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The immunological effect of anticancer drugs combined with *Tamarix mannifera* extracts on colon cancer cells

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Abstract

Background: Colon cancer, which affects both men and women, is the second most lethal kind of cancer and a global health concern. The world's colon cancer death rate is rising as a result of the high meat and alcohol consumption of modern diets and lifestyles, combined with little physical exercise. Therefore, the development of innovative and ecologically safe pharmacological therapy for colon cancer is necessary. Nutraceuticals are now being used to treat a number of chronic conditions, including diabetes, Alzheimer's disease, and colon cancer. Nutraceuticals come from a variety of natural sources, including fruits, vegetables, marine life, and medicinal plants. It has been demonstrated that nutraceuticals may both lower the risk and delay the development of colon cancer. These food ingredients focus on several molecular elements of colon cancer growth. Building on its traditional therapeutic applications, researchers are looking into natural substances with possible anticancer effects, such as the latex of *Tamarix mannifera*. This study investigates the apoptotic effects of *Tamarix mannifera* alone and in conjunction with traditional anticancer medications and chemotherapeutic agents on the LS174T cell line. By comprehending these connections, potentially safer and more effective therapies for colon cancer may be discovered.

Method: For 24 hours, the human colon cancer LS174T cell-line was given a variety of doses of cisplatin, doxorubicin and *Tamarix mannifera* each alone and their combinations. MTT assay was used to assess the cytotoxicity of the agents, then the supernatant was used to measure the level of caspase 3 level.

Results: *Tamarix mannifera* significantly increase cytotoxicity (P-value ≤0.05) at concentrations from 31.25 to 1000 µg/ml, with the most notable increase in cytotoxicity at 1000 compared to 125 µg/ml. Cisplatin also increase cytotoxicity at concentrations from 7.8125 to 250 µg/ml, most notably at 250 compared to 15.625 µg/ml. doxorubicin also increase cytotoxicity at concentrations from 7.81 to 250 µg/ml, most notably at 250 compared to 31.25 µg/ml. Combining *Tamarix mannifera* with cisplatin increase cytotoxicity at various concentrations but showed significant increase cytotoxicity (P-value ≤0.05) at 250 compared to 125 µg/ml. also combining *Tamarix mannifera* with doxorubicin increase cytotoxicity at various concentrations but showed significant increase cytotoxicity (P-value ≤0.05) at 31.25 compared to 500 µg/ml.

Conclusion: *Tamarix mannifera* extract with cisplatin and doxorubicin increase cytotoxicity level(decrease viability of cancer cells), showing potential. However, saturation effects at greater doses imply dosage complexity. Cisplatin's effectiveness, doxorubicin's effectiveness and Tamarix 's potential anti-cancer qualities demand additional research and clinical testing.

Keywords: Caspase 3, *Tamarix mannifera*, combined, anticancer drug, colon cancer, LS174T cell line

Introduction

One of the most deadly types of cancer is colon cancer, which can spread to the liver, lungs, ovaries, and other digestive organs, among other places in the body. As an inhibitor of DNA synthesis, sofar, 5-fluorouracil (5-FU), is the top choice for treating colon cancer [1-2]. Although synthetic chemical anticancer medications increase lifespan, they frequently have negative side effects and off-target effects. This has led to research into the potential therapeutic uses of phytochemicals and nutraceuticals for colon cancer [3]. The word "nutritraceuticals," which comes from the fields of pharmaceuticals and nutrition, can also refer to "functional foods" [4]. Phytochemicals found in food have a rich historical basis and are widely used in contemporary medicine. These substances find application in the pharmaceutical and commercial sectors as food additives, cosmetics, and helpers [5]. Nutraceuticals can regulate the deteriorating of DNA components in cancer cells and control

the transcription of DNA in malignancies. Many therapeutic advantages are associated with them, including the ability to reduce obesity, improve cardiovascular health, lower diabetes, boost immunity, naturally activate antioxidants, and reduce inflammation [6-7]. Different treatment approaches, including radiation, chemotherapy, surgery, and phytotherapy, are appropriate for different stages of colon cancer. Every other type of cancer treatment has serious side effects. Nutraceuticals produced from plants are beneficial in the treatment of colon cancer and also have a positive impact on general health [8]. The nutritional substances have shown less side effects and offered superior therapy (9). The rising number of cases of colon cancer seems to be caused by hereditary factors, dietary changes, and changes in physical activity levels [10]. In healthy cells, reactive oxygen species can lead to issues. O2 – and OH_ are examples of free radicals that can enhance healthy human colonocyte activity and cause colon polyps to develop. Fruits and other plant resources include natural antioxidants that help reduce oxidative damage to colon cells. Plant-derived flavonoid phytochemicals, such as *Tamarix mannifera*, are useful in triggering apoptosis in colon cancer cells. Live microorganisms that have been produced recently, like probiotics, are also significant nutritional supplements for people. Probiotics have positive effects on humans and help to balance the mix of gut microorganisms. Intestinal tract. Dietary practices and environmental variables can change the concentration of lactobacilli and colonic bacteria, which can result in the development of malignant tumors and polyps. Therefore, taking probiotics may prevent the formation of early cancer cells [41]. Nutraceuticals have been shown to offer therapeutic advantages, however this has not yet been proven. However, early characterization and epidemiological research indicate that various dietary regimens help manage and avoid intestinal dysfunction. Supplements added to the diet increase intestinal function and lower the risk of colon cancer. Pro-oxidants are linked to a higher risk of developing cancer. Free radicals have the ability to overexpress proteins linked to them and cause post transcriptional alteration in cancer. Food supplements high in antioxidants are a key treatment and prevention strategy for cancer. The scientific and pharmaceutical communities have recently become interested in medicinal plants, and several papers have shown how beneficial they may be. Due to the drawbacks of the existing cancer treatment, such as its high cost, unfavorable toxicities, and patient recurrence, attention has been focused on their anticancer potential. The comparatively non-toxic nature of medicinal plants makes them an advantageous choice for cancer treatment. The significance of nutrigenomics and Cancer research is interested in the role that nutrition plays in both illness prevention and therapy. Combining phytochemicals with cytostatic medications might potentially reduce dosages, increase the pace of beneficial effects, and provide a synergistic anticancer impact. A viable approach to enhance the effectiveness of anticancer treatment is the combination of chemotherapeutic medications with natural substances [12]. Phytochemicals may, in fact, interact with a variety of molecular targets in tumor cells, enhancing the effectiveness of conventional anticancer medications. Additionally, they may act as a buffer against the adverse effects of chemotherapy drugs on tissues that are not the intended target. Significant progress has been made in creating efficient treatments, such as chemotherapy, but the outcomes haven't always been pleasing. For a very long time, plants have been a major source of life-saving drugs in developing nations. The pharmaceutical industry presently uses a variety of medications made from plants [13]. Plants must thus continue to be at the forefront of drug discovery as they are crucial to the development of novel treatments, such as chemotherapy. A natural substance like *Tamarix mannifera* combined with well-known anticancer medications may increase the apoptotic effects on cancer cells in a synergistic way. In particular, the human colon adenocarcinoma-derived colon cancer LS174T cell line is an essential model system for researching the effects of these combinations on colon cancer cells [14]. The purpose of this work is to clarify the apoptotic effects of *Tamarix mannifera* on the LS174T cell line when used in conjunction with traditional anticancer medications. By comprehending these relationships, we may be able to identify less hazardous and more efficacious treatment approaches for colon cancer.

Materials and Method

Chemicals: The chemicals used in this study are listed in (Table 1) with their suppliers.

Chemicals used in the study

Alcohol spray (ethanol 70%), Dimethyl sulfoxide (DMSO), Fetal bovine serum (FBS), MTT(3-(4,5-Dimethylthiazole-2-yl)-2,5-diphenyl-2H-tetrazolium bromide) dye powder, Phosphate buffer saline tablet, Roswell Park Memorial Institute-1640 (RPMI-1640) powder medium, Sodium bicarbonate powder, Trypsin- Ethylene diamine tetra acetic acid (EDTA) powder.

Drugs

The drugs used in the study is cisplatin and doxorubicin.

Instruments and Tools used in the study

Autoclave, Automatic micropipettes (different sizes), Cell culture flask (25 ml), Cell culture plate (96- wells), Distiller, Double distillation water stills, Electric oven, ELISA Reader, Sterile freezing vial (1.5 ml), Flow cytometer (BriCyte E6), Incubator, Inverted microscope, Laminar air flow cabinet, Microcentrifuge, Millipore filter (0.45, 0.22 µm), Refrigerator, Sensitive Balance, Vortex, Water bath.

Preparation of Solutions and Reagents

1. **Phosphate Buffer Saline (PBS):** Prepared according to Gibco's manual by dissolving a PBS tablet in 500 ml deionized distilled water. The pH is 7.45 and doesn't need adjustment.
2. **Cisplatin Stock Solution:** Contains 50 mg / 50 ml according to Roche. Serial dilutions led to concentrations ranging from 7.8125 to 250 µg /ml.
3. **Doxorubicin Stock Solution:** Contains 50mg/25ml according to Novartis. Serial dilutions led to concentrations ranging from 7.8125 to 250 µg /ml.
4. ***Tamarix mannifera* Stock Solution:** Weight of 25 mg of dry powder that dissolve in 500 ml of hot DW to make hot water extract, then drying this extract in 37c microwave for several days until compelet dry and take 5mg of dry extract and dissolve in DW. Take a280 ml solution with 5000 µg/ml was mixed with serum-free medium for serial dilutions resulting in concentrations between 31.25 to 1000 µg/ml.
5. **Trypsin-(EDTA) Solution:** 10.1 gm of trypsin-EDTA

powder was mixed with 900 ml of DDW, sterilized, and stored at -20 °C.

6. **MTT Solution:** 0.5 g of MTT powder in 100 ml PBS produced a 5 mg/ml solution stored at 4 °C or -20 °C.

Cell lines preparation for cytotoxicity assays

- Cells from the University of Babylon were harvested using trypsin-EDTA when they were healthy and sub-confluent.
- For freezing cells, they were checked for growth and contamination, then frozen in a liquid nitrogen container at -196°C.
- Thawing involved placing frozen cells in pre-warmed sterile DDW, followed by centrifugation and incubation.

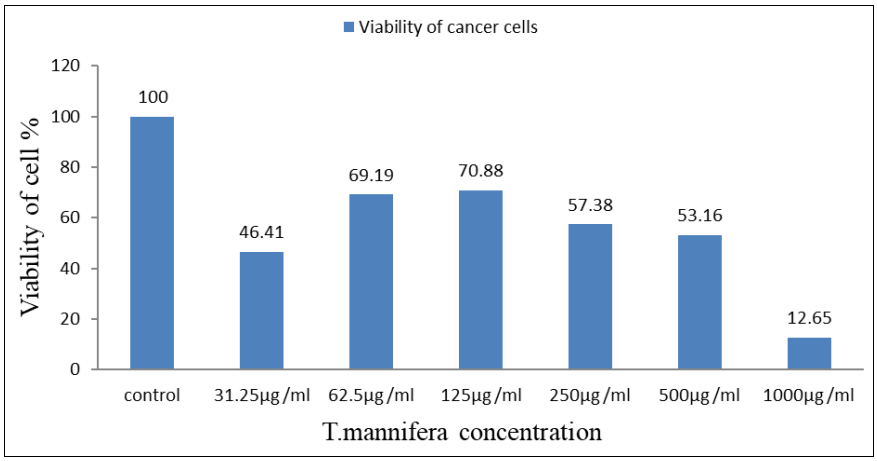
Cytotoxicity Assays

- **MTT Assay Principle:** The MTT assay gauges' viable cell count using 96-well plates. The activity of cell mitochondria converts MTT dye into purple formazan crystals. The resulting color intensity, measured at 570 nm, indicates the number of viable cells. The assay helps evaluate the cytotoxicity of drugs across concentrations
- **Statistical Analysis:** Statistical analysis done by SPSS 22, frequency and percentage used for categorical data, mean, median and SD for continuous data. T test used for evaluation differences between mean and median of continues variables. P-value less or equal to 0.05 is consider significant.

Results

1. The effect of *Tamarix mannifera* on LS174T cell line

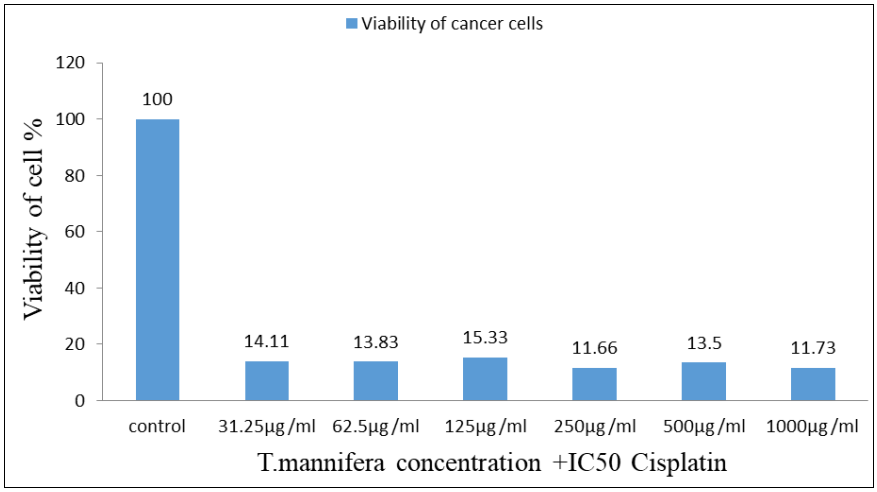
As shown in fig 1; there is significant (P-value ≤0.05) decrease in viability percentage of LS174T cell line in all concentration used *Tamarix mannifera* (31.25, 62.5, 125, 250, 500 and 1000 µg /ml) than control, also there is so significant decrease in viability of cancer cells in concentration 1000 more than 31.25 µg /ml and other concentration. But there is no significant difference in viability percentage at concentration 125 than 62.5 µg /ml and other concentration.



P-value ≤0.05 (significant).

Fig 1: cytotoxicity effect of extract of *Tamarix mannifera* on LS174T cell line.

2. The effect of IC50 Cisplatin + different concentration of *T. mannifera* on LS174T cell line: As shown in Fig 2. There is significant (P-value ≤0.05) decrease in viablility percentage of LS174T cell line in all concentration used this combination (IC50 Cisplatin + different concentration of *T. mannifera*) (31.25, 62.5, 125, 250, 500 and 1000 µg /ml) than control, also there is so significant (synergestic effect) decrease in viability of cancer cells in concentration 250 µg /ml more than other concentration. But there is no significant difference in viability percentage at 125 than 31.25 µg /ml.

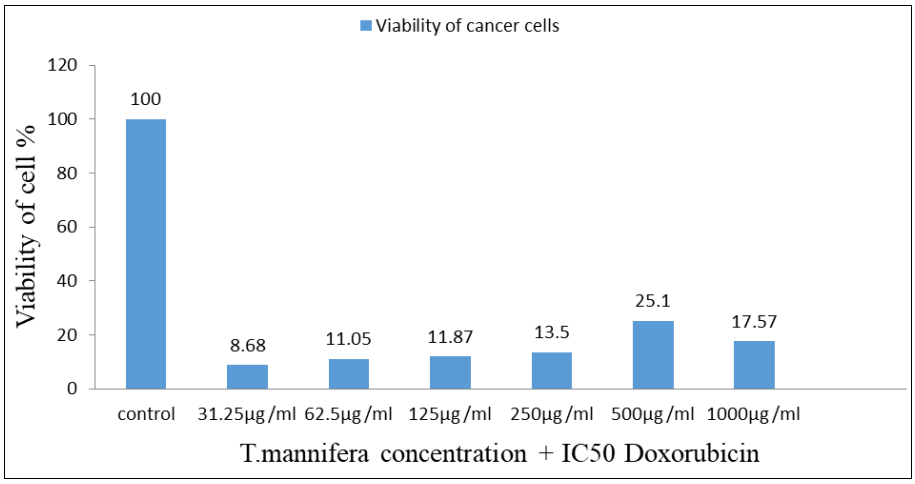


P-value ≤0.05 (significant).

Fig 2: cytotoxic effect of IC50 Cisplatin + different con. *T. mannifera* on LS174T cell line

3. The effect of IC50 Doxorubicin + different concentration of *T. mannifera* on LS174T cell line

As shown in fig 3; The result showed that there is significant (P-value ≤0.05) decrease in viablility percentage of LS174T cell line in all concentration used this combination (IC50 Doxorubicin + different concentration of *T. mannifera*) (31.25, 62.5, 125, 250, 500 and 1000 µg /ml) than control, also there is so significant (Synergestic effect) decrease in viability of cancer cells in concentration 31.25 µg /ml more than other concentration. But there is significant decrease (P-value ≤0.05) in viability percentage at concentration 500 less than 1000 µg /ml and other concentration.



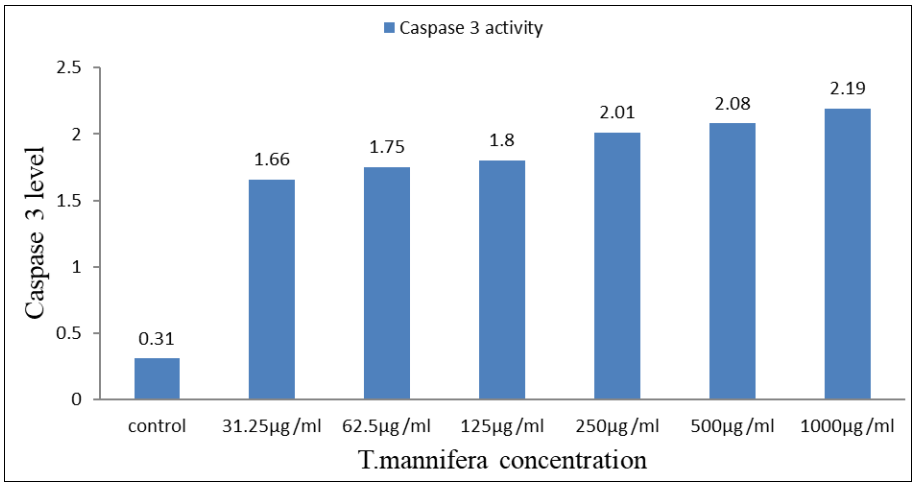
P-value ≤0.05 (significant).

Fig 3: cytotoxic effect of different con. *T. mannifera* +IC50 Doxorubicin on LS174T cell line.

Caspase 3 Kit resualt

4. The effect of *T. mannifera* on LS174T cell line

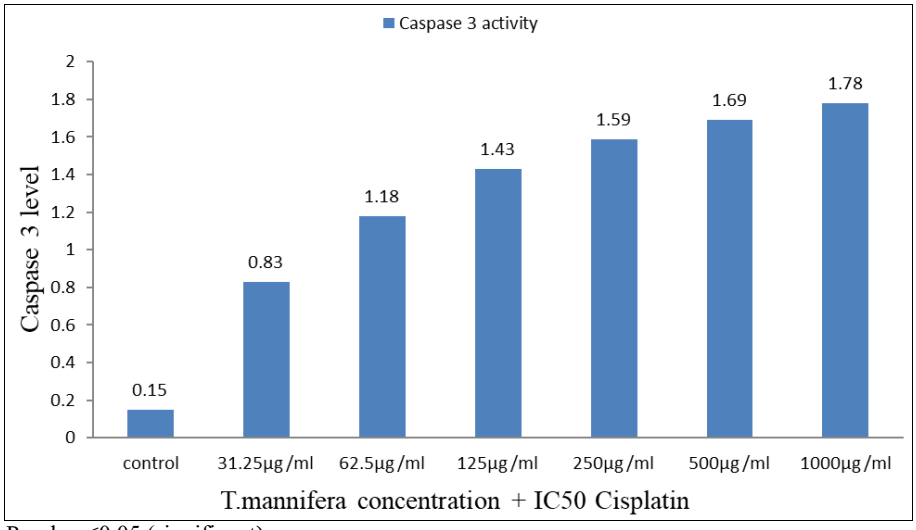
As shown in fig 4; There were significant (P-value ≤0.05) increase in Caspase 3 level as a result to *T. mannifera* effect and the activity increase as the concentration of *T. mannifera* increase.



P-value ≤0.05 (significant).

Fig 4: The effect of *T. mannifera* on Caspase 3 in Colon cancer LS147T Cells.

5. The effect of *T. mannifera* + IC50 Cisplatin on LS174T cell line: As shown in fig 5;There were significant (p≤0.05) increase in Caspase 3 level as the concentration of *T. mannifera* increase in combination with constant concentration of IC50 Cisplatin untile maximum concentration (1000 µg /ml).

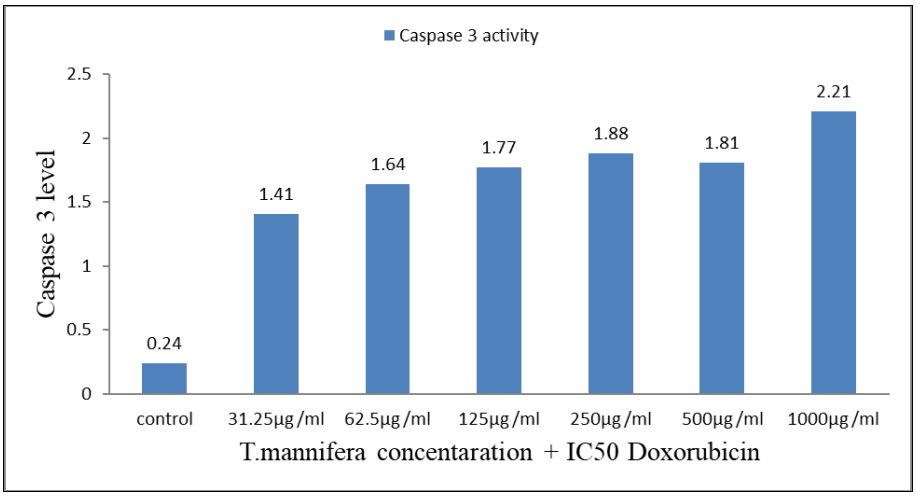


P-value ≤0.05 (significant).

Fig 5: The effect of *T. mannifera* +IC_{50} Cisplatin on Caspase 3 in Colon cancer LS147T Cells.

6. The effect of *T. mannifera* + IC50 Doxorubicin on LS174T cell line;

As shown in fig 6;There were significant (p≤0.05) increase in Caspase 3 level as the concentration of *T. mannifera* increase in combination with constant concentration of IC50 Doxorubicin untile maximum concentration (1000 µg /ml).



P-value ≤0.05 (significant).

Fig 6: The effect of *T. mannifera* +IC50 Doxorubicin on Caspase 3 in Colon cancer LS147T Cells.

Discussion

Despite recent advances in diagnostic, surgical, and therapeutic techniques, colorectal cancer (CRC) remains one of the leading causes of death among cancer patients, mostly from recurrent and metastatic disease [15-16]. Chemotherapy is one of the most common therapeutic options for colon cancer, specifically at the advanced stages and after surgical removal of the tumor [17]. Although this process is to a great extent effective, aggressive treatment can be limited by the severe side effects associated with high doses of chemotherapeutic drugs [18-19]. Chemotherapy for leukemia, lymphoma, and breast cancer is the most prevalent usage of anthracycline medicines, such as doxorubicin (DOX), but not for colorectal cancer. While DOX has been demonstrated to be a more effective adjuvant chemotherapy medication for advanced stages of colorectal cancer (CRC) [20], the high dosage of the medication administered in these situations can result in significant cardiotoxicity [21]. Numerous strategies have been attempted to reduce the cardiotoxicity linked to DOX, including dosage adjustments, the use of DOX compounds, and adjuvant therapy [22]. Apoptosis malfunctioning, angiogenesis initiating, aberrant gene function, altered gene expression patterns, loss of normal cell growth, development, and control, and metastasizing to other healthy tissue or organs are the causes of cancer [23]. Metastasis is the term used to describe the spread of cancer from its original cells or tissue to a different healthy area of a tissue or organ. Cancer differs from infectious and environmental illnesses brought on by antigens not found in our body system, as well as diseases linked to microbes and parasites. Human malignancies can arise from a variety of causes, including genetic or epigenetic variables that cause normal cells to mutate [25]. The study of epigenetics examines how variations in heritable gene expression cause aberrant cells to proliferate [26]. When a medicinal drug is delivered simultaneously, it might have a synergistic impact or work through several routes. The many bioactives might be administered in a way that is comparable to polychemotherapeutic drugs. Polychemotherapeutic

administration did not produce the highest level of results. Owing to restrictions on solubility, permeability, dosage, and adverse effects, in terms of therapeutic response [27]. Nutrients or phytochemicals are extractives from organic plant materials. The body's natural functions can be better regulated by the secondary metabolites. In various illnesses, polyherbals are employed as an immunostimulant. Herbal phytochemicals work through a variety of mechanisms, including the suppression of overexpressed hormones, proteins, enzymes, and amino acids. Additionally, the phytochemicals quicken the synthesis of defense-related enzymes. Through the regulation of many pathways, phytochemicals have demonstrated their ability to generate oxygen and act as antioxidants. These physicochemical properties support increased immunity while having a little negative impact on healthy cells [28]. Phytomedicines have been investigated extensively in the last several decades for the treatment of cancer, offering a substitute for traditional therapy without demonstrating any negative side effects while the patient is receiving treatment [29]. Natural extractives from plants have the potential to reduce toxicity and dosage frequency while still improving the action of chemotherapy drugs. The phytochemicals numerous molecular processes are regulated by dietary fibers, fruits, vegetables, natural products, and plants. A healthy, balanced diet may help reduce the risk of cancer. There was regulation of the chemopreventive and chemotherapeutic action. Via the suppression of angiogenesis, metastasis, apoptosis, cell cycles, and proliferation [30]. The role of inhibition is provided by bioactive chemicals derived from plants, such as flavonoids, vitamins, minerals, alkaloids, terpins, oils, gums, glycosides, saponin, lignins, podophyllotoxin, taxol, polyphenol, and vinca alkaloids, which are primary and secondary metabolites. The phytochemicals actively suppress or overexpress certain protein, enzyme, or other metabolite types in the cancer cell. Normal cells are not harmed by phytochemicals in any way. There are several ways in which phytochemicals might activate the presidential mechanism. In addition to their anticancer properties, phytochemicals also exhibit anti-inflammatory and antioxidant properties. Phytochemical activity potentials exhibit a substantial inhibitory impact on cancer cells through a variety of methods [31]. Numerous studies on the phytochemistry of several Tamarix species have been introduced. A group of phytochemicals, the most significant of which are polyphenolic substances, such as tannins, flavonoids, and phenolic acids.

Significant Increase in Cytotoxicity: There was a marked increase (P-value ≤0.05) in the levels of cytotoxicity after using *T. mannifera* extract at various concentrations. Increase in Caspase3 suggests a potential reduction in tumor activity or size. Dose-dependent Increase in Cytotoxicity (decrease in viability of cancer cells): Notably, there was a significant decrease (P-value ≤0.05) in viability of cancer cells levels (that mean increase in cytotoxicity) at the concentration of 1000 compared to 31.25, indicating a dose-dependent response. This kind of dose-response relationship is vital when considering the therapeutic index and potential side effects of any treatment. Given these promising results, *T. mannifera* extract presents itself as a potential therapeutic agent for colon cancer treatment. However, like any early-stage research, it is crucial to approach these findings with cautious optimism. As always, when discussing plant extracts, it's essential to recognize that while they may harbor therapeutic potential, they can also contain toxic or harmful compounds. The combined use of *T. mannifera* extract and cisplatin and doxorubicin to treat colon cancer appears to demonstrate significant potential based on the findings you've presented. The impact on colon cancer markers provides important insights into its therapeutic efficacy, while the absence of a decrease in cytotoxicity levels between certain concentrations suggests a saturation or threshold effect.

Significant Increase inCaspase3 level: The notable increase in levels of caspase3 at various concentrations when using the combined treatment of *T. mannifera* extract and cisplatin is promising. A promotion in cytotoxicity often signifies a decrease in tumor activity, suggesting the potential efficacy of the combined treatment. Also there is notable increase in levels of caspase3 at various concentrations when using the combined treatment of *T. mannifera* extract and doxorubicin is promising.

Synergistic or Additive Effects: The use of *T. mannifera* in combination with cisplatin and with doxorubicin underscores the broader movement in oncology towards combination therapies. The idea is that using two or more agents together might exhibit synergistic or additive effects, leading to increased efficacy. However, it's also critical to consider potential antagonistic effects or heightened side effects.

***T. mannifera* in Cancer Treatment:** While your data is focused on the combination therapy, it's worth noting that various Tamarix species have historically been studied for their potential anti-cancer properties, attributed to their diverse phytochemicals.

Cisplatin's Established Efficacy: Cisplatin has a well-documented history as a potent anti-cancer agent, especially in solid tumors. Its mode of action and side effect profile are well understood, making it a valuable candidate for combination therapies [32]. Both live-attenuated MV (Edmonton strain) and cisplatin could reduceKi-67P expression in tumor cell line when treated with either one of them. Therefore, they have beneficial effects in reducing the resistance to chemotherapy and radiotherapy [33].

Doxorubicin's Established Efficacy: Doxorubicin is widely used in the treatment of hematological and solid tumors. Unfortunately, their dose-dependent cardiotoxicity, a serious side effect that worsens the patient's prognosis and survival, limits their effectiveness [34]. Furthermore, genetic and epigenetic changes that impact drug sensitivity frequently result in cancer cells developing resistance to these medications [35]. Vitamins are crucial for both the prevention and treatment of cancer. They can help prevent colon cancer. A significant part of DNA synthesis and DNA methylation is played by folic acid. It engages in the single carbon methyl cycle in conjugation with vitamins B6 and B12. Treatment with vitamin B complex was started to lower the incidence of breast, colon, and rectal cancer [36]. In a similar vein, vitamin D receptor molecules are extensively expressed in colon cancer cells and may govern and control aberrant metastasis. Colon cell death mechanism [37].

Other therapeutic agent that used for colonic canser, Bardoxolone inhibited proliferation and induced apoptosis *in vitro* in wide types of human cancer cells [38-39, 3, 4]. It was noticed that bardoxolone at low nanomolar concentrations protects the cells from oxidative stress through the inhibition of reactive oxygen species (ROS) production [40].

Conclusion

With a notable increase in cytotoxicity shown, the combination of *Tamarix mannifera* extract, cisplatin, and doxorubicin offers promise in the treatment of colon cancer. Saturation effects at greater doses, however, may indicate complex dosage. Tamarix may have anti-cancer effects, and the proven effectiveness of doxorubicin and cisplatin calls for more thorough research and clinical trials.

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