Cytotoxic activity of methanolic extract of Artocarpus heterophyllus against A549, Hela and MCF-7 cell lines

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ABSTRACT

Research is focusing on the search for new types of natural chemotherapeutic agents derived from plants which are proving to be excellent sources of new compounds. The present research article was aimed to study the cytotoxic activity of methanolic extracts of Artocarpus heterophyllus plant by various in vitro cytotoxic assays like MTT and SRB against different cell lines like HEK293, A549, HeLa and MCF-7. The IC50 values of methanolic extract of Artocarpus heterophyllus were found 35.26 µgm/ml and 35.27 µgm/ml against A549 cell line by MTT and SRB assay methods respectively whereas this extract was found to be non toxic to normal cells (HEK293), proved that the methanolic extract exhibited significant anti cancer potential with no toxicity on normal cell line. The methanolic extract had no activity against Hela and MCF-7 cell line.

Key words: Cytotoxicity, SRB, MTT, Extraction.

INTRODUCTION

Many medicinal and food plants contain large amounts of chemical components having broad spectrum of pharmacological activities like anti cancer, anti tumor and anti oxidant activities. The anti cancer activities are mainly due to Phenolic acids, flavonoids, and phenolic diterpenes. Natural products are reportedly beneficial to physiological health. Various flavonoids and non-flavonoids have been reported as showing anti cancer, anti tumor and anti oxidant activities. Moraceae is a large family comprising sixty genera and nearly 1400 species, including important group such as Artocarpus, Morus, and Ficus. Artocarpus heterophyllus or Jackfruit (family of Moraceae) is a monoeocious evergreen tree that is grown in several tropical countries. It produces a large pear or barrel-shaped fruit that can grow up to 90 cm long, 50 cm thick and having a weight of 20 kg. Several individual fruits, covered with fleshly and juicy perianths, are found under the spiny surface. The seed is large, oblong and has a slimy membranous testa and a brown tegmen. A.heterophyllus is widely distributed in tropical region and has been used as traditional folk medicine against inflammation, malarial fever and so on. In addition, the function of Artocarpus heterophyllus in human health such as pulp and seed for tonic; root for diarrhoea, fever; wood for muscular contraction; leaves for activating milk in women and animal, anti-syphilis, vermifuge; leaf ash for ulcers and wound. Moraceae plants including A. heterophyllus are rich sources of the isoprenylated phenolic compounds, including flavonoids (Acedo, 1992; Jarrett, 1959; Piga, 2004). The objective of this study is to investigate cytotoxic activity of crude extracts from seeds of Artocarpus heterophyllus on A549, Hela and MCF-7 cell lines using the MTT assay and SRB
assay methods. The overall result indicates the promising baseline information for the potential uses of crude extract from the tegmen of *A. heterophyllus* as an antitumor agent.

**MATERIALS AND METHODS**

**Plant material**

*Artocarpus heterophyllus* seeds were obtained from pujan nursery, Ahmedabad, Gujarat. The seeds were washed with sterile water, dried in shade, finely powdered & stored in air tight bottles.

**Preparation of Plant extract**

10 gm of dried, finely grounded powder of *Artocarpus heterophyllus* seeds was immersed in 150 ml of methanol and extracted by reflux extraction at 40 °C for 3 hours. After extraction, the extract was filtered through Watmann filter paper and evaporated till dryness. 100 Microgram of plant extract was dissolved in the 1 ml DMSO and then 1:3 dilution of test compound was prepared for MTT and SRB assay.

**Phytochemical investigation of *Artocarpus heterophyllus* seeds**

*Test for steroids*: Liebermann –Burchard reaction : Mix 2ml extract with chloroform .Add 1-2 ml acetic anhydride and 2 drops conc. H2SO4 from the side of test tube. First red, then blue and finally green color appears.

*Test for Saponins*: Foam test: shake the drug extract or dry powder vigorously with water. Persistent foam observed.

*Test for Flavonoids*: Shinoda test: To dry powder or extract, add 5ml 95%ethanol, few drops conc. HCl and .5gms magnesium turnings, Pink color observed.

**Cell culture**

Human embryonic kidney cell line (HEK 293T), lung adeno carcinoma cell line (A549 cell line), cervical cancer cell line (Hela cell line) and breast cancer cell line MCF-7 was grown in DMEM (Dulbecco’s modifications of eugal’s medium with L-glutamine & 4.5G/L glucose) supplemented with fetal bovin serum. Pre incubate cells at a concentration of 1× 10⁴ cells/ml using medium containing 10% new born calf serum. To each well of the 96 well microtitre plate, 0.1ml of the diluted cell suspension was added and the plates were gently shaken and incubated for 4 hours at 37ºC in 5% CO2 incubator. The supernatant was removed and 100 µl of Isopropanol was added and the plates were gently shaken to solubilize the formed formazan. The absorbance was measured using a microplate reader at 590 nm with a reference filter of 620 nm. (Mossmann, T., (1983; Knick, V.C. 1995; Skehan, P.2003; Skehan P, 1990).

The percentage cell growth inhibition or percentage cytotoxicity was calculated by following formula:

\[
\text{% Growth inhibition} = 100 \times \frac{\text{Mean OD of control Group} - \text{Mean OD of individual Test Group}}{\text{Mean OD of control Group}}
\]

**Cytotoxicity assay by SRB method**

The monolayer cell culture was trypsinized and the cell count was adjusted to 0.5-1.0 x 10^⁵ cells/ml using medium containing 10% new born sheep serum. To each well of the 96 well microtitre plate, 0.1ml of the diluted cell suspension (approximately 10,000 cells) was added. After 24 hours, when a partial monolayer was formed, the supernatant was flicked off, washed once and 100µl of different test compound concentrations were added to the cells in microtitre plates. The plates were then incubated at 37ºC for 72 hours in 5% CO2 incubator and microscopic examination was carried out and observations recorded every 24 hours. After 72 hours, 25µl of 50% trichloroacetic acid was added to the wells gently such that it forms a thin layer over the test compounds to form a over all concentration 10%. The plates were incubated at 4ºC for one hour. The plates were flicked and washed five times with tap water to remove traces of medium, sample and serum, and were then air-dried. The air dried plates were stained with 100µl SRB and kept for 30 minutes at room temperature. The unbound dye was removed by rapidly washing four times with 1% acetic acid. The plates were then air-dried. 100µl of 10mM Tris base was then added to the wells to solubilize the dye. The plates were shaken vigorously for 5 minutes. The absorbance was measured using microplate reader at a wavelength of 540nm (Vanicha V. 2006; Skehan, P.2003; Skehan P, 1990)

The percentage growth inhibition was calculated using following formula,

\[
\text{% Growth inhibition} = 100 \times \frac{\text{Mean OD of individual Test Group} - \text{Mean OD of control Group}}{\text{Mean OD of control Group}}
\]

**RESULTS AND DISCUSSION**

*In vitro cytotoxic Activity of methanolic extract of *Artocarpus heterophyllus* by MTT assay*

The effect of methotrexate and *Artocarpus heterophyllus*, was performed on four different cell lines by MTT assay. Dose response curves constructed for MTT method between the range of 5.0-100000 n gm/ml. Calculation of IC₅₀ and R² values was done using graphs generated from Microsoft excel 2007 edition .The
susceptibility of cells to the drug exposure was characterized by IC₅₀ values. Results indicate that the antiproliferative effect strengthens with increase in the concentration of drug.

From table 1, observed that the highest cytotoxic activity was found with methotrexate against A549 and HeLa having IC₅₀: 9.70 and 27.90 µ gm/ml respectively. Lower activities were in HEK-293 and MCF-7 having IC₅₀: 45.42 and 49.42 µ gm/ml.

From figure 1a, it showed Methotrexate has the dose-effect co-relation with maximum linearity in case of A549, MCF-7, HEK 293 and HeLa, respectively with increasing with increase in the concentration & attains linearity till IC₅₀ value is reached. Interestingly, observed that methotrexate showed cytotoxic activity against A549, HeLa, MCF-7 cancer cell lines and also the normal kidney cell line i.e., HEK-293T cell line show better results in terms of IC₅₀ and regression due to its antimetabolite activity which inhibit dihydro folate reductase enzyme.

![Fig 1(a) & (b): % Growth inhibition of methotrexate and methanolic extract of Artocarpus heterophyllus against HEK 293, A549, HeLa, and MCF-7 by the MTT assay.](image1)

From table 1, observed that cytotoxic activity of Methanolic extract of *Artocarpus heterophyllus* have found against only A549 cell line with IC₅₀: 35.26 µ gm/ml. Whereas this extract not showed the activity against other cell lines.

From figure 1b, it showed that methanolic extract of *Artocarpus heterophyllus* showed the dose-effect co-relation with maximum linearity in case of A549 of the four cell lines at value being 0.9117. The % inhibition is increasing with linearity till IC₅₀ value is reached. Linearity in case of A549, HEK 293 and MCF-7 cell lines with increasing with increase in the concentration & attains linearity till IC₅₀ value is reached respectively.

On the other hand, Methanolic extract of *Artocarpus heterophyllus* against HeLa and MCF-7 show insignificant regression with non linearity in the values of change of % inhibition with the increase in concentration, due to that they are not reach upto 50% cell growth inhibition. Normal cell line HEK 293 also show non linearity with insignificant regression, which is less toxic to human health.

**In vitro metabolic assay of Artocarpus heterophyllus extracts and methotrexate by SRB assay**

The effect of Methotrexate and *Artocarpus heterophyllus*, extracts was performed on four different cell lines by SRB assay. Dose response curves constructed for SRB method between the range of 5.0-100000 n gm/ml. Calculation of IC₅₀ and R² values was done using graphs generated from Microsoft excel 2007 edition. The susceptibility of cells to the drug exposure was characterized by IC₅₀ values. Results indicate that the antiproliferative effect strengthens with increase in the concentration of drug. The IC₅₀ values of test extract as well as standard drug (methotrexate) were summarised in table 2 and figure 2(a) & (b).

![Fig 2(a) and (b): % Growth inhibition of methotrexate and methanolic extract of Artocarpus heterophyllus against HEK 293, A549, HeLa, MCF-7 by SRB assay](image2)

From table 2, observed that highest activity of Methotrexate have found against HeLa and A549 having IC₅₀: 7.7643 and 16.112 respectively. Lower activities were in MCF-7 and HEK 293 (IC₅₀: 16.531 and 45.671). From figure 2a, it showed that methotrexate has the dose-effect co-relation with maximum linearity in case of HeLa of the four cell lines at value being 0.9747. The % inhibition is increasing with linearity till IC₅₀ value is reached. Linearity in case of A549, HEK 293 and MCF-7 cell lines with increasing with increase in the concentration & attains linearity till IC₅₀ value is reached respectively.

So, methotrexate shows cytotoxic activity against A549, HeLa, MCF-7 cancer cell lines and also the normal kidney cell line.
Table 1: % growth inhibition and IC50 values of methotrexate and methanolic extract of Artocarpus heterophyllus by MTT assay.

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<th>Conc. ng/ml</th>
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<th>A549</th>
<th>HeLa</th>
<th>MCF-7</th>
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<th>A549</th>
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i.e., HEK-293T cell line show better results in terms of IC50 and regression due to its antimetabolite activity which inhibit dihydro folate reductase enzyme.

From table 2, observed that cytotoxic activity of methanolic extract of Artocarpus heterophyllus have found against only A549 cell line IC50: 35.27 µ gm/ml.where as other cell lines have no activity.

From figure 2b, showed that methanolic extract of Artocarpus heterophyllus has the dose-effect co-relation with maximum linearity in case of A549 of the four cell lines at value being 0.9117.The % inhibition is increasing with increase in the conc. linearly. The other cell lines showed insignificant regression with non linearity in the values of change of % cells inhibition not reach up to 50% with the increase in concentration.

**DISCUSSION**

The results of cytotoxicity study of methanolic extract of Artocarpus heterophyllus showed significant cytotoxicity against A549 cell line, whereas this extract had no activity against HeLa, MCF-7 cell lines. This methanolic extract of Artocarpus heterophyllus is non toxic to normal cells, but showed excellent toxicity on cancer cells. The cytotoxicity of methanolic extract of Artocarpus heterophyllus may be due to the presence of flavonoids having mono to poly phenolic groups in the structure. The flavonoids have reported for their cytotoxic activity due to presence of phenolic groups.( Matsuo M., 2005).

The extractive value, total polyphenolic content and anti cancer activity was at its peak in methanolic extract indicating that most of the active components are extracted with methanol. Cytotoxic changes observed was cell aggregation, cell rounding and cell death. The overall results indicates the promising baseline information for the potential uses of the methanol extracts of tegmen of Artocarpus heterophyllus seed as an anti cancer agent.

**CONCLUSION**

From the results obtained from MTT and SRB assay methods, by the comparision of the IC50 values and linearity of the
activity, the methanolic extract of Artocarpus heterophyllus showed excellent cytotoxicity against the A549 cell line, but had no activity against Hela and MCF-7 cell lines, and HeLa cancerous cell line respectively. The IC50 value found for methanolic extract of Artocarpus heterophyllus on A549 cell line was 35.26 µg/ml and 36.119 µg/ml by MTT and SRB assay respectively. From results it found that the methanolic extract had no cytotoxicity against HEK293, whereas methotrexate showed toxicity with IC50 of 45.42 µg/ml and 45.67 µg/ml against HEK 293 by MTT and SRB assay methods respectively. It proved that the methanolic extract of Artocarpus heterophyllus had potential cytotoxicity against lung cancer, but non toxic to the normal cells (HEK293 cell line) as compared to methotrexate.

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REFERENCES


