PRO-APOPTOTIC ROLE OF TECOMA STANS IN MELANOMA CELL LINE (A-375) - AN IN VITRO STUDY

Danda Nishitha, Lavanya Prathap, Selvaraj Jayaraman, Preetha.S

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Authors :-

[1]**Danda Nishitha**
Department of Anatomy
Saveetha Dental College and Hospitals
Saveetha Institute of Medical and Technical sciences, Saveetha University, Chennai.
Email: nishitha.danda2002@gmail.com
[2]**Lavanya Prathap**
Associate Professor
Department of Anatomy
Saveetha Dental college and hospitals,
Saveetha Institute of Medical and Technical sciences, Saveetha University, Chennai.
Email: lavanya.sdc@saveetha.com
[3]**Dr. Selvaraj Jayaraman**
Department of Biochemistry
Saveetha Dental college and Hospitals,
Saveetha Institute of Medical and Technical Sciences,Saveetha University, Chennai.
Email : selvarajj.sdc@saveetha.com
[3]**Preetha. S**
Assistant Professor
Department of Physiology
Saveetha Dental college and hospitals,
Saveetha Institute of Medical and Technical Sciences, Saveetha University,Chennai.
Email: Preethas.sdc@saveetha.com

Corresponding Author :-

Lavanya Prathap
Associate Professor
Department of Anatomy
Saveetha Dental college and hospitals,
Saveetha institute of medical and technical sciences
Chennai.
Email ID: lavanyap.sdc@saveetha.com

ABSTRACT :

Background : Vigorous growth of cells leads to cancer. Tecoma Stans, a flower which consists of anti - cancer properties. One of such anti - cancer properties is the pro - apoptotic role in melanoma cell line (A-375). This is an in vitro study. The intervention is a hydroethanolic extract of Tecomastans on the melanoma cell line. The gene expressions chosen for this study are P53, caspase 3 and caspase 9. Aim of this study is to assess the pro apoptotic role of Tecomastans in melanoma.

Materials and methods : Human skin cancer (A - 375) was brought from NCCS, Pune, India. Cell viability test using MTT assay and Gene expression analysis for P53, caspase 3 and caspase 9 were carried out using MTT and PCR respectively. The results were analyzed using appropriate statistical tools using ANOVA and Duncan's test.

Results : The study suggest in MTT assay, the percentage of cell viability is observed to decrease on induction of Tecoma Stans.Caspase 9, mRNA gene expression decreases on induction of dosages 300mg/ml and 400mg/ml of Tecomastans. P53 gene expression increases on induction of dosages 300mg/ml and 400umg/ml of Tecomastans. Caspase 3 gene expression doesn't show any significance on induction of dosages 300mg/ml and 400mg/ml of Tecomastans.

Conclusion : Tecomastans consists of pro apoptotic property which is one of the anti cancer properties in melanoma when acted on the gene expressions P53, caspase 3 and caspase 9.
Key words : Tecoma stans; apoptosis; melanoma; anticancer; Innovative

INTRODUCTION :

Cancer, a very serious disease. Cell is a small and primary unit of the body. Vigorous growth of cells leads to cancer which is usually uncontrollable and incurable (1). It is not natural and forms lumps. Skin cancer is the vigorous growth of cells. Skin cancer is most often developed when the skin is exposed to the sun(2). Primarily there are three types of cancers. The cell line of melanoma is A - 375(3). Incidence of melanoma is 1,32,000 generally. The five-year survival rate of melanoma stage 0,1 and 2 is 98.4% according to the 2019 survey(4).

Tecomastans is a trumpet flower, also called yellow bells. Origin of tecoma stans is from virgin islands,caribbean, peru and ecuador. Tecomastans is occasionally an invasive weed. Tecoma Stans belong to the family bignoniaceae(5). Tecomastans has lots of uses. Some of the uses of tecoma stans are cure for diabetes, cure for syphilis and cure for stomach pain. From Tecomastans more than 120 chemical compounds have been obtained and were isolated(6). Tecomastans is an anticancer drug which consists of anti-cancer properties(7). The genes which react with tecoma stans during melanoma are P53, caspase 3 and caspase 9. Cell viability can be checked through MTT assay(8). Caspase 3 is an apoptotic gene which increases gene expression and reduces cell cancer(9). One of the treatments used to treat the cancer is chemotherapy. The experience from our previous studies (10) (11,12) (11)(13)(14)(15)(16)(14,16)(17)(18) (19) have conduct us to concentrate on the current topic.

This research is conducted to know the anti-cancer and pro-apoptotic properties of hydroethanolic extract in tecoma stans in melanoma cell line (A-375). Research is rarely performed in this field. So I have taken an attempt to do this research. Tecomastans consist of anti-cancer properties. This property reduces, cures and prevents skin cancer and even other cancers. Some of the treatments for melanoma are excisional surgery, mohs surgery and radiation therapy(20). In normal cancer therapy cancer cells as well as other cells respond together. To solve that tecoma stans is used which is a new way of treatment for cancer(21). Studies at molecular levels were performed by our team of researches which insisted us to proceed this study (22–29),(30),(31),(32),(33,34),(35),(36),(37–41). The primary aim of this research is to analyse the pro apoptotic role of tecoma stans in melanoma.

MATERIALS AND METHODS :

A private dental college and institutions in Chennai are doing an in vitro investigation. The institutional review board has given its approval to this project.

Procedure:

The human melanoma cell lines (A375) were purchased form cellline centre, pune, India. At 37 degrees Celsius and 5% CO2, tissues were grown in RPMI media containing 10% foetal bovine serum, 100 U/ml penicillin and 100 g/ml streptomycin. The MTT test was used to measure cell growth. (A375) tissues were sown in 96-well plates with 5x104/200l and grown overnight.. As a vehicle control, tissues were treated with dimethyl sulfoxide (0.1 percent DMSO). Six duplicate wells were used in each treatment. All of the tissues were then grown for another 48 hours. The experiment was carried out three times. The MTT absorbance in negative control tissues was employed as a 0 percent cell inhibition measurement. The expression status of m RNA was analysed by Polymerase chain reaction for identifying the fold change of caspase 3, Caspase 9, and P53 m RNA expression over control samples. The obtained date were analysed for its significance using one-way analysis of variance (ANOVA) and Duncan's multiple range test with significance at the 0.05 level.

RESULTS :

Effect on Cell Viability:

The MTT assay was used to assess the viability of human skin cancer cells (A-375 cells) after different dosages of tecoma stans were administered. When compared to control, it was discovered to inhibit skin cancer cells by reducing the percentage of viability of cancer cells in a dose-dependent way. Compared to untreated group, the concentration (100-500ug/ml) employed in this investigation showed the greatest reduction of cell growth. (Figure 1).

Effect of Caspase-3 mRNA expression on the A-375 cancer cells:

The mRNA expression of caspase-3 was assessed in a different doses. When compared to the untreated group, there was no significant difference in caspase-3 mRNA expression at a dosage of 300g/ml.

Furthermore, there was no difference in Caspase-3 mRNA expression at a dosage of 400g/ml compared to control.

Effect of Caspase-9 mRNA expression in A-375 cells

Caspase-9 mRNA expression was measured in a dose-dependent manner. At a dosage of 300g/ml, the cancer cells were considerably inhibited, and there was a considerable drop in caspase-9 mRNA expression when compared to control. At a dosage of 400g/ml, there was also a substantial reduction in caspase-9 mRNA expression when compared to control. Thus interestingly, the decrease was in dose dependent manner.

Effect of P53 mRNA expression in A-375 cells

The mRNA expression of P53 was assessed in different doses. At a dosage of 300g/ml, the cancer cells were considerably suppressed, and there was a considerable increase in p53 mRNA expression as compared to control. At a level of 400g/ml, there was a substantial increase in p53 mRNA expression as compared to control. Thus interestingly, the increase was in dose dependent manner.

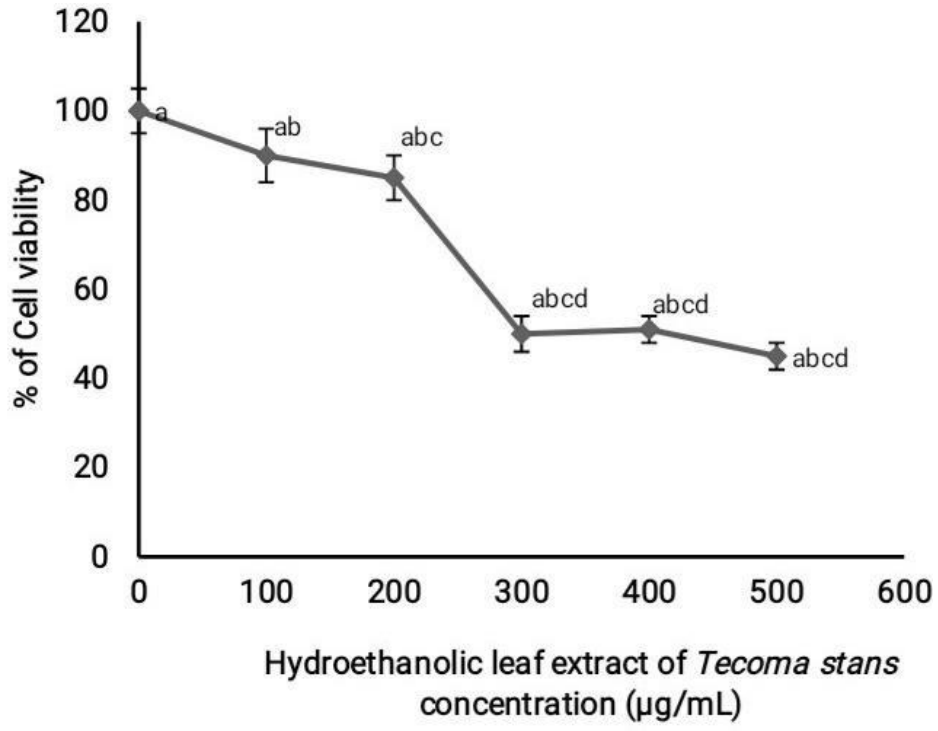

Fig 1 : Effect of hydroethanolic leaf extract of tecomastans on cell viability in A375 cells.

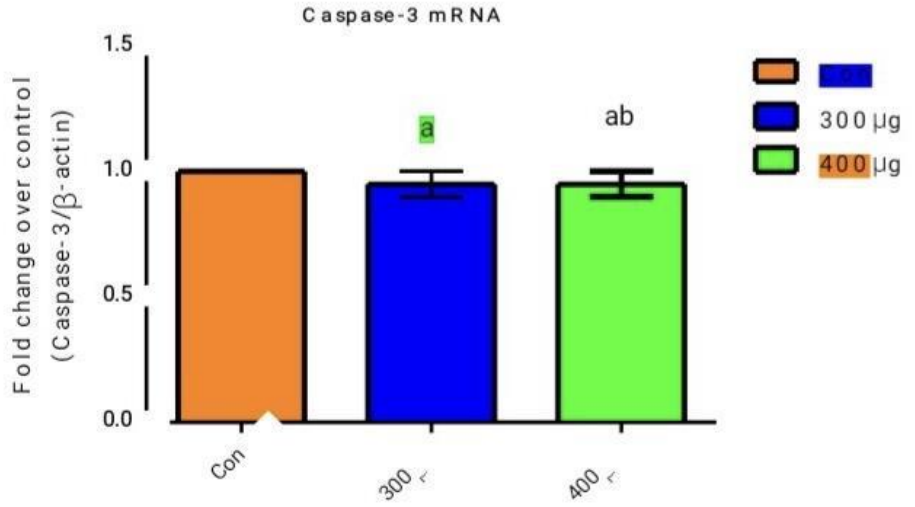

Fig 2 : Effect of Tecomastans on caspase 3 mRNA expression in A-375 cells.

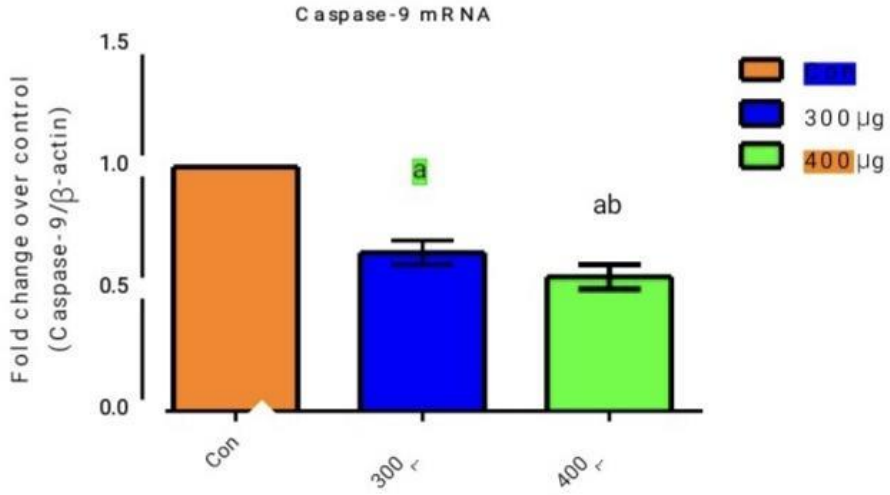

Fig 3 : Effect of Tecomastans on caspase 9 mRNA expression in A-375 cells.

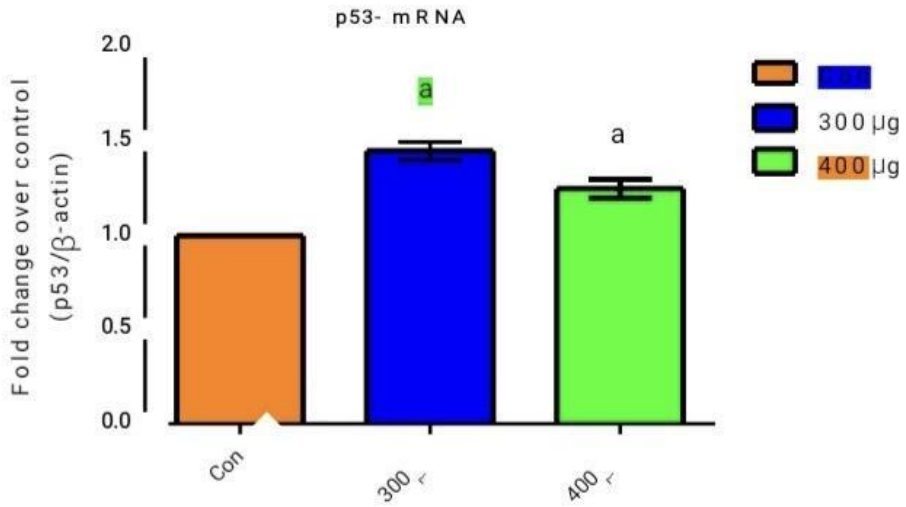

Fig 4 : Effect of Tecomastans on P53 mRNA expression in A-375 cells.

DISCUSSION:

In MTT assay, the percentage of cell viability is observed to decrease on induction of Tecoma Stans. Tecomastans consists of pro apoptotic property which is one of the anti cancer properties in melanoma when acted on the gene expressions P53, caspase 9 and caspase 3. Caspase 3 gene expression doesn't show any significance on induction of dosages 300μg/ml and 400μg/ml of Tecomastans. Caspase 9, the gene expression decreases on induction of dosages 300μg/ml and 400μg/ml of Tecomastans. P53 gene expression increases on induction of dosages 300μg/ml and 400μg/ml of Tecomastans.

Tecomastans is a plant which consists of many properties against cancer. One of such properties is pro - apoptotic property against cancer(7). Then our study is compared to the Xinjian Liu study in the year 2015, it shows that apoptosis is considered as an anti - oncogenic process(42). In our study, In caspase 3, the gene expression is not significant on induction of dosages 300μg/ml and 400μg/ml of tecoma stans. So, it doesn't show a great effect on melanoma. When our study is compared to the Xinjian Liu study in the year 2015,it shows that Caspase 3 promotes carcinogenesis (42).

In our study, In caspase 9, the gene expression decreases on induction of dosages 300μg/ml and 400μg/ml of tecoma stans. When our study is compared to the Razquallah study in the year 1998, it shows that caspase 9 activation can promote pro - apoptotic property against cancer(43). In our study, In P53, the gene expression increases on induction of dosages 300μg/ml and 400μg/ml of tecoma stans. When our study is compared to the Walid.H study in the year 2018, it shows that activation of P53 dependent apoptotic pathway in the response to the parthenolide(44).

Limitation of the study is sample size limitation. More sample size leads to much better results, keen observation and virtuous study. Tecomastans has a lot of future scope for the treatment of cancer and there is a lot of scope for better treatments.

CONCLUSION :

Thus tecomastans have anti - cancer and anti - pro apoptotic properties towards skin cancer through MTT pathway and by the read with specific genes such as P53, caspase 3 and caspase 9.

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CONFLICT OF INTEREST :

All the authors declare that there was no conflict of interest in the present study.

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