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Original Article

Potential Protective Role of *Cyrtopodion Scabrum* in Antioxidant Parameters in Serum and Liver of Rats with 5-FU-Induced Oxidative Damage

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Abstract

Chemotherapy is the main approach for the treatment of cancer; however, it often causes unpleasant oxidative damages. Therefore, the development of an effective alternative/complementary therapy with improved tumor suppression efficiency and lower adverse effects is highly required. Recently, it has been shown that *Cyrtopodion scabrum* extract (CsE) is an effective and selective tumor suppressor medicine. The present study investigated the antioxidant activity of *Cyrtopodion scabrum* homogenate (CsH) and CsE and their effects on attenuating 5-fluorouracil (5-FU)-induced liver dysfunction in rats. A total of 60 male rats (weight: 200-220 g) were divided into six groups and treated for 14 days. The control (group I) and 5-FU (group II) groups received distilled water and 5-FU, respectively. The other four groups were orally administered with CsE, CsH, CsE+5-FU, and CsH+5-FU (groups III to VI), respectively by gavages based on a daily schedule. The 5-FU-induced oxidative damage was evaluated by changes in the weight and food and water intake during the treatment and antioxidant parameters in the liver and serum of the treated rats. The obtained data indicated that the administration of CsH and CsE significantly improved liver function and defense system of antioxidants by attenuating the levels or activities of malondialdehyde, superoxide anion, aspartate aminotransferase, alanine aminotransferase, and alkaline phosphatase and decrease of superoxide dismutase, catalase, glutathione peroxidase, glutathione reductase, glutathione S-transferase, total antioxidant capacity, glutathione, total protein, and albumin in the liver and serum, induced by 5-FU treatment. The obtained data of the current study suggested that CsH and CsE play a protective role in the imbalance elicited by 5-FU and can be used as alternative/complementary supplements with 5-FU to reduce oxidative damages which is the consequence of reactive oxygen species production in cancerous patients.

Keywords: *Cyrtopodion scabrum*, 5-FU, Antioxidant parameters, Oxidative damages

Rôle Protecteur Potentiel du *Cytopodion Scabrum* dans les Paramètres Antioxydants dans le Sérum et le Foie de Rats Présentant des Dommages Oxydatifs Induits par le 5-fluorouracil (5-FU)

Résumé: La chimiothérapie est la principale approche pour le traitement du cancer; cependant, elle provoque souvent des dommages oxydatifs désagréables. Par conséquent, le développement d'une thérapie alternative / complémentaire efficace avec une efficacité de suppression tumorale améliorée et des effets indésirables moindres est hautement nécessaire. Récemment, il a été démontré que l'extrait de *Cyrtopodion scabrum* (CsE)

est un médicament suppresseur de tumeur efficace et sélectif. La présente étude a examiné l'activité antioxydante de l'homogénat de *Cyrtopodion scabrum* (CsH) et de la CsE et leurs effets sur l'atténuation du dysfonctionnement hépatique induit par le 5-fluorouracile (5-FU) chez les rats. Un total de 60 rats mâles (poids: 200-220 g) ont été divisés en six groupes et traités pendant 14 jours. Les groupes témoins (groupe I) et 5-FU (groupe II) ont reçu respectivement de l'eau distillée et du 5-FU. Les quatre autres groupes ont été administrés par voie orale avec CsE, CsH, CsE+5-FU et CsH+5-FU (groupes III à VI) par des gavages basés sur un programme quotidien. Les dommages oxydatifs induits par le 5-FU ont été évalués par les modifications du poids et de la prise de nourriture et d'eau pendant le traitement et les paramètres antioxydants dans le foie et le sérum des rats traités. Les données obtenues ont indiqué que l'administration de CsH et CsE améliorait considérablement la fonction hépatique et le système de défense des antioxydants en atténuant les niveaux ou les activités du malondialdéhyde, de l'anion superoxyde, de l'aspartate aminotransférase, de l'alanine aminotransférase et de la phosphatase alcaline et de la diminution de la superoxyde dismutase, de la catalase, glutathion peroxydase, glutathion réductase, glutathion S-transférase, capacité antioxydante totale, glutathion, protéines totales et albumine dans le foie et le sérum, induites par un traitement au 5-FU. Les données obtenues de la présente étude suggèrent que la CsH et la CsE jouent un rôle protecteur dans le déséquilibre provoqué par le 5-FU et peuvent être utilisées comme suppléments alternatifs / complémentaires avec le 5-FU pour réduire les dommages oxydatifs qui sont la conséquence de la production d'espèces réactives de l'oxygène chez les patients cancéreux.
Mots-clés: *Cyrtopodion scabrum*, 5-FU, Paramètres antioxydants, dommages oxydatifs

Introduction

Cancers are a group of diseases involving abnormal cell growth, with the potential to invade or spread to other parts of the body. They are responsible for 13% of all mortalities around the world and the third leading cause of death in Iran. The prevalence of cancer is predicted to increase up to 45% in 2025 (Amoori et al., 2014; Amirkhah et al., 2017).

Chemotherapy is a common method for cancer treatment, and 5-fluorouracil (5-FU) has played a critical role as the main backbone of chemotherapy for patients with colorectal cancer (CRC) and other gastrointestinal (GI) cancers for over 50 years (Li et al., 2014). It has been used as 5-FU-monotherapy, 5-FU-combination chemotherapy with other cytotoxic agents (including irinotecan or oxaliplatin), or 5-FU-based regimens in combination with various biological agents (including ziv-aflibercept, bevacizumab, ramucirumab, cetuximab, and panitumumab). The main goals of different studies during the years have been the increase of antitumor efficacy and reduction of drug-associated toxicity.

Reactive oxygen species are often released by normal and tumor cells in response to chemotherapy and play a crucial role in the survival and migration of cancer cells (Hagar et al., 2006). Recently, in vitro and in vivo studies have demonstrated that the administration of 5-FU increases oxidative stress (Seghatoleslam et al., 2014), unwanted toxicity in other different organs and tissues, and increase in the risk of therapeutic failure (Amiri et al., 2015). Today, the world is moving toward the extension of traditional medicine (TM) and complementary drug production to increase efficacy and decrease side effects and costs of treatments (Orhan, 2012).

The TM has a long history in the world, and many drugs have been isolated from natural resources. Two of the most important types of TM are herbal therapy and zootherapy that are the use of plants, animals, and their derivatives (Gohari et al., 2018). According to Chinese TM, several studies experimentally demonstrated that a type of lizard named *swinhonis Guenther* has anti-cancer and anti-inflammatory properties (Rashidi et al., 2017). Recently, the antiproliferative effects of *Cyrtopodion scabrum* (also known as house gecko) extract (CsE) has been reported on the human breast and CRC cells (Amiri et al., 2015).

It was also shown that CsE selectively inhibits the growth of various cell lines within the range of 30-

78%, with the highest inhibitory effect on SW742 (CRC), MKN45 (GI cancer), and HepG2 (liver carcinoma) cell lines, respectively. It was suggested that the mechanism of this growth inhibition is probably apoptosis and G2 cell cycle arrest through P21 (Rashidi et al., 2017). The antitumor property of *C. scabrum* was also confirmed in vivo, using the CT26-tumor-bearing mice model. The results (unpublished) showed that the extract of this lizard effectively suppresses tumor growth, with no or fewer side effects, compared to 5-FU.

C. scabrum is a species of genus *Cyrtopodion*, distributed in southwestern, central, eastern, and northern parts of Iran (Rastegar-Pouyani et al., 2008). In recent years, the chemoprotective potential of some natural (Talas et al., 2009) or synthetic organoselenium (Talas et al., 2009) compounds have been studied by the evaluation of antioxidant parameters. With this background in mind, the present study investigated the potential antioxidant activities and protective roles of CsE and *Cyrtopodion scabrum* homogenate (CsH) in 5-FU-induced oxidative damage in rats.

Material and Methods

Pharmacological Materials. The 5-FU was provided by EBEWE Pharma (Ges.m.b.H.Nfg.KG.A 4866 Unterach, Austria). Moreover, ketamine 10% was purchased from Alfasan Company (Woerden, Holland).

Preparation of the Whole-body Homogenate and Aqueous Extract from *C. scabrum*. *C. scabrum* was provided by Razi Vaccine and Serum Research Institute, Shiraz Branch, Shiraz, Iran. For the preparation of CsH, the required amount of *C. scabrum* was homogenized and dissolved in distilled water (DW), then freeze-dried in the Laboratory freeze dryer Alpha 1-2/LD plus (Christ, Germany), and kept at -20°C until use. The CsE preparation was according to a protocol previously described (Amiri et al., 2015). Briefly, the whole animals were crushed by liquid nitrogen, homogenized by a homogenizer (Bodine Electric Company, Chicago, USA), and further extracted to obtain crude sulfated peptide, then dialyzed against DW using a dialysis bag of pore with a size of 1 µm. The solution was then freeze-dried in the Laboratory freeze dryer Alpha 1-2/LD plus (Christ, Germany), and the powder was then kept at -20°C until use.

Experimental Designs. A total of 60 Sprague-Dawley male rats (weight: 200-220 g) were provided from the Animal Center of Shiraz University of Medical Sciences and randomly divided into six groups, each including 10 rats. Group I (control) received 3 ml of intragastric water daily for 14 days. Group II (5-FU-only) was given 3 mL of intragastric water daily for 14 days and received 5-FU (50 mg/kg/BW) on days 10 to 14 by intraperitoneal injection. Groups III (CsH group) and IV (CsE group) were given CsH (1600 mg/kg/BW) and CsE (120 mg/kg/BW) dissolved in 3 ml of DW orally by gavages based on a daily schedule for 14 days, respectively. Groups V (CsH+5-FU group) and VI (CsE+5-FU group) received CsH (1600 mg/kg/BW) and CsE (120 mg/kg/BW) dissolved in 3 ml of DW orally by gavages for 9 days and 5-FU (50 mg/kg/BW) within days 10 to 14 by intraperitoneal injection. The animals were kept in an optimum temperature condition (24±2°C) with a 12-h light/ dark alternating cycle. Body weight and food and water intake were monitored and measured daily during the experiment.

Collection of Blood Samples. At the end of the experiment, on the 15th day, the rats were anesthetized by ketamine 10%. The intracardiac blood samples were collected and centrifuged at 1500×g for 10 min at 4°C. Finally, the sera were immediately collected and stored at -80°C for further use.

Collection and Preparation of Tissue Samples. The livers of the rats were separated and washed immediately with ice-cold NaCl (0.9%) and then weighed and stored frozen at -80°C until use. The liver homogenates were prepared by homogenizing the tissue in ice-cold NaCl (0.9%) to obtain a 10% solution using a homogenizer (Bodine Electric Company, Chicago, USA). The samples were centrifuged immediately at 12000×g for 15 min at 4°C (28).

Finally, the clear supernatant was separated and used for the biochemical analysis.

Biochemical Analysis

Liver Function Assessment. The specific markers of liver function, including the levels of aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP), total protein (TP), and albumin (Alb), in the serum and liver samples of the experimental rats were spectrophotometrically evaluated using commercial diagnostic kits (Pars Azmoon, Iran).

Measurement of Antioxidant Parameters

Glutathione Peroxidase Assay. Glutathione peroxidase (GPx) activity was determined by continuous monitoring of the generation of reduced glutathione (GSH) from oxidized glutathione (GSSG) upon the consumption of nicotinamide adenine dinucleotide phosphate (NADPH) by glutathione reductase (GR) according to the method of Fecondo and Augusteyn (Fecondo and Augusteyn, 1983). The oxidation of NADPH is directly proportional to the GPx activity by a reduction in the absorbance at 340 nm at 37°C for 5 min.

Glutathione Reductase Assay. The GR catalyzes the reduction of GSSG to GSH by the consumption of one molecule of NADPH. The assay was performed following a method previously described (Carlberg and Mannervik, 1985) with minor modifications. The decrease in the absorbance was spectrophotometrically monitored at 340 nm at room temperature for 3min.

Catalase Assay. The CAT activity was determined according to the Aebi's procedure based on the content of H_2O_2 that decomposes (Carlberg and Mannervik, 1985). Briefly, 150 µL of phosphate buffer (pH=7.0 and 66.7 mM), 10 µL of the sample, and 365 µL of DW were added to 75 µL of H_2O_2 (120 mmol/L). Furthermore, the utilization of H_2O_2 was spectrophotometrically followed at 240 nm at 25°C for 2 min. The CAT activity was expressed as H_2O_2 µmol consumed per min per mg enzyme.

Glutathione S-transferase Assay. Glutathione S-transferase (GST) activity is colorimetrically assayed (Habig et al., 1974). Based on this method, GST affects reduced GSH and 1-Chloro-2, 4-dinitrobenzene (CDNB) and causes the production of Glutathione-S-CDNB; then, the absorbance of the product was measured at 320 nm.

Superoxide Dismutase Assay. Superoxide dismutase (SOD) activity was determined as previously described (McCord and Fridovich, 1969). The SOD inhibits the oxidation of epinephrine hydrochloride resulted in a decrease in the absorbance at 480 nm.

Reduced Glutathione Assay. The GSH, as the main antioxidant parameter in the body, was measured by the colorimetric quantification method (Sun et al., 2001). The process is based on the formation of glutathione sulfide-5'-thio-2-nitrobenzoic acid complex from 5,5-dithio-bis-(2-nitrobenzoic acid (DTNB), and the reduction of DTNB causes the development of yellow color in the samples. The GSH levels were measured using a spectrophotometer (SHIMADZU, Japan) at 412 nm. Finally, the total levels of GSH were calculated using a standard curve.

Estimation of Lipid Peroxidation. Malondialdehyde (MDA) is the indirect index of lipid peroxidation. The MDA level was determined (Hagar et al., 2006) in this study. Briefly, 0.5 ml of sample was added to 2 ml of thiobarbituric acid (TBA) reagent (containing 0.375% of TBA, 15% of trichloroacetic acid, and 0.25 mol/L of hydrochloric acid [HCl]). Then, the mixture was boiled for 15 min and cooled and centrifuged at 1700×g at 4°C for 15 min. Finally, the absorbance of the samples was measured at 532 nm.

Determination of Total Antioxidant Capacity. The total antioxidant capacity (TAC) of the samples was determined using a minor modification of the ferric reducing ability of plasma (FRAP) assay of Benzie and Strain (Wootton-Beard et al., 2011). The FRAP reagent was prepared from glacial acetic acid and 300 mM of acetate buffer (pH 3.6), 10 mM of 4, 6-tripryridyl-s-triazine made up in 40 mM of HCl, and 20 mM of ferric chloride. All the solutions were mixed together in the ratio of 10:1:1. The FRAP assay was performed by warming 1 mL of dH_2O to 37°C and then adding 50 µL

of sample and 1.5 mL of reagent and final incubation at 37°C for 10 min. The absorbance was determined at 593 nm, and the total antioxidant capacity of the samples was measured against a standard of known FRAP value, ferrous sulfate (1000 µM).

Estimation of Superoxide Anion (O_2^-) Level. Serum and hepatic O_2^- concentrations were measured by the ability of O_2^- to reduce nitroblue tetrazolium (NBT) to an insoluble formazan (Devi et al., 2000). The NBT assay is a simple, reliable, and sensitive technique. The samples were incubated with 0.1% NBT dissolved in phosphate-buffered saline (pH 7.4) at 37°C for 30 min. Finally, 0.6 ml of glacial acetic acid was added to the samples and the absorbance was read at 560 nm. The level of O_2^- was calculated using a standard curve (Diformazan 1-100 nmol/ml).

Evaluation of Antioxidant Capacity of CsE and CsH. The 2,2-diphenyl-1-picryl-hydrazyl-hydrate (DPPH) method is the first approach for the evaluation of the antioxidant potential of a compound, an extract, or other biological sources. It is a spectrophotometric method (Kedare and Singh, 2011) based on the reduction of DPPH, a stable free radical, to the yellow-colored product, diphenyl-picrylhydrazine, with the reduction of the absorbance at 517 nm. Briefly, 50 µL of various concentrations of CsH and CsE were added to 5 mL of a 0.004% methanol solution of DPPH. After a 30 min of incubation period at room temperature, the absorbance was measured against a blank at 517 nm using an ELX 808 microplate reader (Biotek, Germany). Gallic acid, a strong antioxidant compound, was used as a control, and tests were repeated four times.

Statistical Analysis. Statistical analyses were performed using SPSS software (version 22; Chicago, USA). The Kruskal-Wallis test and Mann-Whitney U test were used to compare the groups. In addition, the Wilcoxon test was applied for the comparison of different parameters on days 9 and 14 of the treatment. Graphs were plotted using GraphPad Prism software (version 8; San Diego, CA, USA). Finally, p-values of ≤ 0.05 were considered statistically significant.

Results

Elimination of 5-FU Effect on Weight and Food and Water intake by CsH and CsE. Table 1 shows the effects of different treatments on the weight and dietary and water intake of the animals on days 9 and 14 and weight of the livers at the end of the experiment. As shown, 5-FU treatment caused weight loss, malnutrition, and decrease in the water intake and significantly reduced the weight of the livers in group II, compared to that reported for the control group. The CsH and CsE gavage feeding not only showed the same profile as the control group in groups III and IV of the experimental rats but also significantly reduced the negative effects of 5-FU on the above-mentioned parameters with no significant changes in the liver weight in groups V and VI, compared to those of group II.

Normalization of Effects of 5-FU on Markers of Liver Function by CsH and CsE. The 5-FU treatment induced hepatotoxicity in the adult male rats by a significant increase ($P<0.05$) in the levels of ALT, AST, and ALP (Figures 1A and 1B) and decrease ($P<0.05$) in the levels of Alb and TP (Figures 2A and 2B) in the serum and liver of the treated rats, compared to the control group. As demonstrated, the treatment with CsH and CsE resulted in a significant increase only in the Alb and TP levels in the liver of the rats of groups III and IV with no significant changes in other parameters, compared to that of the control group. The treatment of the rats in groups V and VI normalized the liver function ($P<0.05$) in the serum and liver in comparison to the treatment of the rats with 5-FU only in group II.

Elevation of Levels or Activities of Nonenzymatic and Enzymatic Antioxidant Parameters by CsH and CsE. As illustrated in Figures 3 and 4, the treatment with 5-FU induced a significant ($P<0.05$) decrease in GSH, TAC, CAT, GPx, GR, GST, and SOD and increase in the MDA and O_2^- levels/activities, compared to the control group. The CsH or CsE treatment resulted in a significant increase in the levels/ activities of GSH (4-6 times),

TAC (two to three times), GPx (eight times), and SOD (two times) in comparison to the control group. A significant decrease (one half) was also observed in MDA and O_2^- levels, compared to that reported for group II. The treatment in the CsH+5-FU and CsE+5-FU groups significantly elevated ($P<0.05$) the activities of antioxidant enzymes and decreased the MDA and superoxide anion levels in the serum and liver of the rats, compared to that of group II.

CsH and CsE as Strong Antioxidant Compounds. According to the results of the DPPH assay, CsH and CsE are very good free radical scavengers. The half maximal inhibitory concentrations (IC_{50}) for CsH and CsE were determined at 242.4±8.3 and 115±4.6 mg/L, respectively. These compounds showed lower antioxidant activities in comparison to the standard gallic acid presenting IC_{50} of 26.3±1.4 mg/L; however, they had strong antioxidant potentials, compared to those reported for many natural compounds.

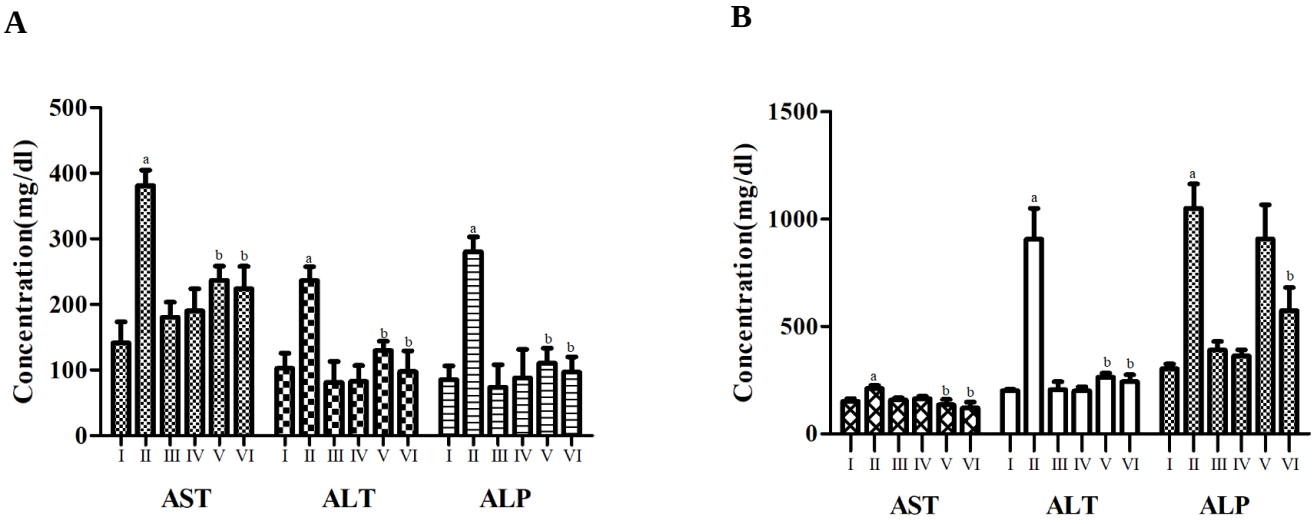

Figure 1. Effects of 5-fluorouracil (5-FU), *Cyrtopodion scabrum* homogenate (CsH), and *Cyrtopodion scabrum* extract (CsE) on the levels of aspartate aminotransferase (AST), alanine aminotransferase (ALT), and alkaline phosphatase (ALP) (mean±standard error of the mean); **A)** liver; **B)** serum; groups: **1:** control; **2:** 5-FU (5-FU 50 mg/kg daily); **3:** CsH (1600 mg/kg daily); **4:** CsE (120 mg/kg daily); **5:** CsH (1600 mg/kg daily) + 5-FU (50 mg/kg daily); **6:** CsE (120 mg/kg daily) + 5-FU (50 mg/kg daily); **a** vs. control and **b** vs. 5-FU group considered significant at $P<0.05$ (Mann-Whitney U test and Kruskal-Wallis test)

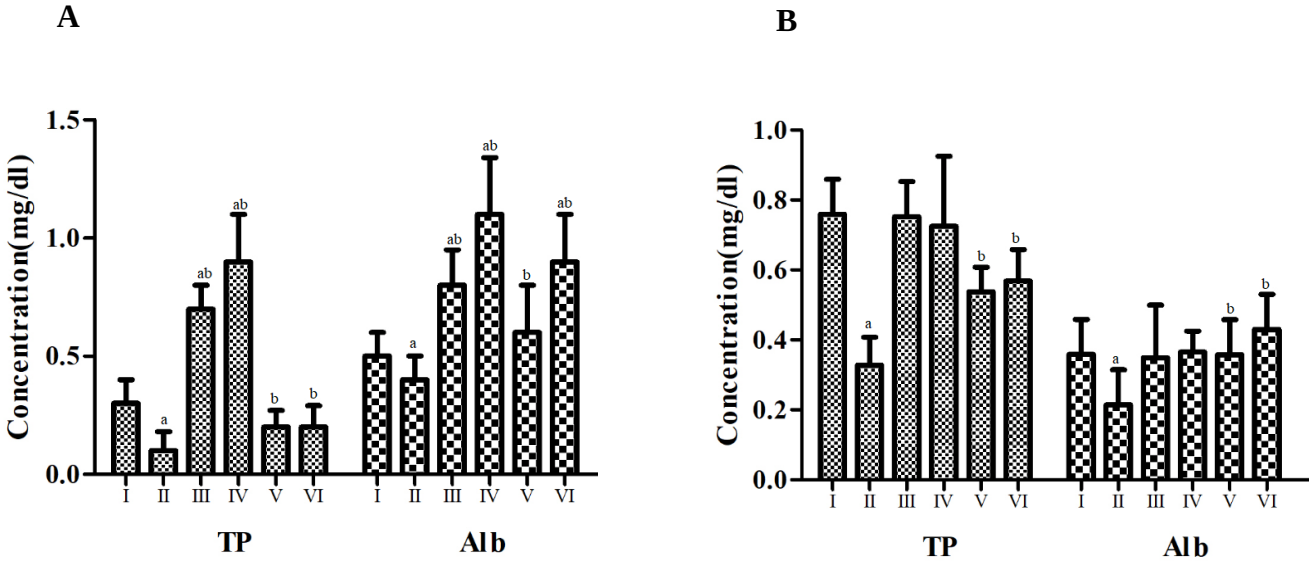

Figure 2. Effects of *Cyrtopodion scabrum* homogenate (CsH) and *Cyrtopodion scabrum* extract (CsE) on the levels of total protein (TP) and albumin (Alb) (mean±standard error of the mean); **A**) liver; **B**) serum; groups: **1:** control; **2:** 5-fluorouracil (5-FU; 50 mg/kg daily); **3:** CsH (1600 mg/kg daily); **4:** CsE (120 mg/kg daily); **5:** CsH (1600 mg/kg daily) + 5-FU (50 mg/kg daily); **6:** CsE (120 mg/kg daily) + 5-FU (50 mg/kg daily; **a** vs. control and **b** vs. 5-FU group considered significant at P<0.05 (Mann-Whitney U test and Kruskal-Wallis test)

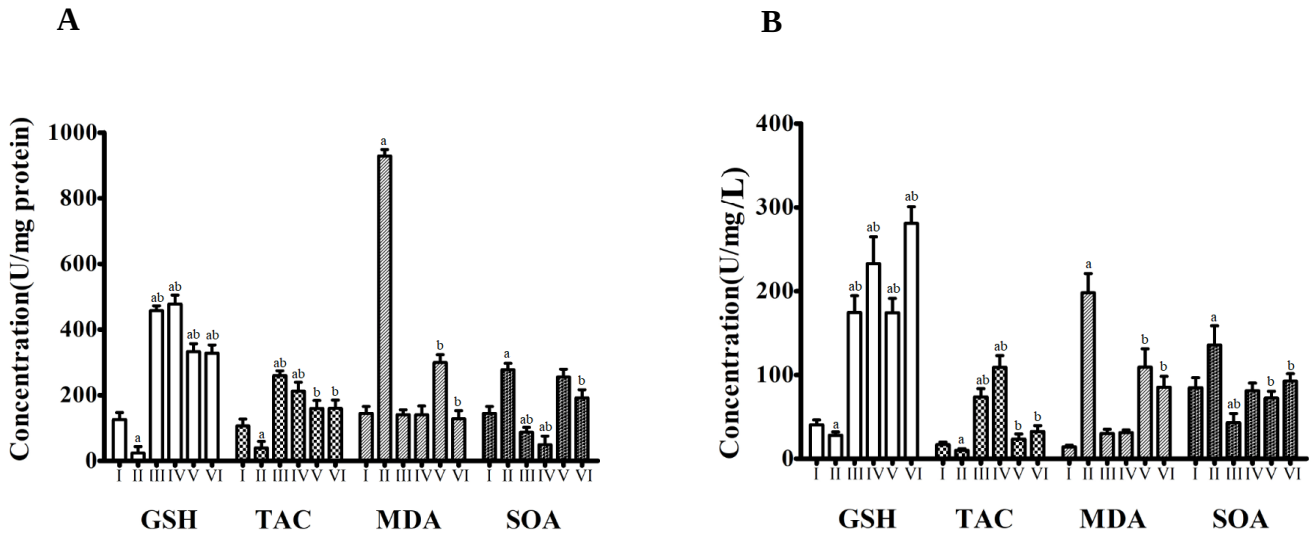

Figure 3. Effects of *Cyrtopodion scabrum* homogenate (CsH) and *Cyrtopodion scabrum* extract (CsE) on the levels of nonenzymatic antioxidant parameters (mean±standard error of the mean); **A)** liver; **B)** serum; groups: **1:** control; **2:** 5-fluorouracil (5-FU; 50 mg/kg daily); **3:** CsH (1600 mg/kg daily); **4:** CsE (120 mg/kg daily); **5:** CsH (1600 mg/kg daily) + 5-FU (50 mg/kg daily); **6:** CsE (120 mg/kg daily) + 5-FU (50 mg/kg daily); **a** vs. control and **b** vs. 5-FU group considered significant at P<0.05 (Mann-Whitney U test and Kruskal-Wallis test); GSH: Reduced glutathione; TAC: Total antioxidants capacity; MDA: Malondialdehyde; SOA: Superoxide anion

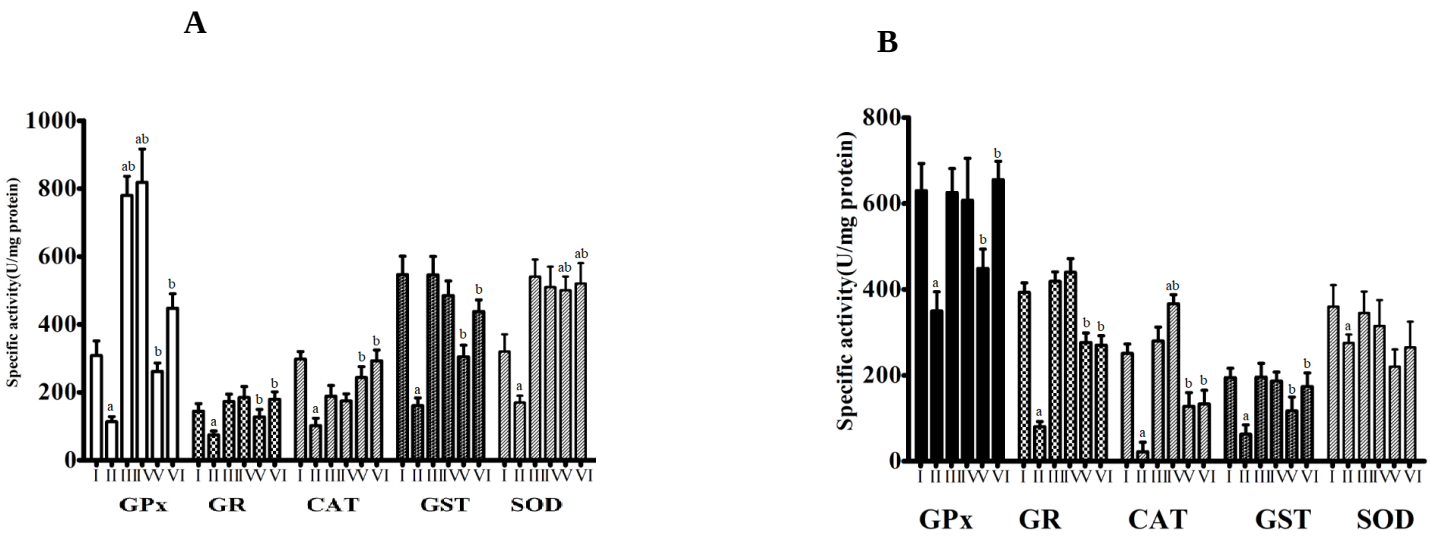

Figure 4. Effects of *Cyrtopodion scabrum* homogenate (CsH) and *Cyrtopodion scabrum* extract (CsE) on the specific activity of antioxidant enzymes (mean±standard error of the mean); **A)** liver; **B)** serum; groups: **1**: control; **2:** 5-fluorouracil (5-FU; 50 mg/kg daily); **3:** CsH (1600 mg/kg daily); **4:** CsE (120 mg/kg daily); **5:** CsH (1600 mg/kg daily) + 5-FU (50 mg/kg daily); **6:** CsE (120 mg/kg daily) + 5-FU (50 mg/kg daily); **a** vs. control and **b** vs. 5-FU group considered significant at P<0.05 (Mann-Whitney U test and Kruskal-Wallis test); GPx: Glutathione peroxidase; GR: Glutathione reductase; CAT: Catalase; GST: Glutathione S-transferase; SOD: Superoxide dismutase

Table 1. Effects of 5-fluorouracil, Cyrtopodion scabrum homogenate, and Cyrtopodion scabrum extract on the averages of body weight and dietary and water intake on days 9 and 14 and weight of the livers on day 14th

		Body weight (g)		Food intake (g/day)		Water intake (ml/day)		Weight of the livers
Groups	***n***	Day 9	Day 14	Day 9	Day 14	Day 9	Day 14	Day 14th
Control	10	237.6±5.0	260.8±5.6	19.4±0.9	19.3±0.3	41.5±7.7	40.9±2.0	8.8±1.3
5-FU	9	236.9±5.4	224.1±3.7^{ab}	19.2±1.4	3.3±4.2^{ab}	44.0±8.2	10.8±8.3^{ab}	6.7±0.8^{a}
CsH	10	244.7±4.8	273.1±4.7	19.7±0.4	20.0±0.0	46.7±10.5	45.7±3.0	8.6±1.6
CsE	10	241.6±4.2	268.2±3.6	19.8±0.9	20.0±0.0	48.7±8.0	45.2±1.7	9.4±1.3
CsH+5-FU	10	245.7±6.6	248.4±6.7^{ab}	19.5±1.3	6.8±2.5^{ab}	44.4±12.7	19.6±4.1^{ab}	6.6±1.1^{a}
CsE+5-FU	9	244.3±4.7	245±4.5^{ab}	19.6±1.3	7.9±2.7^{ab}	44.1±9.8	18.7±3.3^{ab}	7.2±0.9^{a}

1. Data expressed as mean±standard error of the mean; in each column, figures bearing different letter superscripts significantly different at P<0.05 (Mann-Whitney U test and Wilcoxon test) (a: compared to control on day 14; b: compared to 5-fluorouracil group on day 14)
5-FU: 5-fluorouracil; CsH: *Cyrtopodion scabrum* homogenate; CsE: *Cyrtopodion scabrum* extract

Discussion

Cancers are the most devastating and stubborn human diseases in the world. The common chemotherapy methods are not redoubts to cancer therapy, because they also cause many oxidative damages to normal tissues. Therefore, new anticancer complementary and alternative compounds with no or fewer side effects are highly required (Ding et al., 2016). In recent years, TM has introduced plants, animals, or microorganisms as inexhaustible natural resources for the discovery of drugs with a protective role in oxidative damages (Riaz et al., 2018). For example, astragalin, eupatilin, and allicin are defensive compounds extracted from plants with anti-cancer, anti-inflammatory, and anti-oxidant properties (Nageen et al., 2018; Salehi et al., 2019).

In recent years, a series of experiments have been performed investigating the antiproliferative and antitumor properties of *C. scabrum* in vitro and in vivo. It has been previously reported that CsE selectively inhibited the growth of human cancer cells with no significant effect on normal cells. It was suggested that the mechanism of *C. scabrum* is probably apoptosis and G2 cell cycle arrest through P21. It was also shown that this lizard extract effectively cured the tumor in the CT26-tumor-bearing mice model (CRC) with no/fewer side effects on the mice, compared to the 5-FU treatment in animals (unpublished data).

According to several studies reporting the oxidative damages of 5-FU in cancer patients (Burits et al., 2001) and role of the antioxidant system in the suppression of cancerous cells (Wang et al., 2017), it was decided to investigate the antioxidant potential and probable hepatoprotective effects of this Gecko. The results of the present study showed that CsH and aqueous extract of *C. scabrum* (CsE) had strong antioxidant activities, with higher (two times) potency in CsE in comparison to that reported for CsH in the corresponding used doses.

During the injection of 5-FU for 5 days, not only the mice did not gain weight but also the average body weight reduced by 12.8 g, compared to the mice's weight before the treatment. The food and water intake decreased about 6- and 4-fold, respectively, and the weight of the liver reduced 1.4-fold at the end of the treatment in comparison to those reported for the control group. The attenuating effects of 5-FU observed in this investigation are in agreement with the results of other published studies demonstrating the effect of 5-FU administration on a significant reduction in the food intake and body weight with no significant changes in the liver weight of the rats (Wang et al., 2017).

The results of the current study showed that the administration of CsH and CsE in the corresponding doses together with 5-FU inhibited the weight loss and caused an increase in the food and water intake approximately 2.2- and 1.8-fold, respectively, in comparison to those reported for group II. The protective roles of CsH and CsE in the preventing of 5-FU-induced hepatotoxicity in rats were evident when the hepatic biomarkers were estimated at the end of the experiment and compared to the control and 5-FU groups. Hepatotoxicity due to 5-FU was shown by alteration in the liver function parameters. The CsH and CsE played a significant protective role in hepatotoxicity induced by 5-FU and normalized the serum and hepatic biomarkers of liver function. The unfavorable effects of 5-FU on liver function parameters were also shown by other studies.

The 5-FU significantly increased the levels of MDA and O_2^- and decreased the levels of GSH and TAC in the serum and liver representing the oxidative damages. The CsH and CsE increased the level of antioxidant parameters of the body, such as GSH (3.9- and 4.9-fold) and TAC (3.3- and 4-fold), in the serum and liver in comparison to the control, without any oxidative damages to the liver. Furthermore, these antioxidant compounds suppressed the levels of MDA and O_2^- and elevated the levels of GSH and TAC in the serum and liver, compared to those of groups I and II.

There was a significant decrease in the antioxidant enzyme in 5-FU treatment, compared to that of the control group. The SOD acts as the first line of defense against the adverse effects of oxygen radicals in the tissues. The SOD converts the superoxide to H_2O_2 and

O_2, and then CAT and GPx are required to degrade H_2O_2 to H_2O. The GR catalyzed the reduction of GSSG to reduced GSH, which is necessary for resisting oxidative stress and maintaining the reduction of cell environment. The GSTs previously known as ligandins catalyze the conjugation of the reduced form of GSH to xenobiotic substrates in the detoxification process (Mostafavi-Pour et al., 2008; Chen et al., 2018). The treatment with CsH and CsE significantly increased the activities of GPx and SOD in the liver and serum in comparison to the control, and these two antioxidant natural compounds increased the activities of antioxidant enzymes, thereby leading to the protection against the hepatotoxic effects of 5-FU in the serum and liver on 5-FU-treated rats.

According to recent studies carried out by the authors of this article and previous studies regarding the antitumor properties of *C. scabrum*, it is suggested that *C. scabrum* derivatives are the natural compounds with high antioxidants potential and no/fewer toxic effects on the normal tissues and can be used for cancer treatment as alternatives or complementary drugs.

Authors' Contribution

M. D. performed the experiments and drafted the article. A. S., Z. B., S. N. V., M. N., and M. A. helped with performing the study, contributing new reagents/analytical tools, and analyzing the data. M. D. and A. S. designed the study, analyzed the data, wrote, edited, revised, and approved the final version of the manuscript. All the authors read and approved the final manuscript.

Ethics

We hereby declare all ethical standards have been respected in preparation of the submitted article. Ethics ID: IR.SUMS.REC.1397.750

Conflict of Interest

The authors declare that they have no conflict of interest.

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