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Anticancer Potential of *A. marmelos* Fruit Extract in Human Colon Cancer Cell Lines is Mediated through the Regulation of EMT Signalling Molecules

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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Original Research Article

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ABSTRACT

Background and Aim: *A.marmelos* is commonly called as Bael and is used as a medicinal plant mostly in the Ayurveda. Bael has the property of gastrointestinal effect. Bael phytochemicals are radioprotective, chemoprotective and it has efficacious properties in the treatment of cancer and its preventions. The main aim of this study is to estimate an anticancer activity of Bael in colon cancer cells of human.
Materials and Methods: Dimethyl sulfoxide (DMSO), 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT), Phosphate-buffered saline(PBS) are used as materials. The cell line used is HT 29. MTT test was used to check cell viability by calorimetric technique. The HT 29 cell was exposed to different mediums. Then the cell viability is calculated. Real time PCR was used to analysis gene expression in which cDNA synthesis was performed in varied sample volume. Melting curves were acquired for all samples. Data is analyzed by comparative CT method. It was

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analyzed by graph pad prism.
Results: The findings showed that the effect of Bael fruit extraction of HT 29 cells shows a significance of P<0.05. One of the parameters is treated with untreated control cells. The other two parameters are 100µg, 200µg. In Vimentin mRNA and E cadherin mRNA expression one is treated with untreated control cells and 100µg.
Conclusion: This concludes that the properties of the bael can act as an anticancer potential in human colon cancer cell HT 29 through EMT signalling molecules.

Keywords: Bael; anticancer; HT 29 cells; EMT signalling; innovative techniques.

1. INTRODUCTION

A. marmelos is also known as gold apple, Japan sour orange, rock apple, and wood apple [1]. It is a member of the Rutaceae family. It is a tree that grows year after year. It is mostly found in Asia's tropical and subtropical regions [2]. It is used as a traditional Indian system medicine. The bark, roots are used as expectorant [3]. It has many properties such as demulcent, anti-inflammatory, hypoglycemic and gastroprotective as their properties [4]. The bael is also referred to as a healing tree. The bael consisting of tannins and essential oils show varied pharmacological effects [5]. Antimicrobial effects are exhibited by the leaf, root and fruit. All parts of the bael have antiviral activity. Leaf produces immunomodulatory effect and antineoplastic effect [6]. The fruit possesses antitoxic and antidiuretic [7]. This fruit is mostly found in India, Bangladesh, Burma and Srilanka. The tree will have a bitter arid and sour taste [8]. Cancer is one of the major health issues [9]. This fruit is basically involved in curing cancer which acts as an anticancer agent. It has been an efficient plant since the charak [10]. There are about 100 phytochemical compounds which are isolated from this plant. The leaf of the bael is found to be the highest accumulatory in bioactive properties [11]. Colon cancer is one of the most suffered disorders. It is most commonly found in the rectum. Colorectal cancer develops from the deepest layer and spreads to the outer layers [12]. Sometimes it develops slowly over a period of several years. Chemopreventives are mostly advocated in the use of phytochemicals. Molecular changes that undergo cancer development showed the use of ayurvedic medicine [13].

Previous studies showed that *A.marmelos* is also used in drug development. Various animal models are also being used by the crude extraction of bael. Bael leaf inhibits the growth of many cancer cells like HT 29, melanoma and breast cancer cell lines. Anti proliferative activity present in bael which contains the eugenol and citral. Eugenol has cytotoxic effects on tumor HepG2 hepatoma cells, tumor Caco-2 colon cells, and a human tumor cell line [14]. In mice, it also plays a significant role in preventing DMBA-induced skin cancers [15].

Colon cancer depending on the stages it may be treated by ionization, radiation or chemotherapy which is not much effective. so that the side effects can be overcomed by using this fruit extract bael. Our team has a wealth of research and knowledge that has resulted in high-quality publications [16-31].

The goal of this research is to investigate into the anticancer properties of A.*marmelos* fruit extract in human colon cancer cell lines, which is mediated through EMT signalling molecules.

2. MATERIALS AND METHODS

Dimethylsulfoxide(DMSO), 3-(4,5-dimethylthiazol-2-yl) - 2,5-diphenyltetrazolium bromide (MTT) were purchased from Sigma Chemical PvtLtd, 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 benzimidazolecarbocyanine iodide) and Real Time PCR kit was purchased TAKARA (Meadowvale Blvd, Mississauga, ON L5N 5S2, Canada).

2.1 Cell Lines and Cell Culture

The National Centre for Cell Sciences (NCCS) in Pune, India, provided the human colon cell line (HT-29). Cells were grown at 37°C with 5% CO2 in DMEM media (Thermo Fisher Scientific, CA, USA) containing 10% foetal bovine serum (Thermo Fisher Scientific, CA, USA), 100 U/ml penicillin, and 100 µg/ml streptomycin (Thermo Fisher Scientific, CA, USA).

2.2 Cell Viability by MTT Assay

The ability of live cells to convert MTT, a tetrazolium chemical, into purple formazan crystals via mitochondrial reductases was used to determine cell viability (Mosmann, 1983). HT-29 Human colon cell lines (1 $\times 10^4$/well) were treated to various concentrations of *A.marmelos* fruit extract (100-500µg) over 48 hours with HT-29 cells. After the treatment, each well was filled with 100 µl of 0.5 mg/ml MTT solution and incubated for an hour at 37°C. The crystals were then dissolved in dimethyl sulfoxide (100 µl) and incubated for an hour in the dark. Using a Micro ELISA plate reader, the colour intensity was created and measured at 570 nm. The percentage of control cells cultivated in serum-free medium was used to calculate the number of viable cells. Without any treatment, cell viability in the control media was indicated as 100%. The formula for calculating cell viability is: percent cell viability = [A570 nm of treated cells/A570 nm of control cells] x 100.

2.3 Gene Expression Analysis by Real Time-PCR

For RNA extraction, samples from each group were immersed in 2 mL Trizol (Invitrogen, Carlsbad, CA, USA) and kept at 80°C until further processing (Ref.). The manufacturer recommended using Superscript II reverse transcriptase (Invitrogen) to make cDNA from 2 µg RNA in a 10 µl sample volume. A total volume of 20 µl was used for the real-time PCR array analysis, which included 1 µlcDNA, 10 µlqPCR Master Mix 2x (Takara, USA), and 9 µl ddH2O. Reactions were run on a Bio-Rad CFX96 Touch Real-Time PCR Detection System (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 72°C followed by a melting curve and continued melting). Melting curves were obtained for all samples for quality control objectives. Melting curve analysis was used to determine the specificity of the amplification product for each primer pair (Ref.). Using CFX Manager Version 2.1, the data were processed using the comparative CT method (Complete), and the fold change was determined using the 2CT method provided by Schmittgen and Livak (2008). (Bio Rad, USA).

2.4 Statistical Analysis

To examine the significance of individual variations across the control and experimental groups, the collected data were statistically analysed using one-way analysis of variance (ANOVA) and Duncan's multiple range test with computer-based software (Graph Pad Prism version 5). Duncan's test was used to assess significance at the $p<0.05$ level.

3. RESULTS

EMT signalling components such as Vimentin mRNA and E-cadherin mRNA were observed to be decreased in 300 µg/ml and 400 µg/ml treated cells, indicating that *A.marmelos* fruit extract promotes cytotoxicity in HT 29 cells (Fig. 1). The presence of bioactive chemicals in *Aegle marmelos* may play a role in the modulation of EMT signalling molecules in colon cancer cells, according to this study. As the percentage of *A.marmelos* of fruit extraction increases, the cell viability falls. In the untreated cells it has 100 %viability. When the concentration is increased from 100 µg to 500 µg, the viability % decreases. According to the current investigations on cancer cell lines, *A.marmelos* fruit extract and its bioactive components showed antiproliferative efficacy in vitro and in vivo.

4. DISCUSSION

The decrease in cell viability is more severe in the current study when the concentration is at 300-400 µg/ml, whereas Sherin et al. [32], Antiproliferative effects of Sallylmercaptocysteine on colon cancer cell line SW - 480 and HT 29 in *Allium sativum* where there is a significance of $p<.0.001$ in untreated control cells and in case of *A.marmelos* there is a significance of $p<0.05$ in untreated control cells. When the extract concentration is 200-250 µg/ml, the percentage of cell viability decreases more dramatically [33]. From the study of Ali imran et al. [34], *Camellia sinensis* acts against HT116 colon cancer cells that have the cell viability concentration (50-250/ml). The dose 50 µg/mL caused 96.7% inhibition in the viability of HCT 116 cells whereas in this study HT 29 colon cancer cell viability concentration was 0-500 µg/mL. The dose 50 µg/mL caused 74% inhibition. From the study of Kandasamy Sasikumar et al. [35], When the concentration of the extract reaches 45 µg/ml, the efficacy of oleanolic acid against HCT-116 cells is significant, with a drop in the percentage of cell viability. When the extract concentration reaches 400 µg/ml, the percentage of cell viability is significant, according to this study. From the study of Vinodkumar Nelson et al. [36], when the concentration is 500 µg/ml,

Eclipta alba has a substantial proportion of cell viability against HCT-116 colon cancer cells. When the concentration reaches 400 µg/ml, the percentage of cell viability is substantial in this investigation. An extract made from the leaves of *Annona muricata* suppresses colon cancer cell multiplication and promotes apoptosis [37]. *Lafoensiapacari* also inhibits colon cancer by using the HRT-18 colon cancer cells [38]. *Aegle marmelos* are also helpful in curing other cancers using human cancer cell lines MCF-7, H-450 [39].

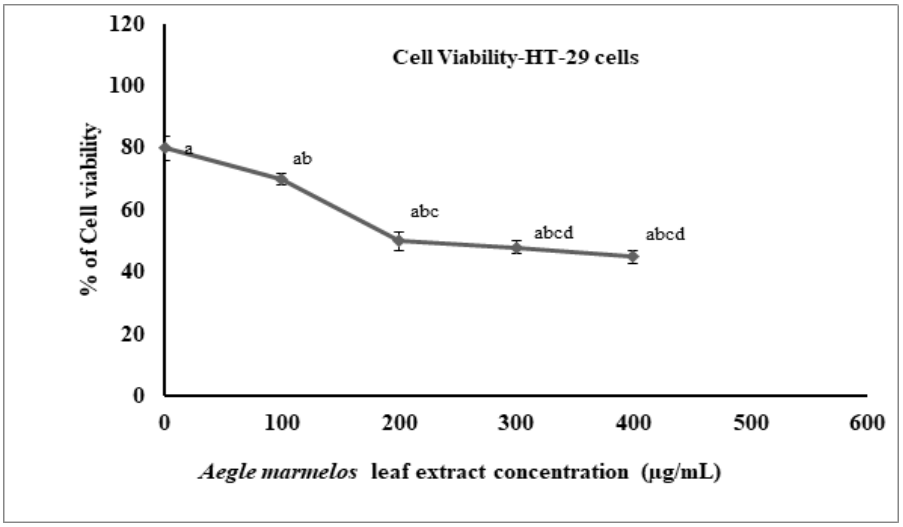

Fig. 1. Assessment of Cell viability

In HT-29 cells, the effect of Aegle marmelos fruit extracts on cell viability. The mean ± SEM of 6 observations is represented by each bar. Significance at p< 0.05, a-compared with untreated control cells, b-compared with 100µg treated HT-29 cells, c-compared with 200µg treated cells

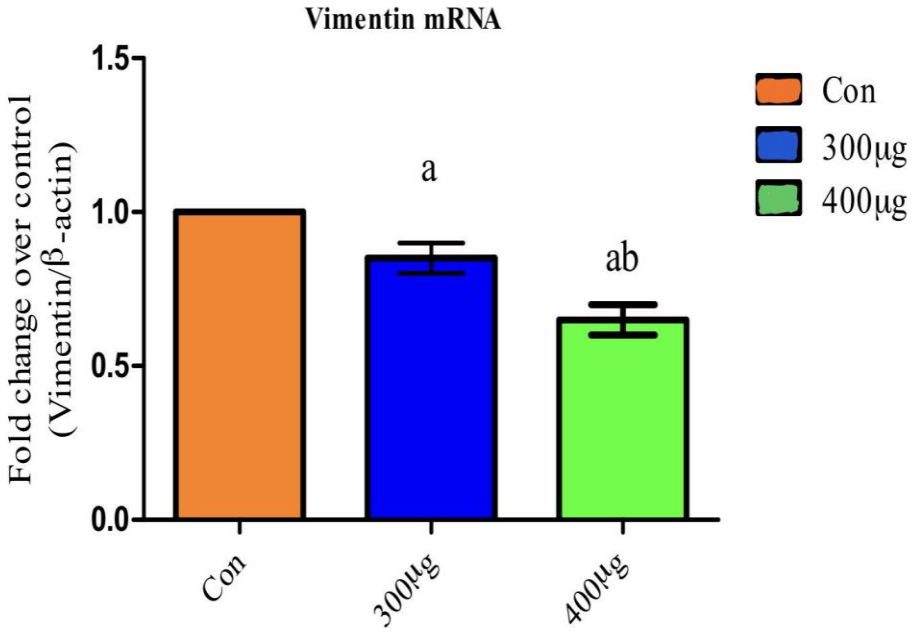

Fig. 2. Vimentin mRNA expression (Fold change over control). Orange colour denotes control, Blue colour denotes 300µg, Green colour denotes 400 µg treatedHT-29 Cells

In HT-29 cells, the effect of A.marmelos fruit extract on Vimentin mRNA expression. A mean ± SEM of 6 observations is represented by each bar. Significance at p< 0.05, a-compared with untreated control cells, b-compared with100µg treated HT-29 cells

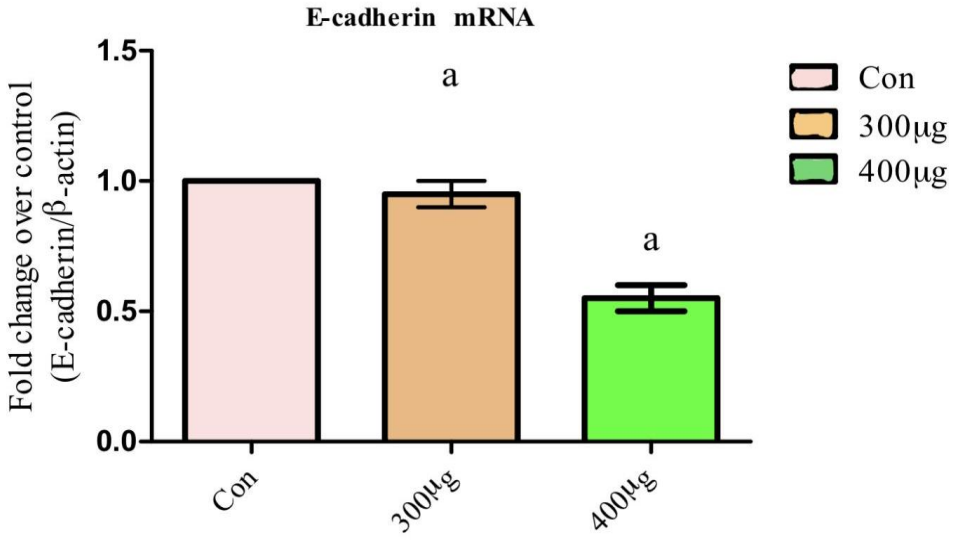

Fig. 3. E-cadherin mRNA expression (Fold change over control), Pink colour denotes control, Peach colour denotes 300µg ,Green colour denotes 400 µg treatedHT-29 Cells

In HT-29 cells, the effect of A.marmelos fruit extract on E-Cadherin mRNA expression. A mean ±SEM of 6 observations is represented by each bar. Significance at p<0.05. a-compared with untreated control cells, b-compared with 100 µg treated HT-29 cells.

The study's weakness is that it was based solely on the anticancer and antiproliferative properties of *Aegle marmelos* fruit extracts. Additional research can be done to assess the anticancer potential of other sections of the plant as well as various molecular activities to confirm the activity.

5. CONCLUSION

The fruit extract of A.marmelos has an influence on the EMT signalling molecules of the HT-29 colon cancer cell line, according to this study. Colon cancer is still a major public health concern. Anticancer factors derived from plants may be beneficial in colon cancer cells and could be used as anticancer medications.

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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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