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EVALUATION OF CYTOTOXICITY AND ANTI-CANCER ACTIVITY OF *Solanum torvum* STEM EXTRACT ON NORMAL VERO AND HUMAN BREAST CANCER MCF - 7 CELL LINE

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AUTHORS' CONTRIBUTIONS

This work was carried out in collaboration among all authors. Author RS designed the study, performed the statistical analysis, wrote the protocol and wrote the first draft of the manuscript. Authors DS and KR managed the analyses of the study. Author DS managed the literature searches. All authors read and approved the final manuscript.

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ABSTRACT

Limited plants have been screened for their complete biological and pharmacological nature. In the present research investigation an attempt was made to decipher the medicinal value of aqueous extract of *Solanum torvum* (*S.torvum*) stem. Phytochemical screening of the aqueous extract of *S.torvum* stem in the present study ascertain the presence of flavonoids, phenols, saponins, alkaloids, coumarins, sterols, proteins and reducing sugars. Further, the potentiality of aqueous extract of *S.torvum* stem was assessed for its cytotoxic effect on Normal Vero cell line and anticancer activity on Human breast adenocarcinoma cell line by 3-(4,5dimethylthiazole-2-yl)-2,5-diphenyl tetrazolium bromide assay and compared with normal drug target doxorubicin. A 24-hour incubation cell proliferation study reduced the cell viability of MCF-7 breast cancer cell lines. *In vitro* studies on cytotoxicity analysis on Vero cell line revealed that the aqueous stem extract of *S.torvum* show mild toxicity and further it was found to be effective in the prevention of cell proliferation by MCF-7 cell lines.

Keywords: Phytochemical analysis; doxorubicin; cell viability; tryphan blue; selectivity index.

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1. INTRODUCTION

Cancer is still a growing health problem world-wide characterized by their regular proliferation of the cells, as a cell progresses from normal to cancerous tissue, the biological imperative to survive and perpetuate drives fundamental changes in cells behavior [1]. In 2012, it was estimated that there were 14 million new cases and 8.2 million cancer related deaths and by the year 2050, this could increase to 27 million cases, 17 million deaths and 75 million persons living with cancer [2]. Breast cancer is the second leading cause of mortality among women. Breast cancer is generally treated in recent days by radiotherapy, hormonal or chemotherapy. Intensive therapy with chemotherapeutical agents usually results in adverse effects [3].

Doxorubicin (Dox) is one of the most effective agents in the treatment of breast cancer patients, even if it shows severe side effects in the form of typhlitis, cardiotoxicity, nephrotoxicity, hepatotoxicity and other toxicities. Dox is an anthracyclin antibiotic, which remains an important agent in many chemotherapy regimens [4]. Although Dox is currently considered to be one of the most effective agents in the treatment of human breast cancer, its chemotherapeutic use is associated with severe side effects to non tumor tissues, such as the heart, liver, and kidney, thus greatly limiting its clinical application [5].

Nature becomes a great source of medicinal treatment for millions of years. Much of the world's biological diversity remains unexplored as a source of novel biological compounds and the search for new bioactive agents from natural sources, including extreme environmental niches is expanding. Unique bioactive compounds have many pharmacological activities. Medicinal plants occupy an important position for being the paramount sources of drug discovery in the modern era. The medicinal value of herbal plants lies in bioactive substances called phytochemicals that produced infinite physiological action of human body immense therapeutic quality. Experimental animal studies indicate that many phytochemicals in plants are potential antioxidants and possess anticancer properties [6].

Solanum is the largest genus in the family Solanaceae, comprising of about 2000 species distributed in the subtropical and tropical regions of the world. *S. torvum* belongs to the family Solanaceae and is distributed throughout the Southern parts of India. It is common name is Turkey berry and known as Sundaikkai in Tamil and Bhankatiya in Hindi. This is a shrub which grows upto 5 mt tall and cultivated in the tropics for its tasty immature fruits [7]. Many plants of this family are economically significant species and have received considerable attention in chemical and biological studies.

Several pharmacologically active potential chemical compounds which include flavonoids, steroidal saponins, steroidal alkaloids, sterols, lignans, phenolic compounds, coumarins terpenes, and glycosides have been identified from this genus which present wide range of pharmacological activities to different tumors such as breast cancer, colorectal cancer and prostate cancer cell lines [8]. Secondary metabolite compounds like alkaloids, sapogenin, chlorogenin, solasodine, solamargine, solanine and tomatidine were isolated from leaf and stem of *Solanum* species [9,10].

Clinical research revealed that nutraceutical factors can defend cancers. The antioxidant power of herbal resources and their bioactive compounds, have been linked to their potentialities to inhibit multiplication of oncogenic cells by minimizing oxidative stress, which may play key role in the progression of cellular disintegration underlying tumor growth. Research reports have identified that antioxidant supplementation may inhibit breast cancer recurrence and mortalities [11]. Pharmacological studies reveal that the stem, roots and fruits of *S. torvum* have cytotoxic, anti-tumor, anti-bacterial, anti-viral and anti-inflammatory properties [12].

Studies reported earlier on *S. torvum* highlights extensively on the pharmacological properties of its fruits using various solvent systems on cancer cell lines and was found to be extremely effective in the prevention of cell proliferation of the mammary gland breast adenocarcinoma cell lines [13]. Studies pertaining to the pharmacologic action of aqueous extract of stem of *S. torvum* on Vero and MCF-7 cell lines are scanty. In this study an attempt was made to find the effect of aqueous extract of *S. torvum* stem on normal Vero and MCF-7 cell lines to unravel the cytotoxic effect and the anticancer activity respectively.

2. MATERIALS AND METHODS

2.1 Collection and Identification of Plant Material

Stems of *S. torvum* used for the study were collected from in and around Kanchipuram District, Tamil Nadu during the months of February and March, 2018. Fresh plant specimen collected was authenticated by Dr.P.Jayaraman, Director, Plant Anatomy Research Center, Tambaram, Chennai (Registration No.PARC/2018/3855).

2.2 Extraction of Plant Material

The plants were freshly collected, the stem portions were separated from the shoots. The stems were washed with running tap water and rinsed in distilled water. The stems were shade dried for two week complete dryness. The dried stems were powdered, using mechanical grinder. They were ground well to fine powder and then transferred into air-tight containers until further use.

2.3 Cold Extraction

5 g of *S. torvum* dried stem powder was soaked in 50ml of distilled water in a 250ml conical flask. The flask was plugged with cotton wool and aluminium foil. The conical flask was placed in a shaker for 24 h. The extract was filtered using Whatman filter paper to get the crude plant extract of the stem. The filtered extract in the form of concentrated paste were used for the study. The aqueous extract was evaluated for preliminary phytochemical screening [14].

2.4 Procurement and Maintenance of Cell Lines

Normal Vero (African green monkey kidney) and MCF-7 cell lines were procured from National Centre for Cell Sciences (NCCS), Pune, India. The cell lines procured were maintained at Life Teck Research Centre, Arumbakkam, Chennai, Tamilnadu, India. The cells were maintained in Minimal Essential Media (MEM) and were supplemented with 10% Fetal Bovine Serum (FBS), Penicillin (100 IU / ml) and Streptomycin (100 µg/ml) in a humidified atmosphere of 5% CO_2 at 37°C.

2.5 Cell Viability Assay: *In vitro* Studies

Aqueous extract of *S. torvum* stem was analyzed for its cytotoxicity in Normal Vero cell line and for Anticancer activity in MCF-7 cell line based on the principle of (MTT) assay [15].

2.6 Maintenance of Vero and MCF-7 Cell Lines

Cells (1×10^5/well) were plated in 24-well plates and incubated in 37°C with 5% CO_2 condition. After the cell reaches the confluence, the prepared sample plant extract was added and incubated for 24 h. After incubation, the sample was removed from the well and washed with phosphate-buffered saline (pH 7.4) 100 µl/well without serum. 5mg/ml of 0.5% (MTT) was added and incubated for 4 h. After incubation, 1.0 ml of 0.1% DMSO was added to all the wells. The absorbance at 570 nm was measured with UV-spectrophotometer using DMSO as the blank in triplicates. Measurements were performed and the concentration required for a 50% inhibition (IC_{50}) was determined. Standard drug Doxorubicin was used to compare the cell viability of the stem extracts. Cell control and sample control is included in each assay to compare the complete the cell viability assessments.

The % cell viability was calculated using the following formula:

% Cell Viability =
Absorbance of sample / Absorbance of control cells × 100

2.7 Morphological Studies of Vero and MCF-7 Cells after 24 h Incubation

The aqueous stem extract-treated cell lines (Normal Vero and MCF-7) were observed and photographed under inverted animal cell culture microscope (LABOVERT-FS) under 40 x objective.

2.8 Cell Viability Analysis - Trypan Blue Dye Exclusion Method

A simple cell count method was performed to assess live and dead cells using hemocytometer and Trypan blue, a vital dye. This assay was based on the assumption that the dead cells will take up the dye and viable cells will not take up dye [16]. Cell count was performed for MCF-7 cell lines treated with aqueous stem extract by staining with trypan blue dye. The dead and live cells counted were obtained from the IC_{50} concentration at the end of 24 h incubation. The percentage of viable cells (live cells) and non-viable cells (dead cells) from the aqueous extract treated cell line were calculated. The percentage growth inhibition was calculated as using the following formula

% Growth Inhibition (Dead cells) =
Total Cells - Dead Cells / Total Cells × 100

2.9 Determination of Selectivity index (SI):

The degree of selectivity of the aqueous extract of *S. torvum* stem is expressed by its SI value. The SI value was calculated based on the effect of the extraction Normal Vero cell line and MCF- 7 cell line [17]. The SI value of the extract was calculated using the formula:

SI = CC_{50} normal vero cell line / IC_{50} cancer cell line

Cytotoxic concentration of (CC_{50}) of normal Vero cell line and inhibitory concentration (IC_{50}) of MCF-7 cell line.

3. RESULTS

Phytochemical analysis of the aqueous extract of *S. torvum* stem showed the presence of various specific phytoconstituents such as flavonoids, alkaloids, phenols, saponins, coumarins, sterols, proteins and reducing sugars (Table 1).

3.1 Cytotoxicity and Anti-Cancer Activity of Aqueous Stem Extract of *S. torvum* on Normal Vero

3.1.1 Cell line and MCF -7 cell line

The percentage cell viability shown by Vero cell line treated with aqueous stem extract was 97.96% at 7.8 µg/ml and 52.03% at1000 µg/ml. The IC_{50} was also recorded at 1000 µg/ml during 24 h of incubation (Fig. 1). Normal Vero cells treated with doxorubicin showed maximum cell viability at 1000 µg/ml with a minimal cell death. The results of Vero cell lines were statistically significant ($p< 0.05$) when compared among the various concentrations treated with stem extracts.

The MCF-7 cells treated with the stem extract showed percentage cell viability of79.78 % at 7.8 µg/ml and 29.25 % at 1000 µg/ml. The IC_{50} is value of 50.17 % reported at 125 µg/ml concentration at 24 h of incubation (Fig. 2). The reference standard drug doxorubicin treated with the stem extracts showed percentage cell viability of 55.89 % at 7.8 µg/ml and 12.29 % at 1000 µg/ml. The IC_{50} value of 49.17 % was reported at 125 µg/ml.

The statistical significance was calculated among the various concentrations of Doxorubicin ($p< 0.05$) treated with stem extracts , MCF-7 treated with stem extracts ($p< 0.05$) and between the various concentrations of doxorubicin and MCF-7 treated with stem extracts ($p< 0.05$).

Table 1. Phytochemical screening of aqueous extract of S. *torvum* stem

S.No	Phytochemical compounds	*S.torvum* stem extract
1	Flavanoids	+
2	Alkaloids	+
3	Phenols	+
4	Coumarin	+
5	Triterpenes	-
6	Saponins	+
7	Steroids	+
8	Proteins	+
9	Reducing Sugars	+
10	Anthraquinones	-
11	Anthocyanins	-
12	Tannins	-

Presence of the compound (+) and absence of the compound (-)

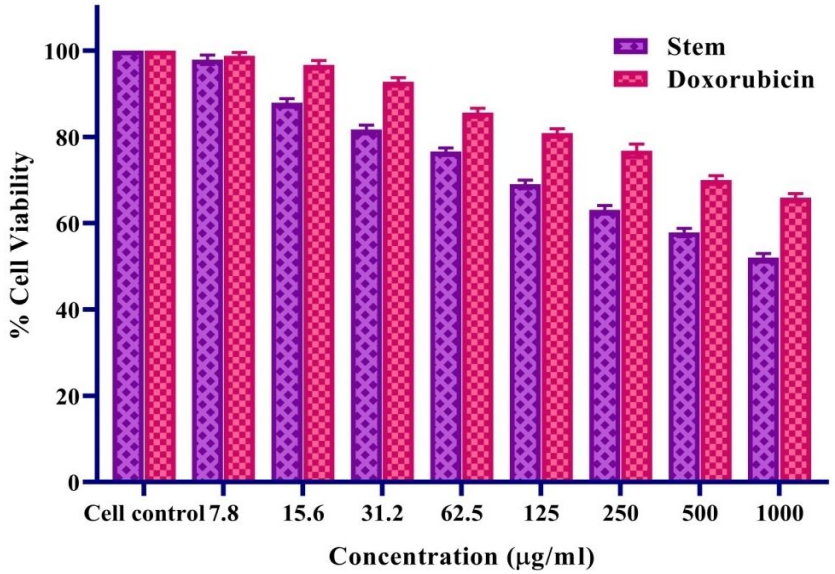

Fig. 1. Graph representing the cytotoxicity of *S. torvum* aqueous stem extract on normal Vero cell line at 24 h incubation compared with Doxorubicin. Values expressed as Mean ± SD (n = 3)

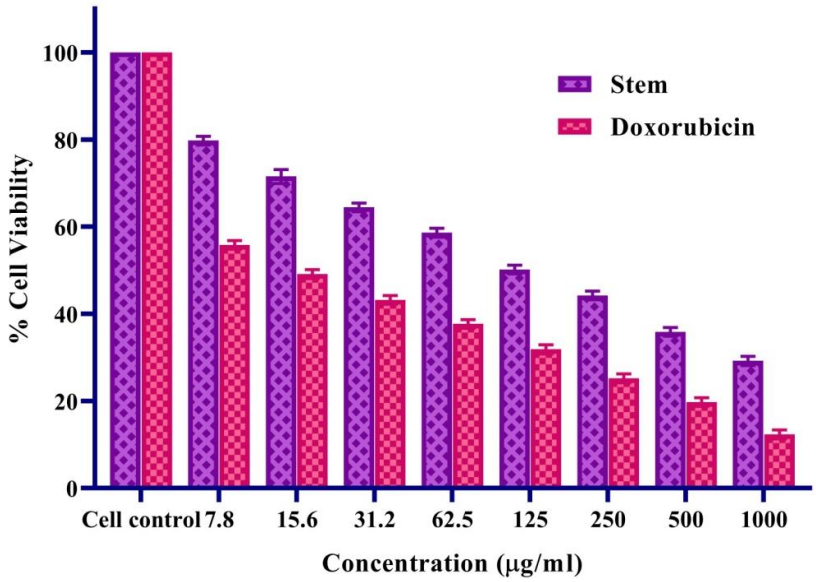

Fig. 2. Graph representing the anticancer activity of *S. torvum* aqueous stem extract on MCF-7 cell line at 24 h incubation compared with Doxorubicin. Values expressed as Mean ± SD (n = 3)

3.2 Morphological Studies of Vero Cells Treated with Aqueous Extract of *S. torvum* stem

Vero cells are uniformly spread in confluent layer with long and elongated shape in appearance. At highest concentration of 1000 µg /ml of stem extract the treated cells lose their normal cytostructure and show polygonal shape with shrunken cells at the end of 24 h incubation (Fig. 3).

3.3 Morphological Studies of MCF-7 Cells Treated with Doxorubicin

MCF-7 cells treated with DOX show changes in cell cytostructure and loss of cells as the concentration increases which substantiates a dose-dependent effect (Fig. 4).

3.4 Morphological Studies of MCF-7 Cells Treated with Aqueous Extract of Stem

MCF-7 cells treated with different concentrations of the extract show reduction in number of cells as the concentration increases. Cells show loss of regular shape and size. Majority of cells are with flattened structures with cell to cell contact disappearing (Fig. 5).

3.5 Cell Count of Live and Dead Cells by using Trypan Blue Dye Exclusion Method

MCF-7 breast cancer cell line treated with aqueous extract of stem show variation in the number of live and dead cells. The cell viability was 53.84% and cell death was 46.16% at IC_{50} concentration of 125µg /ml (Table 2).

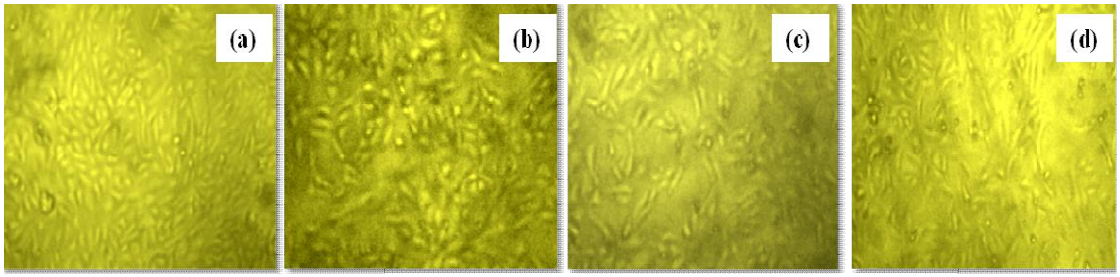

Fig. 3. Photomicrograph of *S. torvum* aqueous extract of stem on the morphology of normal Vero cell line at different concentrations (a) Control (b) 7.8 µg/ml (c) 125 µg/ml (d) 1000µg/ml

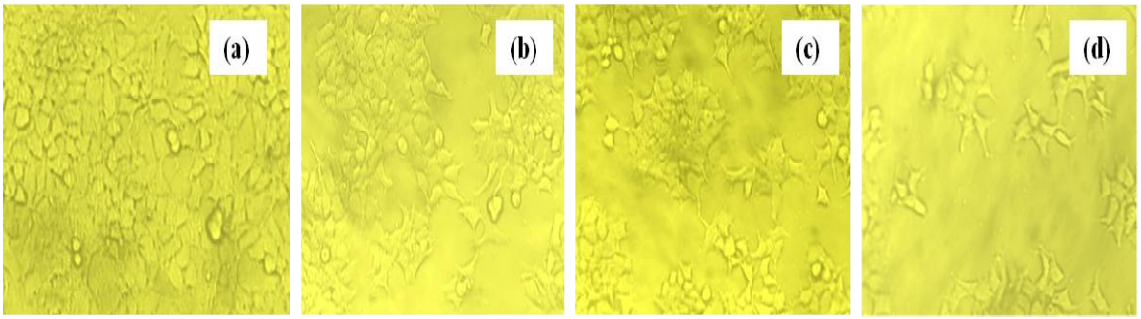

Fig. 4. Photomicrograph representing anticancer effect of Doxorubicin on MCF-7 cell line at different concentrations (a) Control (b) 7.8 µg/ml (c) 125 µg/ml (d) 1000µg/ml

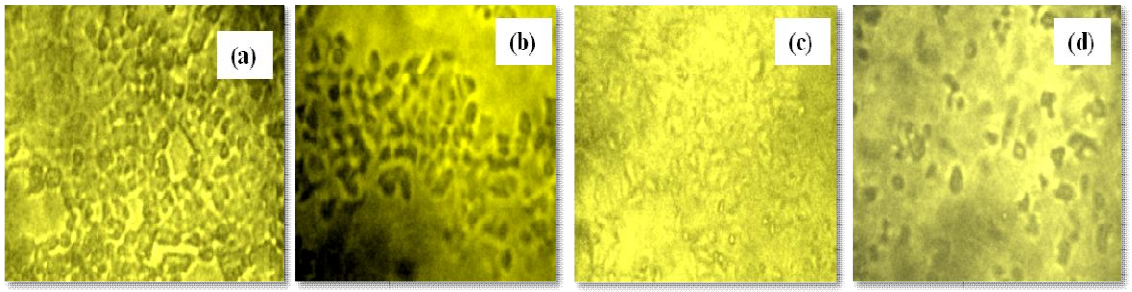

Fig. 5. Photomicrograph of *S. torvum* aqueous extract of stem on the morphology of MCF-7 cell line at various concentrations (a) Normal MCF-7 Cell line (b) 1000µg/ml (c)125 µg/ml (d) 7.8 µg/ml

Table 2. Cell count to observe live and dead cells of MCF-7 cell line treated with aqueous extract of *S. torvum* stem by Trypan blue dye at IC_{50} concentration

No of live cells	142
No of dead cells	123
% Cell viability	53.84
% Cell death	46.16

3.6 Selectivity Index (SI)

The SI for (CC_{50}) of Vero cell line and (IC_{50}) of MCF-7 cell line for cells treated with aqueous extract of stem was 8 after 24 h of incubation (Table 3).

Table 3. SI of (CC_{50}) of normal Vero cell line and (IC_{50}) of MCF-7 cell line treated with aqueous extract of stem of *S. torvum* at 24 h of incubation

CC_{50} of Vero (µg/ml)	1000
IC_{50} of MCF-7(µg/ml)	125
SI	8

4. DISCUSSION

The major health concern of the 21st century is cancer, which has no boundaries and can affect any human organ. It is a very complex disease to understand because it has many cellular physiological systems such as cell signaling and apoptosis. The most common cancer treatments are chemotherapy, hormone therapy, radiation and surgery. Chemotherapy, the most popular treatment provides patients with systemic anti-cancer drugs to manage uncontrolled cancer cell proliferation [18].

The raw materials from the herbals form the base for drug production in pharmaceuticals. Understanding of the interaction of various constituents of medicinal herbs, would help in formulating and designing drug to act on the cancerous cells without harming the normal cells of the body [19,20].

In the present study cytotoxicity analysis on Vero cell line revealed that the aqueous stem extract of *S. torvum* exhibited 50% toxicity at the maximum concentration. The CC_{50} was found at 1000 µg/ml for Normal Vero cell line when treated with the aqueous extract of *S. torvum* stem at 24 h of incubation showed 50.32 % of cell viability and 49.68% cell death respectively.

The IC_{50} was found at 125 µg/ml for human breast cancer MCF-7 cell line when treated with the aqueous extract of *S. torvum* stem after 24 h of incubation. The MCF-7 cell line treated with aqueous extract of stem

show 51.70 % of cell viability and 48.30% cell death respectively. The extract was effective in reducing the cell viability of MCF-7 breast cancer cell lines. The IC_{50} was determined based on inhibitory concentration that induced 50% inhibition on the growth of the treated cells as compared to the untreated cells.

The phytochemical analysis in the present study shows presence of phytoconstituents such as flavonoids, alkaloids, phenols and saponins in the aqueous extract of stem which may have contributed to the anti-proliferative effect. The observed cytotoxic and anticancer action of the aqueous extract of *S. torvum* stem are in accordance with earlier studies carried out on the properties of *Solanum* phytoconstituents [21]. The phytocompounds isolated from Solanaceae family have been reported to possess several medicinal values, antioxidant and anticancer properties [22,23]. Flavonoids which prevent oxidative cell damage have shown to possess anti-proliferative role in cancer cells. This compound has profound effects on signal transduction mechanisms in cell proliferation and angiogenesis [24].

Solanum genus have also been shown to contain steroidal glycoalkaloids and steroidal saponins with significant cytotoxic and anti-tumor activities that have a close structural relationships [25]. Saponins react with cholesterol rich plasma membrane of cancer cells and inhibit their proliferation. Studies have demonstrated that several classes of compound including phenolic compounds, terpenes and coumaric acid may possibly act synergistically to inhibit cell proliferation and induce apoptosis in cancer cells [26]. The anti-proliferative effect observed in MCF-7 cell line in the present study can be attributed to the combined integrated mechanism of action of the phytochemicals present in *S. torvum* stem aqueous extracts. The qualitative analysis of these phytochemicals from the extract also substantiates this role.

Considerable amount of sterol content in aqueous extract of *S. torvum* stem have been reported. The anticancer activity of *S. torvum may* be attributed partially to steroidal alkaloid and steroidal saponins substances present in the stem [27].

The cytotoxic action indicates that the aqueous stem extract probably contain secondary metabolites or novel compounds which may inhibit cellular division in cancer cells [28]. Studies on *S. erianthum* revealed high cytotoxic and anticancer activity which is attributed to presence of compounds like flavonoids and phenols [29].

Aqueous extract of *S. torvum* stem was found to be extremely effective in the prevention of cell proliferation of breast adenocarcinoma cell lines. The extract if tested in animal models or administered in human may prevent cell proliferation by possible mechanism which could directly combine with cell receptor and elicit cellular apoptosis. Plant derived phytochemicals coupled with chemotherapy has gained much importance now-a-days in alleviating the proliferation of various carcinomas with minimal side effects [30].

Cell viability was also performed by staining the cells treated with trypan blue. The dead and live cells were counted from IC_{50} concentrations of 24 h incubation. About 53.84% live cells and 46.16% dead cells were identified. Trypan blue is water soluble dye and it is insoluble in membrane lipids. Chromophore is negatively charged and does not interact with cells unless the membrane is damaged. This could have been the possible mechanism in the present study when dead or non-viable cells show membrane damage and take up the dye whereas the viable cells do not take up the dye and are transparent as revealed from the cell count of the study [31].

Morphological alterations in Vero and MCF-7 cell lines clearly validated that extract of *S. torvum* induced drug dosage response in the present study. Microscopic observations clearly revealed Vero cells with regular shape and size at lower concentrations of the extract and with loss of their normal cytostructure at higher concentration. MCF-7 cells are seen in clusters, spindle shaped at lower concentrations. Cells with flattened structures are seen with increase in concentration of the extract at 24 h of incubation. The progressive changes seen are in a dose-dependent manner.

Cell morphological analysis indicated a significant loss of cell structure and disruption of cellular organelle integrity following treatment of the MCF-7 cell lines with aqueous extract of stem which can be correlated to non-viable cells exposed to trypan blue. Similar morphological changes were observed in MCF-7 cells treated with *Pongamia glabra* seed oil extract [32] and alkaloid extract of leaf from *Excoecaria agallocha* [33].

The degree of selectivity of the compounds is expressed by its SI value. The greater SI value (above 2) of a compound suggests selective toxicity and differential action against the target cells, while a compound with SI value (less than 2) indicates general toxicity of the pure compound which can also cause cytotoxicity in normal cells [34]. The IC_{50} values are used to determine the SI of each extract

which represents the overall activity between normal cell line and cancer cell line. The SI value for the present study on Normal Vero cell lines and MCF-7 breast cancer cell lines is calculated as 8 for cells treated with aqueous extract *S. torvum* of stem. 'Therapeutic index' is an important parameter to select samples for developing drugs [35,36].

Chemotherapy is routinely used for cancer treatment although its success is quite limited, due to severe side effects caused by drug resistance, targeting of healthy cells and metabolic stress. Dox increases the oxidative stress, which kills cancer cells and induces an inflammatory microenvironment, with cellular toxicity. Dox alone is not a preferable drug. A myriad of plant products have shown very promising anticancer properties *in vitro*, but they have yet to be evaluated in humans. However, some phytochemicals have shown a chemopreventive effect and the ability to sensitize cancer cells [37].

The results of the present study suggest that the uptake of the bioactive compounds from aqueous extract of *S. torvum* stem may suppress growth of cancer cells *in vitro* in breast cancer cell line MCF-7 and may prevent breast cancer development and proliferation induced by carcinogens. Isolation and characterization of specific bioactive compounds from the stem of this plant will be promising for its further development as an anticancer drug through molecular docking studies and in-silico predictions.

5. CONCLUSION

Information on ethnopharmacognosy is to gain knowledge for an effective method in the discovery of new anti-infective molecules from medicinal herbal plants.MCF-7 breast cancer cells serves as an excellent source of *in vitro* model for studying the mechanism of tumor response as well as complex relationships between binding and biological systems. This medicinal plant *Solanum torvum* can be considered for drug development and synthesis. The present information related with these secondary biomolecules will lead to new sources in search of lead molecules for curing cancer related ailments.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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