RESEARCH ARTICLE

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Potential Anti-Inflammatory and Growth Inhibitory Effect of *Cyrtopodion scabrum* Extract on Colon Cancer; An *in vivo* Study

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Abstract

Background: The use of complementary and/or alternative medicine to increase the efficacy and decrease the side effects of current cancer treatment is highly required. In this *in-vivo* study, we aimed to investigate the anti-tumor activity and probable side effects of a natural treatment, *Cyrtopodion scabrum* extract (CsE), in a model of tumor bearing mice. **Methods:** We established 28 female CT26-tumor bearing balb/c-mice model. We divided them randomly into four groups (n=7): Negative control received distilled water (DW) and the three treatment groups were administered with 5-FU and two different doses (300 and 600 mg/kg) of the gecko aqueous extract, respectively. The changes in the tumor volumes and weights during and after treatment, along with the blood cell counts; spleen and thymus indices were assessed in the treatment groups. We have also measured the serum TNF-α, VEGF, AST, ALT and GSH, as well as the physical activities of the experimental mice. **Results:** We found that the means of tumor weights and volumes in both CsE and 5-FU treated groups were significantly lower than the untreated group ($p<0.05$). Serum TNF-α and VEGF levels in both CsE treated groups were remarkably lower than 5-FU and untreated groups ($p<0.05$). The 5-FU treatment caused a remarkably decrease in serum GSH, RBC count, WBC count, thymus index, and spleen index , while CsE treatment maintained these quantities, with no significant changes, compared to the control group. AST and ALT were not significantly changed in none of the treated groups compared to control. **Conclusion:** Altogether, data suggest *C. scabrum*, as an effective and safe anti-cancer natural source, which could be used as an alternative/ complementary medicine for the treatment of patients who suffer from colon cancer.

Keywords: *Cyrtopodion scabrum*-natural anti-tumor medicine- anti-inflammation- side effect- 5-FU

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Introduction

Cancer is one of the major public health problems in the world (Torre et al., 2016). The statistics show an estimated 19.3 million new cases and 10 million cancer deaths worldwide in 2020 and a global cancer burden of 28.4 million cases in 2040, a 47% rise from 2020 (Sung et al., 2021). Colorectal cancer (CRC), the third most common malignancy, is predicted to increase by 60% to more than 2.2 million new cases and 1.1 million deaths by 2030 (Arnold et al., 2017). The incidence of this type of cancer is the fourth and third among Iranian men and women, respectively (Halimi et al., 2020; Mansori et al., 2018). Chemotherapy, one of the main powerful treatments for cancer, has been shown to increase the survival rate by 10% (Blauwhoff-Buskermolen et al., 2016). It not only kills cancer cells, but also affects normal cells intensively. Various side effects including cardiocytotoxicity, nephrotoxicity, myelosuppression, neurotoxicity, hepatotoxicity, and gastrointestinal toxicity have been reported to be associated with chemotherapy and strongly impair the quality of life of cancer patients (Liu et al., 2021). Therefore, finding less toxic and safer drugs against cancer is urgent, and many scientists have turned the spotlight to the use of natural agents such as herbal extracts or animal products. For almost thousands of years, the traditional use of natural products including zoo therapy and herbal therapy has represented a source of effective drugs (Fakher et al., 2019; Gohari et al., 2018; Seghatoleslam et al., 2014; Tavakoli et al., 2015).

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In Traditional Chinese Medicine (TCM), the whole-body extract of a kind of Chinese gecko (*swinhonis Gūenther*) has been used as a drug to treat various diseases such as cancer for hundreds of years (Duanet al., 2018; Jeong et al., 2012; Lee et al., 2019; Yang and Wang, 2020). In our previous studies, we also showed that a kind of gecko, named *Cyrtopodion scabrum* (so-called rough-tailed home gecko), has anti-proliferative effects on the breast, colorectal (Amiri et al., 2015), stomach, and liver cancer cells (Rashidi et al., 2017). *C. scabrum* is distributed around the Indus Valley to the eastern borders of the Caspian Sea, including Iran, Turkey, Pakistan, Iraq, etc. (Rastegar-Pouyani et al., 2010). Our recent studies revealed that the *C. scabrum* extract (CsE) had antitumor properties and growth inhibitory effects of 30-78% on different human cancer cells with a significant impact on SW742, MKN45 and HepG2, and no significant effect on the normal cells. We also found that treatment with CsE induced apoptosis in cancer cells and the observed anti-cancer effect of CsE might occur through TP53 up-regulation, but P53-independent transcriptional activity. According to our last interesting findings, *C. scabrum* homogenate and extract significantly attenuated the 5-fluorouracil (5-FU)-induced liver dysfunction in rats through strengthening antioxidant defense system, resulting in liver function improvement (Amiri et al., 2015; Diba et al., 2021; Rashidi and Seghatoleslam, 2021).

We designed the present study to investigate the potential *in-vivo* anticancer properties and side effects of CsE, which are still unclear. We also compared the effects of CsE with 5-FU, as the main backbone of chemotherapy for colorectal cancer patients which have not been reported previously. We also aimed to identify a new inexpensive anticancer drug with natural sources which might be an efficient noninvasive therapy compared with current treatment strategies.

Materials and Methods

Cell culture

The CT26 cell line was purchased from National Center for Cell Sciences, Pasteur Institute of Iran (Tehran). The cells were cultured as monolayers in RPMI-1640 medium (Bioidea, Iran) supplemented with 10% FBS (Shellmax, China), 1% Penicillin Streptomycin (Shellmax, China) and maintained in a humidified atmosphere of 5% CO_2 at 37°C.

Establishment of CT26-tumor bearing mice

28 female BALB/c mice (5-7-week-old and weight of 16-18 g) were purchased from Pasteur Institute of Iran (Tehran). The mice were housed under standard conditions with light: dark cycle 12:12 h, humidity 23-32%, temperature 22-25°C, and allowed access to food and water ad libitum. All the procedures were approved by Institutional Animal Ethics Committee of Shiraz University of Medical Sciences, Shiraz, Iran (Ethical code: IR.SUMS.REC. 1395.S772). The CT26 model was established by subcutaneous injection of 3×10^6 viable cells to the armpit region of BALB/c mice, as previously reported (Zhang et al., 2013). Tumor growth was monitored daily during 2 weeks for tumor development. When the tumor volume reached approximately 90-100 mm3, the mice were considered ready for starting the treatments.

Preparation of the Cyrtopodion scabrum extracts (CsE)

C. scabrum was provided by Razi Research Institute of Vaccine and Serum, Shiraz Branch, Iran. The identification of the specimens had been performed by a taxonomist (F.Torki, the head of the department at FTEHCR). The extract was prepared according to the protocol described previously by Amiri et al., (2015). Briefly, the animals were weighted, cleaned, and crushed by liquid nitrogen; then, homogenization, water extraction, and ethanol precipitation were done to obtain aqueous crude extract (CsE). It was then dialyzed, freeze dried by freeze dryer Alpha 1-2/ LD plus (Christ, Germany), and kept at -20°C for later use.

Experimental design

The established tumor-bearing mice (n=28, 7-9 weeks, 20±3 gr) were randomly divided into four experimental groups as follows: Group I, the negative control group, was given 0.2 ml DW orally by gavages. Groups II and III received CsE (300 and 600 mg/kg BW- according to the pilot study) orally by gavages in a daily schedule (0.2 ml of DW consisting of required amount of CsE). Group IV was given daily intraperitoneal injections of 5-FU (2 mg/kg BW) (Cipla, Iran).

Animal body weight and the tumor size were measured on the first day, and the changes were monitored daily until the end of the treatment. The duration of treatment was determined based on the tolerability of the growing tumors by the animals in the control group, their ability to move, body weight loss, infirmity and weakness, in the mice of 5-FU group. Thus, in this experiment, the treatments were terminated on the day 12, according to the above-mentioned items. At the end of the treatment, the mice were anesthetized using CO_2. Blood sampling was performed from the heart, and the sera were collected and stored at −20°C until use for biochemical and immunological measurements. The final tumor size and volume were also measured. The mice were sacrificed, and the whole-body skin of the tumor-bearing mice was removed for complete detection (Figure 1A).

Anti-tumor assay

The length and width of the tumors were measured daily using a caliper (Heng Liang, China) and the tumors' volumes were calculated using the standard formula:

$$V=ab^2/2$$

where a, is the length, and b is the width of the tumors.

Moreover, final body and tumor weights were measured after dissection and the tumor volumes were also evaluated after dissection by immersion method. The anti-tumor activity of the treatments was evaluated using the following formula:

Tumor inhibitory rate (%) = (Average tumor weight of treatment groups / Average tumor weight of control group) ×100

Immune function analysis

To evaluate the impact of the treatments on the immune organs, we calculated the thymus and spleen indexes, as follows:

Organ index = average weight of organ (mg) / body weight (g)

The number of WBCs was also analyzed using neubauer counting chamber, after dilution of the blood samples with Marcano solution (2% acetic acid + 1% methylene blue + 97% DW).

Serum TNF-α, as an important pro-inflammatory cytokine, was also measured using mouse TNF-α (IBL, Germany) ELISA kit, following the manufacturer's instructions.

Hematologic and non-hematologic toxicity study

On day 13, the blood samples were collected via cardiac puncture under CO_2 anesthesia. To study hematologic toxicity of CsE, we analyzed the whole blood for red blood cell (RBC) count using neubauer-counting chamber. Also, to study the probable side effects of CsE on the liver, we analyzed the sera for aspartate transaminase (AST) and alanine transaminase (ALT) activity using colorimetric assay kits (MAN, Iran). Moreover, the body weight was also measured to evaluate the effect of CsE treatments on the body weight of the experimental mice.

Determination of antioxidant state

The level of GSH, as a main antioxidant parameter in the body, was evaluated in the sera of experimental mice, using Ellman's method with minor modification (Rostampour et al., 2017). The process was based on the formation of GS-TNB complex from DTNB (5, 5' dithiobis 2-nitrobenzoic acid) which developed yellow color because of the DTNB reduction. The GSH level was measured using spectrophotometer (SHIMADZU, Japan) at 412 nm. The total level of GSH was calculated using a standard curve.

Analysis of VEGF angiogenic marker

As a preliminary study to explore the anticancer mechanism of CsE, serum level of VEGF, as a main angiogenic marker, was analyzed using mouse VEGF ELISA kit (IBL, Germany), following the manufacturer's instructions.

Statistical analysis

All data were expressed as mean ± SD from duplicate experiments and analyzed using SPSS software (Version 16.0; Chicago, USA). Mann-Whitney U test was also applied to analyze the significant groups with one factor. $P<0.05$ was considered statistically significant.

Results

In this study, we evaluated the *in-vivo* antitumor efficacy of CsE in CT26-tumor-bearing mice and compared it to 5-FU chemotherapy treatment. We also studied its effects on hemato-immunological indices and liver function as well.

Effects of CsE on tumor growth and body weight in CT26-tumor bearing mice

Daily changes in the tumor volume were measured and summarized in Figure 1. These results revealed unchanged tumor growth and volume in the groups treated with 5-FU and two doses of CsE compared to the control group, suggesting a significant antitumor effect of both CsE and 5-FU.

On day 12, the mice were sacrificed, and the final body weight, tumor volume, and tumor weight were measured after dissection. The results are summarized in Table1.

Based on the results, the tumor volume and weight were decreased dramatically in the treated groups compared to the control group ($p<0.05$), with no significant differences between CsE and 5-FU treated groups. The tumor growth suppression was approximately 63.36 and 65.99% for the mice treated with two doses of CsE and 78.11% for 5-FU treated mice, compared to the control group. The dissected tumors were also photographed, and the tumor size and volume in the different groups were compared (Figure 2 A,B). As demonstrated, the sizes of the dissected tumors in the treatment groups diminished significantly, and hopefully they were about to be cured in some cases. In the 5-FU group, the tumor growth was suppressed compared to the control group, but two of the mice died due to the side effects of the chemotherapy. We also observed that although 5-FU, as a chemotherapeutic agent, dramatically suppressed the tumor weight, it simultaneously reduced the body weight of mice compared to the control group ($p=0.017$), which indicated that 5-FU had a strong toxicity on the body. Meanwhile, the body weights of CsE-treated mice were significantly higher than those in the 5-FU group, with no significant differences with the control mice. These results suggest that CsE had no toxicity to the organism (Table 1).

Tables 1. The Effects of CsE on Body Weight, Tumor Weight, Calculated Volume and Inhibitory Rate at the End of the Treatments

Groups	Body weight (g)	Tumor weight (g)	Tumor volume (%)	Inhibitory Rate (%)
Control	18.80±3.1	5.52±2.67	5.18±2.50	-
CsE 300	20.00±3.4	1.87±1.27[a]	1.24±1.01[a]	65.99
CsE 600	23.60±4.5	2.02±1.98[a]	1.90±1.40[a]	63.36
5-FU	12.83±2.9[a]	1.2±0.90[a]	1.31±0.84[a]	78.11

The values (n=7) are presented as mean±SD; [a], indicates significant at $P < 0.05$ vs Control group

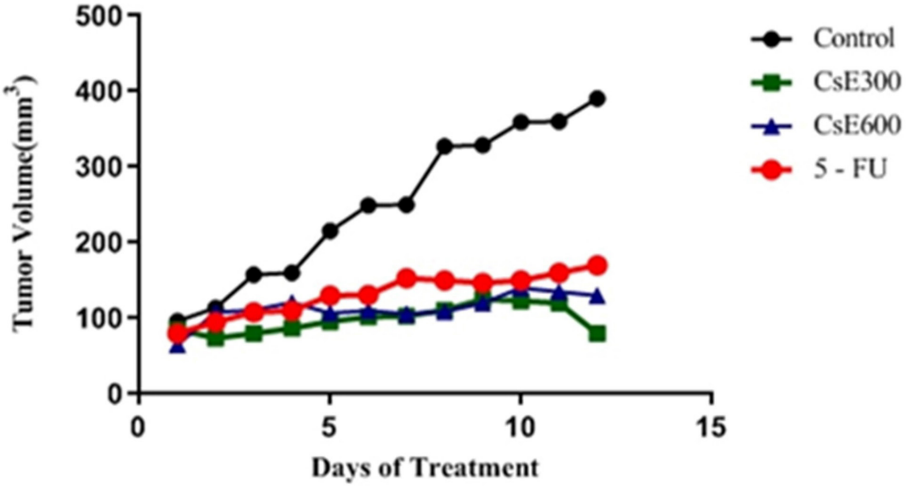

Figure 1. The Effect of CsE on Tumor Growth during 12-day Treatments in the CT26-Tumor-Bearing Mice. The lengths and widths of the tumors were measured individually every day and presented as an average for each group in tumor growth curve.

Table 2. The Effects of CsE on the Immune Function in the Tumor-Bearing Mice

Groups	WBC count ($\times 10^3$ /ml)	Spleen index (mg/g)	Thymus index (mg/g)
Control	7.22±1.5	5.16±2.5	16.45±4.5
CsE 300	5.35±0.82	4.98±0.94	14.01±3.7
CsE 600	5.57±1.2	4.60±0.68	14.85±2.5
5-FU	0.72±0.16[a]	1.60±0.94 [a]	8.9±4.7[a]

The values (n=7) are presented as mean±SD; [a], indicates significant at $P < 0.05$ vs Control group

The effect of CsE on immune function

As summarized in Table 2, WBC count in the experimental groups revealed no significant changes in the CsE treated groups, but a significant (P=0.004) decrease was observed in the 5-FU-treated group when compared to the control group.

There was no significant difference between the thymus and spleen index of the treatment groups of CsE compared to the untreated control group, but these indexes decreased significantly ($P<0.05$) in the 5-FU-treated group.

The serum level of TNF-α was significantly lower in the CsE treated groups compared to the control and 5-FU

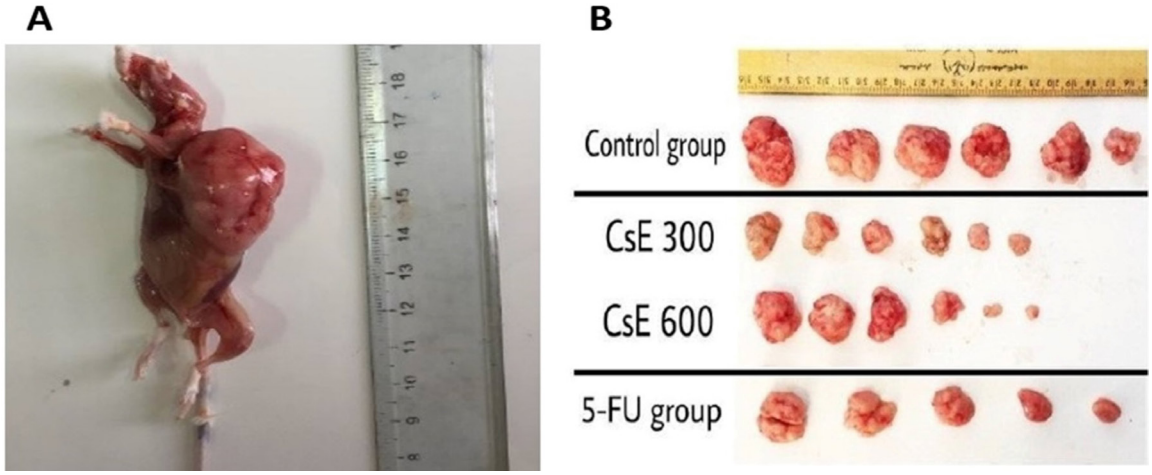

Figure 2. (A) Photograph of the whole body of the tumor-bearing mice, on day 13, after removing the skin. (B) Photograph of the dissected tumors from the mice of each group on day 13.

Table 3. The Potential Toxicity of CsE in the Tumor-Bearing Mice

Groups	RBC count ($\times 10^9$/ml)	Serum AST Level (U/L)	Serum ALT Level (U/L)	Serum GSH Level (mmol/ml)
Control	0.31±.12	181.22±25.3	62.7±7.7	5.70±1.7
CsE 300	0.40±.38	164.00±24.1	55.83±10.7	5.90±2.1
CsE 600	0.32±.13	166.00±29.1	60.80±3.5	5.90±.98
5-FU	0.15±.10[a]	198.17±42.5	67.78±10.9	2.44±.88[a]

The values (n=7) are presented as mean±SD; [a], indicates significant at $P < 0.05$ vs Control group

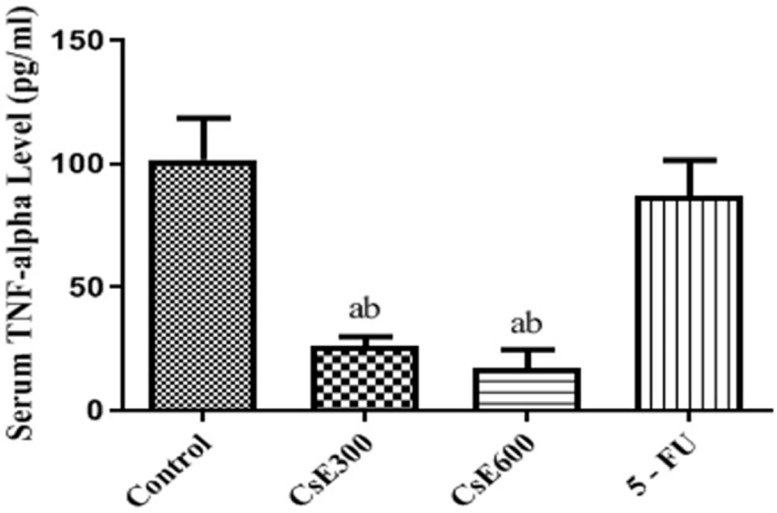

Figure 3. The Effect of CsE on the Serum TNF-α Level in the Tumor-Bearing Mice. a, indicates significance at P < 0.05 vs. Control group; b, indicates significant difference vs. 5-FU group.

groups (p<0.05), but the difference between the control and 5-FU groups was not significant (Figure 3).

The Effect of CsE on organ function

We further studied the safety and potential toxicity of CsE on the hematologic (RBC) and nonhematologic (serum ALT and AST) parameters, in response to the CsE and 5-FU treatment. As shown in Table 3, CsE treatment did not make any significant change in the RBC count compared to the control group, while treatment with 5-FU significantly (p=0.010) reduced the RBC count compared to the control group. Thus, unlike 5-FU, treatment with CsE was not associated with hematological toxicity. However, no significant changes, which indicate liver toxicity, were observed in the serum AST and ALT levels in the treatment groups compared to the control one.

The Effect of CsE on GSH level

Serum GSH level, as a main antioxidant parameter, was also measured in the CT26-tumor- bearing mice. As shown in Table 3, while the serum GSH level in the 5-FU group significantly decreased (P<0.05), CsE treatment did not shown reduction, compared to the control group.

The Effect of CsE on VEGF angiogenic marker

Serum VEGF levels, as the main regulator which induces tumor angiogenesis, are represented in Figure 4. According to the results, CsE treatment significantly decreased the VEGF levels compared to the control and 5-FU groups (p<0.05), while the difference between the control and 5-FU groups was not significant.

Discussion

The selective anticancer properties and growth inhibitory effects of *C. scabrum*, a genus of the rough-tailed geckonid lizard of Iran, was approved for the first time in our previous studies (Amiri et al., 2015; Rashidi et al., 2017). The present study was designed to have a better understanding of its anti-tumor properties, potential

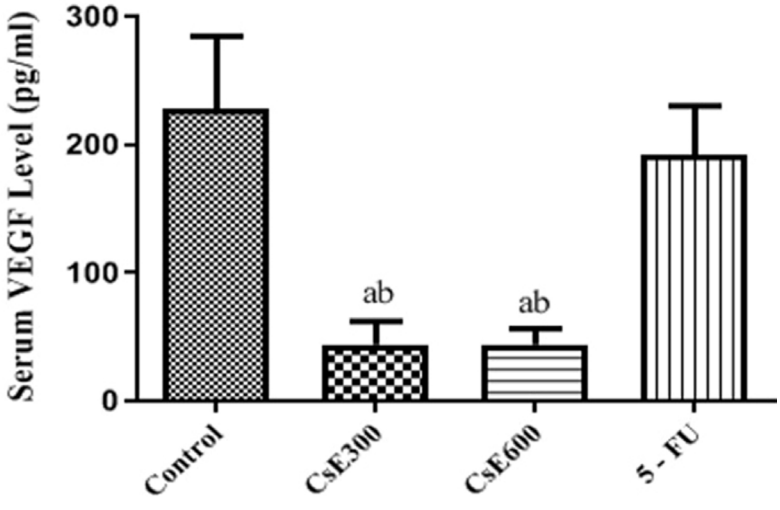

Figure 4. The effect of CsE on the serum VEGF level in the tumor-bearing mice. a, indicates significance at P < 0.05 vs. Control group; b, indicates significant difference vs. 5-FU group.

toxicity, and possible mechanism for tumor suppression, *in-vivo*. For this purpose, we selected a colon cancer cell line, CT26, to establish BALB/c tumor-bearing mice. CT26 tumor-bearing mice have been used extensively in the literature, as a model of colorectal cancer (Tuo et al., 2020; Wei et al., 2018). The results obtained from this study indicated that the tumors of groups subjected to different concentrations (300 and 600 mg/kg BW) of CsE as well as 5-FU-treated group shrank significantly, compared to control group. These results are consistent with those of Liu et al.'s study on anti-tumor activities of one kind of Gecko powder on the tumor model of the mouse S180 sarcoma (Liu et al., 2008). While the unwanted side effects of 5-FU treatment are evident, we extended our investigation on CsE treatment to explore whether it may have negative side effects on the treated groups as well. Therefore, we evaluated some immune and hematopoietic parameters in the treatment groups. Immune system and hematopoietic system are two important sensitive systems that are the target of harmful effects of drugs on living organisms. It is well known that the thymus and spleen, as the main immune organs, play important roles in the immunity of the host. Therefore, a higher organ index indicated a stronger immune capability (Yin et al., 2007; Zhu et al., 2016). The toxic effect of drugs on hematopoietic system can be evaluated through measurement of blood parameters such as the total WBC and RBC count. The results obtained from this part of the study indicated that 5-FU, as a conventional chemotherapy agent, had adverse effects by the significant reduction in WBCs and RBCs in the peripheral blood and a decline in the spleen and thymus indexes as well. In close agreement with our results, Li et al., (2018) and Gelen and Şengül, (2018). Also reported immune suppression and cytopenia followed by 5-FU-treatment. Unlike the results of 5-FU-treated group, CsE exerted its antitumor effects without any immune suppression or cytopenia, indicating its prominence in immune safety. Moreover, the GSH marker, as a representative of antioxidant state, significantly reduced in the 5-FU-treated group compared to the CsE-treated and control groups and there was no difference in the GSH level in the CsE treatment groups compared to the control group. Taken together, these observations strongly indicate that CsE, as a natural source-based remedy, is a safer antitumor agent compared to 5-FU chemotherapy drug.

In order to further confirm the safety of CsE, we measured the activity of hepatic enzymes, ALT and AST, to assess the potential liver function or liver damage (Moreno, 2009). Fortunately, CsE consumption revealed no hepatotoxicity in the animals. Moreover, the mice treated with CsE demonstrated good physical activity and normal weight gain, while in the 5-FU group, two mice died and the rest had become very weak; in some cases, they were even unable to eat normally; these results also approved the safety of CsE, as a natural antitumor treatment, compared to 5-FU.

To uncover the mechanism of tumor suppression and the possible role of gecko extract, as an anti-angiogenic and/or anti-inflammatory factor, we measured serum TNF-α and VEGF of the treatment groups. The results obtained from our study showed that the level of these factors decreased significantly following CsE, but not 5-FU treatment, which suggests that the antitumor mechanisms of CsE is different from that of 5-FU. In close agreement with our results, there was the reduced level of TNF-α and VEGF following some Gecko species extract treatments in some *in-vivo* and *in-vitro* experiments (Song et al., 2012; Tang et al., 2015). Therefore, based on the results of our study, CsE treatment can lead to effective cancer treatment due to the reduction of pro-inflammatory cytokine TNF-α together with VEGF. However, there is a complicated interplay between inflammation and carcinogenesis. Most recent reports revealed a direct causal link between inflammation and carcinogenesis. During inflammation, cytokines are released by immune and stromal cells of the tumor by cell-to-cell signaling. Elevated expression of TNF-α, as a pro-inflammatory cytokine, is the characteristic of many malignant tumors and is associated with poor prognosis (Wu and Zhou, 2009). Inflammation could lead to not only the induction of neoplastic transformation or promotion of tumor growth, but also recurrence after therapy. Inflammatory conditions also increase the production of growth factors such as VEGF, which increase angiogenesis and results in tumor growth and proliferation (Avraamides et al., 2008; Li et al., 2007).

Limitations

Further biochemical and Molecular studies are required for investigating the exact antitumor mechanisms of CsE treatment as well as exploring the active component of the extract. In addition, more extensive research should be conducted to verify and optimize CsE for using in Clinical trial.

In conclusion, to the best of our knowledge, this is the first *in-vivo* experimental study to examine the anti-tumor effects of *C. scabrum* compared to 5-FU, an important chemotherapy agent. The most remarkable result emerging from our data is that *C. scabrum* is a valuable source of anti-cancer materials with no unwanted side effects, which possibly exerting its anti-tumor effects through anti-inflammatory and anti-angiogenic mechanisms.

Author Contribution Statement

A.S. designed the study and Z.B. and S.M.S. participated in the design of the work. Z.B., G.N., F.Kh. and F.Ko conducted the experiments and analyzed the data. M.R., F.Z. and S.M.S. provided conceptual and technical guidance for all aspects of the study. A.S. and Z.B. wrote the manuscript with input from all the authors. All the authors contributed to the manuscript revisions.

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Ethical approval

All the procedures were approved by Institutional Animal Ethics Committee of Shiraz University of Medical Sciences, Shiraz, Iran (Ethical code: IR.SUMS.REC. 1395.S772).

Availability of data

data are available from the corresponding author on reasonable request.

The present study follows the guidance of Critical Appraisal Tool (AIMRDA) developed for the Peer-Review of Studies Assessing the Anticancer Activity of Natural Products (Ahmad et al., 2022).

Disclosure of interest

The authors declare that they have no conflicts of interest.

References

Ahmad R, Riaz M, Aldholmi M, et al (2022). Development of a Critical Appraisal Tool (AIMRDA) for the Peer-Review of Studies Assessing the Anticancer Activity of Natural Products: A Step towards Reproducibility. *Asian Pac J Cancer Prev*, **22**, 3735-40.

Amiri A, Namavari M, Rashidi M, Fahmidehkar MA, Seghatoleslam A (2015). Inhibitory effects of *Cyrtopodion scabrum* extract on growth of human breast and colorectal cancer cells. A*sian Pac J Cancer Prev*, **16**, 565-70.

Arnold M, Sierra MS, Laversanne M, et al (2017). Global patterns and trends in colorectal cancer incidence and mortality. *Gut*, **66**, 683-91.

Avraamides CJ, Garmy-Susini B, Varner JA (2008). Integrins in angiogenesis and lymphangiogenesis. *Nat Rev Cancer*, **8**, 604-17.

Blauwhoff-Buskermolen S, Versteeg KS, de van der Schueren MA, et al (2016). Loss of muscle mass during chemotherapy is predictive for poor survival of patients with metastatic colorectal cancer. *J Clin Oncol*, **34**, 1339-44.

Diba M, Seghatoleslam A, Namavari M, et al (2021). Potential protective role of *cyrtopodion scabrum* in antioxidant parameters in serum and liver of rats with 5-FU-induced oxidative damage. *Arch Razi Inst*, **76**, 95-105.

Duan YM, Jin Y, Guo ML, Duan LX, Wang JG (2018). Differentially expressed genes of HepG2 cells treated with gecko polypeptide mixture. *J Cancer*, **9**, 2723.

Fakher S, Seghatoleslam A, Noorafshan A, et al (2019). The Impact of Echium Amoenum Distillate, Aragh Gav-Zaban, on Naturally Boosting Fertility: Potential Ameliorative Role in Male Mice Reproductive Parameters. *Iran J Med Sci*, **44**.

Gelen V, Şengül E (2018). Hematoprotective effect of naringin on 5-fu toxicity in rats. *Chem Re J*, **3**, 127-30.

Gohari A, Noorafshan A, Akmali M, Zamani-Garmsiri F, Seghatoleslam A (2018). Urtica dioica distillate regenerates pancreatic beta cells in streptozotocin-induced diabetic rats. *Iran J Med Sci*, **43**, 174.

Halimi L, Bagheri N, Hoseini B, et al (2020). Spatial analysis of colorectal cancer incidence in Hamadan Province, Iran: a retrospective cross-sectional study. Appl Spat Anal Polic, 13, 293-03.

Jeong AJ, Chung CN, Kim HJ, et al (2012). Gecko proteins exert anti-tumor effect against cervical cancer cells via PI3-Kinase/Akt pathway. *Korean J Physiol Pharmacol*, **16**, 361-5.

Lee JE, Lee DG, Park SY, et al (2019). Gekkonidae, Lizard tail extracts elicit apoptotic response against non-small cell lung cancer via inhibiting Akt signaling. *Biomed Pharmacother*, **116**, 109050.

Li H, Fan X, Houghton J (2007). Tumor microenvironment: the role of the tumor stroma in cancer. *J Cell Biochem*, **101**, 805-15.

Li Y, Zhu S, Zhang Y, et al (2018). High fat diet-induced obesity exacerbates hematopoiesis deficiency and cytopenia caused by 5-fluorouracil via peroxisome proliferator-activated receptor γ. *Exp Hematol*, **60**, 30-9.

Liu F, Wang JG, Wang SY, et al (2008). Antitumor effect and mechanism of Gecko on human esophageal carcinoma cell lines in vitro and xenografted sarcoma 180 in Kunming mice. *World J Gastroenterol*, **14**, 3990.

Liu YQ, Wang XL, He DH, Cheng YX (2021). Protection against chemotherapy-and radiotherapy-induced side effects: A review based on the mechanisms and therapeutic opportunities of phytochemicals. *Phytomedicine*, 80, 153402.

Mansori K, Mosavi-Jarrahi A, Motlagh AG, et al (2018). Exploring spatial patterns of colorectal cancer in Tehran City, Iran. *Asian Pac J Cancer Prev*, **19**, 1099.

Moreno Reyes MR (2009). Iodine, selenium deficiency and Kashin-Beck disease. Comprehensive Handbook of Iodine, pp 685-700.

Rashidi M, Seghatoleslam A (2021). Induction of Apoptosis with *Cyrtopodion Scabrum* Extract in Colon Cancer Cells: A Preliminary Study on Targeting P53 Signaling Pathway. *Middle East J Cancer*, **12**, 466-72.

Rashidi M, Seghatoleslam A, Namavari M, et al (2017). Selective Cytotoxicity and apoptosis-induction of *Cyrtopodion scabrum* extract against digestive cancer cell lines. *Int J Cancer Manag*, **10**, e8633.

Rastegar-Pouyani N, Khosravani A, Oraie H (2010). A new record of *Cyrtopodion scabrum* (Heyden, 1827) from the Caspian Sea Coastal Region, Guilan Province, Northern Iran. *Herpetol Notes*, **3**, 61-3.

Rostampour F, Ghasemi H, Mousavi-Bahar SH, et al (2017). Total antioxidant capacity, lipid peroxidation, thiol group and catalase activity in patients with kidney stone. *Avicenna J Med Biochem*, **5**, 60-4.

Seghatoleslam A, Mashkour N, Namavari M, Azarmehr B, Nejabat M (2014). The potential effects of herbal distillates with hot and cold temperament on cell metabolic activity and growth: a preliminary in vitro study. *J Pharm Biomed Sci*, **4**.

Song Y, Wang JG, Li RF, et al (2012). Gecko crude peptides induce apoptosis in human liver carcinoma cells in vitro and exert antitumor activity in a mouse ascites H22 xenograft model. *J Biomed Biotechnol*, **2012**.

Sung H, Ferlay J, Siegel RL, et al (2021). Global cancer statistics 2020: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. *CA Cancer J Clin*, **71**, 209-49.

Tang Z, Huang SQ, Liu JT, Jiang GX, Wang CM (2015). Anti-angiogenic activity of gecko aqueous extracts and its macromolecular components in CAM and HUVE-12 cells. *Asian Pac J Cancer Prev*, **16**, 2081-6.

Tavakoli MB, Kiani A, Roayaei M (2015). The effects of

fenugreek on radiation induced toxicity for human blood T-cells in radiotherapy. *J Medical Signals Sens*, **5**, 176.
Torre LA, Siegel RL, Ward EM, Jemal A (2016). Global Cancer Incidence and Mortality Rates and Trends An UpdateGlobal Cancer Rates and Trends An Update. *Cancer Epidemiol Biomarkers Prev*, **25**, 16-27.
Tuo Y, Zhang Z, Tian C, et al (2020). Anti-inflammatory and metabolic reprogramming effects of MENK produce antitumor response in CT26 tumor-bearing mice. *J Leukoc Biol*, **108**, 215-28.
Wei Z, Chen G, Zhang P, et al (2018). Rhizopus nigricans polysaccharide activated macrophages and suppressed tumor growth in CT26 tumor-bearing mice. *Carbohydr Polym*, **198**, 302-12.
Wu Y, Zhou BP (2009). Inflammation: a driving force speeds cancer metastasis. *Cell Cycle*, **8**, 3267-73.
Yang Y, Wang L (2020). Effect of Gecko Active Ingredients on Transplanted Tumor of Colon Cancer in Mice. *Rev Cient Fac Cienc Vet*, **30**, 570-8.
Yin Y, Fu W, Fu M, He G, Traore L (2007). The immune effects of edible fungus polysaccharides compounds in mice. *Asia Pac J Clin Nut*, **16**.
Zhang S, Nie S, Huang D, Li W, Xie M (2013). Immunomodulatory effect of Ganoderma atrum polysaccharide on CT26 tumor-bearing mice. *Food Chem*, **136**, 1213-19.
Zhu L, Cao J, Chen G, et al (2016). Anti-tumor and immunomodulatory activities of an exopolysaccharide from Rhizopus nigricans on CT26 tumor-bearing mice. *Int Immunopharmacol*, **36**, 218-24.