Indian Journal of Pharmacy and Pharmacology 2022;9(4):242–246

Content available at: https://www.ipinnovative.com/open-access-journals

Indian Journal of Pharmacy and Pharmacology

Journal homepage: https://www.ijpp.org.in/

Original Research Article

Terminalia chebula possesses *in vitro* anticancer potential

Arti Heer[1], Vikas Sharma[1,*], Navneet Kour[1], Shivangi Sharma[2]

[1]*Division of Biochemistry, Faculty of Basic Sciences, Sher-e-Kashmir University of Agricultural Sciences and Technology of Jammu, Jammu and Kashmir, India*
[2]*Dept. of Chemistry, University of Jammu, Jammu & Kashmir, India*

ARTICLE INFO

Article history:
Received 18-05-2022
Accepted 23-07-2022
Available online 12-11-2022

Keywords:
Terminalia chebula
SRB assay
Human cancer cell lines
Anticancer drug

ABSTRACT

Introduction: Cancer is one of the major human diseases and causes large suffering / economic loss worldwide. These days various new strategies are being developed to treat human cancer, but the sure-shot, perfect cure is yet to be brought into the world of medicine. In the search of potential anticancer agents from natural products, the present investigation was carried out to evaluate the in vitro anticancer efficiency of *Terminalia chebula.*

Materials and Methods: The anticancer activity was determined by the cytotoxic potential of test material at 100 μg/ml via SRB assay. Cells were allowed to grow for 24 h on 96 – well flat bottom tissue culture plates and cells were further allowed to grow in the presence of test material for 48h. Cell growth was terminated by addition of 50% (w/v) tricarboxylic acid and cells were stained with SRB dye. Excess dye was removed by washing with 1% (v/v) acetic acid and bound dye was dissolved in Tris buffer. OD was taken at 540 nm and growth inhibition of 70% or above was considered active.

Results: The methanolic extract of *Terminalia. chebula* fruit showed in vitro anticancer effect in the range of 75%-95% against four human cancer cell lines of colon, melanoma, prostrate and lung origin.

Conclusion: The methanolic fruit extract of harad can be explored for lead molecule in the development of anticancer drug to provide a great service to cancer patients.

1. Introduction

Terminalia chebula commonly known as Harad or Myrobalan and belonging to family Combretaceae, contains various biochemical compounds such as tannins, chebulinic acid, ellagic acid, gallic acid, punicalagin, flavonoids[1] and has been reported to possess anti-oxidant,[2] anti-diabetic,[3] anti-cancer,[4] anti-mutagenic,[5] anti-viral,[6] anti-bacterial[7,8] and radioprotective[9] properties. In vivo and in vitro anticancer potential of the ethanolic extract of *T. chebula* fruit was determined against Ehrlich Ascites Carcinoma (EAC) induced cancer in swiss albino mice. The anticancer activity was assessed using *in vitro* cytotoxicity, mean survival time, tumor volume and hematological studies. The high dose of extract (200 mg/kg, orally) significantly reduced the tumor growth which was demonstrated by increased lifespan of the mice and restoration of hematological parameters.[10] Acetone extract of fruit was used to determine anticancer activity towards HeLa cell line. The viability of cells was determined by MTT (3,4,5-dimethylthiazol-2-yl)-2-5-diphenyltetrazolium bromide) assay. Acetone extract (IC_{50} at 0.113 mg/ml) showed effective anticancer activity compared to that of cisplatin as control. The presence of polyphenolics in the extract was determined using HPLC method.[11] Chebulinic acid, an ellagitannin widely present in *T. Chebula* showed

* *Corresponding author.*
E-mail address: vikas.skuast@gmail.com (V. Sharma).

https://doi.org/10.18231/j.ijpp.2022.044

many bioactivities including inhibition of cancer cell growth like human leukemia K562 cells.[12] *In-vitro* anti-cancer activity of Chebulinic acid on colon adenocarcinoma HT-29 cancer cell line was studied. Cell lines were examined by using MTT cell growth inhibition assay. Results showed that the maximum percentage inhibition of cancer cell lines for chebulinic acid was found to be 41.2% at a dose of 200 μg/ml. Hence, chebulinic acid can be used as a potent anti-cancer agent.[13] In the present investigation, the fruit of hard was evaluated against eight human cancer cell lines from six different tissues.

2. Materials and Methods

2.1. Chemicals

RPMI-1640 medium, dimethyl sulfoxide (DMSO), EDTA, fetal bovine serum (FBS), sulphorhodamine blue (SRB) dye, phosphate buffer saline (PBS), trypsin, gentamycin, penicillin and 5-flurouracil were purchased from Sigma Chemical Co., USA. All other chemicals were of high purity and obtained locally with the brand Sigma-Aldrich Chemicals Pvt. Ltd. and S.D. Fine Chemicals Pvt. Ltd. from Ramesh Traders, Panjthirthi-Jammu, J&K.

2.2. Fruit material and preparation of extracts

Terminalia was authenticated at site and enough quantity of fresh fruits were collected.The freshly collected fruits were chopped, shade-dried and ground into powdered form. The methanolic extracts of all the fruits were prepared by percolating the dried ground plant material (100 g) with 99% methanol and then concentrating it to dryness under reduced pressure. Stock solutions of 20 mg/ml were prepared by dissolving methanolic extract in DMSO.[14] Stock solutions were prepared at least one day in advance and were not filtered. The microbial contamination was controlled by addition of 1% gentamycin in complete growth medium i.e. used for dilution of stock solutions to make working test solutions of 200 μg/ml.

2.3. Cell lines / cultures and positive controls

The human cancer cells were obtained from National Centre for Cell Science, Pune, India and National Cancer Institute, Frederick, USA. These human cancer cells were further grown and maintained in RPMI-1640 medium. Doxorubicin, 5-Fluorouracil, Mitomycin-C, Paclitaxel and Tamoxifen were used as positive controls.

2.4. In vitro assay for cytotoxic activity

Extracts were subjected to in vitro anticancer activity against various human cancer cell lines.[15] In brief, the cells were grown in tissue culture flasks in growth medium at 37^{o}C in an atmosphere of 5% CO_2 and 90% relative humidity in a CO_2 incubator (Hera Cell, Heraeus; Asheville, NCI, USA). The cells at sub-confluent stage were harvested from the flask by treatment with trypsin (0.05% trypsin in PBS containing 0.02% EDTA) and suspended in growth medium. Cells with more than 97% viability (trypan blue exclusion) were used for determination of cytotoxicity. An aliquot of 100 μl of cells (10^5cells/ml) was transferred to a well of 96-well tissue culture plate. The cells were allowed to grow for 24 h. Extracts (100 μl/well) were then added to the wells and cells were further allowed to grow for another 48 h.

The anti-proliferative SRB assay which estimates cell number indirectly by staining total cellular protein with the dye SRB was performed to assess growth inhibition. The SRB staining method is simpler, faster and provides better linearity with cell number. It is less sensitive to environmental fluctuations and does not require a time sensitive measurement of initial reaction velocity.[16] The cell growth was stopped by gently layering 50 μl of 50% (ice cold) trichloro acetic acid on the top of growth medium in all the wells. The plates were incubated at 4^{o}C for 1 h to fix the cells attached to the bottom of the wells. Liquid of all the wells was then gently pipetted out and discarded. The plates were washed five-times with distilled water and air-dried. SRB 100 μl (0.4% in 1% acetic acid) was added to each well and the plates were incubated at room temperature for 30 min. The unbound SRB was quickly removed by washing the cells five-times with 1% acetic acid. Plates were air-dried, tris buffer (100 μl, 0.01 M, pH 10.5) was added to all the wells to solubilize the dye and then plates were gently stirred for 5 min on a mechanical stirrer. The optical density (OD) was recorded on ELISA reader at 540 nm. Suitable blanks (growth medium and DMSO) and positive controls (prepared in DMSO and distilled water) were also included. Each test was done in triplicate and the values reported were mean values of three experiments.

The cell growth was determined by subtracting average absorbance value of respective blank from the average absorbance value of experimental set. Percent growth in presence of test material was calculated as under:

1. OD Change in presence of control = Mean OD of control – Mean OD of blank.
2. OD Change in presence of test sample = Mean OD of test sample – Mean OD of blank.
3. % Growth in presence of control = 100/OD change in presence of control.
4. % Growth in presence of test sample = % Growth in presence of control × OD change in presence of test sample.
5. % Inhibition by test sample = 100 – % Growth in presence of test sample.

Table 1: Growth inhibitory effect of Terminalia chebula fruit on human cancer cell lines

Extract	Conc. (μg/ml)	Human cancer cell lines from six different tissues							
		Breast MCF-7	Breast T-47D	Colon SW-620	Colon HCT-116	Lung A-549	Melanoma MDA-MB-435	Ovarian OVCAR-5	Prostate PC-3
		Growth Inhibition (%)							
Methanolic	100	20	11	62	95	75	88	41	88
	50	*	*	*	10	61	66	*	00
	10	*	*	*	5	41	00	*	00
	1	*	*	*	00	24	00	*	2
Positive controls	Conc. (μM)	Growth Inhibition (%)							
Doxorubicin	1	-	-	71	-	-	-	-	-
5-Fluorouracil	20	-	-	-	65	-	-	70	-
Mitomycin-C	1	-	-	-	-		-	-	63
Paclitaxel	1	77	72	-	-	71	-	-	-
Tamoxifen	1	-	-	-	-	-	75	-	-

Growth inhibition of 70% or more in case of extracts has been indicated in bold numbers
Mark (-) indicates that particular human cancer cell line was not treated with that particular positive control
Symbol (*) means not further evaluated / calculated

The growth inhibition of 70% or above was considered active while testing extracts, but in testing of active ingredients at different molar concentrations, the growth inhibition of 50% or above was the criteria of activity.

Fig. 1: Terminalia chebula (harad)

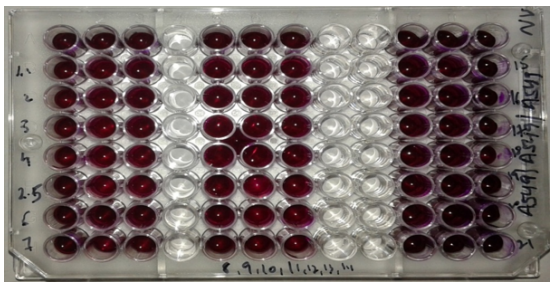

Fig. 2: 96-Well TCP after addition of SRB dye

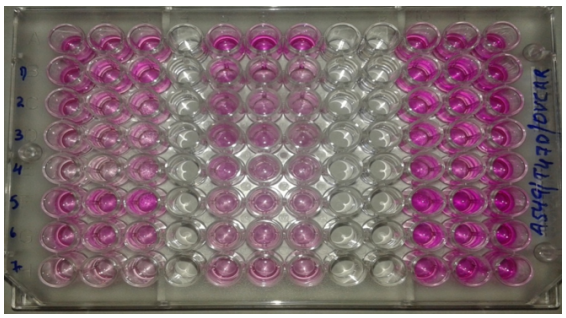

Fig. 3: 96-Well TCP afteraddition of tris buffer

3. Results

Terminalia chebula was selected from Jammu region for testing *in vitro* anticancer efficacy. This plant has numerous medicinal properties and is used in the traditional system of medicine in India. Systematic bioassays were performed against eight human cancer cell lines from six different origins, which were obtained from National Cancer Institute, Frederick, U.S.A and National Centre for Cell Science, Pune, India. The human cancer cell lines along with particular tissues were Breast: MCF-7, T-47D; Colon: HCT-116, SW-620; Lung: A-549; Melanoma: MDA-MB-435; Ovary: OVCAR-5; Prostate: PC-3. Standard protocols

as given in *"Materials and Methods"* were employed for the extraction of powdered dried plant material and the methanolic extract was prepared / employed for bioassay. Standard drugs for cancer that served as positive controls in the present investigation included the Doxorubucin, 5-Flurouracil, Mitomycin-C, Paclitaxel and Tamoxifen. Results revealed that the methanolic extract from the fruit part of *Terminalia chebula* showed *in vitro* anticancer effect against four human cancer cell lines. The fruit displayed 95% growth inhibition of colon (HCT-116) cancer cell line, 88% growth inhibition of melanoma (MDA-MB-435) cancer cell line, 88% growth inhibition of prostrate (PC-3) cancer cell line and 75% growth inhibition of lung (A-549) cancer cell line. Further, the methanolic extract of harad showed 20% growth inhibition of MCF-7 - a cell line from breast origin, 11% growth inhibition of T-47D - a cell line from breast origin, 62% growth inhibition of SW-620 - a cell line from colon origin and 40% growth inhibition of OVCAR-5 - a cell line from ovary origin, which was not considered active. When evaluated at lower concentrations, the fruit extract did not showed significant growth inhibition against any of the human cancer cell lines.

4. Discussion

Cancer is becoming a big load on families and economies. The cancer cases are on rise in Jammu and Kashmir with lung cancer becoming most prominent due to smoking. Cancer research has, therefore, become a major area of scientific research supporting the foundations of modern biology to a great extent. Chemotherapy is a major treatment modality for cancer, but most of the drugs used in cancer chemotherapy exhibit cell toxicity and can induce genotoxic, carcinogenic and teratogenic effects in non tumor cells. Therefore, the research for alternative drugs of natural origin, which are less toxic, endowed with fewer side effects and more potent in their mechanism of action, is an important research line. Medicinal plants have long history for the treatment of various diseases including cancer and active principles from these plants are used to control the advance stages of malignancies in clinical settings. These natural products now have been contemplated of exceptional value in the development of effective anticancer drugs with minimum host cell toxicity. A number of exciting researches suggest that traditional medicinal plants contain an abundance of polyphenolic compounds, terpenoids, sulphur compounds, pigments and other natural antioxidants, that have been associated with protection from or treatment of conditions such as cancer.

Therefore, natural products have been a prime source of highly effective conventional drugs for the treatment of many forms of cancer. Accordingly, this research work has two fold importance: First, in Jammu subtropics, the *in vitro* anticancer efficiency of harad against colon, melanoma, prostrate and lung cancer cells have been reported and secondly, the results from the investigation forms a good basis for the selection of this plant from Jammu region for further phytochemical and pharmacological analysis to offer new drugs from natural sources which would be less toxic and more potent for the efficient management of cancer.

5. Conclusion

This promising methanolic extract from the fruit part of harad can be explored for lead molecule in the development of anticancer drugs to provide a great promise and service to cancer patients. Further, isolation and characterisation of active ingredients with anticancer potential is required from this particular traditional medicinal plant.

6. Abbreviations

1. EAC: Ehrlich Ascites Carcinoma
2. HPLC: High-performance liquid chromatography
3. SRB: sulphorhodamine blue
4. FBS: Fetal Bovine Serum
5. PBS: Phosphate Buffer Saline
6. DMSO: Dimethyl Sulfoxide

7. Source of Funding

None.

8. Conflict of Interest

None.

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Author biography

Arti Heer, Ph.D Scholar

Vikas Sharma, Associate Professor

Navneet Kour, Ph.D Scholar

Shivangi Sharma, Ph.D Scholar

Cite this article: Heer A, Sharma V, Kour N, Sharma S. *Terminalia chebula* possesses *in vitro* anticancer potential. *Indian J Pharm Pharmacol* 2022;9(4):242-246.