Tecoma stans Hydroethanolic Leaf Extract its Antioxidant and Cytotoxic Activity Against Colon Cancer (HT-29)

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ABSTRACT :-

BACKGROUND :-
Colon cancer is known to be the third most deadly and fourth most commonly diagnosed cancer in the world. In this research we study the anti-cancer properties of the plant leaf extract of *Tecoma stans.* There has always been a need for anti-cancer agents, since the commercially available synthetic drugs have their own limitations due to undesirable side effects. AIM :- To analyse the antioxidant and cytotoxic activity of Hydroethanolic leaf extract of Tecoma stans in colon cancer cell lines.

MATERIALS AND METHODS :-

Human colon cancer cell line (HT -29) was brought from NCCS, Pune, India. Cell viability test and Gene expression analysis were carried out for P-I3 and Akt mRNA Gene expression using MTT and PCR respectively. The results were analyzed using appropriate statistical tools.

RESULT :-
Results suggest that maximum reduction of cell proliferation was at dosage of (100-500 µg/ml) of the Hydro ethanolic leaf extract of *Tecoma stans* used in the study when compared to untreated group. At doses of 300 g/ml and 400 g/ml, the cancer cells were severely suppressed and mRNA expression of P-I3 and Akt was significantly reduced as compared to control.

CONCLUSION :-
The research carried out revealed that leaf extracts of *Tecoma stans* have diverse pharmacological spectrum and contain anti-cancer behavious. Research on this plant is increasing day by day because of its potent pharmacological uses. However further investigations involving the isolation on screening models of constituents are needed for further confirmation of various potential of *Tecoma stans*

Key words :- *Tecoma stans;* Colon cancer; Anti oxidant; Anti proliferative;Innovative technique

INTRODUCTION :-

We live in an era with improved living standards worldwide where we can find access to numerous healthcare that has considerably improved the diagnosis and treatment of diseases [1]. These measures happened to create an impact on the average life expectancy in most parts of the world. Even though deaths caused by communicable diseases have somewhat decreased around the world, the mortality caused due to cancer has increased vividly. It has the ability to spread, infiltrate and destroy normal body tissue. As a result, it is the second prevalent cancer.. The main issue with colon cancer is that it is asymptomatic, which means that the signs aren't always obvious. The stage of the disease at diagnosis has little bearing on survival. [3]. However, the rate differs by region. It is lowest in Africa and Asia and highest in Europe, North America, and Australia. Dysplastic adenomatous polyps are the most common cause of colon cancer. There is a two-step process that requires the inactivation of several genes that suppress tumour and repair DNA, as well as the activation of an oncogene. This gives the colonic epithelial cell a selective growth advantage. [4] The experience from our previous studies [5 6,7 68910119,111213 14] have led us to concentrate on the study.

There are many scientific and alternative treatments for colon cancer. The treatments show their effectiveness based on what stage and the security the cancer is detected. Evidences support the use of aggressive surgery and adjuvant treatment and therapies . However most of the available treatment options that are commonly used are prone to cause more harm and side-effects [15]. The choice of treatment method for colon cancer is very important because each tumour responds to different methods differently. With much research work, alternative medicine offers many methods that help a number of people since natural methods are preferred over synthetic and harmful drugs that contain plenty of side-effects. One such source is the antioxidant and cytotoxic activity of hydro ethanolic leaf extract of *Tecoma stans* that contains anti-cancer properties [16,17]. *Tecoma stans* is a non-

toxic herb and is also used as a remedy for several medical conditions like diabetes. Despite the traditional use of this plant, its pharmacological effect is proven to process anti-cancerous properties. [18]. Along with anti-cancer properties it also contains antidiabetic, anti-oxidant, antispasmodic, antimicrobial and antifungal properties [19]. Studies at molecular levels were performed by our team of researches which insisted us to proceed this study [20–27],[28],[29],[30],[31,32],[33],[34],[35–39]Therefore this study is being undertaken to analyse the activity of hydro ethanolic leaf extract of *Tecoma stans* against colon tumor.

MATERIALS AND METHODS :-

Procedure :-

Cell line centre, Pune, India, provided the human colon cancer cell line HT29. Tissues were cultured in RPMI media with 10% foetal bovine serum, 100 U/ml penicillin, and 100 g/ml streptomycin at 37 degrees Celsius and 5% CO2.The MTT test was used to measure cell growth. (HT-29) tissues were sown in 96-well plates with 5x104/200l and grown overnight. 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 P-I3 and Akt mRNA Gene expression m RNA expression over control samples. The samples were assessed using (ANOVA) and Duncan's multiple range test with p value at 0.05.

RESULT :-

Tecoma stans was found to reduce the abnormal proliferation of cells by reducing its cell viability at concentration (100-500 µg/ml. [Fig 1, Table 1]. Effect of tecoma stans on P-I3 and Akt, the cells were reduced in its proliferation and the reduction in in mRNA expression is observed. [Fig 2] [Fig 3]

Table -1 :- Assessment of Cell Viability

Hydro ethanolic leaf extract of *Tecoma stans* concentration (µg/mL)	**Cell viability %**
0	100
100	90
200	60
300	50
400	45
500	45

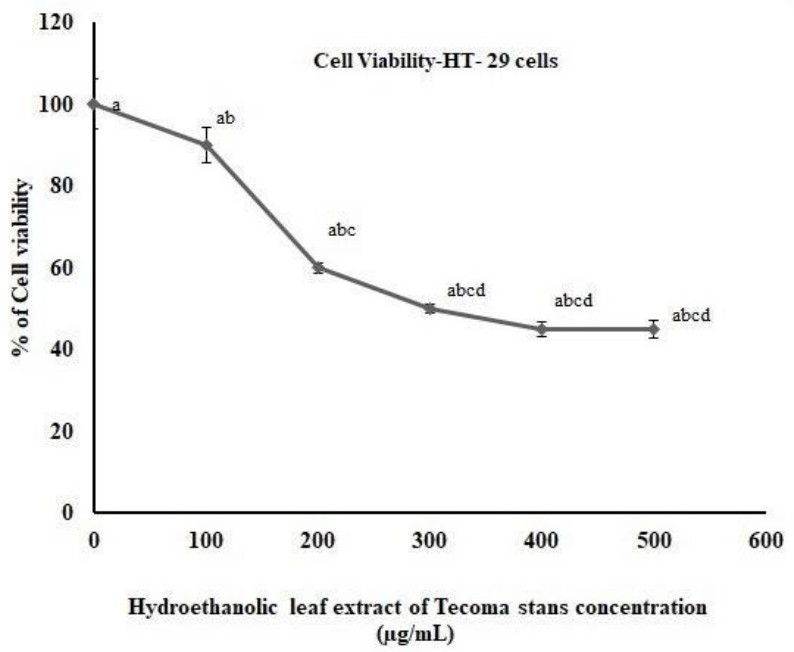

Fig 1 :- Effect of serotonin on cell viability in HT-29 cells.

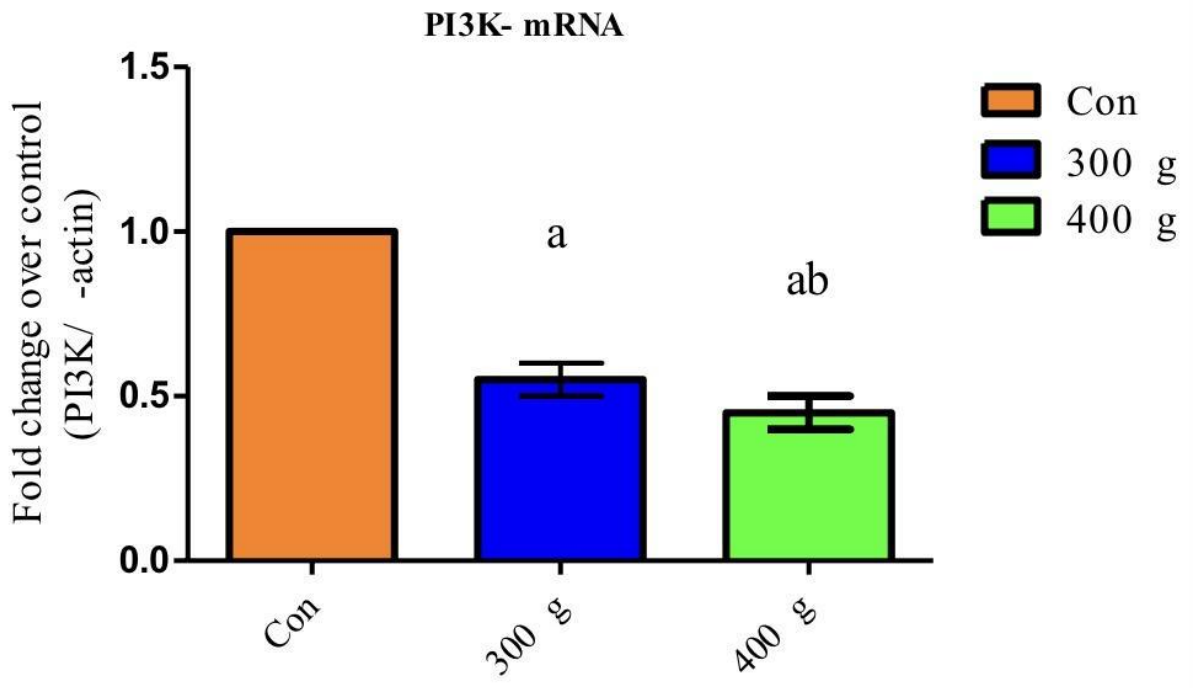

Fig 2 :- Effect of dopamine on P-I3 mRNA expression in HT-29 cells.

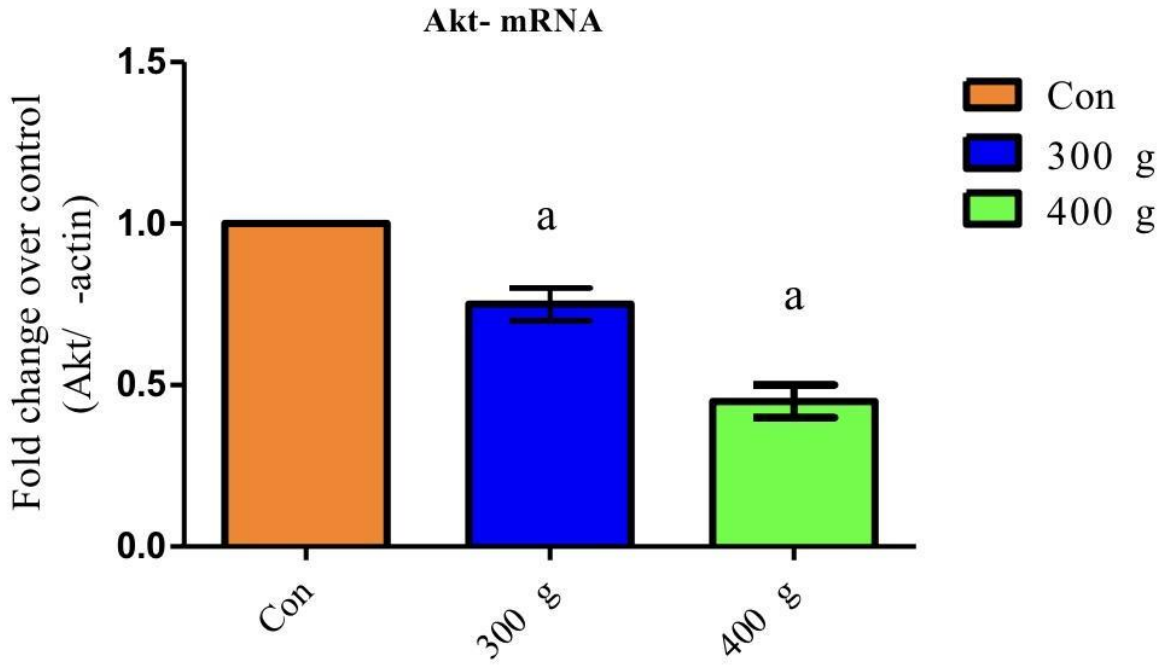

Fig 3 :- Effect of dopamine on Akt mRNA expression in HT-29 cells.

DISCUSSION :-

In this study we come across natural aids for the prevention of colon cancer. The intervention compound used for this research is the hydroethanolic leaf extract of Tecoma stans due to its anticancer property. Results revealed that maximum reduction of cell proliferation was at concentration (100-500 µg/ml) of the Hydro ethanolic leaf extract of *Tecoma stans* used in the study when compared to untreated group. The cancer cells were significantly decreased and showed significant reduction in mRNA expression of P-I3 and Akt when compared to control at a dose of 300 µg/ml and 400 µg/ml. Hence the research carried out proved that *Tecoma stans* contain anti-cancer properties along with numerous pharmacological uses.

For millennia, the subject of what causes cancer has piqued people's interest. People who migrated to other countries were thought to get cancers that were more common in their adopted countries than in their original countries. Most cancers are thought to be caused by synthetic substances, according to popular belief.[40] In contrast to that this research involves a natural anti-cancer plant extract that also possesses anti-oxidant and cytotoxic properties. The plant extracts used in our study is *Tecoma stans* due to its anticancer properties. Patients preferred to be treated with natural and herbal extracts rather than synthetic medication that causes numerous side-effects [41].

In a 2017 study, Robinson JP et colleagues discovered that the presence of phenolic chemicals causes cancer cells to undergo a cascade-based apoptosis, resulting in cytotoxicity. Lung cancer was the type of cancer that was studied in this study. The cell viability of a lung cancer cell line was shown to diminish as the concentration of the plant extract was increased. It was discovered to have the maximum concentration at 100 g/ml, but in our investigation, it was proven to inhibit colon cancer cells by lowering the percentage of viability of cancer cells in a dose-dependent way when compared to control cells. When compared to control, the concentration (100-500 g/ml) employed in the study had the greatest reduction of cell proliferation. The ability of the Tecoma stans

plant extract to be an efficient anti-cancer agent was demonstrated when cell viability decreased as the concentration of the extract increased. The extract's antioxidant and free radical scavenging activities could be the reason for its anti-cancer benefits. [42]. In 2018, approximately 1,096,000 instances of colon cancer are projected to be diagnosed, and approximately 704,000 cases of rectal cancer are expected. Colon cancer is more frequent in men than in women, and it is three to four times more likely in affluent countries than in underdeveloped countries. [43] Per 100,000 of colon cancer diagnosed in both genders which is 19.7, 23.6 is detected in males and 16.3 is detected in females. The same statistics for women are detected to be 20.9 and 5.9 respectively [44]. One of the genes used to carry out the experiment was P-I3, from the study conducted it was understood that the function of this gene is to enable acid cell growth and proliferation and can be interpreted that it leads to increase in gene expression and favours cancer cell proliferation.

Contrasting to the findings of our research, in this study it was derived that P-I3 inhibited growth and aids the death of many cancer cell lines. [45] Similarly, this study has also used the HT-29 cell line and MTT assay in order to measure cell viability and cell proliferation. It was observed in our study, that the hydro ethanolic leaf extract of *Tecoma stans*, showed an enhanced cytotoxic activity on HT-29 cell line after drug treatment, likewise in the study conducted by Pal I et al 2013, On the same cell line, a combination of Celecoxib and GSK690693 caused cytotoxicity. Celecoxib, unlike Tecoma stans, has adverse effects. Pal I et al 2013, by his research could interpret that with the involvement of GSK690693, there is a reduction in the high dose of Celecoxib. [46,47] Therefore novel potent anti-cancer drugs without considerable side effects from natural sources are under evaluation.

CONCLUSION :-

The research carried out revealed that leaf extracts of *Tecoma stans* are important therapeutic plant with a diverse pharmacological spectrum and contain anti-cancer properties. Research on this plant is increasing day by day because of its potent pharmacological uses. However further investigations involving the isolation of screening models of constituents are needed for further confirmation of various potential of *Tecoma stans.*

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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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