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Study on the Anti-cancer Potential of *Aegle marmelos* Fruit Extract Pro and Anti Apoptotic Molecule in Human Melanoma Cell Line-A375

G. V. Venkatakarthikeswari [a], R. Gayatri Devi [b*#], J. Selavaraj [c†] and A. Jothi Priya [b#]

[a] Saveetha Dental College and Hospital, Saveetha Institute of Medical and Technical Science (SIMATS), Saveetha University, Chennai, India.
[b] Department of Physiology, Saveetha Dental college and Hospital, Saveetha Institute of Medical and Technical Science (SIMATS), Saveetha University, Chennai, India.
[c] Department of Biochemistry, Saveetha Dental College and Hospital, Saveetha Institute of Medical and Technical Science (SIMATS), Saveetha University, Chennai, India.

Authors' contributions

This work was carried out in collaboration among all authors. Author GVV managed the literature search, data collection, analysis, manuscript drafting. Authors RGD, AJP and JS prepared the data verification and manuscript. All authors read and approved the final manuscript.

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Original Research Article

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ABSTRACT

Aegle marmelos is also known as bael which is commonly found in south East Asia and Indian-sub continent. The origin of bael is India. Bael is also known as the golden apple, Bengal-quince in India. In the ancient medical system, *Aegle marmelos* play an important role and its extract is also useful in inflammation, diabetes, cancer and asthma. The leaves are used for anti-inflammatory, nervous disorder, control blood sugar and fruit is used to treat antiviral, anti-diabetics, and brain and heart tonic. In addition, studies have proved that bael is used for the treatment and prevention of cancer. The main aim of this study is to assess the anti-cancer potential of Aegle marmelos fruit extract pro and anti apoptotic molecules in human melanoma cell line-A375. In the present study, Human Melanoma A375 cells will be produced, grown and will be passed in different culture flasks,

[#]Assistant Professor,
[†]Associate Professor
**Corresponding author: E-mail: gayatridevi@saveetha.com;*

then RNA isolation was done and by reverse transcriptase process, RNA gets converted into cDNA. This cDNA will be used for the amplification of growth factor beta using gene specific primers by commercially available real time PCR kit. The anticancer potential effect was found in 400µg/ml, as the concentration increases, the cell viability is decreased. The current study explains the potential application of bael in pharmacological and medicinal uses in near future.

Keywords: Aegle marmelos; bael; fruit; leaves; anti-diabetic; anti-cancer; anti-inflammatory; innovative technology.

1. INTRODUCTION

According to modern Ayurvedic sources, *Aegle marmelos* belong to the Rutaceae family and are morphologically deciduous, 6-9 metres in height with trifoliate leaves, and bark is shallow, furrowed with spines, short flowers, slightly pear shaped fruits [1]. About 80% of the world population depends on traditional medicines for health needs. Bael fruits possess various nutrients like vitamin C, B1, B2, B3, proteins and minerals like calcium, potassium [2]. Modern allopathic medicine has developed many cost diagnostic methodologies which also cause side-effects like constipation, diarrhea [3]. Availability of natural products which have low side effects and are highly effective [4]. Bael possesses a broad range of therapeutic effects like anti-ulcerative, hepatoprotective, cardioprotective, radioprotective effects [5].

Cancer is a painful and aggressive killer disease. Nowadays 60% of drugs used for the treatment of cancer have been isolated from natural products [6]. Cancer is mainly caused by genetic mutation in DNA [7]. Nowadays various methods used to treat cancer like chemotherapy may cause side effects like hair loss. Malignant Melanoma is also known as skin cancer which is developed from melanocytes that produce melanin [8]. There are four types of melanoma cancer-superficial spreading, nodular, lentigo maligna and acral lentiginous [9]. In male it commonly occurs on the backside of their body and for females it occurs on legs [10]. Symptoms are changes in skin colour, increase in mole size, itchiness. The drugs used in melanoma cancer do not kill all cancer cells and the cells which survey are responsible for drug resistance and increases mortality rate. The advantage of traditional medicine is being cost effective, easily available [11]. The ripened fruit is useful for the treatment of anti-viral, treatment of rectum inflammation. The unripened fruit is useful for astringent dysentery [12]. The essential oil extract from bael is useful for several biological activities like radiation protection, chemoprevention, antipyretic. The role of apoptotic is regulated by (BCL-2, BCL-XL) [13] and pro-apoptotic is regulated by (BID, BIM) [14-15].

Our team has extensive knowledge and research experience that has translate into high quality publications [16–34].

The main aim of this study is to assess the anti-cancer potential of Aegle marmelos fruit extract pro and anti apoptotic molecules in human melanoma cell line-A375.

2. MATERIALS AND METHODS

Dimethyl sulfoxide (DMSO), 3-(4,5-dimethy lthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) were purchased from Sigma Chemical Pvt Ltd, USA. Trypsin-EDTA, fetal bovine serum (FBS), antibiotics-antimycotics, RPMI 1640 medium and phosphate buffered saline (PBS) were purchased from Gibco, Canada. (5,5,6,6-tetrachloro-1,1,3,3-tetraethyl benzimidazole carbocyanine iodide) and Real Time PCR kit was purchased TAKARA (Meadowvale Blvd, Mississauga, ON L5N 5S2, Canada).

2.1 Cell Lines and Cell Culture

The Human Melanoma cell line (A375) was purchased from the National Centre for Cell Sciences (NCCS), Pune, India. Cells were cultured in DMEM medium (Thermo Fisher Scientific, CA, USA) containing 10% fetal bovine serum (Thermo Fisher Scientific, CA, USA), 100 U/ml penicillin and 100 µg/ml streptomycin (Thermo Fisher Scientific, CA, USA) at 37°C with 5% CO2.

2.2 Cell Viability by MTT Assay

Cell viability was assayed using a modified colorimetric technique that is based on the ability of live cells to convert MTT, a tetrazolium compound into purple formazan crystals by

mitochondrial reductases (Mosmann, 1983). Briefly, A375 Human Melanoma cell lines (1 ×104/well) were exposed to different concentrations of *Aegle Marmelos* fruit extract(100-500µg) with A549 cells for 48 h. At the end of the treatment, 100 µl of 0.5 mg/ml MTT solution was added to each well and incubated at 37 ◦C for an hour. The crystals formed were dissolved in dimethyl sulfoxide (100 µl) and incubated in dark for an hour. Then the intensity of the color developed was assayed using a Micro ELISA plate reader at 570 nm. The number of viable cells was expressed as the percentage of control cells cultured in serum-free medium. Cell viability in the control medium without any treatment was represented as 100%. The cell viability is calculated using the formula: % cell viability = [A570 nm of treated cells/A570 nm of control cells] × 100.

2.3 Gene Expression Analysis by Real Time-PCR

Samples from each group were submerged in 2 ml Trizol (Invitrogen, Carlsbad, CA, USA) for RNA extraction and stored at −80°C until further processed. cDNA synthesis was performed on 2 µg RNA in a 10 µl sample volume using Superscript II reverse transcriptase (Invitrogen) as recommended by the manufacturer. Real-time PCR array analysis was performed in a total volume of 20 µl including 1 µl cDNA, 10 µl qPCR Master Mix 2x (Takara, USA) and 9 µl ddH2O. Reactions were run on an CFX96 Touch Real-Time PCR Detection System (Bio-Rad, USA) using universal thermal cycling parameters (95°C for 5 min, 40 cycles of 15 sec at 95°C, 15 sec at 60°C and 20 sec at 72°C; followed by a melting curve: 5 sec at 95°C, 60 sec at 60°C and continued melting). For quality control purposes, melting curves were acquired for all samples. The specificity of the amplification product was determined by melting curve vs analysis for each primer pair. The data were analyzed by comparative CT method and the fold change is calculated by 2−ΔΔCT method described by Schmittgen and Livak (2008) using CFX Manager Version 2.1 (Bio Rad, USA).

2.4 Statistical Analysis

The obtained data were analyzed statistically by one-way analysis of variance (ANOVA) and Duncan's multiple range tests with computer-based software (Graph Pad Prism version 5) to analyze the significance of individual variations among the control and experimental groups. The significance was considered at $p<0.05$ level in Duncan's test.

3. RESULTS

The point finding showed that the *Aegle marmelos* fruit induces cytotoxicity in A375 cells. M-RNA expression of anti-apoptotic molecules such as BCL-2, BCL-XL found to be decreased in 300 and 400 µg/ml treated cells (Fig. 1). Effect of *Aegle marmelos* fruit extract on mRNA expression in A375 cells showed significant difference between control and treatment with A375 cancer cells. *Aegle marmelos* fruit extraction Bcl-xL mRNA showed significant difference between control and expression in A375 cells. That extract has significantly decreased the mRNA expression in A375 cancer cells ($p <0.05$) (Figs. 2 and 3).

4. DISCUSSION

Oncologists square measure typically face a huge drawback in deciding the effective doses of chemo and radiation therapies to attenuate the biological science and genetic damages on the healthy cells [13]. Most importantly one of the most vigorous cancers is melanoma cancer with a high mortality rate [35]. These cells reproduce faster than normal cells and migrate to various parts of the human body easily [36]. From the study of Tripti singh, Berberis vulgaris of A375 and Hs294 human melanoma cells has significance of ($P<0.01$-0.001) in A375 cells which has no significant effect on cell viability [37] whereas in this current study the significance is at $P<0.05$ in A375 cells. From the study of Ayoub khalfaui, Algerian Asphodelus tenuifolius A375 melanoma cells in which the decrease in the percentage of cell viability is significant when the concentration of the extract is 50µg/ml [38].

In malignant melanoma cells, *S. frutescens* extract is effective in inducing apoptosis. The viability of A375 and Colo-800 human melanoma cells was lowered by *S. frutescens* extract in a dose- and time-dependent manner [28] Significant increases in the expression of the CASP9 and FASLG genes in response to *S. frutescens* extract suggest that the extract may trigger apoptosis through both intrinsic and extrinsic mechanisms [39]. In this study, the percentage of cell viability is significant when the concentration of the extract is 400µg/ml. The chemical composition and relative quantity of biological active components determine the

cytotoxicity of the plant extracts tested on A431 cells [9]. The A375 cell line was sensitive, but not as sensitive as the A431 cell line [40]. In the present study, 500µg treated A375 cells showed reduced mRNA expression and the extract showed potent anticancer activity.

This study clearly indicates that plant extract has a significant role in modulation of intrinsic apoptotic signaling in melanoma cells which might be due to presence of marmelosin, tannins present in bael. In accordance with present studies on various cancer cell lines have demonstrated that bael and their bio-chemical compounds showed anti-proliferative activity in vitro and in vivo condition.

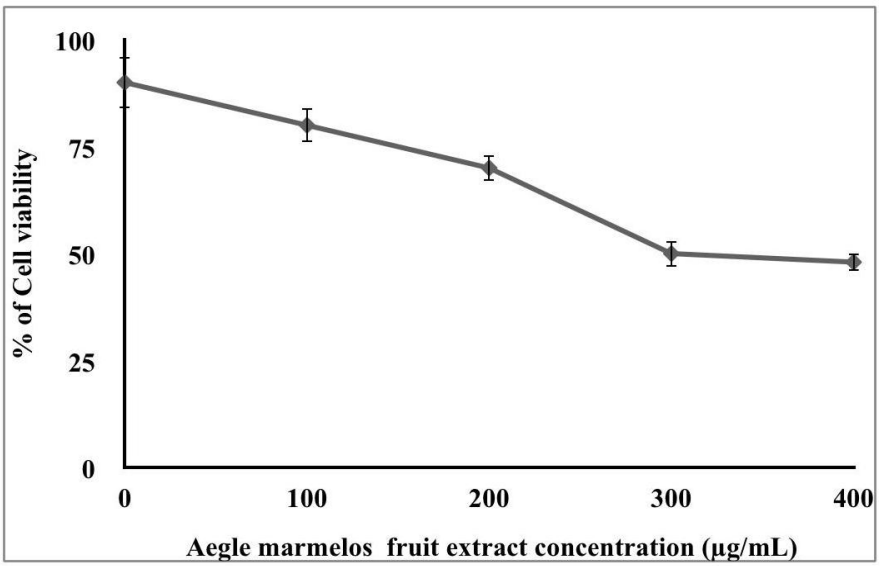

Fig. 1. Cell viability A375 cells. Effect of *Aegle marmelos* fruit extract on cell viability in A375cells. Each bar represents a mean ± SEM of 6 observations. Significance at p< 0.05, a-compared with untreated control cells, b-compared with 100µM treated A375 cells, c-compared with 200µg treated cells

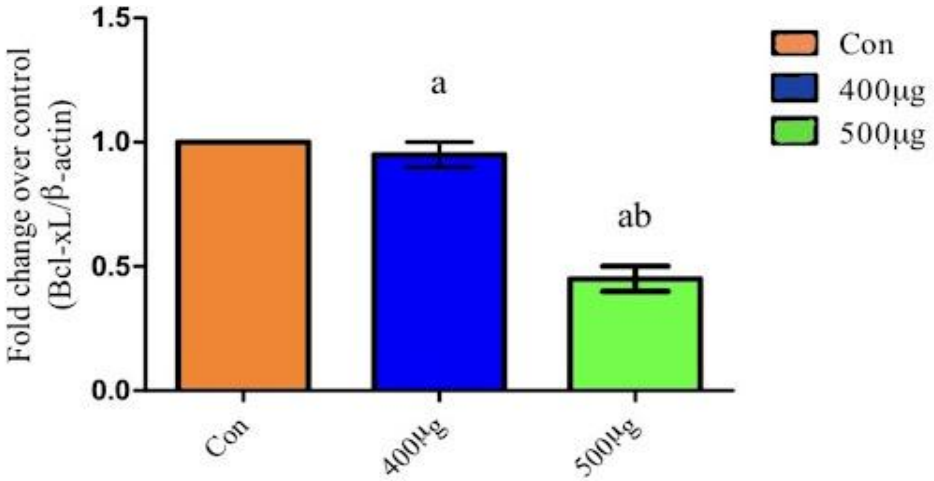

Fig. 2. BCL 2 mRNA expression (fold change over control). Orange color denotes control, Blue color denotes 400µg and green denotes 500µg treated A375 cells. Effect of *Aegle marmelos* fruit extraction Bcl-2 mRNA expression in A375cells. Each bar represents a mean ± SEM of 6 observations. Significance at p< 0.05, a-compared with untreated control cells, b-compared with100µg treatedA375 cells

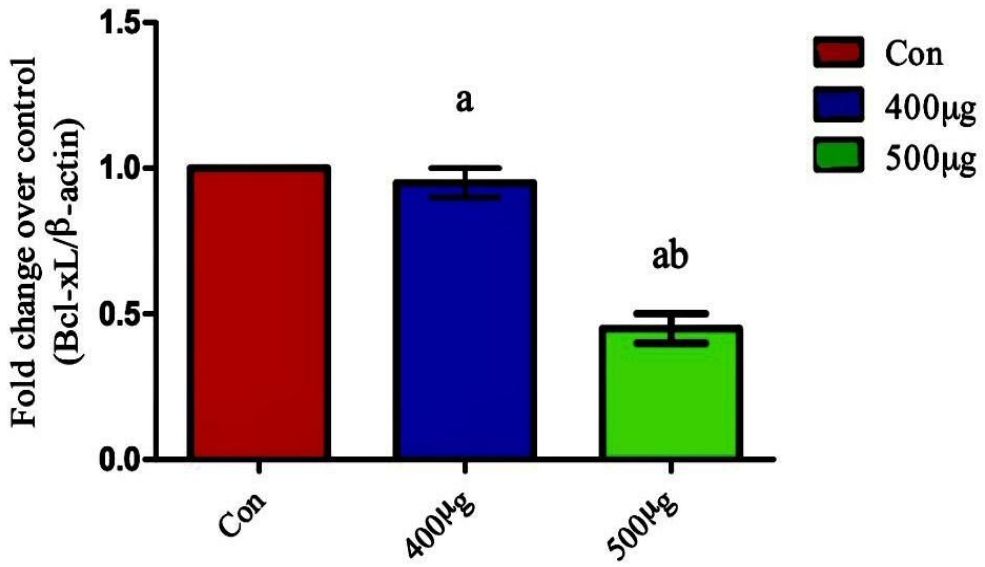

Fig. 3. BCL-XL mRNA expression (fold change over control). Red colour denotes control, Blue color denotes 400µg and green denotes 500µg treated A375 cells. Effect of *Aegle marmelos* fruit extraction Bcl-xL mRNA expression in A375 cells. Each bar represents a mean ± SEM of 6 observations. Significance at p<0.05, a-compared with untreated control cells, b-compared with 100 µg treated A375 Cells

This study only dealt with anticancer and anti proliferative activity of fruit extract alone. Further studies required to check other parts of the plant and also proceed in molecular techniques to confirm the activity.

5. CONCLUSION

From this study concluded that *Aegle marmelos* may be used as a potential anti-cancer, anti-proliferation activity. Further studies need to be analysed on signaling molecules involved in cancer progression in order to accretion potential of *Aegle marmelos*.

CONSENT

It is not applicable.

ETHICAL APPROVAL

It is not applicable.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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