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Effects of *Froriepia subpinnata* extract on serum biochemicals and histopathological changes of liver in rats treated with trichloroacetic acid

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Abstract

Hepatocellular carcinoma is one of the challenges in health system in occurrence of which oxidative stress plays an important role. Considering *Froriepia subpinnata* (Anarijeh=FS) antioxidant effects, this study aimed to investigate its effect on preventing the occurrence of liver toxicity induced by Trichloroacetic acid (TCA) in animal model. FS hydroalcoholic extract was prepared from the aerial parts by maceration method. Forty-eight rats were divided into 8 groups as: control animals, treated with TCA (500 mg/kg) TCA+FS treated groups (100, 200, 400 mg/ kg), FS treated group 400 mg/kg, and doxorubicin (DOX) treated group (at 2.5 mg/ kg) and TCA + DOX treated group. After 28 days, blood was collected and serum was isolated. then Malondialdehyde (MDA), Glutathione peroxidase (GPx), Total Antioxidant Capacity (TAC), alanine aminotransferase (ALT), aspartate aminotransferase (AST), Alkaline phosphatase (ALP), tumor necrosis factor-alpha (TNF-alpha) were measured followed by liver tissue examination of histopathologically by light microscope. TCA significantly increased the amount of MDA and FS, at different concentrations, (100, 200, and 400mg/kg) decreased it compared to other groups ($P \leq 0.05$). The amount of TNFα was decreased by TCA but DOX increased considerably and FS treatment did not change the effect of TCA on TNFα level. Serum level of GPX, TAC, ALT, AST and ALP did not change by either TCA or FS treatment. TCA damaged liver tissue and caused hepatocyte degeneration, sinusoidal stenosis and vacuolization of cytoplasm. FS protected liver tissue in a dose dependent manner and at the dose of 400 mg/kg had better effect on reducing tissue damages. FS has a protective effect against histopathological changes induced by TCA in rat's liver tissue.

Key words: *Froriepia subpinnata*, Trichloroacetic acid, Oxidative stress, Hepatotoxicity

Introduction

Hepatocellular carcinoma (HCC) is responsible for 5.5% of all cancer cases globally and the second leading cause of cancer-related deaths worldwide (Seyfizadeh et al. 2019; Tupal et al. 2020). More than 90% of HCCs arise in the context

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of hepatic inflammation. Chronic liver inflammation leads to oxidative/nitrosative stress and lipid peroxidation (LPO), generating excess reactive oxygen species (ROS) and reactive nitrogen species (RNS) (Campos et al. 2020). Oxidative stress lead to damage of the liver which causes release of liver enzymes such as alanine aminotransferase, aspartate aminotransferase, and alkaline phosphatase into the plasma(Chen et al. 2020). On the other hand, one of the most important indicators of oxidative stress is malondialdehyde (Cherian et al. 2019). Moreover, ROS and RNS, as by-products of metabolism, are continuously produced in biological systems and cause damage to DNA, proteins and lipids. Recent studies indicate the role of ROS in enzymatic reactions, message transduction, and activation of nuclear transcription factors (Kruk et al. 2019). Mitochondrial electron transfer chain, nicotinamide adenine dinucleotide phosphate (NADPH) oxidase in phagocytic cell membranes, endoplasmic reticulum cytochrome P450 monooxygenase, xanthine oxidase, Fenton and Haber-Weiss reaction are responsible for ROS production in cell systems (Snezhkina et al. 2019). ROS modifies the function of proteins by regulating oxidation-reducing proteins, gene expression, redox-sensitive binding proteins, redox-sensitive enzymes, and regulating protein turnover(Zhang et al. 2016). Cancer cells produce more ROS than normal cells due to hypoxia, mutations in nuclear and mitochondrial genes, activation of oncogenes, and loss of tumor suppressor genes (Schumacker, 2006). In cancer cells, low to moderate levels of ROS are essential for cell development, differentiation, and survival, but at high levels it leads to cell death (Weinberg et al. 2010). Recent evidence suggests the role of ROS as a messenger in tumor cell invasion, angiogenesis and metastasis. Therefore, trying to reduce the ROS and NOS can be an adjunctive therapeutic strategy in the treatment of liver cancer. On the other hand, synthetic antioxidants had side effects and attempts are being made to use natural antioxidants for this purpose (Wang et al. 2010).

Trichloroacetic acid (TCA) is produced in the liver by metabolizing trichlorethylene, which is one of the most widely used organic solvents in the industry and is considered the 16th most toxic substance in the list of toxic substances in the United States (Yoo et al. 2015). TCA is commonly used in many educational and research laboratories and have various toxic effects on organs causing tumors (Mather et al. 1990). TCA side effects and toxicity probably caused through oxidative stress parameters, for example, in a study by Alzergy and Elgharbawy (2017), it was shown that TCA causes serious damage to liver tissue via oxidative stress, and *Juniperus phoenicea L.* with antioxidant properties could be effective in reducing this toxicity. Plants containing chemicals such as flavonoids, tannins, phenolic acids, terpenoids are the source of potentially natural antioxidants. These compounds could have anti-inflammatory and anti-cancer activity (Huang et al. 2009). In the recent years, many studies have been conducted on herbal antioxidant sources which decrease the lipid peroxidation process and production of ROS (Devasagayam et al. 2004).

Froriepia subpinnata (FS) belongs to Apiaceae also called the Umbelliferae family (Parsley family) with approximately 400 genera. Moreover, FS genera is Froriepia including three species: Froriepia subpinnata, Froriepia nuda and Froriepia gracillima, among which F. subpinnata is the only species growing naturally in northern Iran (Bahrami et al. 2021). It is an edible biennial plant which grows up to 150 cm with anti-flatulence, antiseptic, antispasmodic, anti-cancer, antifungal, antimicrobial and diuretic properties (Mozaffarian, 2007). In spite of the wide uses of *FS* in traditional medicine, there are

only a few documents on chemicals and biological activities of this beneficial plant (Bahrami et al. 2021; Mohammadzadeh et al. 2018; Morteza-Semnani et al. 2009; Rustaiyan et al. 2001). Considering the importance of the toxic effects of TCA and its effect on various parameters including inflammatory factors and oxidative stress indicators, in this experimental study the role of hydroalcoholic extract of *FS* aerial organs against possible changes in oxidative stress indicators, tissue necrosis factor (TNF) and biochemical factors related to liver function was investigated in the serum of rats exposed to TCA.

Materials and Methods

Animals and environmental conditions

In this study, forty eight adult male Wistar Rats were purchased from laboratory animal center, Babol University of Medical Sciences, Iran. All of the rats were kept in the same environmental and nutritional conditions. The temperature of the storage room was 22-25° C also 12 hours of light and 12 hours of darkness were applied. Rats had free access to normal feed (pellet) and water, moreover, similar conditions were considered for them.

Preparation of hydroalcoholic extract

To prepare the hydroalcoholic extract of FS, first, the aerial parts of FS were collected from forest of north of Iran – Mazandaran –Babol in spring and dried in the shade and then grinded to powder. Later, the extract was prepared by digestion with ethanol (80%). After evaporation of the solvent by evaporator apparatus, the dry matter was determined, and the obtained extract was stored in the refrigerator at 4 ° C for long-term storage.

Experimental groups and dose selection

Rats were divided into 8 groups of six. Group 1 consisted of control animals, group 2 was treated with TCA (Scharlau- Spain) at a dose of 500 mg/kg orally for 5 consecutive days (TCA group), groups 3, 4,5 ,were first, treated with 500 mg/kg TCA orally for 5 consecutive days, and then given the hydroalcoholic extract of FS at the doses of 100, 200 and 400 mg/kg orally for 28 days, receptively (TCA + FS100, TCA + FS200, TCA + FS400). Group 6 was treated only with 400 mg/kg FS extract per day for 28 days (FS 400). Group 7 was treated with doxorubicin at a dose of 2.5 mg/kg intraperitoneally once a week for 4 weeks (DOX). Finally group 8 was first treated with 500 mg/kg of TCA for 5 consecutive days, and then with 2.5 mg/kg intraperitoneally doxorubicin once a week for 4 weeks (TCA + DOX). After 28 days, the rats were anesthetized and blood samples were taken from their eyes. The rats were scarified and their liver samples were taken for histopathological examination.

Serum biochemistry

The method provided by Koroluk et al. (1988) was used for catalase measurement. The reaction of MDA to thiobarbituric acid produces a red colored complex that color intensity can be measured by spectrophotometry and has a direct relationship with the amount of MDA and ultimately with the oxidative stress (Placer et al. 1966). Enzyme activities, including ALT, AST, ALP, and TNFα were determined using commercial assay kits (Karmania pars gene, Iran), Gpx and total antioxidant capacity (TAC) were determined using commercial assay kits (Navand lab kit, Iran).

Histopathological examination

For histopathological examination, first a slice of the liver was fixed in formalin solution for 2 weeks, then was set in paraffin, sectioned to 5 μm thickness, deparaffinized, and rehydrated using standard techniques. The extent histological change was assessed with hematoxylin and eosin and recorded with a light microscope.

Statistical analyses

Mean± SEM was used for data expression. One-way analysis of variance followed by the LSD multiple comparison tests was used for results comparison. The data were analyzed with SPSS ver. 19. P-value ≤0.05 was considered statistically significant.

Ethical consideration

All experiments complied with the ethical guidelines for the care and use of laboratory animals. Also the protocol of study was approved by Imam Khomeini International University of Qazvin University.

Results

Results of biochemical factors

In ALT, difference was observed among control, TCA and TCA+DOX groups. Also, a significant decrease in liver AST was observed in the TCA group compared to the control group (P≤0.05). A significant decrease liver AST was observed in the TCA grouped compared to 100 and 400 mg/kg *FS* treated groups (P≤0.05). Hepatic ALP showed significant increase in the TCA group treated with different concentrations of FS (100, 200, and 400 mg / kg) in comparison with control, TCA and TCA+DOX groups (P≤0.05). More details are shown in table 1.

Results of inflammatory and oxidative stress factors

TNF-α showed a significant decrease in all groups except the DOX treated group compared to the control group. On the other hand, there was no significant difference between the FS treated groups (100, 200, and 400 mg/kg) with the TCA and TCA + DOX groups. Also, there was no significant difference in the catalase and total antioxidant capacity levels between groups. More details are shown in table 2.

Table1: Mean ±SEM of serum enzyme activity related to liver toxicity. Different letters show statistical differences between groups (p≤0.05)

Group/ Variable	ALT(U/L)	AST(U/L)	ALP(IU/L)
Control	37.4±3.57^{a}	130.83±20.25^{ac}	259.23±32.10^{a}
TCA	38.75±2.06^{ac}	110.25±6.29^{b}	272±19.81^{a}
TCA+ A100	66.33±4.93^{d}	129.5±6.75^{ac}	697±36.09 ^{b}
TCA+ A200	51±2.44^{bf}	123±4.58^{abc}	594.33±90.23 ^{c}
TCA+ A400	57.33±8.32^{b}	137±6.55^{c}	417±35.56 ^{d}
A400	46.25±2.36^{ef}	117.33±5.5^{ab}	303.5±49.26 ^{a}
DOX	44.25±4.57^{ce}	126.66±11.7^{ac}	468.5±75.67 ^{d}
TCA+ DOX	37.25±6.07^{a}	120.33±4.04^{ab}	313.66±80.25 ^{a}

Table2: Inflammatory and oxidative stress factors (mean ±SEM). Different letters show statistical differences between groups (p≤0.05)

Group/ Variable	TNF (pg/ml)	GPX (nmol/min/ml)	TAC(micromollar)	Catalase (nmol/min/ml)
Control	31.02±6.6^{a}	5580±1985 ^{a}	1.49±0.35 ^{a}	0.14±0.02 ^{ab}
TCA	15.30±1.36^{b}	4875±175.11 ^{ab}	1.43±0.17 ^{a}	0.14±0.01 ^{ab}
TCA+ A100	20.07±5.73^{b}	4715±644.43 ^{ac}	1.48±0.12 ^{a}	0.13±0.02 ^{ab}
TCA+ A200	15.78±1.79^{b}	2828.33±830.32^{d}	1.35±0.09 ^{a}	0.12±0.03 ^{ab}
TCA+ A400	13.64±1.42^{b}	10405±1969.59^{e}	1.41±0.17 ^{a}	0.11±0.02 ^{ab}
A400	15.19±1.25^{b}	10121.66±716.26^{e}	1.49±0.23 ^{a}	0.15±0.03 ^{a}
DOX	67.65±21.78^{c}	3852.5±497.75^{cbd}	1.41±0.05 ^{a}	0.12±0.007 ^{ab}
TCA+ DOX	14.23±1.84^{b}	4138.33±323.31^{abd}	1.53±0.13 ^{a}	0.11±0.03 ^{b}

A significant decrease in MDA was seen in the TCA groups with different concentrations of FS (100, 200, and 400mg/kg) compared to the control, TCA, TCA+DOX, and DOX groups (Figure1). Regarding glutathione peroxidase, a significant difference was seen between glutathione peroxidase of other groups ($P \leq 0.05$).

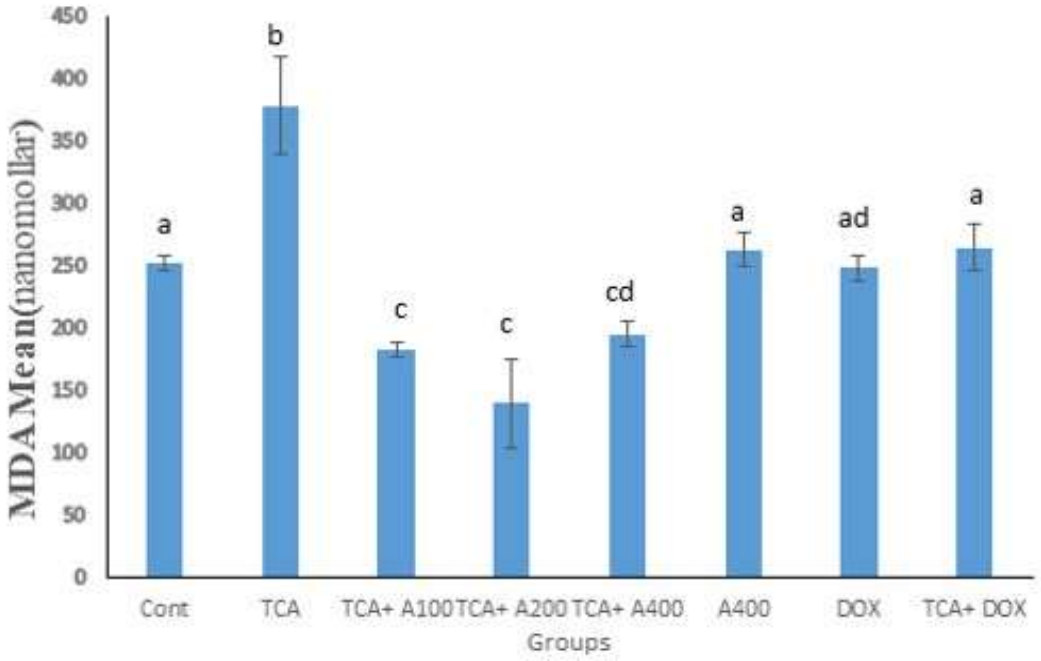

Figure1: Mean ±SEM of MDA. Different letters show statistical differences between groups ($P \leq 0.05$)

Histopathological results

Histological investigation showed that in TCA, DOX and TCA+DOX treatment causes loss of the radial arrangement of hepatocyte cords, hepatocyte degeneration, resulting in sinusoidal stenosis and vacuolization of cytoplasm. Also, the results showed that the FS at 100 mg/kg concentration has no significant effects on the TCA caused changes, although the FS with 100 mg/kg was able to partially neutralize the effects of TCA through the reduction of necrotic, hypertrophied hepatocyte and congestion in portal vessels. It seems that the dose of 400 mg/kg of FS had significant effects in reducing the impression of TCA, because in the group treated with this dose, the radial arrangement of the hepatocyte cords is completely clear, the hepatocytes and Kupffer cells are of normal size, and there is effect congestion in portal vessels. More details are shown in figure 2.

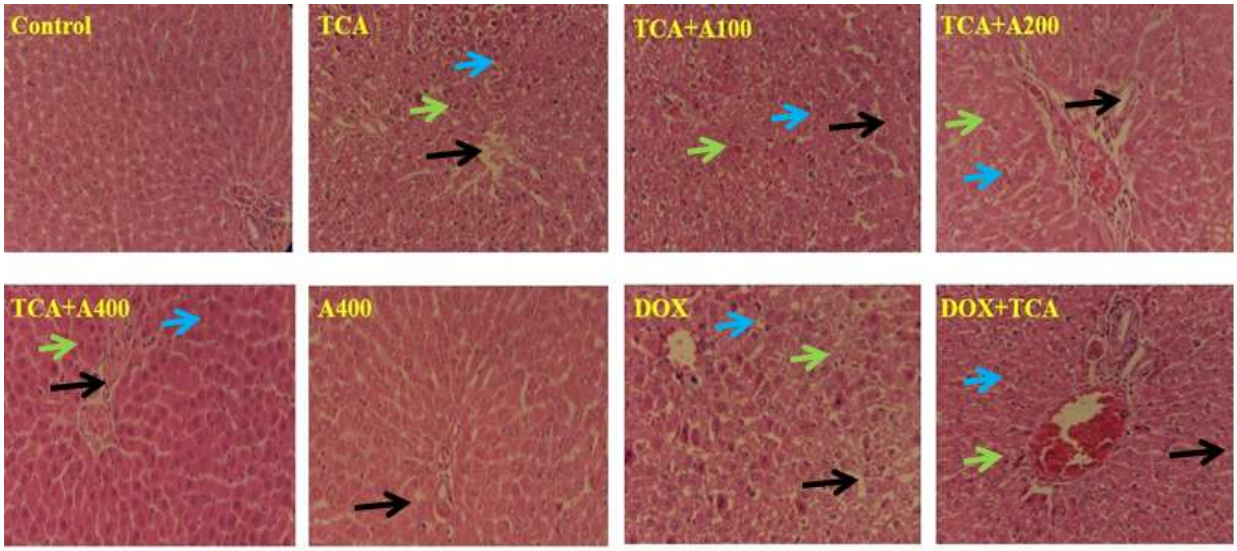

Figure2: Liver tissue morphology in the studied groups. H & E (× 10). (black arrow: vacuolization, green arrow: degeneration, blue arrow: sinusoidal stenosis)

Discussion

In the present study, TCA treatment (500 mg/kg), for 5 days, led to liver tissue damage and cell necrosis, focal hypertrophy of hepatocytes, narrowing of sinusoids and cytoplasmic vacuolation of hepatocytes in animal model. Moreover, an increase in serum MDA and decrease in serum TNFα in TCA treatment group compared to the control group were observed, but no effect on antioxidant factors such as the activity of glutathione peroxidase, catalase, total antioxidant levels and liver enzyme (ALT&ALP) was reported. Also in DOX treated rats increase of TNF was reported.

TCA is a chemical carcinogen that may cause experimental hepatocellular carcinoma (Caldwell and Keshava, 2006). Exposure to TCA for a short period causes pre-neoplastic lesions in the form of dysplastic tissue changes, vascular congestion, ballooning of liver cells and liver cell foci with extensive vacuolation (Abdel-Hamid et al. 2011; Alzergy et al. 2018). Previous studies have suggested increased oxidative stress and inflammation (Abdel-Hamid et al. 2011), hypo-methylation (Tao et al. 2004) as mechanisms were involved in the carcinogenic and metastatic effects of TCA. Our results show that TCA significantly increased MDA compared to the control group. Lipid peroxidation, determined by the MDA index, is a key marker for oxidative stress estimation patients with liver cancer (Lorente et al. 2016). The oxidative effect of TCA can be considered by the increase of MDA along with the decrease of GSH level (as a non-enzymatic antioxidant) and the decrease of CAT and SOD activities.

In our study, the antioxidant factors including catalase, glutathione peroxidase enzymes and total antioxidant capacity in TCA treatment group have no significant difference compared to the control group. Although the dose of TCA used in the present study was similar to many previous studies, the observations were different from other studies. TCA causes inflammation and oxidative stress and is used to induce liver carcinoma in animal models (Fouad et al. 2013) through potential mechanisms including DNA hypo-methylation, peroxisome development, oncogene activation, and inhibition of intercellular communication (Hari Babu et al. 2012).

In addition, TCA significantly reduced TNFα compared to the control group. This may be related to long time (28 days) TCA

administration while the TNFα increases in acute phase of inflammation. However, doxorubicin significantly increased the TNFα. TNFα is an inflammatory factor that increases in the acute phase of inflammation. Fouad et al. (2013) investigated the possible anticancer effect of carnosine compared to doxorubicin in hepatocellular carcinoma (HCC) induced by TCA (500 mg/kg per day, for 5 days) in rats. After induction of HCC, rats were treated with carnosine (10 mg/kg/day, i.p.), or doxorubicin (2.5 mg/kg, i.p., once weekly) for 2 weeks. Carnosine significantly reduced serum ALT and hepatic lipid peroxidation, nitric oxide and TNF-α and significantly improved the total antioxidant status in TCA-treated Rats. The effects of doxorubicin on oxidative and inflammatory stress were lower than carnosine. However, carnosine and doxorubicin significantly induced apoptosis biomarkers, Bax, cytosolic cytochrome C and caspase-3. Furthermore, carnosine and doxorubicin reduced histopathological dysplastic changes and alpha-fetoprotein expression in the liver of Rats with HCC. Fouad et al. (2013) concluded that carnosine has protective effect on TCA-induced HCC in rats through antioxidant, anti-nitrative and anti-inflammatory and induction of apoptosis mechanisms. Moreover, Ibrahim et al. (2021) investigated the effect of Diacerein (DIA), an interleukin (IL)-1β inhibitor, on TCA-induced preneoplastic changes in animal model. In this study, animals were treated with 1 mg/kg TCA orally for 5 days. Serum liver enzymes, oxidative stress parameters, inflammatory interlukin-1β (IL-1β), and angiogenesis marker (VEGF & HIF-1α) were evaluated along with histopathological changes and caspase-3 expression. The results showed that at the histological level, DIA improves the liver precancerous lesions through the modulation of the IL-1β-HIF-1α-VEGF pathway (Ibrahim et al. 2021). In the current study, no significant change in the activity of liver damage related enzymes (ALT, AST, ALP) with TCA treatment was observed, which is contrary to the results of many studies. In the study of Sweeney et al. (2009) results showed significant decrease in body weight of TCA-treated mice compared to control mice. Weight loss may be attributed to the cytotoxic effect of TCA, damage liver tissue. But we did not record rat´s weight change at the end of our study. Liver damage caused by chemicals, led to secretion of ALT, AST, ALP to blood circulation system due to the change in the permeability of the liver cells membrane (Mabrouk et al. 2016).

Ni et al. (1996) reported that the biotransformation of TCA by cytochrome P450 led to production of dichloroacetic acid free radical which causes oxidative DNA damage and lipid peroxidation, so TCA induces hepatic oxidative stress. ALT activity usually increases in TCA hepatotoxicity. ALT is the most specific and sensitive biomarker for liver damage because it is mostly found in liver tissue and is located in hepatocyte cytosol. Although ALT isoenzymes are expressed in variety of tissues, elevated serum ALT activity is considered the "gold standard" clinical marker for liver injury (Davis, 1992; Wedemeyer et al. 2010). On the other hand, the significant increase in ALP enzyme activity as a marker for TCA-treated rats may be due to the obstruction of the bile duct, resulting in failure to excrete the enzyme (Wiwanitkit, 2001). Liver activity changes via TCA treatment. Mokhamer et al. (2022) reported that TCA treatment (500 mg/kg/day for 5 days) significantly increased the activity of ALT, AST, and ALP, as well as total serum bilirubin levels, while the activity of the antioxidant enzymes SOD and catalase, as well as the level of glutathione decreased. Total bilirubin is a sensitive biomarker for liver cell damage. Decreased biliary secretion of conjugated bilirubin compared to increased bilirubin caused by hemolysis leads to an increase in serum bilirubin. In liver tumors,

hemolysis and liver dysfunction lead to hyper-bilirubinemia (Gowda et al. 2009).

Sweeney et al. (2009) reported a significant increase in serum total bilirubin levels via TCA treatment, which may be due to jaundice caused by hepatocellular damage or ductal obstruction in HCC. In the current study, TCA treatment caused liver damage, so that different degrees of necrosis, degeneration and focal hypertrophy of hepatocytes were observed, which seems to be the initiation of cell tumorigenesis. The result of the present study is consistent with the results of those reaserchers that showed TCA treatment led to disordered liver structure with thick fibrous tissue septa and lymphocyte infiltration, as well as dilation and congestion of liver sinusoids with large vesicular nuclei. Oxidative stress, cytochrome P450 dysfunction, inflammation and mitochondrial dysfunction are considered as the main mechanisms explaining liver damage (Zhang et al. 2018). Fouad et al. (2013) investigation showed that TCA caused significant liver damage, which was observed in the form of vacuolization, irregular dysplasia of liver cells, polymorphic hyper- chromatic nucleus with more than one nucleus, increased nucleus-to-cytoplasmic ratio, and dense nucleus. In the study of Hari Babu et al. (2012) the histo-pathological changes caused by TCA included vacuolation of hepatocytes, irregular dysplasia, polymorphous hyper-chromatic nucleus with more than one nucleus. Increased *FS* extract treatment reduces the tissue damage caused by TCA in a dose-dependent manner. In addition, it significantly reduced MDA as the oxidative stress index, which is probably related to the antioxidant properties of *FS*. Although studies related to the pharmacological properties of *FS* are limited, few studies confirm the anti-inflammatory and antioxidant properties of this plant. Bahrami et al. (2021) analyzed the chemical composition of the hexane extract of the aerial parts of *FS* by GC/MS and reported 21 compounds (80.60%) of the total including phytosterols and hydrocarbons. Also, a significant amount of flavonoids in the methanolic extract of *FS* (27.235 ± 0.048 µg/ml) was estimated by AlCl3 colorimetric method. Two flavonoids, rutin and catechin, were identified in the methanol extract of *FS* by HPLC (Abu-Odeh and Talib, 2021). So, *FS* has a variety of useful natural compounds that make it a promising plant for agricultural and medicinal purposes.

FS has a protective effect against histopathological changes induced by TCA in rat's liver tissue.

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Conflict of interest

Authors have no conflict of interest.

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اثرات عصاره *Froriepia subpinnata* بر تغییرات بیوشیمیایی سرمی و هیستوپاتولوژیک کبد در موش‌های صحرایی تیمار شده با تری کلرواستیک اسید

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چکیده

کارسینوم سلول‌های کبدی یکی از چالش‌های سیستم سلامت است و استرس اکسیداتیو نقش مهمی در بروز آن دارد. با توجه به اثرات آنتی‌اکسیدانی گیاه اناریجه (FS) *Froriepia subpinnata* ، این مطالعه با هدف بررسی اثر آن در جلوگیری از بروز سمیت کبدی ناشی از تری کلرواستیک اسید (TCA) در مدل حیوانی انجام شد. عصاره هیدروالکلی اناریجه از اندام هوایی به روش خیساندن تهیه شد. چهل و هشت سر موش صحرایی به ۸ گروه تقسیم شدند: حیوانات کنترل، گروه‌های تحت درمان با TCA (۵۰۰ میلی‌گرم بر کیلوگرم)، گروه‌های تیمار شده با TCA + FS (۱۰۰،۲۰۰،۴۰۰ میلی‌گرم بر کیلوگرم)، گروه تیمار شده با اناریجه (۴۰۰ میلی‌گرم بر کیلوگرم)، گروه DOX تحت درمان با دوکسوروبیسین (۲/۵ میلی‌گرم بر کیلوگرم) و گروه تحت درمان با TCA + DOX. پس از ۲۸ روز، خون جمع‌آوری و سرم جدا شد، سپس مالون دی‌آلدئید (MDA)، گلوتاتیون پراکسیداز (GPx)، TAC (ظرفیت تام آنتی‌اکسیدانی)، آلانین آمینوترانسفراز (ALT)، آسپارتات آمینوترانسفراز (AST)، آلکالین فسفاتاز (ALP)، فاکتور نکروز تومور-آلفا (TNF-alpha) اندازه‌گیری شد و بافت کبد با میکروسکوپ نوری مورد بررسی هیستوپاتولوژیک قرار گرفت. TCA به طور معنی‌داری مقدار MDA را افزایش داد و TCA با غلظت‌های مختلف FS (۱۰۰، ۲۰۰ و ۴۰۰ میلی‌گرم بر کیلوگرم) مقدار MDA را در مقایسه با سایر گروه‌ها کاهش داد ($P\leq ۰/۰۵$). مقدار TNFα توسط TCA کاهش یافت، اما با DOX به طور قابل توجهی افزایش یافت و تیمار FS اثر TCA بر سطح TNFαرا تغییر نداد. سطح سرمی GPX، TAC، ALT، AST و ALP با تیمار TCA یا FS از نظر آماری تغییری نکرد TCA به بافت کبد آسیب رسانده و باعث انحطاط سلول‌های کبدی، تنگی سینوسی و واکوئل شدن سیتوپلاسم شد. FS به صورت وابسته به دوز از بافت کبد محافظت کرد اما در دوز ۴۰۰ میلی‌گرم بر کیلوگرم اثر بهتری در کاهش آسیب بافت داشت. FS یک اثر محافظتی در برابر تغییرات هیستوپاتولوژیک ناشی از TCA در بافت کبد موش صحرایی دارد.

کلمات کلیدی: اناریجه، تری کلرواستیک اسید، استرس اکسیداتیو، سمیت کبدی

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