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

Diba, M. 1, Seghatoleslam, A. 2*, Namavari, M. 3, Assadi, Sh. 1, Vakili, S. N. 4, Babaei, Z. 1, Akmali, M. 1

1. Department of Biochemistry, School of Medicine, Shiraz University of Medical Sciences, Shiraz, Iran
2. Research Center for Traditional Medicine and History of Medicine, School of Medicine, Shiraz University of Medical Sciences, Shiraz, Iran
3. Razi Vaccine and Serum Research Institute, Shiraz Branch, Agricultural Research, Education and Extension Organization (AREEO), Tehran, Iran
4. Department of Virology, School of Medicine, Shiraz University of Medical Sciences, Shiraz, Iran

Corresponding Author: seghatoleslama@yahoo.com

Abstract

Chemotherapy is the main approach for the treatment of cancer but is often causes unpleasant oxidative damages. Therefore developing an effective alternative/complementary therapy with improved tumor suppression efficiency and lower adverse effects is highly required. Recently we showed that *Cyrtopodion Scabrum* extract is an effective and selective tumor suppressor medicine. In this study, the antioxidant activity of *Cyrtopodion Scabrum* homogenate (CsH) and extract (CsE), and their effects on attenuating 5-FU-induced liver dysfunction in rat was investigated. 60 Male rats (200-220 gr) were divided into six groups and treated for 14 days. The control (group I) and 5-FU (group II) groups were received distilled water and 5-FU, respectively. Four groups were administered by CsE, CsH, CsE+5-FU and CsH+5-FU (groups III to VI), orally by gavages in a daily schedule. 5-FU-induced oxidative damage was evaluated by changes in the weight, food and water intake during treatment and the antioxidant parameters in the liver and serum of the treated rats. Data indicated that the administration of CsH and CsE improved liver function and antioxidants
defense system significantly by attenuating the levels or activities of MDA, Superoxide anion, AST, ALT, ALP and decrease of SOD, CAT, GPx, GR, GST, TAC, GSH, TP, and Alb in the liver and serum, induced by 5-FU treatment. Our data suggests that CsH and CsE play a protective role against the imbalance elicited by 5-FU and can be used as alternative/complementary supplement with 5-FU to reduce oxidative damages which is the consequence of ROS (Reactive oxygen species) production in cancerous patients.

**Keyword:** Cyrtopodion Scabrum, 5-FU, antioxidant parameters, oxidative damages

**Introduction**

Cancer is a group of diseases involving abnormal cell growth, with the potential to invade or spread to other parts of the body. It is responsible for 13% of all deaths in the world and is the third leading cause of death in Iran. The prevalence of cancer is predicted to proceed up to 45% in 2025 (Amirkhan et al., 2017; Amoori et al., 2014). 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 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 in different research during years have been the increasing of antitumor efficacy and reducing drug-associated toxicity.

Reactive oxygen species (ROS) are often released by normal and tumor cells in response to chemotherapy, and plays 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 the oxidative stress (Seghatoleslam et al., 2014), unwanted toxicity in other different organs and tissues, and increasing the risk of therapeutic failure (Amiri et al., 2015). Today the world is moving towards the extension of traditional medicine and complementary drug production to increase efficacy and decrease side effects and costs of the treatments (Orhan et al., 2012).
Traditional medicine (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., 2017). According to traditional Chinese medicine (TCM), several studies demonstrated experimentally that a type of lizard named *swinhonis Guenther* has anti-cancer and anti-inflammatory properties (Rashidi et al., 2017). Recently, we have reported the anti-proliferative effects of *Cyrtopodion scabrum* (also known as home gecko) extract (CsE) on the human breast and colorectal cancer cells (Amiri et al., 2015). We also showed that CsE selectively inhibits the growth of various cell lines between 30-78%, with the highest inhibitory effect on SW742 (colorectal cancer), MKN45 (gastric cancer), and HepG2 (liver carcinoma) cell lines, respectively. We suggested that the mechanism of this growth inhibition probably is apoptosis and G2 cell cycle arrest through P21 (Tavakoli et al., 2015). The anti-tumor property of *C. scabrum* was also confirmed in vivo, using CT26-tumor-bearing mice model. The results (unpublished) showed that the extract of this lizard suppresses tumor growth effectively, with no or less side effects compared with 5-FU. *C. scabrum* is a species of genus *Cyrtopodion*, distributed in southwestern, central, eastern parts and north of Iran (Rastegar-Pouyani et al., 2011). In the recent years, the chemoprotective potential of some natural (Talas et al, 2015) or synthetic organoselenium (Talas et al, 2009) compounds have been studied by evaluation of antioxidant parameters.

In this study we investigated the potential anti-oxidant activity and protective roles of C.Scabrum extract (CsE) and homogenate (CsH) against 5-FU-induced oxidative damage in rat.

Materials and Methods

**Pharmacological materials**

5-Fluorouracil (5-FU) produced by EBEWE Pharma (Ges.m.b.H.Nfg.KG.A 4866 Unterach, AUSTRIA). Ketamine 10% was purchased from AlfasanCompany (WOERDEN, HOLLAND).

**Preparation of the whole body homogenate (CsH) and aqueous extract (CsE) from C.Scabrum**
C. Scabrum was provided by Razi Research Institute of Vaccine & Serum, Shiraz Branch, Iran. For CsH preparation the required amount of C. Scabrum was homogenized and dissolved in distilled water, then freeze dried in the Laboratory freeze dryer Alpha 1-2/ LD plus (Christ, Germany) and kept at -20°C until use. CsE preparation was according to the protocol described previously (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 distilled water (DW) using dialysis bag of pore size 1-µm. The solution then freeze dried in the Laboratory freeze dryer Alpha 1-2/ LD plus (Christ, Germany), and the powder were then kept at -20°C until use.

**Experimental designs**

A total of 60 Sprague-Dawley male rats (200-220 g of weight) were obtained from the Animal Center of Shiraz University of Medical Sciences and randomly distributed into 6 groups, each including 10 rats. Group I (Control) received 3 ml intragastric water daily for 14 days. Group II (5-FU-only group) was given 3 ml intragastric water daily for 14 days, and on the day 10 to 14, received 5-FU (50 mg/kg BW) 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 distilled water, orally by gavages, in a daily schedule for 14 days, respectively. Groups V (CsH+5-FU group) and VI (CsE+5-FU group) were administered by CsH (1600 mg/kg BW) and CsE (120 mg/kg BW) dissolved in 3 ml distilled water orally by gavages for 9 days, and 5-FU (50 mg/kg BW) from the day 10th to 14th 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 day 15th, 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%), 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 centrifuged immediately at 12000×g for 15 min at 4°C (28). Finally the clear supernatant were separated and used for the biochemical analysis.

**Biochemical analysis**

**Liver function assessment**

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

**Measurement of anti-oxidant parameters**

**Glutathione peroxidase (GPx) assay**

GPx activity was determined by continuous monitoring of the generation of reduced GSH from oxidized glutathione (GSSG) upon the consumption of NADPH by glutathione reductase (GR) according to the method of Fecondo & Augusteyn (Fecondo et al., 1983). The oxidation of NADPH is directly proportional to the GPx activity by reductionin the absorbance at 340 nm at 37°C for 5 min.
**Glutathione reductase (GR) assay**

GR catalyzes the reduction of GSSG to GSH by consumption of one molecule of NADPH. The assay was performed following the method described previously (Carlberg et al., 1985) with minor modifications. The decrease in the absorbance was monitored spectrophotometrically at 340 nm at RT for 3 min.

**Catalase (CAT) assay**

CAT activity was determined according to the Aebi’s procedure based on the content of H$\text{2O}_2$ that decomposes (Carlberg et al., 1985). Briefly, 150 µL of phosphate buffer (pH = 7.0 and 66.7 mM), 10 µL of sample and 365 µL of distilled water were added to 75 µL of H$\text{2O}_2$ (120 mmol/L) and the utilization of H2O2 was followed spectrophotometrically at 240 nm at 25°C for 2 minutes. The CAT activity was expressed as µmol H2O2 consumed per min per mg enzyme.

**Glutathione S-transferase (GST) assay**

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

**Superoxide dismutase (SOD) assay**

SOD activity was determined as described previously (Fridovic et al., 1969). SOD inhibits the oxidation of epinephrine hydrochloride resulted in a decrease in the absorbance at 480 nm.

**Reduced Glutathione (GSH) assay**

GSH, as the main antioxidant parameter in the body, was measured by colorimetric quantification method (Sun et al., 2001). The process is based on the formation of GS-TNB complex from 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 standard curve.

**Estimation of lipid peroxidation**

Malondialdehyde (MDA) is the indirect index of lipid peroxidation. The MDA level was determined (Hagar et al., 2006) as follows: Briefly, 0.5 ml of sample was added to 2 ml of TBA reagent (containing: 0.375% TBA, 15% trichloroacetic acid, 0.25 mol/L HCL), then, the mixture was boiled for 15 min, 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 anti-oxidant capacity (TAC)**

The TAC of the samples, was determined using a minor modification of the FRAP assay of Benzie and Strain (Peter et al., 2011). FRAP reagent was prepared from glacial acetic acid and 300 mM acetate buffer (pH 3.6), 10 mM 4, 6-tripryridyl-s-triazine (TPTZ) made up in 40 mM HCl and 20 mM ferric chloride. All solutions were mixed together in the ratio of 10:1:1. FRAP assay was performed by warming 1 mL of dH₂O to 37 °C, 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 were measured against a standard of known FRAP value, ferrous sulfate (1000 μM).

**Estimation of superoxide anion (O₂⁻) level**

Serum and hepatic O₂⁻ concentrations were measured by the ability of O₂⁻ to reduce nitroblue tetrazolium (NBT) to an insoluble formazan (Sandhya et al., 2000). NBT assay is a simple, reliable and sensitive technique. The samples were incubated with 0.1% NBT dissolved in PBS (pH 7.4) at 37°C for 30 min. finally, 0.6 ml glacial acetic acid was added to the samples and the absorbance was read at 560 nm. The level of O₂⁻ was calculated using standard curve (Diformazan 1-100 nmol/ml).

**Evaluation of antioxidant capacity of CsE and CsH**
DPPH method is the first approach for evaluating the antioxidant potential of a compound, an extract or other biological sources. It is a spectrophotometric method (Sagar et al., 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 the CsH and CsE were added to 5mL of a 0.004% methanol solution of DPPH. After a 30min incubation period at room temperature the absorbance was measured against a blank at 517nm using an ELX 808 microplate reader (Biotek, Germany). Gallic acid, a strong antioxidant compound, was used as a control and tests were repeated for four times.

Statistical analysis

Statistical analyses were performed using SPSS22 software (Chicago, USA). Kruskal-Wallis and Mann-Whitney U tests were used to compare the groups. Also, the Wilcoxon test was used for the comparison of different parameters on the days 9 and 14 of the treatment. Graphs were plotted using graph pad prism8 software (San Diego, CA, USA). Finally, P-values ≤ 0.05 were considered as significant.

Results

CsH and CsE eliminate the 5-FU influence on the weight, food and water intake

Table 1 shows the effects of different treatments on the weight, dietary and water intake of the animals on the days 9th and 14th, and weight of the livers at the end of the experiment. As shown, 5-FU treatment causes weight loss, malnutrition and decrease in the water intake and reduced weight of the livers in the group II significantly when compared to the control. CsH and CsE gavage feeding not only showed the same profile as control group in the 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 on the liver weight in the groups V and VI compared with the group II.

CsH and CsE normalize the effects of 5-FU on the markers of liver function

DPPH method is the first approach for evaluating the antioxidant potential of a compound, an extract or other biological sources. It is a spectrophotometric method (Sagar et al., 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 the CsH and CsE were added to 5mL of a 0.004% methanol solution of DPPH. After a 30min incubation period at room temperature the absorbance was measured against a blank at 517nm using an ELX 808 microplate reader (Biotek, Germany). Gallic acid, a strong antioxidant compound, was used as a control and tests were repeated for four times.

Statistical analysis

Statistical analyses were performed using SPSS22 software (Chicago, USA). Kruskal-Wallis and Mann-Whitney U tests were used to compare the groups. Also, the Wilcoxon test was used for the comparison of different parameters on the days 9 and 14 of the treatment. Graphs were plotted using graph pad prism8 software (San Diego, CA, USA). Finally, P-values ≤ 0.05 were considered as significant.

Results

CsH and CsE eliminate the 5-FU influence on the weight, food and water intake

Table 1 shows the effects of different treatments on the weight, dietary and water intake of the animals on the days 9th and 14th, and weight of the livers at the end of the experiment. As shown, 5-FU treatment causes weight loss, malnutrition and decrease in the water intake and reduced weight of the livers in the group II significantly when compared to the control. CsH and CsE gavage feeding not only showed the same profile as control group in the 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 on the liver weight in the groups V and VI compared with the group II.

CsH and CsE normalize the effects of 5-FU on the markers of liver function
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 (Fig 1, A and B) and decrease (P < 0.05) in the levels of albumin and total protein (Fig 2, A and B) in the serum and liver of the treated rats when 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 the groups III and IV with no significant changes on the other parameters compared to the control. Interestingly, the treatment of rats in the groups V and VI normalized the liver function (P<0.05) in the serum and liver when compared to 5-FU- only treated rats in group II.

**CsH and CsE elevate the levels or activities of Non-enzymatic and Enzymatic antioxidant parameters**

As seen in the Figure 3 and 4, treatment with 5-FU induced a significant (P < 0.05) decrease in the GSH, TAC, CAT, GPx, GR, GST and SOD and increase in the MDA and O$_2^-$ levels/activities compared to the control. Interestingly, the treatment CsH or CsE resulted in a significant increase in the levels/activities of GSH (4-6 times), TAC (2-3 times), GPx (8 times) and SOD (~2 times) compared to control. A significant decrease (~one half) was also observed in MDA and O$_2^-$ levels compare to group II. The treatment in the groups CsH+5-FU and CsE+5-FU elevated significantly (P < 0.05) the antioxidant enzymes activities and decreased the MDA and superoxide anion levels in the serum and liver of the rats compared to group II.

**CsH and CsE are strong antioxidant compounds**

According to the DPPH assay results, CsH and CsE are very good free radical scavengers. The IC50 for CsH and CsE was determined as 242.4±8.3 mg/L and 115±4.6 mg/L, respectively. These compounds showed a lower antioxidant activity in comparison with the standard gallic acid that presented IC50 values of 26.3±1.4 mg/L, but have strong antioxidant potential compared with many natural compounds.

**Discussion**
Cancers is the most devastating and stubborn human disease in the world. The common chemotherapy methods do not redoubt to cancer therapy, because they have very oxidative damages on normal tissues as well. Thus, the new anti-cancer complementary and alternative compounds with no or less side effects are highly required (Ding et al., 2016). In recent years, Traditional Medicine has introduced plants, animals or microorganisms as an inexhaustible natural resource for discovery of many drugs with protective role against oxidative damages (Riaz et al, 2018). For example, Astragalin, Eupatilin and Allicin are defensive compounds that extracted from plants and have anti-cancer, anti-inflammatory and anti-oxidant properties (Nageen et al, 2018; Salehi et al, 2019).

In the recent years, we have performed a series of experiments investigating the anti-proliferative and anti-tumor properties of *Cyrtopodion Scabrum*, *in vitro* and *in vivo*. We have reported previously that *C. scabrum* extract selectively inhibited the human cancer cells growth with no significant effect on the normal cells. We suggested that the mechanism of *Cyrtopodion scabrum* probably is apoptosis, and G2 cell cycle arrest through P21. We also showed that this lizard extract effectively cured the tumor in the CT26-tumor-bearing mice model (colorectal cancer) with no/lesser side effect on the mice compared with 5-FU treated animals (unpublished data).

According to several studies reporting the oxidative damages of 5-FU in cancer patients (Burits et al., 2001) and the role of antioxidant system in the suppression of cancerous cells (Wang et al., 2017), we decided to investigate the antioxidant potential and the probable hepato-protective effects of this Gecko.

Our results showed that the homogenate (CsH) as well as the aqueous extract (CsE) of *C. Scabrum* have strong antioxidant activity, with higher (two times) potency in CsE compared to CsH in the corresponding doses used.

During the injection of 5-FU for 5 days, not only the mice did not get weight but also the average of the body weight declined for ~12.8 gr compared to the mice weight before treatment. The food and water intake
was decreased for about 6 and 4 fold, respectively, and the weight of the liver for 1.4 fold, at the end of the treatment in comparison with the control group I.

The attenuating effects of 5-FU observed in this investigation is in agreement with the other published studies demonstrating the effect of 5-FU administration in a significant reduction in the food intake and body weight with no significant changes in the liver weight of the rat (Wang et al., 2017).

The results of our 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 folds, respectively in comparison to the group II.

The protective roles of CsH and CsE in the preventing of 5-FU induced hepatotoxicity in rats are evident when the hepatic biomarkers were estimated at the end of the experiment and compared to the control and 5-FU group. Hepatotoxicity due to 5-FU was revealed by alteration in the liver function parameters. CsH and CsE played a significant protective role against 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 showed by other studies by Gelen et al. as well.

5-FU increased the levels of MDA and \( \text{O}_2^- \) and decreased the levels of GSH and TAC in serum and liver significantly representing the oxidative damages. 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 control, without any oxidative damage on the liver. Also, these antioxidant compounds suppressed the levels of MDA and \( \text{O}_2^- \) and elevated the levels of GSH and TAC in the serum and liver compared with groups I and II.

We observed a significant decrease in the antioxidant enzyme upon 5-FU treatment compared to the controls. SOD acts as the first line of defense against the adverse effects of oxygen radicals in the tissues. SOD converts the superoxide to H2O2 and O2, and then CAT and GPx are required to degrade H2O2 to H2O. Glutathione reductase catalyzed the reduction of glutathione oxidized to reduced glutathione, which
is necessary for resisting oxidative stress and maintaining the cells environment reduction. GST previously known as ligandins, catalyzes the conjugation of the reduced form of glutathione (GSH) to xenobiotic substrates in the detoxification process (Chen et al., 2018; Mostafavi-Pour et al., 2008). Treatment with CsH and CsEin increased the activities of GPx and SOD in the liver and serum in comparison to the controls significantly, and these two antioxidant natural compounds increased the activities of antioxidant enzymes, thus leading to the protection against the hepatotoxic effects of 5-FU in serum and liver on 5-FU-treated rats.

According to our recent and previous studies regarding the anti-tumor properties of C.Scabrum, we suggest that C.Scabrum derivatives are the natural compounds with high antioxidants potential and no/less toxic effects on the normal tissues and can be used for cancer treatment as alternative or complementary drugs.

Acknowledgements

The authors would all like to acknowledge RCC department of vice chancellor for research of Shiraz University of Medical Sciences, for English editing of the manuscript. This article was extracted from the MSc thesis written by MohamadrezaDiba.

Funding

All part of the present study was financially supported by research grant no. 97-01-01-17099, from Shiraz University of Medical Sciences, Shiraz, Iran.

Authors’ contributions

MD performed the experiments and drafted the article. SA, ZB, SNV, MN, MA helped with performing the research, contributing new reagents/analytical tools and analyzing the data. MD and AS designed research, analyzed data, wrote, edited, revised and approved the final version of the manuscript. All authors read and approved the final manuscript.
References


The effects of 5-FU, CsH and CsE on the levels of AST, ALT and ALP (Mean ± SEM). 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 were considered significant at P-value < 0.05 (Mann-Whitney U and Kruskal-Wallis test). AST: Aspartate amino transferase. ALT: Alanine amino transferase ALP: Alkaline phosphatase.
Figure 2: The effects of CsH and CsE on the levels of total protein and albumin (Mean ± SEM). 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 were considered significant at \( P_{\text{value}} < 0.05 \) (Mann-Whitney U and Kruskal-Wallis test). Alb: Albumin. TP: Total protein.
Figure 3:

The effects of CsH and CsE on the levels of non-enzymatic antioxidant parameters (Mean ± SEM). 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 were considered significant at \( P_{\text{calc}} < 0.05 \) (Mann-Whitney U and Kruskal-Wallis test). GSH: Reduced glutathione. TAC: Total antioxidants capacity. MDA: Malondialdehyde. SOA: Superoxide anion.
Figure 4:

The effects of CsH and CsE on the specific activity of antioxidant enzymes (Mean ± SEM). 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 were considered significant at P<0.05 (Mann-Whitney U and Kruskal-Wallis test). GPx: Glutathione peroxidase. GR: Glutathione reductase. CAT: Catalase. GST: Glutathione S-transferase. SOD: Superoxide dismutase.
Table 1: The effects of 5-FU, CsH and CsE on the averages of body weight, dietary and water intake on the day 9 and day 14 and weight of the livers on the day 14.

<table>
<thead>
<tr>
<th>Groups</th>
<th>n</th>
<th>Body weight(gr)</th>
<th>Food intake(gr/day)</th>
<th>Water intake(ml/day)</th>
<th>Weight of the livers</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Day 9(^{th})</td>
<td>Day 14(^{th})</td>
<td>Day 9(^{th})</td>
<td>Day 14(^{th})</td>
</tr>
<tr>
<td>Control</td>
<td>10</td>
<td>237.6 ± 5.0</td>
<td>260.8 ± 5.6</td>
<td>19.4 ± 0.9</td>
<td>19.3 ± 0.3</td>
</tr>
<tr>
<td>5-FU</td>
<td>9</td>
<td>236.9 ± 5.4</td>
<td>224.1 ± 3.7(^{ab})</td>
<td>19.2 ± 1.4</td>
<td>3.3 ± 4.2(^{ab})</td>
</tr>
<tr>
<td>CsH</td>
<td>10</td>
<td>244.7 ± 4.8</td>
<td>273.1 ± 4.7</td>
<td>19.7 ± 0.4</td>
<td>20.0 ± 0.0</td>
</tr>
<tr>
<td>CsE</td>
<td>10</td>
<td>241.6 ± 4.2</td>
<td>268.2 ± 3.6</td>
<td>19.8 ± 0.9</td>
<td>20.0 ± 0.0</td>
</tr>
<tr>
<td>CsH+5-FU</td>
<td>10</td>
<td>245.7 ± 6.6</td>
<td>248.4 ± 6.7(^{ab})</td>
<td>19.5 ± 1.3</td>
<td>6.8 ± 2.5(^{ab})</td>
</tr>
<tr>
<td>CsE+5-FU</td>
<td>9</td>
<td>244.3 ± 4.7</td>
<td>245 ± 4.5(^{ab})</td>
<td>19.6 ± 1.3</td>
<td>7.9 ± 2.7(^{ab})</td>
</tr>
</tbody>
</table>

1. Data are expressed as mean ± SEM. In each column, figures bearing different letter superscripts are significantly different at P < 0.05 (Mann Whitney U and Wilcoxon test)(a: Compare with control on the day 14. b: Compare with 5-FU group on the day 14).