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Synergistic Effects of *Zygophyllum coccineum* and Taurine on SW480 Colon Cancer Cells

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ABSTRACT

Colorectal cancer is a leading cause of cancer-related deaths worldwide, necessitating the development of novel therapeutic strategies. Since conventional treatments often have limited specificity and significant side effects, this study aimed to investigate the cytotoxic potential of *Zygophyllum coccineum* extracts (aqueous and alcoholic) and taurine, both individually and in combination, against SW480 colon cancer cells. For this purpose, SW480 cells were treated with varying concentrations of aqueous and alcoholic extracts of *Z. coccineum*, taurine, and combinations thereof. Cell viability was assessed using the MTT assay. The expression of apoptosis-related genes p53 and Bcl2 was quantified using real-time PCR. Results showed that the alcoholic extract of *Z. coccineum* exhibited greater cytotoxicity towards SW480 cells than the aqueous extract. Taurine alone showed a dose-dependent effect on cell viability. Notably, combining taurine (62.5 µg mL^{-1}) with both alcoholic and aqueous extracts significantly enhanced cytotoxicity compared to the extracts alone. Real-time PCR analysis demonstrated that treatment of SW480 colon cancer cells with *Z. coccineum* extracts (both aqueous and alcoholic) and taurine led to a significant upregulation of the pro-apoptotic gene P53, along with either an increase or modulation of the anti-apoptotic gene Bcl2. The highest induction of P53 expression was observed with the combination of alcoholic extract and taurine (14.75-fold), while the lowest Bcl2 expression was also recorded in this group (1.07-fold). This pattern indicates a synergistic effect in enhancing apoptotic pathways and controlling cell proliferation, suggesting potential anti-cancer efficacy of these treatments. In conclusion, *Z. coccineum* extracts, particularly when combined with taurine, demonstrate significant cytotoxic activity against SW480 cells. The synergistic effect of the extract and taurine suggests a potential therapeutic strategy for colorectal cancer by promoting apoptosis.

Keywords: Apoptosis gene, SW480 colon cancer, Taurine, Zygophyllum plant extract

INTRODUCTION

Cancer encompasses a diverse range of diseases characterized by the uncontrolled growth and division of abnormal cells. These cells can invade and destroy healthy tissues, as well as spread throughout the body. Cancer remains a leading cause of mortality worldwide, with approximately 10 million deaths reported in 2020 alone. Specific cancers contributing significantly to this figure include lung cancer (1.8 million deaths), colorectal cancer (916,000 deaths), liver cancer (830,000 deaths), stomach cancer (769,000 deaths), and breast cancer (685,000 deaths). Fortunately, advancements in early detection and treatment methods are continually improving cure rates for many cancer types [1]. A wide range of events can influence the chance of developing cancer, and oversimplifying most cancers to a single cause is inaccurate. Nevertheless, it has been discovered that a variety of substances, such as radiation, chemicals, and viruses, can cause cancer in both people and laboratory animals [2].

Numerous cancer therapy methods are linked to a range of adverse effects, including inadequate specificity and sensitivity. Chemotherapy is one of the many methods used today to treat cancer, but due to the non-selectivity of the drugs employed in this procedure, a significant portion of healthy cells will be lost along with cancerous cells. The primary challenge in cancer treatment is eliminating tumor cells while preserving healthy cells in the process. Cytotoxic chemical testing and raw plant extract screening are necessary in order to develop anticancer drugs utilizing natural resources like plants [3].

Previous research has shown that plants produce many different chemical compounds, most of which appear to have no direct relationship to their growth. These substances are called secondary metabolites. Among these compounds' significant components are tannins, flavonoids, terpenoids, alkaloids, and pigments. Secondary metabolites affect hematopoietic cells, lipids, and cardiovascular systems and have varying physiologic effects, including anti-inflammatory, contraceptive, and anticancer effects [4, 5]. Extensive research has demonstrated the beneficial effects of plants in the treatment of cancer [6].

Zygophyllum coccineum is a succulent plant belonging to the Zygophyllaceae family. *Z. coccineum* is a plant that many researchers have mentioned as being widespread in desert areas, which are considered its preferred environment and also characterized by its tolerance to high concentrations of salinity as well as its tolerance to drought. It has the ability to produce primary and secondary metabolic compounds that work as chemical compounds, phytoalexins, and antioxidants. It has been mentioned in some research on its medical importance [7]. In alternative medicine, this herb, which also has strong antioxidant properties, has been used for a long time as an anthelmintic, diuretic, or pain reliever and as a treatment for diabetes, high blood pressure, burns, infections, gout, and rheumatic fever [8, 9]. Additionally, earlier research has shown anti-microbial [10, 11] and *Z. coccineum*'s in vitro cytotoxic activities, particularly against MCF-7 and HeLa cell lines [10]. It's rich content of bioactive secondary metabolites such as flavonoids, alkaloids, and phenolic compounds, which may promote apoptosis and inhibit tumor progression [12].

The world is looking for new anti-cancer and antimicrobial drugs to fight the spread of chemotherapy-resistant microbes and cancer cells. The anti-cancer and antimicrobial bioactions of plants from different climates and environments, such as halophytes, are thought to hold new structural templates that can be used to get to the active ingredient [13, 14]. Meat and seafood are the main sources of taurine, an aminosulfonic acid that is conditionally necessary. While taurine is rich in certain algae and widely distributed in the animal kingdom, it is only found in trace amounts in a small number of plants, fungi, and bacteria. Humans and other mammals classified as omnivores or carnivores have high taurine concentrations. There are up to 70 g of taurine in a 70 kg male. Taurine has been shown to modulate cell proliferation, apoptosis, and oxidative stress in cancer cells. Its reported mechanisms include mitochondrial regulation, antioxidant activity, and modulation of signal transduction pathways involved in cell survival [15-17].

There is a study that proved that adding a certain amount of taurine to drinking water increases the life expectancy of mice with transplanted tumors by approximately 40% due to the rate of tumor growth inhibition [18]. Furthermore, it has been discovered that serum taurine levels in breast cancer patients are substantially lower than those in high-risk and control groups [19]. In addition to improving the body's endocrine system and helping to regulate body metabolism, taurine can also stimulate the pancreas and increase pituitary hormone release. It also enhances immunity and reduces weariness in the organism. In addition to having a protective impact on cisplatin-induced alterations in rabbit primary renal tubular epithelial cells, taurine can aid in the recovery of acute hepatitis. Certain visceral damage will result after chemotherapy; however, taurine can mitigate this harm. Furthermore, research on colorectal cancer has shown that taurine can both induce and impede cell death [20].

In this study, the SW480 cell line was selected as an in vitro model due to its well-characterized genetic profile, high proliferative capacity, and frequent use in studies of cytotoxicity and apoptosis. It serves as a representative model for early-stage colorectal cancer. Although both *Z. coccineum* and taurine have demonstrated individual anticancer effects in prior research, no studies to date have investigated their combined use. The possibility of a synergistic interaction between these agents remains unexplored, particularly in colorectal cancer models. Therefore, this study aims to investigate the cytotoxic and pro-apoptotic effects of aqueous and alcoholic extracts of *Z. coccineum* and taurine, both individually and in combination, on SW480 colon cancer cells.

MATERIAL AND METHODS

SW480 colon cancer cells were obtained from the Cell Bank of the Type Culture Collection of the Chinese Academy of Sciences Committee (Shanghai, China). The RPMI 1640 culture medium, which contained 10% fetal bovine serum (FBS; Gibco Life Technologies, New York, NY, USA), was used to cultivate the cells in vitro at 37°C. Taurine was obtained from Shanghai Macklin Biochemical Co., Ltd. (China). Taurine treatment groups (with concentrations of 62.2, 125, 500, 1000, and 2000 µg mL^{-1}) and the plant were obtained from the desert of Samawah Governorate. The selected taurine concentrations were chosen to span a wide dose-response range, based on prior in vitro studies demonstrating dose-dependent anti-proliferative and pro-apoptotic effects of taurine in colorectal cancer cell lines such as HT-29 and LoVo [21, 22]. The extract treatment groups used the same concentrations as the taurine treatment groups. The effect of taurine with plant extract on SW480 colon cancer cell proliferation was observed after 24 hours.

Plant Extract

Fresh leaves of Zygophyllum coccineum were thoroughly cleaned to remove dust and other contaminants and repeatedly rinsed with distilled water. The leaves were then shade-dried at room temperature for 7 days in a well-ventilated, dark area to prevent photodegradation of phytochemicals. Once completely dried, the leaves were ground into a fine powder using a mechanical grinder and stored in tightly sealed, dark glass bottles at room temperature until extraction.

Preparation of Alcohol Extract of the Plant

The alcoholic extract was prepared following the method described by Moustafa *et al*. [23] with slight modifications. A total of 75 g of the dried leaf powder was soaked in 450 ml of 100% methanol and left to stand for 24 hours at room temperature. The mixture was then filtered using Whatman No. 1 filter paper. The resulting extracts were then dried in a rotary evaporator and stored in an airtight container at 4°C until further use.

Preparation of Aqueous Extract of the Plant

The aqueous extract was prepared based on the method reported by El-Shora *et al*. [24]. Dried leaves were oven-dried at 60°C for 48 hours to ensure complete dehydration without degradation of active constituents. A total of 200 g of powdered leaves was soaked in 1 L of distilled water and kept on an orbital shaker at 150 rpm for 24 hours at room temperature (23–28°C). The extract was then filtered through Whatman No. 1 filter paper, and the resulting solution was stored at 4°C. Different concentrations were prepared from the stock solution for experimental use.

In Vitro Cytotoxicity Assay

An in vitro cytotoxicity assay was conducted by performing a 24-hour cell growth test using 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT). A 96-well microplate with a cell line split into three groups was filled with MTT powder that had been dissolved in PBS solution. In each group, the following concentrations were used: 62.5, 125, 250, 500, 1000, and 2000 µg ml^{-1}. Alcoholic plant extract was administered to Group 1, aqueous plant extract was administered to Group 2, and taurine was administered to Group 3. In another subsequent stage of treatment, mixtures of 62.5 taurine and concentrations of both plant extracts (250, 500, and 1000 µg ml^{-1}) were administered to the two groups with different concentrations. Each experimental condition was tested in triplicate (n=3), and each well was measured in three replicates. Optical density (OD) was measured at 570 nm using a microplate reader. The background absorbance from blank wells (containing media without cells) was subtracted, and the resulting values were normalized to the untreated control group, which was set to 100% cell viability.

Detection of Gene Expression P53 and Bcl2

Real-time PCR was used with the appropriate primers. The specific primers used for determination of P53, Bcl2 and Housekeeping gene for normalizing the results [25] as follow for P53, Forward: 5'- CCTCAGCATCTTATCCGAGTGG -3, Reverse: 5-TGGATGGTGGTACAGTCAGAGC-3; Bcl2, Forward: 5'- ATCGCCCTGTGGATGACTGAGT-3, Revers:5-GCCAGGAGAAATCAAACAG -AGGC-3, Housekeeping gene, Forword: 5-GCGAGAAGATGACCCAGAT-3 and Reverse: 5-GAGGCGTACAGGGATAGC-3. The RNA extraction transcript levels of the interested gene were examined by the molecular detection method, which is quantitative real-time PCR. The RNA extraction has been carried out according to the manual protocol using AccuZolTM Total RNA Extraction Solution (Bioneer, South Korea). RNA concentrations, quality, and purity were assessed using a NanoDrop spectrophotometer (Quawell, UK). The A260/A280 ratios ranged between 1.9 and 2.0, indicating high-purity RNA suitable for downstream applications. Using NCBI/primer-BLAST, primers were created for each gene, and BLAST and a single peak dissociation curve were used to confirm the primers' specificity for each gene under investigation. The expression of p53 and Bcl2 genes in SW480 cell lines in both treated and untreated cells treated with taurine and plant extract was measured using a one-step KAPA real-time PCR kit.

Perform Real Time-PCR

Using the primers of each gene in the reverse transcription (RT) phase for cDNA synthesis and amplification, a one-step SYBR green kit (one-step SYBR Fast, KAPA Biosystems, USA) was utilized for quantitative reverse transcriptase PCR (qRT-PCR). According to each gene's optimal temperature, the thermal profile is 42°C for 5 minutes to synthesize cDNA, 95°C for 3 minutes to deactivate reverse transcriptase, and 40 cycles at 94°C for 15 seconds, 30 seconds at 60°C, 59°C, and 58 °C for 20 seconds to anneal primers. Using the ΔΔCt method, the fold expression of P53 gene and Bcl2 was evaluated in relation to the housekeeping gene [26].

Statistical Analysis

Statistical analysis was performed using one-way ANOVA followed by Tukey's post hoc test to determine differences between treatment groups. A p-value <0.05 was considered statistically significant. All results are presented as mean ± standard deviation (SD). For every sample, three duplicate sets of data were gathered. The threshold cycle (Ct) values were used to evaluate the expression of mRNA. The Ct values were computed using the 2−ΔΔCT method in accordance with Livak and Schmittgen [26] and were normalized with the Housekeeping gene expression levels and the relative quantity of mRNA specific to the target genes.

RESULTS

The results showed that the cell viability percent of the SW480 colonic cancer cell line treated with an alcoholic extract of *Z. coccineum* after 24 hours of incubation was significant ($p<0.05$) as compared to the control group (cancer cells without treatment) in the increased concentrations (125, 250, 500, 1000, and 2000) µg mL^{-1}, while there were no significant effects ($p>0.05$) at the concentration of 62.5 µg mL^{-1} (Figure 1). The data in Figure 2 showed the cell viability percent in the treatment with a water extract of *Z. coccineum* after 24 incubations. When the colon cells were treated after 24 hours with the aqueous plant extract, there was a significant effect of the extract in the concentrations of 62.5, 1000, and 2000 µg mL^{-1} and no significant effects ($p>0.05$) in the concentrations of 125, 250, and 500 µg mL^{-1}. Figure 3 shows that the cell viability percent in treatment taurine explains the effect at concentrations of 62.5, 250, and 2000 µg mL^{-1} and no significant effects ($p>0.05$) at the other concentrations.

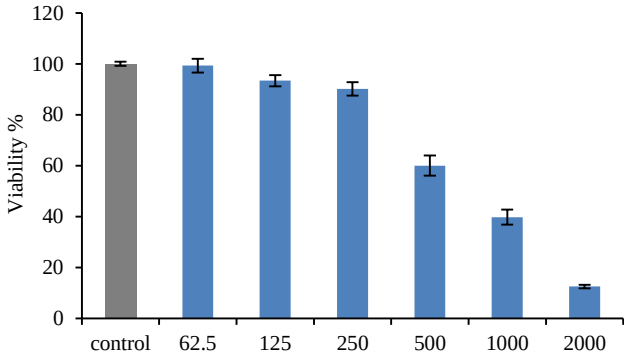

Fig. 1 The cell viability percent of the SW480 colonic cancer cell line treated with an alcoholic extract of *Z. coccineum* after 24 hours

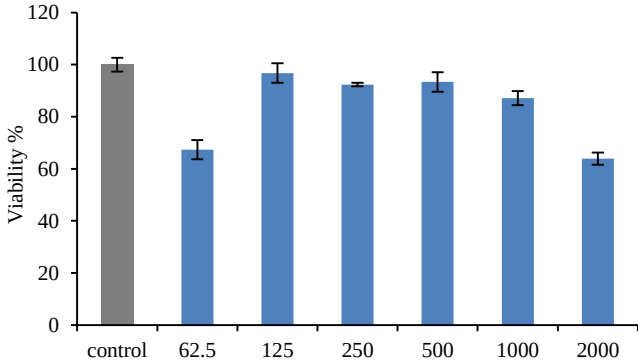

Fig. 2 Cell viability percent of SW480 colonic cancer cell line by water extract of *Z. coccineum* treatment after 24 hours

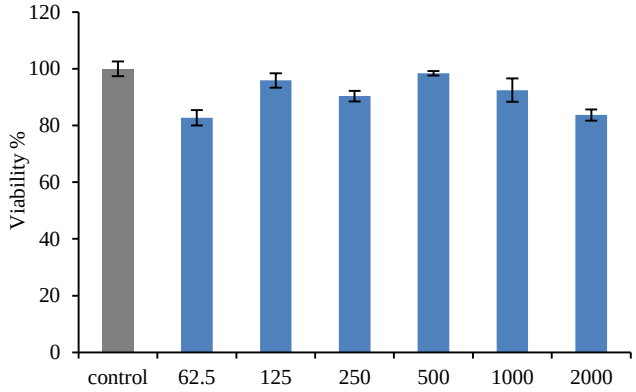

Fig. 3 The cell viability percent of the SW480 colon cancer cell line by taurine treatment after 24 hours

The current study also showed that when a mixture of taurine at a concentration of 62.5 µg mL^{-1} (because this concentration best affects cell viability) was used with the alcoholic plant extract at concentrations of 62.5, 125, 250, 500, and 1000 µg mL^{-1}, the result was, as shown in Figure 4, that there was a significant difference ($P<0.05$) compared to the control group at concentrations of 62.5, 500, and 1000 µg mL^{-1}, and there were no significant effects ($P>0.05$) at concentrations of 125 and 250 µg mL^{-1}. When we mixed taurine at a concentration of 62.5 µg mL^{-1} with the aqueous plant extract at concentrations of 62.5, 125, 250, 500, and 1000 µg mL^{-1}, the result was that there was a significant difference ($p<0.05$) in the concentrations of 62.5, 250, 500, and 1000 µg mL^{-1}, and no significant effects ($p>0.05$) appeared only at concentrations of 125 µg mL^{-1}, as shown in Figure 5.

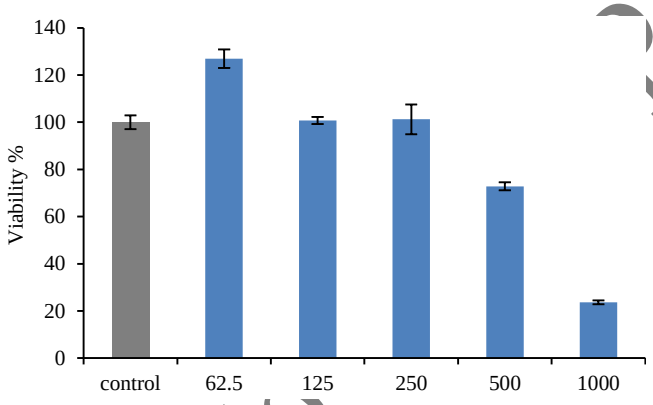

Fig. 4 The effect of *Z. coccineum* (alcoholic extract) combined with taurine on SW480 colon cancer cells (48h)

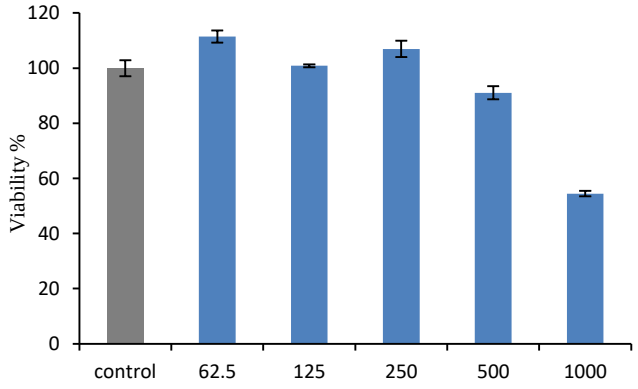

Fig. 5 The effect of *Z. coccineum* (aqueous extract) combined with taurine on SW480 colon cancer cells (48h)

Expression of P53 and Bcl2 Genes in Treated SW480 Colonic Cell Line with Alcoholic Extract of *Z. coccineum*

The expression levels of the p53 and Bcl2 genes in the SW480 colonic cell line after being treated with an alcoholic extract of *Z. coccineum* were determined, and the expression of the gene in the untreated SW480 colonic cell line was detected by quantitative real-time PCR. Three concentrations have been identified, which include high, medium, and low concentrations. The results that were obtained determined p53 and Bcl2 gene expression in SW480 colonic cell lines after being treated with an alcoholic extract of *Z. coccineum* and also in non-treated SW480 colonic cells. P53 expression increased in treated cells at a concentration of 250 µg mL^{-1} while being reduced in control cells, while Bcl2 expression was higher than P53 in treated cells at the same concentration (250 µg mL^{-1} as explained in Figure 6. As for the statistical analysis, a significant difference was found at the $p<0.05$ level in the two concentrations of 250 and 1000 µg mL^{-1} in p53 and also in Bcl2 gene expression compared to the control group.

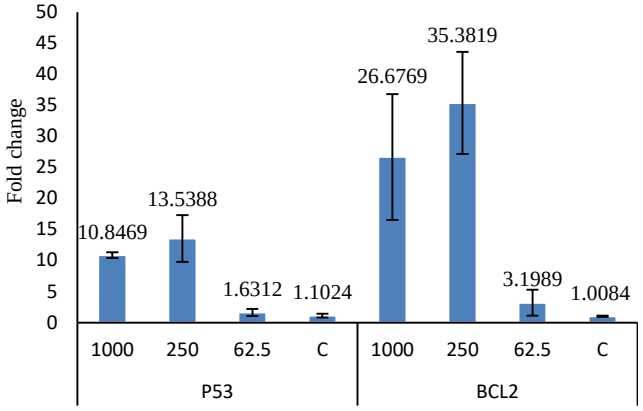

Fig. 6 The level of p53 and Bcl2 gene expression in SW480 colonic cell lines after being treated with alcohol extract of *Z. coccineum* and control (c)

Expression of P53 and Bcl2 Genes in Treated SW480 Colonic Cell Line with Water Extract of *Z. coccineum*

Expression levels of the p53 gene have been detected in the SW480 colonic cell line after treatment with a water extract and also in the non-treated SW480 colonic cell line, detected by quantitative real-time PCR. Also, the same concentrations were identified in alcohol treatment. The results that were obtained determined p53 gene and Bcl2 expression in SW480 colonic cell lines after being treated with water extract of *Z. coccineum*, and also in non-treated SW480 colonic cells. P53 and Bcl2 gene expression increases in treated cells with the three concentrations, while it is reduced in control cells, as explained in Figure 7. The results of the statistical analysis of the expression genes showed a significant effect ($P<0.05$) only in P53 with a concentration of 250 µg mL^{-1} compared to the control group.

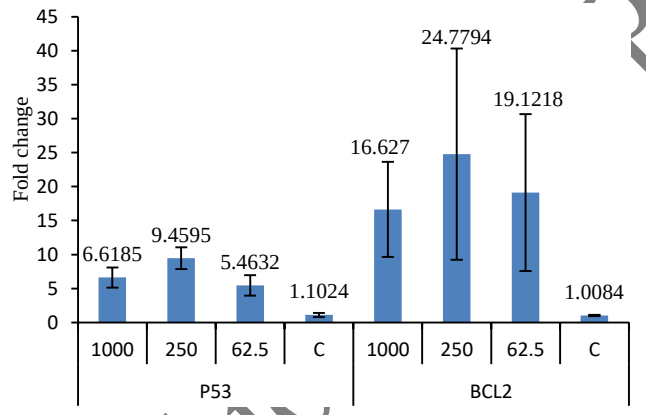

Fig. 7 The level of p53 and Bcl2 gene expression in SW480 colonic cell lines after being treated with water extract of *Z. coccineum* and control (c)

Expression of P53 in Treated SW480 Colonic Cell Line with Taurine

Expression levels of the p53 gene and Bcl2 have been detected in the SW480 colonic cell line after treatment with taurine in the same steps as treatment with extract. P53 and Bcl2 increase expression in treated cells with a concentration of 250 µg mL^{-1} while being reduced in control cells, as explained in Figure 8. In statistical analysis, the significant effect of p53 gene expression was only at a concentration of 250 and 62.5 µg mL^{-1}, while Bcl2 had a significant effect only at a concentration of 250 µg mL^{-1}.

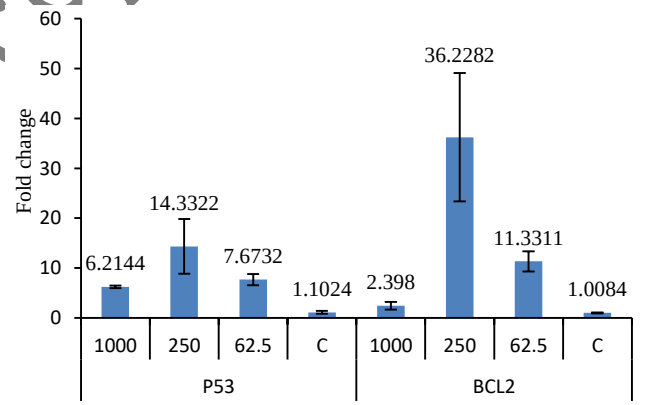

Fig. 8 The level change of p53 and Bcl2 gene expression in SW480 colonic cell lines after treatment with taurine and control (c)

Expression of P53 and Bcl-2 Genes in SW480 Colon Cancer Cells Treated with a Combination of Alcoholic Extract of *Z. coccineum* and Taurine

The effect of treatment with 62.5 µg mL^{-1} taurine and alcoholic plant extract on the gene expression levels of p53 and Bcl2 was assessed using real-time PCR (Figure 9). Treatment of SW480 cells with the combination significantly upregulated p53 expression at all tested

concentrations of the alcoholic extract (62.5, 250, and 1000 µg/mL) compared to untreated cells. In contrast, there was no significant change in Bcl2 expression in treated cells compared to the control. Statistical analysis confirmed a significant effect of the combination treatment on p53 gene expression across all concentrations of the alcoholic extract when combined with 62.5 µg mL^{-1} taurine, relative to the control cell line.

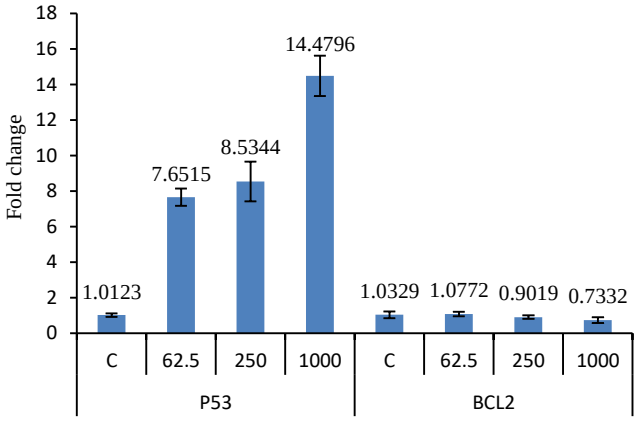

Fig. 9 Gene expression in treated SW480 colonic cell line with a combination of alcoholic extract of *Z. coccineum* and taurine

Expression of P53 and Bcl-2 Genes in SW480 Colon Cancer Cells Treated with a Combination of Water Extract of *Z. coccineum* and Taurine

Results Real-time PCR indicated the expression level of the P53 and Bcl2 genes in the treatment of the SW480 cell line with 62.5 µg mL^{-1} taurine and water plant extract led to upregulation of the P53 gene (Figure 10). This treatment utilized cell lines that were also upregulated significantly and increased in concentrations (250 and 1000 µg mL^{-1}) compared with untreated cells, but below that of the alcoholic extract. As for the statistical analysis, a significant difference was found only at concentrations of 1000 µg mL^{-1} in p53 gene expression compared to the control group. Bcl2 gene had no effect in the treatment of the SW480 cell line with 62.5 µg mL^{-1} taurine and water plant extract

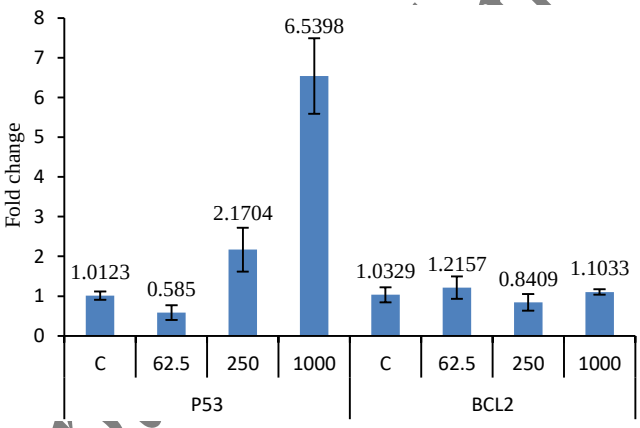

Fig. 10 Gene expression in treated SW480 colonic cell line with a combination of water extract of *Z. coccineum* and taurine

DISCUSSION

Colon cancer is responsible for the majority of cancer-related deaths globally [27, 28]. Today's colorectal cancer patients have far better chances of survival due to advancements in cancer treatment [29]. However, chemotherapy-induced drug resistance is frequently developed as a result of colorectal cancer treatment, hastening the disease's progression [30, 31]. Numerous herbal extracts and mixes have been shown through significant investigation to potentially have anticancer and chemopreventive effects through the induction of apoptosis and disruption of the cell cycle [32]. More than 60% of anti-cancer medications on the market today are derived from naturally occurring plant compounds, according to Latif *et al.* [33]. It has recently been demonstrated that plants can effectively treat cancer [34]. Plants can contain bioactive compounds with anti-inflammatory, anticancer, and antioxidant qualities. As a result, cytotoxicity experiments were executed on treated colon cancer cells with *Z. coccineum* extract and taurine, as well as cytotoxicity experiments on untreated colon cells.

In order to investigate the anti-proliferative activity of *Z. coccineum* extract and taurine on colon cancer cells, they were treated with different concentrations for 24 hours. When compared to colon cancer cells that were not treated, the extract and taurine demonstrated respectable selective cytotoxic effects against colon cancer cells. We measured the optical density at a wavelength of 570 nm and assessed the number of live cells using methyl thiazolyl tetrazolium (MTT). Cell viability should rise with growth, fall with anticancer treatments, and stay constant (or plateau) with cytostatic treatments. Since the control sample produces healthy cells with 100% vitality, it can be used to estimate the cell viability percentage [35]. The stain indicated the number of viable cells, and the treated cells' decreased growth rate in comparison to the untreated cells showed the cytotoxic effect. The most effective technique to measure mitochondrial dehydrogenase activity in living cells is the MTT test. This technique is a straightforward, secure, sensitive, and quantitative colorimetric assay. A plant extract can permanently lessen the capability of cancer cells. Additionally, at higher concentrations of the alcoholic extract of *Z. coccineum*

(2000 µg mL^{-1}), the three highest doses significantly reduced the viability of SW480 cells compared to the control. The cytotoxic effect on colon cancer cells was notably higher than that observed in untreated cells (Figure 1). Therefore, very high concentrations of the alcoholic extract of *Z. coccineum* may cause unwanted side effects in colon cancer cells. Nonetheless, *Z. coccineum* exhibits anti-cancer activity against the SW480 colon cancer cell line, showing a stronger cytotoxic effect at higher doses.

Another study by Mohammed *et al.* [36] showed that the phytochemicals present in the methanol extract of *Z. coccineum* were evaluated against cancer cell lines and found to possess anticancer activity. The results are confirmation of our findings, but they used different cancer cell lines and different fractions and solvents. The cell viability percent of the SW480 colonic malignancy cell line treated with an alcoholic extract of *Z. coccineum* following 24 hours of hatching was noteworthy ($p<0.05$) when contrasted with the control group (cancer cells without treatment) in some fixations (with concentrations of 500, 1000, and 2000 µg mL^{-1}) and there were critical cell cytotoxic impacts in these concentrations. A study by Elbadry *et al*. [10] explained that extracts made from methanol showed strong cytotoxic activity with cell line viability of 48.15%, 67.59%, and 35.19%, respectively. Also, a study by El-Afify *et al*. [35] found that the methanol extract of *Z. coccineum* showed high cytotoxicity for both tumor cells and an increased potential anticancer effect. Therefore, *Z. coccineum*'s biological activity lends support to its application as a conventional treatment for a range of illnesses. Herbicides and chemotherapy did not have the same beneficial effects on cancer cells as medicinal plant extracts [34] and Additionally, a study by Kooti *et al.* [3] found that plant compounds have antioxidant qualities and can inhibit DNA damage, cell cycle arrest (particularly at the G2/M), induce apoptosis, inhibit angiogenesis in tumor cells, and have novel and potent anticancer effects, this result is congruent to the results of the current study when using the water plant extract alone can increase apoptosis when the upregulation p53, significant effect the a concentration of 250 µg mL^{-1} and the effect of Bcl2 gene expression did not significantly change in the case increase or decrease.

Bcl2 is a key anti-apoptotic gene, and its unchanged expression in the presence of significant upregulation of p53 suggests a shift in the apoptotic balance toward cell death. The lack of significant Bcl2 downregulation may indicate that the treatments predominantly activate the intrinsic apoptotic pathway via p53 without strongly suppressing anti-apoptotic signals, or that Bcl2 suppression is not the primary mechanism in this context. This pattern is consistent with studies showing that certain plant extracts and taurine can induce apoptosis primarily through p53 activation, while Bcl2 levels remain unchanged or are modulated only slightly [22, 37]. Hence, the non-significant change in Bcl2 expression supports the notion of a p53-mediated pro-apoptotic response, possibly independent of Bcl2 downregulation.

Our findings indicate that *Z. coccineum* extracts have a specific effect on the regulation of p53 in a colon cancer cell line. The upregulation of p53 gene expression was observed at higher extract concentrations, which is consistent with the findings of Al-Muswie *et al.* [29], who reported similar effects of a plant extract on colon cancer cells. In our study, the effect of taurine on cell proliferation—whether inhibitory or stimulatory—varied depending on its concentration, as shown in Figure 3. Due to the limited understanding of taurine biosynthesis in humans and the possibility of deficiency resulting from inadequate intake, taurine is regarded as a nutritionally essential compound.

According to previous research, variations in systemic taurine levels may serve as indicators for the development and progression of certain tumors into malignancy [22, 38]. Furthermore, the combination of the plant extract and taurine enhanced the expression of specific genes. Researchers have established that taurine can cause apoptosis and limit the growth of colorectal and breast cancer cells. Taurine has also been found to have an inhibitory effect on dimethylbenzanthracine-induced breast cancer in rats [39]. A study by Tu *et al*. [40] demonstrates that the "inhibitory effect of taurine on the proliferation of A549 cells significantly increased in a time- and concentration-dependent manner when compared with the control group". When colon cancer cells were treated with taurine, there was a change in the vitality of the cells compared to the control cells. 62.50 for taurine, the best concentration had an effect on cell vitality, while the effect of taurine at a concentration of 1000 increased cell vitality.

According to studies by Reddy *et al.* [41], taurine can prevent colorectal cancer by boosting the activities of glucuronyl transferase, NAD(P)H reductase, and glutathione transferase. The result of our study on the use of taurine alone is close to the result of a study by Zhang *et al.* [22] demonstrated taurine did not reduce the activity of human embryonic kidney and the expression level of p53 gene was significantly increased compared with the control group. A study by Hernández-Benítez *et al.* [42] demonstrated that the effect of taurine in increasing the number of neural precursor cells may result either from a protective action that improves cell viability, or from enhanced cellular proliferation. Alternatively, taurine might be speeding up cell proliferation by influencing the pace of cell migration. On the plate, cells move toward one another more quickly or by strengthening cell adhesion, and it has been suggested that this proximity may foster conditions that are conducive to proliferation [43] and also studied by Jeong *et al.* [44] ‘who mentioned that the effect of taurine on an increase in Bacl2 expression is an indicator of the level of anti-apoptosis’. In the study by Jeon *et al.* [45], the cell growth pattern of taurine treatment was far higher than that of the controls. Hao *et al.* [46] is a study that demonstrates that taurine promotes protein synthesis and proliferation of C2C12 cells in a dose-dependent manner.

Our study showed the apoptosis increased in the mixture of plant extract and taurine at a concentration of 62.5 µg mL^{-1} was affected, as was observed in the increase in gene expression of p53, and there was a clear and significant difference in the effect compared to the gene expression of Bcl2, which is considered an antiapoptosis, this result from effect of taurine in the combination with other therapeutic correspondence with result in the study [47].

Our results partially align with prior studies on Z. coccineum, where methanolic extracts showed potent cytotoxicity against MCF-7 and HCT-116 cells ($IC_{50} \approx 3.2–3.5$ µg mL^{-1}) by Mohammed *et al.* [36]. In our study, higher concentrations were needed to elicit cytotoxicity in SW480 cells, suggesting cell line-specific sensitivity. Taurine’s modest cytotoxic effect in our assay is consistent with earlier findings in colon cancer models, where it induced apoptosis via p53 and Bax/Bcl-2 modulation Zhang *et al.* [22], though without strong suppression of Bcl-2. Unlike prior studies combining taurine with chemotherapeutics like cisplatin or 5-FU, our findings show for the first time a potential synergistic effect between taurine and *Z. coccineum* extract in colorectal cancer cells, warranting further mechanistic investigation.

CONCLUSION

In conclusion, this study demonstrates that *Z. coccineum* extracts, particularly the alcoholic extract, exhibit cytotoxic activity against SW480 colon cancer cells. The combination of *Z. coccineum* extract with taurine synergistically enhances this effect, leading to increased cytotoxicity and modulation of p53 gene expression, indicative of apoptosis induction. These findings suggest that *Z. coccineum*, in combination with taurine, holds promise as a potential therapeutic strategy for colorectal cancer by promoting apoptosis in cancer cell lines. However, the study is limited by the lack of in vivo validation and mechanistic exploration beyond p53 expression. Future research should focus on in vivo studies, detailed apoptotic pathway analysis, and evaluating combination therapies to confirm and expand upon these findings.

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