines were incubated in RPMI medium along with methanol at a concentration of 0.1% v/v.
3.	Test	*Colo 320* cell line with cultured medium and MEFB extract at different concentration

5. A no. of untarnished (viable) but also discoloured (nonviable) cellulars have been collected individually within haemocytometer. In order to be able to acquire the whole number of selected cell cultures for every millilitre like equal volume, multiply a whole number of selected cellular was multiplied through 2 (i.e, the dilution factor). To acquire the whole amount of cells for every millilitres like equal volume, the entire number of organism but also nonviable cellular were decided to add but also magnified through 2.

Seeding of Cells

COLO 320 cellular have been cultivated to achieve an 80-90% final concentration utilizing RPMI 1640 intermediate. Now since achieving the specified final concentration, civilization has been gathered as well as subjected to centrifugation there as 3000 RPM as the min to just get organelle bead. A bead has been re-suspended through 1ml like subculturing media [14]. Cell density was resolute through Tryphan turquoise analysis was conducted through combining 50 μlitres like civilization as well as 50 μlitres like 0.4% tryphan blue dye. Eventually cellular implanted through 24 fine panels just at accumulation 10000 cellular/milliliters but also sub cultured there as 5% carbon dioxide Incubation about as 37^0C such as 24 hour shifts.

Drug Treatment

Cellular have been preserved through 24 fine panels through triplicate per each accumulation, (10, 25, 50, 75, 100μg/ml) of MEFB as well as treated with various concentrations but also healthy controls have been allowed to treat as for intermediate methanol. The cells treated have been sub cultured such as 24 hour shifts through 5% carbon dioxide business incubation about as 37^0c.

In-vitro Cytotoxic Assay

After 24 hour shifts fermentation, that whole cellular have been gathered out of each well here in eppendorff but also centrifuge tube there as 3000 rpm such as 10 min to be get organelle bead, towards the bead 50μlit of every intermediate as well as tryphan turquoise assay. % like inhibition of growth has been measured while using the following equations concept:

$$\% \quad of \quad growth \quad inhibition = \frac{No.of\ cells\ in\ Control\ cells/\ ml - No.\ of\ cells\ in\ testcells/ml}{Control\ cells/ml} \times 100$$

Micro Culture Tetrazolium Assay

Plant Material Used

Methanolic Extract Obtained from the Prop Roots *of Ficus benghalensis.*

Principle

Such a test depends upon the capabilities of cellular membranes succinate dehydrogenase enzymatic through living organisms to scale back a yellow coloured soluble in water adsorbent 3 (4, 5 dimethyl thiazol yl) 2, 5 diphenyl tetrazolium bromide within such an irresolvable purple coloured formazan item whose coloured has been calculated by means like ELISA reader at 540 nm. Just cell viability as for effective cellular membranes minimize substantial quantities like MTT, until minimisation like MTT only can eventuate through metabolically active cellular [Figure 1].

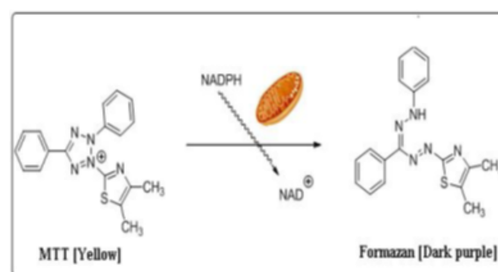


Figure 1: Reduction of MTT to a Formazan Compound by Mitochondrial

Enzymes Cell Lines

Person colon cancer adenocarcinoma-*colo 320* were have been acquired through sugen Life Sciences Pvt. Ltd., Tirupati from such a verified distributor, the stock civilization of such cell cultures seem

to be cultivated through RPMI -1640 for 10% inactive newly born human serum, amoxicillin (100 IU/ml), Streptomycin (100μg/ml)) below pressurized atmospheric composition like 5% Carbon dioxide about as 37^0C till the collinear. A cellular have been disconnected along 0.2% trypsin but also 0.02% EDTA through phosphate buffer solution saline flush, a stock culture was grown in 25cm 2 tissue culture flasks and cytotoxicity experiments were held in 96 well microtiter plates [15].

Procedure

1. Cell culture within exponentially growing stage have been chosen, cleaned, trypsinized but also suspension such as entire agar medium i.e., RPMI 1640.
2. Cellular have been plated about as10,000 cells/well through ninety six fine microplates as well as sub-cultured just that 24hrs where a biased single layer was established.
3. They really were subjected to multiple concentration levels of a extricate (1-100μg/ml). Regulate of well did receive just the maintenance intermediate.
4. A panels have been sub cultured about as 37°C inside a humid organizer as for 5% CO_2 for a period of 48 hr shifts but also cellular have been sporadically did check such as granular permissions, shrinking as well as bulging. Now since 48 hr shifts, a standard solution through of well has been glanced off it but also 50μl like MTT pigment has been decided to add to every well.
5. A panels seem to be tenderly rattled as well as sub-cultured such as 4 hr shifts about as 37^0C through 5% CO_2 incubating. A supernatant was discarded as well as 50 μl like DMSO has been got to add.
6. A panels have been tenderly rattled of about solubilise an established precipitate. A absorbance was recorded there as 540nm [16].

A proportion like inhibition of growth has been calculated by using the following equation,

$$Percentage\ growth\ inhibiton = \frac{Mean\ OD\ of\ individual\ test\ group}{Mean\ OD\ of\ control\ group}$$

Value like absorption have been transformed into the proportion like viable remnant cells. Generally an inhibitory activity accumulation 50% (IC_{50}) has been selected even as finest physiological indicator like cytotoxic activity. A IC_{50} values signifies an accumulation of an experiment that helps in extracting a certain lowered 50% like organelle inhibitory activity [17].

Statistical analysis

IC_{50} has been determined by calculating through linear programming technique just using formula

$$IC_{50} = \frac{50-A}{B-A} \times (D-C) + C$$

Where A = The first point on the curve, demonstrated through % inhibitory activity, which is less than 50%

B = The first point on the curve, expressed in percent inhibition, that is greater than or equal to 50%

C =The concentration of inhibitor that gives A% inhibition

D = The concentration of inhibitor that gives B% inhibition

The experimental data obtained were statistically examined through normal one-way ANOVA pursued through Tukey's multitude comparative experiment employing the trial version of Graph Pad Prism [18], San Diego version (Prism graph pad version 8.0.2 (263, graph pad prism, Inc. La Jolla, CA USA). Statistical significant has been arranged about as p,0.0001.

RESULTS AND DISCUSSION

The Percentage yield of prop roots extracts of *Ficus benghalensis.*

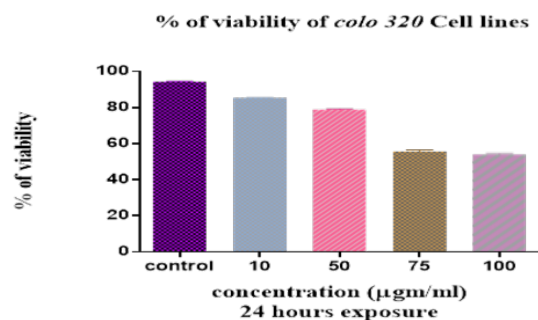


Figure 2: Effect of Methanolic Extract of *Ficus benghalensis* on Viability of Colo 320 Cell Lines by Tryphan Blue Assay

Single exposure of incubation of *Colo 320* cell lines to different concentrations of MEFB (10, 25, 50, 75, 100 μg/ml) for 24 hours by Tryphan blue assay resulted in an increasing production reliant minimisation there in the viability like cells when compared with that of control ($p<0.0001$).

Micro Culture Tetrazolium Assay

Single exposure of incubation of *Colo 320* cell lines to different concentrations of MEFB (10, 25, 50, 75,

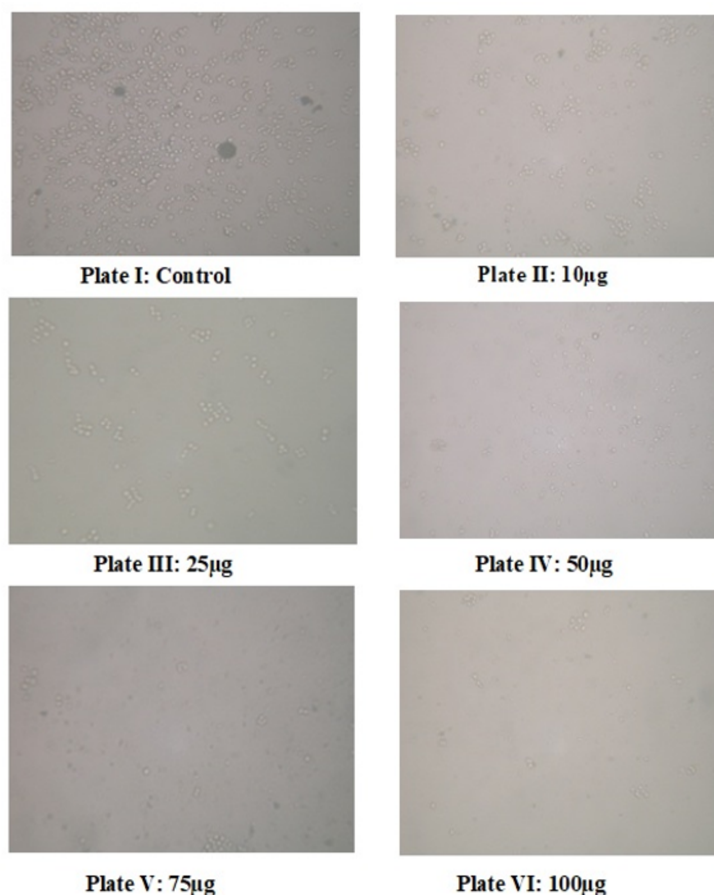


Figure 3: Images of *Colo 320* Cell Lines Treated with MEFB

Table 2: Percentage Yield of Prop Roots Extract of *Ficus benghalensis*

S. No.	Name of the test substance	% Yield (mg/100 gm)
1.	Methanolic prop root extracts of Ficus benghalensis	4.75 ± 2.13

Table 3: Effect of Methanolic Extract of *Ficus benghalensis* on Viability of *Colo 320* Cell Lines by Tryphan Blue Assay

S.No.	Groups	Concentration of MEFB (μg/ml)	% Cell viability
1	Control		94.43±0.3480
2	Test	10	85.30±0.1538^{a}
		25	82.17±0.5487^{a}
		50	78.83±0.4410^{a}
		75	55.73±0.8192^{a}
		100	54.07±0.5207^{a}

All values were expressed as Mean ± SEM of (n=3) performed by ANOVA; a = p<0.0001; All groups are compared with control.

Table 4: Effect of Methanolic Extract of *Ficus benghalensis* on Viability of *Colo 320* Cell Lines by MTT Assay

S.No.	Groups	Concentration of MEFB (μg/ml)	% Cell Viability
1	Control		95.07±0.233
2	Test	10	87.17±0.441[a]
		25	82.73±0.3712[a]
		50	78±0.5774[a]
		75	64.07±0.3528[a]
		100	47.87±1.139[a]

The whole values are presented just like Mean ± SEM of (n=3) performed by ANOVA; a = p< 0.0001; All groups are compared with control.

100 μg/ml) for 24hours by MTT assay resulted in a significant dose dependent reduction in the viability of cells when compared with that of control (p<0.0001).

IC_{50} value of Methanolic extract of *Ficus benghalensis* was calculated by linear interpolation method using the formula:

$$IC_{50} = \frac{50-A}{B-A} \times (D-C) + C$$

where A = The first point on the curve, expressed in percent inhibition, that is less than 50%

B = The first point on the curve, expressed in percent inhibition, that is greater than or equal to 50%

C = The concentration of inhibitor that gives A% inhibition; and

D = the concentration of inhibitor that gives B % inhibition.

$$IC_{50} = \frac{50-35.9}{52.1-35.9} \times (100-75) + 75 = 87.03\mu g/ml$$

Traditionally the complexity of carcinoma biology has required a kind enormous amount of which research has led to significant developments through our understanding of disease pathology. This complexity of the disease is unable to match with the prevention and therapeutic approaches to shut bottom a wide array of immortalized cellular through trying to act on several different methodologies but also paths.

Colon carcinoma is among the most divesting malignancies on the earth which arises as from the epithelial cell lining of the digestive mucosal and undergoes sequenced point mutation through particular DNA sequence alignment, thereby disrupting the entire methodologies like proliferation as well as self revival. All through ancient civilisations, individuals have depended through environment of about accommodate their own essential necessities, which it's a some are used for diagnosis like broad scope like illnesses cancer is one among those with which has taken a large share from the nature.

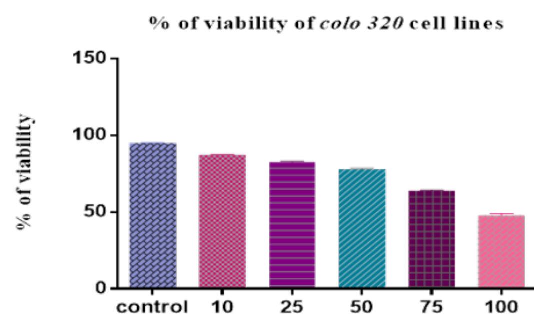


Figure 4: Effect of Methanolic Extract of *Ficus benghalensis* on Viability of Colo 320 Cell Lines by MTT Assay

Ficus benghalensis is a tropical plant belongs to family like Moraceae. A number of preclinical studies have demonstrated various pharmacological activities of the extracts derived from Ficus species. Many novel agents have revolutionized the therapy of colorectal cancer, unfortunately most of them met with severe adverse effects. In this regard our present effort is a preliminary one to detect anti-cancer activity of a widely distributed tree in the Indian subcontinent, viz. *Ficus benghalensis* employing *in vitro* cytotoxic assays like Tryphan blue and MTT assay.

The specimen was taxonomically authenticated and the crude powdered form of shade dried tender prop roots of Ficus tree was extracted with methanol. Phytochemical screening was reported from earlier studies hence not gone for the screening.

The methanol predicted excavation methodology used for the present research has indeed been regularly tried to follow for exploring therapeutically valuable phytochemicals. Further the solvent used for extraction i.e methanol was likely possess antibacterial activity and thus prevent the culture from contamination and development of fungal spores. However as much improved excavation methods have been needed to fix an anticancer activity in

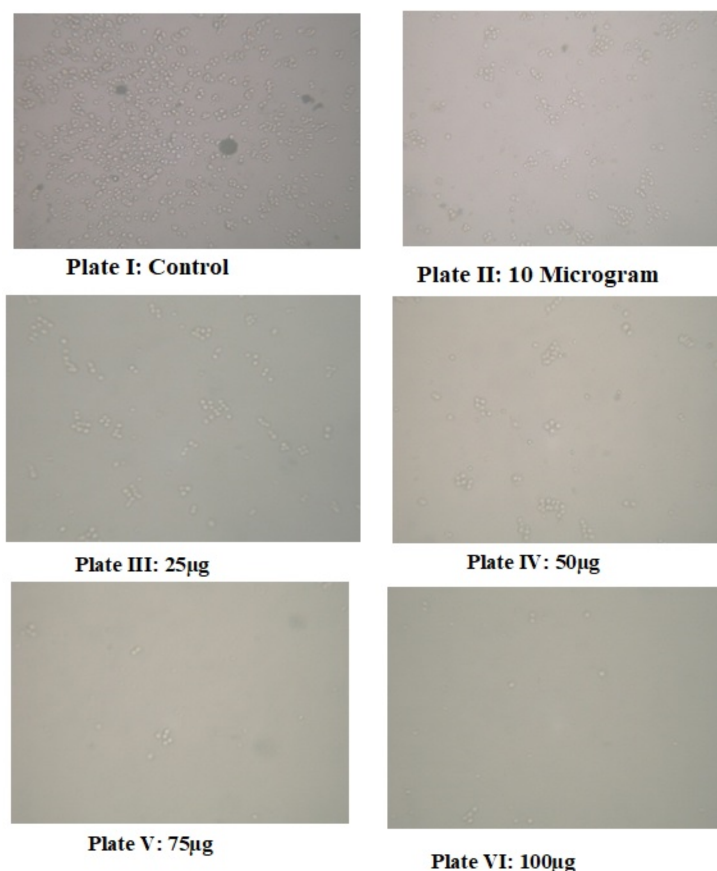


Figure 5: Images of *Colo 320* Cell Lines Treated with MEFB

future studies.

Preclinical safety testing of recent drug candidates may be a crucial step in pharmaceutical drug development and depends on an ordered series of *in-vitro, in-vivo* and *in-silico* tests before administration to humans.

Currently, *in-vivo* assessing may be an important part like safety evaluation, but also may be the regulatory standards before such a substance will advancement into the clinical research. However, through current history, numerous in-vitro immunoassays have now been developed but also implemented such as early-stage monitoring toward order to filter beyond particles the next possibilities as a toxic effect and that in some instances planning to replace as well as minimizing utilization with certain throughout *vivo* experiments as adhesion to a 3Rs, one set of values by ICH a certain underlines a partial substitute, reducing but also refinement of an utilization like living creatures through data analysis.

Cell civilization methodologies play an important role within discovery of modern anti-cancer agents. The vast majority of cytotoxic anti-cancer substances with use presently have been chosen on the basis like living creatures screening program system generally going to employ mouse model as for transplantable tumours. It is not always possible to extrapolate the results of *in vivo* animal studies to humans due to mysterious biology of cancer cells in different system.

The ICH guidelines stress the need to reduce the number of animals, refinement of existing procedures and replacement of animals have paved the way for alternative screening procedures like employing cell lines in disease research. Further the high throughput nature of *in vitro* cytotoxic and anti-proliferative assay form an indispensible link in different components of drug discovery [Table 2].

In these *in-vitro* assays, we have employed *colo 320* cell lines which were economically effective and practical in identifying growth inhibitors as potential therapeutic agents. The *colo 320* cell line grows in suspension and is easy to monitor its growth in micro culture plate.

It is possible to screen compounds at only one single concentration and obtain statistically meaningful results with *colo 320* cell lines. Further the growth

kinetic data show that they are having a mean doubling time of 12 hours. In the present study, we have chosen two most widely used *in-vitro* methods for assessing cytotoxic potential of root extract viz: Tryphan blue and MTT assay [Table 3 & Figure 2].

Tryphan blue is a vital stain which when added to the culture leaves non-viable cells with a distinctive blue colour while visible cells appear unstained. This relies on the other fact cell viability have internal cell membrane and hence need not take in the dye from the surrounding medium. The significant dose-dependent decrease in the number like cell viability inside this microarrays indicates that such leaf extract could be liable for troubling a membrane permeability. Hence the cells with altered membranes allow the dye to enter inside. The perturbation might be due to redistribution of phosphatidyl serine to the external side of the cell membrane which is the main biochemical feature of apoptosis. The very little reduction of viability in the control group (i.e., *colo 320* cell lines with ethanol) might be attributed to the effects of ethanol slightly damaging the cell membrane. This group served the purpose to rule out the effects of ethanol with which the extract was prepared [Figure 3].

A MTT microarrays was involves the reduction like MTT (3-[4, 5- dimethyl thiazol-2yl]-2,5- diphenyl tetrazolium bromide) by mitochondrial dehydrogenase system to water insoluble purple coloured formazan in actively respiring cells. Hence this *in vitro* assay was frequently recommended such as analyzing a cytotoxic activity like xenobiotics, analysing proliferation percentages but also trying to analyse cellular activity like development in 96 well plate format. This method truly distinguishes between healthy cells and cells that are alive but loosing function i.e., MTT reduction can be attributed to the mitochondrial activity, although non-mitochondrial, cytosolic and microsomal enzymes were also responsible for reduction of MTT but to a lesser extent.

This means a major as well as significant dose-dependent minimisation within production like cell cultures. This reduction of cell viability may be due to loss like mitochondrial internal transmembrane potential which is generally observed in the earlier stage of apoptosis. Implosion of such potential has been did believe of about also overlapped with beginning like cell membrane permeation transformation micropore, resulting in the release like cytochrome C through into cytosol. Through cytosol, cytochrome C merges to Caspase-9, Apaf-1 but also dATP to form that apoptosome intricate which then in flip stimulates Caspase-9, Caspase-3, Caspase-7 and leads to apoptotic cell death.

Thus the human colorectal adenocarcinoma cell cultures could provide the conceptual model as research resulting in a better understanding of regulation like distinctiveness but in a wider perspective, a regulation like normal cell distinctiveness. Future research with all these cell cultures as well as chosen variants may could shine some light upon that cellular biology of *Colo 320* and ven perhaps results in new medicinal therapies.

IC_{50} is one important and most commonly determined parameter in *in vitro*cytotoxic assays. IC_{50} value can be defined as the concentration of the test substance at which it reduces the number of surviving cells by 50% and is estimated from the dose dependent curves. The IC_{50} value of the methanolic extract of *Ficus benghalensis* on *colo 320* cell cultures has been discovered to also be 87.03μg/ml through MTT assay.

A minimisation with in survival rate like *colo 320* cell lines by MEFB for a single exposure can be due to various bioactive secondary metabolite that either simply or in synergy with other constituents may have exerted the cytotoxic effect such as flavonoids, glycosides, triterpenoids Etc. Flavones have now been illustrated to inhibit pervasiveness in several types of cultured human carcinoma cells, whereas lesser or no cytotoxic to normal human cellular [Table 4]. A molecular basis like anti-proliferation could require an inhibitory activity of a prooxidant system that creates tumour cell advancement as well as studies established that the formation of growth promoting oxidizing agents acts as the huge catalyst like tumour cell advancement but also progress monitoring phases. In addition inhibitory activity like polyamine biosynthetic pathway can be a making contribution process to an antineoplastic operation like flavones. Ornithine decarboxylase is indeed a rate-limiting enzymatic through polyamine biosynthetic pathway, which has now been interrelated only with percentages like DNA synthesis as well as cellular proliferation in many connective tissues. Numerous experimentations show that having flavones could impede ornithine decarboxylase stimulated through tomour promoter but also then affect a subsequent decrease through polyamine as well as inhibitory activity like DNA/synthesis of proteins. Further more, it is established that flavones also are effective in suppressing signalling like protein tyrosine kinases, protein kinase C etc., which are involved in cell proliferation. The other probable mechanisms for inhibition of cancer cell proliferation by extract can be mitotic block, cell cycle arrest, tubilin structure disruption [Figure 4].

The current investigation implied a certain *colo 320* cellular when allowed to treat as for MEFB may undertake phytoconstituents specialized programmed cell death but also didn't stimulate unspecific necrotic death as control group without extract has not significantly affected cell viability. Therefore this study form an important preliminary base to carry further investigation to isolate, characterize bioactive natural agents for the treatment of human colorectal adenocarcinoma with lower toxicity and higher effectiveness and could serve as a new source for lead molecule [Figure 5].

CONCLUSION

Tissue culture advancements had also practically revolutionized cancer biology through trying to discover cytotoxic particles. With us present research is indeed a preliminary attempt to evaluate cytotoxic possibilities methanolic prop root extract of *Ficus benghalensis* against colorectal adenocarcinoma cell lines *Colo 320* employing two widely known *invitro* assays viz Tryphan blue and MTT assay. Research demonstrates one significant dose-dependent decrease within the number like viable *Colo 320* cells upon single exposure for 24hours signifies that now the extricate might be able to alter membrane permeability as well as going to function like mitochondrial dehydrogenase synthase. Even further research have been suspected of being involved of about accurately identify phytochemical constituents of methanolic extract of *Ficus benghalensis* liable for cytotoxic activity as well as elucidation like probable explanation like activity could result In the event like encouraging instinctual agent of natural origin for a diagnosis of colorectal adenocarcinoma.

ACKNOWLEDGEMENT

I would like to thank Principal sir (Dr. Kamal Hassan), St. Mary's Group of Institutions, Deshmukhi (Village), Pochampally (Mandal), Yadadri Bhuvanagiri (Dist), Telangana-508284, India.

Conflict of Interest

The authors attest that they have no conflict of interest in this study.

Funding Support

The authors declare that there is no financial support for the current study.

REFERENCES

[1] Roderick A F Macleod and Hans G Drexler and. Cytogenetic Analysis of Continuous Cell Lines. pages 51–70, 2005. Methods in Molecular Biology, ISBN: 978-1-59259-838-0.

[2] E Y H P Lee and W J Muller. Oncogenes and Tumor Suppressor Genes. *Cold Spring Harbor Perspectives in Biology*, 2(10):a003236, 2010.

[3] M D Garrett. Cell cycle control and cancer. *Current Science*, 81(5):515–522, 2001.

[4] J Bartek and J Lukas. Chk1 and Chk2 kinases in checkpoint control and cancer. *Cancer Cell*, 3(5):110–117, 2003.

[5] L Bergeron, G I Perez, G Macdonald, L Shi, Y Sun, A Jurisicova, S Varmuza, K E Latham, J A Flaws, J C M Salter, H Hara, M A Moskowitz, E Li, A Greenberg, J L Tilly, and J Yuan. Defects in regulation of apoptosis in caspase-2-deficient mice. *Genes and Development*, 12(9):1304–1314, 1998.

[6] C N Bernstein, J F Blanchard, E Kliewer, and A Wajda. Cancer risk in patients with inflammatory bowel disease. *Cancer*, 91(4):854–862, 2001.

[7] L Bouchier-Hayes. The role of caspase-2 in stress-induced apoptosis. *Journal of Cellular and Molecular Medicine*, 14(6a):1212–1224, 2010.

[8] J M Brown and L D Attardi. The role of apoptosis in cancer development and treatment response. *Nature Reviews Cancer*, 5(3):231–237, 2005.

[9] M Castedo, J. L Perfettini, T Roumier, K Andreau, R Medema, and G Kroemer. Cell death by mitotic catastrophe: a molecular definition. *Oncogene*, 23(16):2825–2837, 2004.

[10] S Elmore. Apoptosis: A Review of Programmed Cell Death. *Toxicologic Pathology*, 35(4):495–516, 2007.

[11] D S Fabricant and N R Farnsworth. The value of plants used in traditional medicine for drug discovery. *Environmental Health Perspectives*, 109(suppl 1):69–75, 2001.

[12] P Ferrari, M Jenab, T Norat, A Moskal, N Slimani, A Olsen, and E Riboli. Lifetime and baseline alcohol intake and risk of colon and rectal cancers in the European prospective investigation into cancer and nutrition (EPIC). *International journal of cancer*, 121(9):2065–2072, 2007.

[13] G J Finlay and B C Baguley. The use of human cancer cell lines as a primary screening system for antineoplastic compounds. *European Journal of Cancer and Clinical Oncology*, 20(7):947–

954, 1984.

[14] J Groden, A Thliveris, W Samowitz, M Carlson, L Gelbert, H Albertsen, G Joslyn, J Stevens, L Spirio, M Robertson, L Sargeant, K Krapcho, E Wolff, R Burt, J P Hughes, J Warrington, J Mcpherson, J Wasmuth, D Le Paslier, and R White. Identification and characterization of the familial adenomatous polyposis coli gene. *Cell*, 66(3):589–600, 1991.

[15] D Hanahan and R A Weinberg. The hallmarks of cancer. *Cell*, 100(1):57–70, 2000.

[16] I M Hisamuddin and V W Yang. Genetics of colorectal cancer. *MedGenMed Medscape General Medicine*, 6(3):1–13, 2004.

[17] R R Huxley, A Ansary-Moghaddam, P Clifton, S Czernichow, C L Parr, and M Woodward. The impact of dietary and lifestyle risk factors on risk of colorectal cancer: A quantitative overview of the epidemiological evidence. *International Journal of Cancer*, 125(1):171–180, 2009.

[18] F Ianzini, A Bertoldo, E A Kosmacek, S L Phillips, and M A Mackey. Lack of p53 function promotes radiation-induced mitotic catastrophe in mouse embryonic fibroblast cells. *Cancer cell international*, 6(1):1–8, 2006.

Copyright: This is an open access article distributed under the terms of the Creative Commons Attribution-NonCommercial-ShareAlike 4.0 License, which allows others to remix, tweak, and build upon the work non-commercially, as long as the author is credited and the new creations are licensed under the identical terms.

Cite this article: Badugu Kranti Kumar, Kondoju Arun Kumar Chary, Kiran, Gajarlu Shivani, Abdul Rahaman S K, Boidi Mamatha. **Evaluation of *In-Vitro* Cytotoxic Potential of *Ficus benghalensis* Tender Prop Roots Extract**. Future J. Pharm. Health. Sci. 2022; 2(3): 159-169.

© 2022 Pharma Springs Publication.