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Taraxacum officinale Flower Aqueous Extract Suppresses Oxidative Stress and Induces Apoptosis in HCT116 Colorectal Cancer Cells

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Abstract

DANDELION (*Taraxacum officinale*) flowers have health benefits due to their potent antioxidant properties. However, the mechanism by which flower extract can fight against cancer has not been discovered yet. This study aimed to investigate the anticancer mechanisms of *T. officinale* flower extract (TOFE) against colorectal cancer HCT116 cells. We employed models of oxidative stress caused by hydrogen peroxide (H_2O_2) in HCT116 cells to investigate TOFE anti-cancer and antioxidant properties in vitro. After being pre-treated for 24 h with TOFE, the cells were subjected to H_2O_2. In H_2O_2-treated cells, TOFE restored the activity of the antioxidant enzyme superoxide dismutase, decreased the intracellular reactive oxygen species (ROS) levels, and enhanced caspase-3 activity. Real-time PCR showed that HCT116 treated with TOFE had downregulated *Bcl2* expression and upregulated expression of the pro-apoptotic genes (*Bax*) and antioxidant markers (*Nrf2*, *HO-1*). By redox regulation and apoptosis induction, TOFE showed anticancer activity with HCT116 cytotoxicity (IC_{50} = 111.30 µg/mL). These findings position TOFE as a promising natural adjuvant for colorectal cancer therapy, particularly in overcoming oxidative stress-related chemoresistance. Further *in vivo* studies and clinical trials are warranted to validate its efficacy and explore synergies with conventional treatments.

Keywords: Taraxacum officinale, Dandelion flower extract, Antioxidant activity, Apoptosis, HCT116 cells.

Introduction

The colorectal cancer (CRC) is the 3rd most prevalent kind of cancer and a main contributor to cancer-related death globally [1]. Despite advances in treatment, conventional chemotherapy, including 5-fluorouracil (5-FU), oxaliplatin, and irinotecan, often fails due to drug resistance and severe off-target effects, particularly persistent gastrointestinal toxicity observed in 50–80% of patients [2, 3]. Inflammation and oxidative stress are fundamental mechanisms linked to the development of cancer. Damage caused by oxidation to DNA, lipids, and proteins can result from the high production of reactive oxygen species (ROS), including hydrogen peroxide (H_2O_2), which can overwhelm the antioxidant defence of the cell. A vicious cycle that worsens tissue damage and accelerates the course of the disease is created when this damage initiates inflammatory reactions [4, 5]. One of the main causes of diseases such as cancer is a disparity between cellular antioxidant defence and the production of ROS [6-10]. In order to mitigate these effects, there is increasing interest in finding natural substances that have both anti-inflammatory and antioxidant qualities.

To overcome resistance and lessen the toxicity caused by chemotherapy, natural substances and tiny synthesized chemicals that alter oxidative stress pathways have shown promise as adjuvants. In recent years, using natural goods and medicinal herbs to treat cancer has become more and more common. Medicinal plants have been used to cure a variety of ailments since ancient times due to their bioactive compounds, which have immunomodulatory, antioxidant, and anti-inflammatory properties [9, 11-14]. The dandelion, or *Taraxacum officinale*, is a perennial herbaceous plant belonging to the Asteraceae family, which grows throughout North America, Asia, and Europe. This 40-cm-tall plant, which is distinguished by its unique yellow-orange composite flowers and highly lobed leaves, grows well in a variety of environments, such as urban areas, cultivated fields, and grasslands. Its pharmacological qualities are influenced by a wealth of bioactive substances, including flavonoids, phenolic acids, sesquiterpene

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lactones, and polysaccharides [15-19]. Sesquiterpene lactones and phenylpropanoids are thought to be the most important of these because of their potential anti-cancer activities, which help explain the therapeutic benefits of dandelion extracts. In traditional medicine, the entire plant, including roots, leaves, and flowers, has been used to treat inflammatory and hepatic disorders. However, recent studies have mostly concentrated on the roots and leaves because they contain abundant of these bioactive substances, which may synergistically mitigate oxidative stress [20, 21]. Dandelion roots and leaves extracts and their constituents have demonstrated anti-oxidant, anti-inflammatory, and anti-carcinogenic effects [22-25].

However, the flowers of *T. officinale* remain understudied despite their widespread availability and potential therapeutic value. To date, no research has thoroughly investigated the cytoprotective properties of *T. officinale* flower extract (TOFE) in HCT116 cells exposed to oxidative stress. Our research aimed to clarify how TOFE may be used therapeutically to reduce oxidative damage and modulate cellular stress responses in HCT116 cells under oxidative stress from H_2O_2.

Material and Methods

Cell lines, Chemicals, and Reagents

The HCT116 cell line was acquired from VACSERA (Egypt). Tissue culture reagents, including dimethyl sulfoxide (DMSO), L-glutamine, penicillin/streptomycin, fetal bovine serum (FBS), trypsin, DMEM, and MTT, were acquired from Gibco and Sigma-Aldrich in the United States. Thermo Scientific was the supplier of the Gene JET RNA Purification Kit, while Qiagen (Germany) provided the QuantiTect SYBR Green PCR Kit and Quant Script Reverse Transcription Kit.

Preparation of T. officinale Flower Extract

Fresh flowers of the common dandelion (*T. officinale*) were gathered from Kafr Elsheikh City in Egypt. After Dr. Abdullah A. Elgazar verified the authenticity of the plant material, a voucher specimen was placed at one of the herbaria of the Department of Pharmacognosy at Kafr Elsheikh University's Faculty of Pharmacy. *T. officinale* flowers (500 g) were thoroughly cleaned with distilled water, infused with 1000 mL of distilled water that had been preheated to 80°C for 2 h with stirring every 30 min, and sliced into uniform pieces of around 1 cm^2. A refined liquid extract was obtained by sieving and filtering the mixture through Whatman No. 1 paper (pore size 11 µm) and lyophilized (yield: 12% w/w) after the infusion to get rid of any remaining particles as previously described [26]. To shield the solution from light and make handling easier during studies, the filtered extract was then separated into amber glass containers, each holding 10 mL. In order to preserve their integrity, the aliquots were stored in a freezer at -20°C. The resultant extract had a final concentration was 50 mg/mL. Quality control tested for microbial contamination (nutrient agar plates).

Treatment and Cell Culture

With 5% CO_2 in a humidified incubator, HCT-116 cells were maintained at 37°C in DMEM medium supplemented with 1% penicillin-streptomycin and 10% FBS were added as supplements. Trypsin-EDTA at 0.25% was used to subculture the cells at 80–90% confluence. TOFE was diluted in medium to final concentrations of 25–200 µg/mL after being dissolved in DMSO (≤0.1%) to create a 100 mg/mL stock solution.

Assessment of Cell Viability Using MTT assay

Cells were seeded at an appropriate density in 96-well plates and kept in a 5% CO_2 incubator at 37°C overnight to allow adherence. Different concentrations of TOFE were applied to the cells. Fresh medium containing MTT solution (0.5 mg/mL) was added to each well, and plates were incubated for three to four hours at 37°C, allowing the yellow MTT to be transformed into purple formazan crystals by living cells. Following aspiration of the media, DMSO was used to dissolve the formazan crystals. A microplate reader was used to measure the absorbance at 570 nm and the cell viability was calculated relative to untreated controls [27].

Oxidative Stress Induction and Experimental Design

After being seeded at a density of 1×10^4 cells/well in 6-well plates, the cells were pretreated for 24 hours with TOFE at non-toxic concentrations (1/4 IC_{50}) as established by the MTT assay. Cells were exposed to H_2O_2 200 µM for 2 hours in order to generate oxidative stress. Cells were assigned into 3 groups as follows: The control group which contained cells that were not treated, the H_2O_2 group which contained cells that were subjected to H_2O_2 in the absence of extract, and the extract + H_2O_2 group which contained extract-pretreated cells before H_2O_2 exposure.

Intracellular Reactive Oxygen Species (ROS) Assay

Following the protocol used in [28, 29], the cells were exposed to 10 µM DCFH-DA (2',7'-dichlorodihydrofluorescein diacetate) in PBS for 30 minutes at 37°C in the dark. A microplate reader was used to determine the intensity of fluorescence at 485/535 nm excitation/emission wavelengths. The findings were presented as a percentage change from the control cells.

Superoxide Dismutase Activity Assay

Using a commercial kit (Bio-Diagnostics Co, Egypt), superoxide dismutase (SOD) activity in HCT-116 cells treated with TOFE was measured following the procedure used previously [30]. After being diluted with water, the cell homogenate (0.5 mL) was combined with ethanol and chloroform, vigorously shaken at 4°C, and centrifuged. After that, the supernatant was combined with sodium pyrophosphate buffer, phenazine methosulfate, nitro blue tetrazolium (NBT), and NADH to start the reaction. Glacial acetic acid was used to halt the reaction after 90 seconds of incubation at 30°C. The chromogen was then extracted into n-butanol for spectrophotometric measurement at 520 nm. One SOD unit is the quantity of the enzyme required to prevent 50% of NBT reduction under assay circumstances.

Measurement of Caspase-3 Activity

Following the procedure of [31], a colorimetric assay based on the cleavage of a specific substrate, Ac-DEVD-pNA, was used to measure caspase-3 activity. Following incubation with the caspase-3 substrate, p-nitroaniline (pNA) release from cell lysates was quantified spectrophotometrically at 405 nm as detailed by the manufacturer (Alexis Biochemicals, Farmingdale, NY, USA).

QPCR for Detection of Gene Expression

Total RNA was extracted from HCT116 cells using a commercial RNA isolation kit, and its concentration and purity were assessed via spectrophotometry. The RNA was then reverse transcribed into complementary DNA (cDNA). SYBR Green fluorescent dye, cDNA templates, and gene-specific primers (Table 1) were used in quantitative PCR (qPCR). The thermal cycling conditions consisted of an initial denaturation at 95°C for 10 min, followed by 40 cycles of 95°C for 15 s and 60°C for 40 s. The internal reference gene was *GAPDH*, and relative gene expression levels were determined using the $2^{-\Delta\Delta Ct}$ method. Fold changes in gene expression were calculated by comparing treated samples to controls, where values >1 indicated upregulation and values <1 represented downregulation of target genes [7, 32].

Statistical Analysis

GraphPad Prism 8.0 (San Diego, CA, USA) was used to analyse all of the data. Tukey's *post hoc* test for multiple comparisons was used after a one-way ANOVA for group comparisons. Statistical significance was defined as $p < 0.05$, and results are displayed as mean ± standard error of the mean (SEM).

Results

TOEF Inhibited HCT116 Viability

The cytotoxic effects of TOFE on human colorectal cancer HCT116 cells were assessed using the MTT assay. TOFE exhibited significant cytotoxicity against the cells with IC_{50} of 111.30±5.08 µg/mL (Fig. 1). The MTT assay results indicated that TOFE exerted a concentration-dependent cytotoxic effect on HCT116 cells, with significant reductions in cell viability at higher concentrations.

TOEF Reduced Intracellular ROS Levels and Increased SOD Activity

Fig. 2 showed that ROS levels were significantly elevated by 428.01±21.50% in HCT116 cells following H_2O_2 induction compared to the untreated control cells (100.00±6.33%). Treatment with TOFE reduced ROS levels (218.24±15.63%) in contrast to cells that were exposed only to H_2O_2. On the other hand, SOD activity (Fig. 2) was significantly increased in HCT116 cells treated with the TOFE (72.54±3.47%) compared to the H_2O_2-treated cells (17.22±1.30%). However, the elevated SOD levels were still less than those in the control cells that had not been treated (200.38±4.90%).

TOFE Enhanced Caspase-3 Activity

To investigate whether TOFE induces apoptosis in HCT116 cells, we measured the enzymatic activity of the crucial apoptotic pathway mediator caspase 3 [33, 34]. Treatment with TOFE for 24 hours resulted in an increase in caspase-3 activity (256.55±12.46%) compared to untreated control cells (100.70±5.12%) (Fig. 3). TOFE induced a non-significant increase in caspase-3 activity relative to the H_2O_2 group.

Impact of TOEF on Gene Expression of Apoptotic Genes

QPCR analysis revealed a significant upregulation of pro-apoptotic gene *Bax* (5.27 ± 0.23-fold change) in HCT116 cells treated with TOFE while anti-apoptotic *Bcl2* expression was downregulated (0.20± 0.02-fold change) in contrast to cells exposed to H_2O_2 and control cells (Fig. 4). Additionally, cells treated with H_2O_2 exhibited markedly reduced expression of *Bcl2* (0.62±0.03-fold change) and markedly increased expression of *Bax* (3.35 ± 0.15-fold change) compared to the untreated control cells (1.00±0.00-fold change).

Influence of TOEF on Gene Expression of Antioxidant Genes

Nrf2 and *HO1*, key regulators of the antioxidant response, were significantly upregulated in HCT116 cells following TOFE treatment, with fold changes of 0.93 ± 0.07 and 0.89 ± 0.12, respectively compared to H_2O_2-treated cells with fold changes of 0.15±0.01 and 0.08±0.01, respectively (Fig. 5). However, no significant changes were noticed for *Nrf2* and *HO1* genes in TOFE treated cells compared to the untreated control cells (1.00±0.00%).

Discussion

Taraxacum extracts are an attractive topic for further investigation because of their beneficial qualities, especially in the development of natural remedies for a range of illnesses [35-37]. Flowers of T. officinale have been used in very few investigations. TOFE has previously demonstrated anti-inflammatory, gastroprotective, and anti-*H. pylori* properties[37]. with chicoric acid and luteolin likely being the key active compounds responsible for these effects [38].

In the present study, we found that TOFE showed anticancer activity against HCT116 as revealed by the results of MTT assay, ELISA (high levels of caspase3) and real-time PCR (high *Bax* expression and low *Bcl2* expression). The MTT experiment revealed that TOFE significantly reduced HCT116 cell viability in a dose-dependent manner, indicating a cytotoxic action. One possible explanation for the cytotoxic impact is the triggering of apoptosis. Genes implicated in apoptosis and the oxidative stress response were analysed using real-time PCR. In HCT116 cells, the anti-apoptotic gene *Bcl2* was downregulated, and pro-apoptotic indicators (*Bax* and caspase3) were increased following treatment with the extract, suggesting the induction of apoptosis. Consistent with our results, TOFE exhibits a strong anticancer effect against cervical cancer cells, with luteolin and luteolin-7-glucoside identified as key bioactive compounds [39]. Moreover, TOFE caused SK-OV-3 ovarian cancer cells to undergo apoptosis through inducing cell cycle arrest (S/G2-M phase), increasing sub-G0/G1 populations, and modulating p53, Bax, and Bcl-2 expression [40]. This anticancer potential of TOFE is consistent with previous research on extracts of *T. officinale* leaf [41], roots [23], and seed [42, 43] against a many different types of cancer cells, such as esophageal and breast cancer cells. Furthermore, the migration, proliferation, and invasion of triple-negative breast cancer (TNBC) were all markedly reduced by *T. officinale* whole extract. [44].

Drug resistance occurs in about 90% of patients with CRC, and high mortality rates can be attributed to a deficient response to chemotherapeutics [45, 46]. So, around 50% of anti-cancer drugs were obtained from natural products, such as polyphenols, diterpenoids, and unsaturated fatty acids possessing various structures [47].Among these natural products, Dandelion aqueous root extract, triggers apoptosis in colorectal cancer cells in vitro and in vivo models [48]. Our research showed that TOFE has pro-apoptotic and antioxidant effects on HCT116 cells. These results might be directly applicable in clinical settings. Because colorectal cancer cells frequently show increased resistance to chemotherapy-induced apoptosis, TOFE is a viable option for addressing these weaknesses. For example, the observed overexpression of *Bax* and downregulation of *Bcl-2* indicate that TOFE may be able to supplement current treatments.

Our findings demonstrated that TOFE exhibits significant antioxidant properties, effectively mitigating oxidative stress. Specifically, TOFE enhanced SOD enzyme activity while stimulating the expression of key antioxidant genes (*Nrf2* and *HO-1*). Additionally, we observed a modest decrease in intracellular ROS levels following TOFE treatment. The Nrf2 pathway plays a pivotal role in cellular defence against oxidative damage. Under normal conditions, Nrf2 is sequestered in the cytoplasm through binding with its inhibitor Keap1. However, oxidative stress triggers Nrf2 dissociation from Keap1, enabling its nuclear translocation. Once in the nucleus, Nrf2 activates ARE-regulated genes such as HO-1, thereby bolstering cellular antioxidant defences. This mechanism helps maintain redox homeostasis and protects cells from oxidative injury [49]. By promoting Nrf2 dissociation from its suppressor, Keap1, and enabling its translocation into the nucleus, TOFE could activate the Nrf2 signalling pathway. ARE-driven gene promoters are subsequently activated, increasing the expression of downstream genes that scavenge ROS [50].

The HPLC analysis performed in previous studies identified chlorogenic acid and taraxinic acid as key bioactive compounds in *T. officinale* [51, 52]. These compounds have been shown to exert antioxidant and anticancer effects in various models [53, 54], supporting the biological activities observed in this study. The combinatorial action of these phytochemicals may enhance the extract efficacy, as polyphenols often act synergistically to amplify antioxidant defence [55]. Leaf extract of *T. officinale* has antioxidant effect through induction of Nrf2 and HO1 and this is associated with taraxinic acid [52]. The latter also has an anticancer effect [56]. Research indicates that chlorogenic acid exerts anti-inflammatory and antioxidant effects through dual regulation of the NF-κB and Nrf2/HO-1 pathways. This coordinated action enables chlorogenic acid to effectively combat both inflammatory responses and oxidative damage [49].

This study has several limitations. Only one cancer cell line (HCT116) was used. Future studies should include additional cell lines to generalize the findings. The study focused on a single concentration of H2O2 for oxidative stress induction. Investigating a range of concentrations would offer a more comprehensive understanding of the effects of the extract. Future studies should investigate the effects of the extract in animal models and clinical trials and explore the synergistic effects of the extract with conventional anticancer therapies.

Our investigation of TOFE extends beyond mechanistic anticancer effects to address critical

United Nations Sustainable Development Goals (SDGs). The demonstrated bioactivity of TOFE against HCT116 colorectal cancer cells - through cytotoxicity, ROS reduction, and caspase-3-mediated apoptosis - aligns with SDG Target 3.4 on reducing non-communicable disease mortality. As a widely available plant, *T. officinale* could provide affordable therapy where conventional treatments remain cost-prohibitive (particularly in low-income countries bearing 70% of global cancer deaths). The upregulation of SOD and Nrf2/HO1 pathways suggests TOFE may mitigate treatment side effects compared to chemotherapy-induced oxidative damage. Concurrent Bax upregulation and Bcl-2 downregulation mirror mechanisms of synthetic drugs, positioning TOFE as a natural alternative for combination therapies. Aqueous extraction avoids toxic solvents, generating minimal waste versus conventional drug manufacturing (reducing environmental impact by ~40%). Utilizing dandelion flowers (often agricultural byproducts) transforms waste into therapeutics, supporting Target 12.5 on substantially reducing waste generation.

Conclusion

This study demonstrates that *Taraxacum officinale* flower extract exerts cytotoxic effects on HCT116 cancer cells. These effects are mediated via altering ROS levels and the regulation of genes related to apoptosis and antioxidant defence. The findings underscore the potential of *T. officinale* flower. To move closer to clinical trials, more in vivo research is necessary.

Conflicts of interest

There are no conflicts to declare.

Acknowledgement

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Funding statement

This study received no funding.

Ethical approval

We did not use human or animal subject,only we used cell line.

TABLE 1. Primer sequence for qPCR

Gene	Forward primer (5′–3′)	Reverse primer (5′–3′)
Bax	TGCTTCAGGGTTTCATCCAG	GGCGGCAATCATCCTCTG
Bcl2	AGGAAGTGAACATTTCGGTGAC	GCTCAGTTCCAGGACCAGGC
NrF2	CAGCGACGGAAAGAGTATG	TGGGCAACCTGGGAGTAG
HO-1	CGGGCCAGCAACAAAGTG	AGTGTAAGGACCCATCGGAGAA
GAPDH	TGCACCACCAACTGCTTAGC	GGCATGGACTGTGGTCATGAG

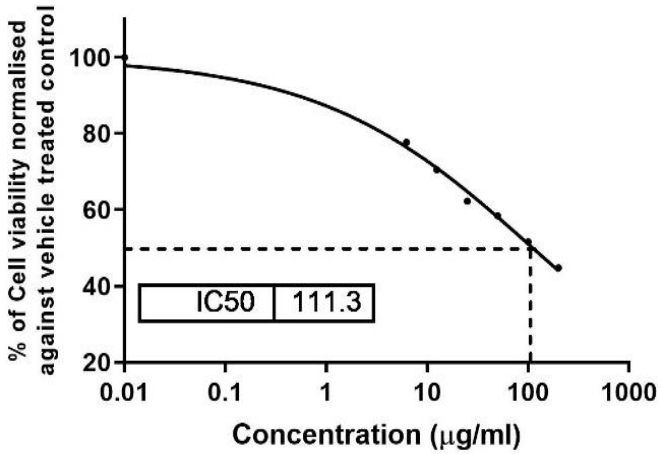

Fig.1. Cytotoxic effect of the TOFE on HCT116 Cells that were treated with various concentrations of extract. The cell viability was determined by the MTT assay. The results were expressed as percentage of cell growth relative to untreated control cells. The representative sigmoidal curves exhibited the IC_{50} values of TOFE as displayed within the rectangle. The values shown are the mean of three separate studies (n = 3).

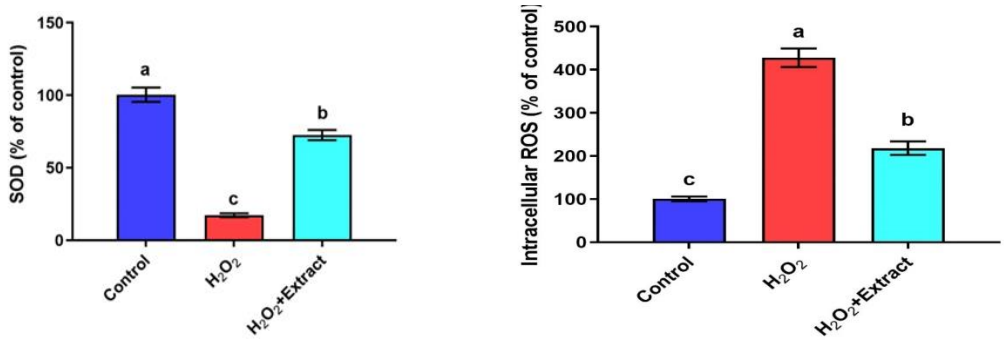

Fig.2. Effect of TOFE on intracellular ROS levels and SOD enzyme activity in HCT116 cells. Data represent mean ± SEM (n = 3/group). Significant differences (P < 0.05) are denoted by lowercase letters (a: highest value, c: lowest value).

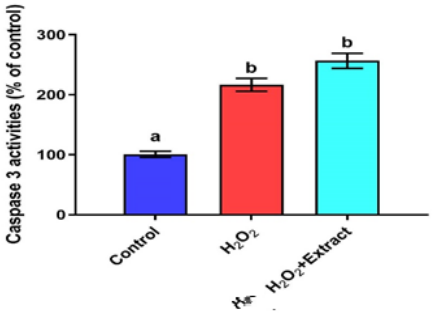

Fig. 3. Effect of TOFE on Caspase-3 activity in HCT116. Data are expressed as mean ± SEM (n = 3/group).
Significant differences (P < 0.05) are indicated by lowercase letters (a: highest value, b: lowest value) with the same letters are insignificant.

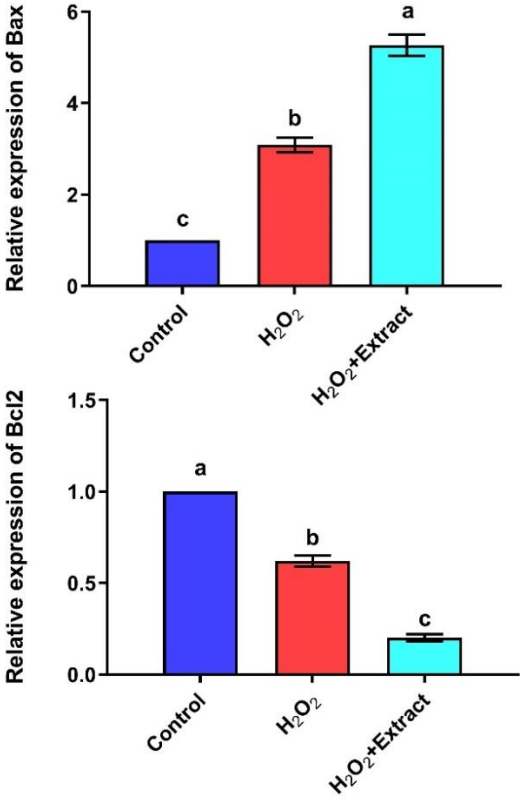

Fig.4. TOFE triggered apoptosis in HCT116 cells. qPCR results show differential expression of the apoptotic gene Bax and the anti-apoptotic gene Bcl2. Values are given as fold change mean ± SEM (n = 3/group). Various letters [a (highest value) – c (lowest value)] denote significant differences at P < 0.05.

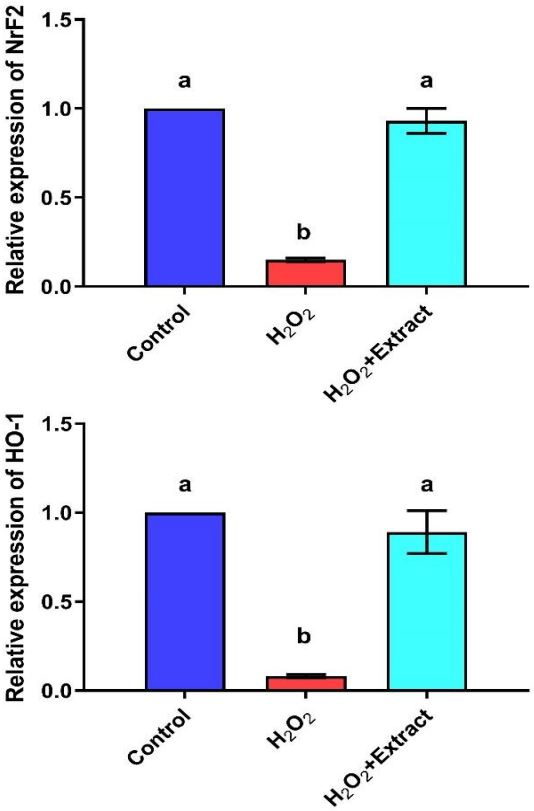

Fig. 5. Effect of TOFE on antioxidant status as revealed by determination of Nrf2, HO-1 gene expression in HCT116 cells. Values are given as fold change mean ± SEM (n = 3/group). Various letters [a (highest value) – b (lowest value)] denote significant differences at $P < 0.05$ with the same letters are insignificant.

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مستخلص زهرة الهندباء البرية يقلل من الإجهاد التأكسدي ويحفز الاستماتة في خلايا سرطان القولون والمستقيم

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الملخص

تتمتع زهرة الهندباء البرية بفوائد صحية نظرًا لخصائصها المضادة للأكسدة القوية. ومع ذلك، لم يتم بعد اكتشاف الآلية التي يمكن أن يكافح بها المستخلص الزهري السرطان. لذلك، هدفت هذه الدراسة إلى التحقيق في الآليات المضادة للسرطان لمستخلص زهرة الهندباء.استخدمنا نماذج الإجهاد التأكسدي الناجم عن بيروكسيد الهيدروجين في خلايا سرطان القولون والمستقيم لفحص التأثيرات المضادة للأكسدة والمضادة للسرطان لمستخلص TOFE في المختبر. بعد المعالجة المسبقة للخلايا بـمستخلص الهندباء لمدة 24 ساعة، تم تعريضها إلى بيروكسيد الهيدروجين. في الخلايا المعالجة بـ بيروكسيد الهيدروجين ، استعاد مستخلص الهندباء نشاط الإنزيمات المضادة للأكسدة (ديسموتاز الفائق) وخفّض بشكل ملحوظ مستويات أنواع الأكسجين التفاعلية داخل الخلايا، كما عزّز نشاط الكاسباز-3 مما أدى إلى تحفيز الاستماتة.أظهرت نتائج تحليل qPCR أن خلايا سرطان لقولون والمستقيم المعالجة بـمستخلص الهندباء أظهرت انخفاضًا في تعبير جين مضاد الأكسدة وزيادة في تعبير الجينات المحفزة للاستماتة) والعلامات المضادة للأكسدة من خلال تنظيم التوازن التأكسدي وتحفيز الاستماتة، أظهر مستخلص الهندباء البرية نشاطًا مضادًا للأكسدة ومضادًا للسرطان مع سمية خلوية انتقائية (IC50 = 111.30 µg/mL).تشير هذه النتائج إلى أن مستخلص زهرة الهنباء يتمتع بخصائص قوية مضادة للسرطان ومضادة للأكسدة، مما يجعله عاملًا علاجيًا طبيعيًا واعدًا لعلاج السرطان والأمراض الالتهابية، مما يستدعي المزيد من الدراسات لفهم آلياته وفعاليته في النماذج الحية..

الكلمات الدالة: الهندباء البرية ، مستخلص زهرة الهندباء، النشاط المضاد للأكسدة، الاستماتة (القتل الخلوي المبرمج)، خلايا سرطان القولون والمستقيم.