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Evaluation of the hepatoprotective effect of *Taraxacum officinale L* (Dandelion) root extract in experimental chronic liver failure

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Abstract
Background. Chronic liver failure (CLF) is a major health problem worldwide and prevention is the major therapeutic goal.
Aims. The current study was designed to evaluate the hepatoprotective effects of T. officinale root extract in a pre-established model of chronic liver failure in rats by analyzing the liver tissue biopsy.
Methods. The rat model was induced through immune liver fibrosis triggered by human serum albumin. The liver biopsy was assessed for portal inflammation and fibrosis, for intralobular and periportal degeneration/necrosis, and the Histological Activity Index was calculated. The immunopositivity for 3-nitrotyrosine analysis was also performed.
Results. The results highlighted the hepatoprotective effect of the ethanolic T. officinale root extract, reduced necro-inflammatory changes, Histological Activity Index and 3-nitrotyrosine expression, with no significant effect on fibrosis from the liver tissue in experimental human serum albumin induced chronic liver failure.
Conclusions. Ethanolic T. officinale root extract has a hepatoprotective activity in experimental CLF by reducing necro-inflammatory alterations and 3-nitrotyrosine expression.
Keywords: chronic liver failure, Taraxacum officinale, oxidative stress, nitric oxide, 3-nitrotyrosine.

Introduction

Today, mortality from chronic liver disease (CLD) is a worldwide concern because it has increased, it mainly affects individuals of working age, and it is expected to become the leading cause of mortality by 2020 (Thiagarajan et al., 2020). In CLD, hepatic injury is the consequence of viral, metabolic, toxic, or autoimmune injuries (Gual et al., 2017). Lately, the etiology of CLD has changed, from the predominance of chronic hepatitis B or C towards the predominance of alcohol and obesity induced chronic liver diseases (Thiagarajan et al., 2020). Another underestimated primary etiology of many chronic diseases, including CLD, is the reduced daily physical activity (Booth et al., 2012). Many studies have indicated that the diet-induced inflammatory process can also trigger or influence CLD (Barrea et al., 2018).

The liver is an important organ for the immunologic and inflammatory processes of the body. Histological analysis found that in CLD there is a high risk of progression toward chronic liver failure (CLF), cirrhosis, and hepatocellular cancer, because hepatocyte injury progresses and maintains a chronic inflammatory response (Furman et al., 2019; Shen et al., 2019). In CLD, many studies indicated oxidative stress as a common underlying mechanism of hepatocellular injury (Stauber et al., 2014; Pfingstgraf et al., 2021; Luangmonkong et al., 2018). In the liver exposed to harmful stimuli, Kupffer cells (KC) are activated and produce most of the reactive oxygen species (ROS) (Mansouri et al., 2018). In physiological and pathological conditions, low production of ROS by mitochondria is needed for metabolic adaptation, and moderate ROS regulate the release of inflammatory mediators. A high, excessive release of ROS can activate

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apoptosis or autophagy, causing tissue injury. In the liver, KC releasing ROS stimulate hepatocyte death by necrosis and apoptosis (Gual et al., 2017), and activate hepatic stellate cells (HSC), triggering their phenotypic switch into cells that produce excessive amounts of extracellular matrix and lead to liver fibrosis. Moreover, the activated liver cells produce at the same time nitric oxide (NO), a freely diffusible molecule that reacts with superoxide and forms peroxynitrite, a reactive nitrogen species (RNS). Peroxynitrite further reacts with tyrosine residues from proteins, and 3-nitrotyrosine results. Together, ROS and RNS will damage proteins, lipids, and mtDNA, creating a vicious cycle of ROS production (Macdonald et al., 2018).

Because there is currently no effective therapy to stop CLD associated ROS production and reverse advanced liver fibrosis, many studies are looking for a new prevention therapy for CLF (Patten & Shetty, 2018). Consequently, efforts to identify dietary factors that reduce the inflammatory process might decrease disease severity and progression (Barrea et al., 2018).

Phytotherapy is considered to be the mother of modern medicine. Plant products are still used as primary or complementary treatments. Many studies have proved that plants are rich sources of polyphenols with antioxidant, anti-inflammatory, and antiapoptotic activities. Therefore, they are part of the human diet in order to reduce the risk of chronic diseases, such as CLD. The Mediterranean diet is rich in compounds such as polyphenols, which have anti-inflammatory and antioxidant effects (Anania et al., 2018).

Taraxacum officinale (dandelion) is a perennial herbaceous plant belonging to the Asteraceae family, and it is found in Eurasia and other areas of the world (Luangmonkong et al., 2018). *T. officinale* (TO) is currently part of the Mediterranean diet. In phytomedicine, TO has been used for liver and gallbladder disorders (Abdel-Magied et al., 2019; Pfingstgraf et al., 2020), but histological changes and the molecular mechanism of these changes have not been completely elucidated (Gerbino et al., 2018).

Hypothesis

In a previous study, we found that TO root extract reduced blood liver injury enzymes and systemic oxidative stress markers in experimental CLF. Therefore, the current study was designed to evaluate the hepatoprotective effects of *T. officinale* root extract in a pre-established model of CLF in rats by analyzing the liver tissue biopsy.

Material and methods

Research protocol

a) Period and place of the research

T. Officinale F.H. Wigg. roots (TO) harvested from the Alexandru Borza Botanical Garden of "Babes-Bolyai" University Cluj-Napoca, Romania in June 2020 and deposited in the "Alexandru Borza" Botanical Garden Herbarium (Voucher CL: 669002) were ground after being dried in a shaded place, and then extracted as previously described (Pfingstgraf et al., 2021). The study was performed in the Department of Pathophysiology of "Iuliu Haţieganu" University of Medicine and Pharmacy Cluj-Napoca, between September-December 2020.

b) Subjects and groups

The current study used male Wistar rats aged 3 months, with a weight of 200–250 g, provided by the Animal Facility of Iuliu Haţieganu University of Medicine and Pharmacy, Cluj-Napoca. The animals were randomly divided into six groups (n = 5): CONTROL, a negative control group; chronic liver failure (CLF); CLF treated with Silymarin (CLF-SIL) (200 mg/kg b.w./day) (Jalali et al., 2017); CLF treated with *T. Officinale* extract, as follows: CLF–TO100%, treated with undiluted extract (200 mg dry plant material/kg b.w./day), CLF–TO50%, treated with a half of a dose of extract (100 mg dry plant material/kg b.w./day), and CLF–TO25% treated with a quarter of a dose of extract (50 mg dry plant material/kg b.w./day). The daily dose of TO was dissolved in corn oil (1 ml/day/animal) (Gao et al., 2019). The control group and the CLF group were treated with physiological saline (1 ml/day). During the study, the animals were kept in a room with a 12/12 h dark/light cycle, controlled temperature (22 ± 2 °C) and humidity (50 ± 5%), with free access to food and water. The study used a CLF rat model induced through immune liver fibrosis triggered by HSA (8 g/L) and incomplete Freund's adjuvant as previously described (Wu et al., 2009; Wang et al., 2012; Pfingstgraf et al., 2020). At the end of the experiment, the animals were euthanatized by cervical dislocation under general anesthesia induced by xylazine (10 mg/kg b.w.) and ketamine (70 mg/kg b.w.) (Francischi et al., 2017), and hepatic biopsies were harvested for histological assessment and 3-nitrotyrosine analysis. The study protocol was approved by the Sanitary Veterinary and Food Safety Authority Cluj-Napoca (no. 19/ 13.12.2016).

c) Applied tests

The histological assessment was performed on two liver samples collected from the left lateral and right medial lobes harvested from each animal (Ruehl-Fehlert et al., 2003). As previously described, liver biopsies were fixed for 24 h in 10% phosphate-buffered formalin, processed and embedded in paraffin wax. Then, for the histological assessment of portal inflammation and fibrosis, intralobular and periportal degeneration/necrosis, and Histological Activity Index (HAI) calculation, two serial sections of 3 μm were cut from each tissue sample, and stained with hematoxylin and eosin (H&E) (Knodell et al., 1981; Mester et al., 2019).

For the immunopositivity for 3NT analysis, the samples were prepared as previously described, and a primary mouse monoclonal [clone 39B6] antibody to 3NT was used (Pfingstgraf et al., 2021). Further, liver tissue 3NT was evaluated and scored as previously described: grade 0, with no 3NT staining; grade 1, positive 3NT staining in less than 10% of hepatocytes/10 high power fields; grade 2, positive 3NT staining in more than 10% but less than 50% of hepatocytes/10 high power fields; grade 3, positive 3NT staining in more than 50% of hepatocytes/10 high power fields (García-Monzón et al., 2000).

d) Statistical processing

Data were expressed as mean ± standard deviation (SD), and the different experimental groups were compared by using the one-way ANOVA test and the

post hoc Bonferroni-Holm test. A $p<0.05$ was considered statistically significant. Data were analyzed using the IBM SPSS Statistics, version 20 (SPSS Inc. Chicago, IL, USA).

Results

The control group biopsy had normal structures and no significant changes were found, HAI was 0 (Table I) and 3-NT immunoexpression was negative (Fig. a1), as previously described (Stauber et al., 2014). In CLF animals, the normal liver architecture was lost, severe chronic intralobular inflammation ($p<0.001$), a moderate amount of mature fibrous tissue ($p< .001$), and bile duct hyperplasia (Fig. 1A) were found. In the periportal area, hepatocyte coagulative necrosis ($p< .001$) was associated with a surrounding macrophage, lymphocyte, and neutrophil infiltrate ($p< .001$). The 3-NT immunostaining score was positive in more than 10% of hepatocytes ($p< .001$) of the CLF group (Fig. a1). In the CLF rat liver, HAI was importantly increased compared to the control group ($p< .001$) (Table I).

The TO treatments significantly reduced periportal necrosis ($p< .001$), with no significant difference between TO doses. Periportal inflammation and intralobular necrosis were also reduced, the highest TO dose having the best effect ($p< .001$). HAI and fibrosis were lowered by the TO treatment ($p< .01$) in a dose dependent way, the higher concentration having the best effect (Fig. 1C, D, E, Table I). SYL was a better inhibitor of HAI (Table I), intralobular necrosis and fibrosis than all TO doses ($p<0.001$) (Fig. 1B). The 3-NT expression was significantly reduced in the hepatic lobules of the CLF-TO100% ($p< .01$) (Fig. c1) and CLF-TO50% ($p = .05$) (Fig. b1) compared to CLF. A minimal expression of 3-NT was identified in hepatocytes of the CLF-SYL group (Fig. b1). There were no important differences of 3-NT expression between CLF-TO25% (Fig. 1c) and CLF groups (Table I).

Table I

Histological assessment and 3-nitrityrosine evaluation in the liver biopsies of the study groups.

Study groups	Periportal necrosis	Intralobular necrosis/ degeneration	Periportal inflammation	Fibrosis	HAI	3NT score
CLF-TO100%	0.20±0.02b	0.60±0.05b,c	0.20±0.04b,c	0.80±0.04b,c	1.80±0.84b,c	1.20±0.04b,c
CLF-TO50%	0.20±0.04b	0.80±0.04b,c	0.40±0.04b	1.20±0.10b,c	3.00±0.80b,c	1.40±0.0,b,c
CLF-TO25%	0.20±0.04b	0.80±0.04b,c	0.40±0.10b	1.80±0.10c	3.40±0.7,c	1.60±0.05c
Silymarin	0.20±0.02b	0.20±0.04b	0.40±0.05b	0.60±0.05b	1.20±0.45b	1.00±0.04b
CLF	1.20±0.04	1.00±0.02	0.80±0.04	1.80±0.10	3.80±0.30	1.60±0.05

Results are expressed as mean ± SD; b $p< 0.05$, versus CLF; c $p< 0.05$, versus Silymarin; CLF-TO 100% - chronic liver failure treated with 200 mg *T. officinale*/kg b.w./d; CLF-TO 50% - chronic liver failure treated with 100 mg *T. officinale*/ kg b.w./d; CLF-TO 25% - chronic liver failure treated with 50 mg *T. officinale*/ kg b.w./d; Silymarin - chronic liver failure treated with 200 mg silymarin/ kg b.w./d; CLF - chronic liver failure; HAI – histological activity index; 3NT – 3-nitrotyrosine.

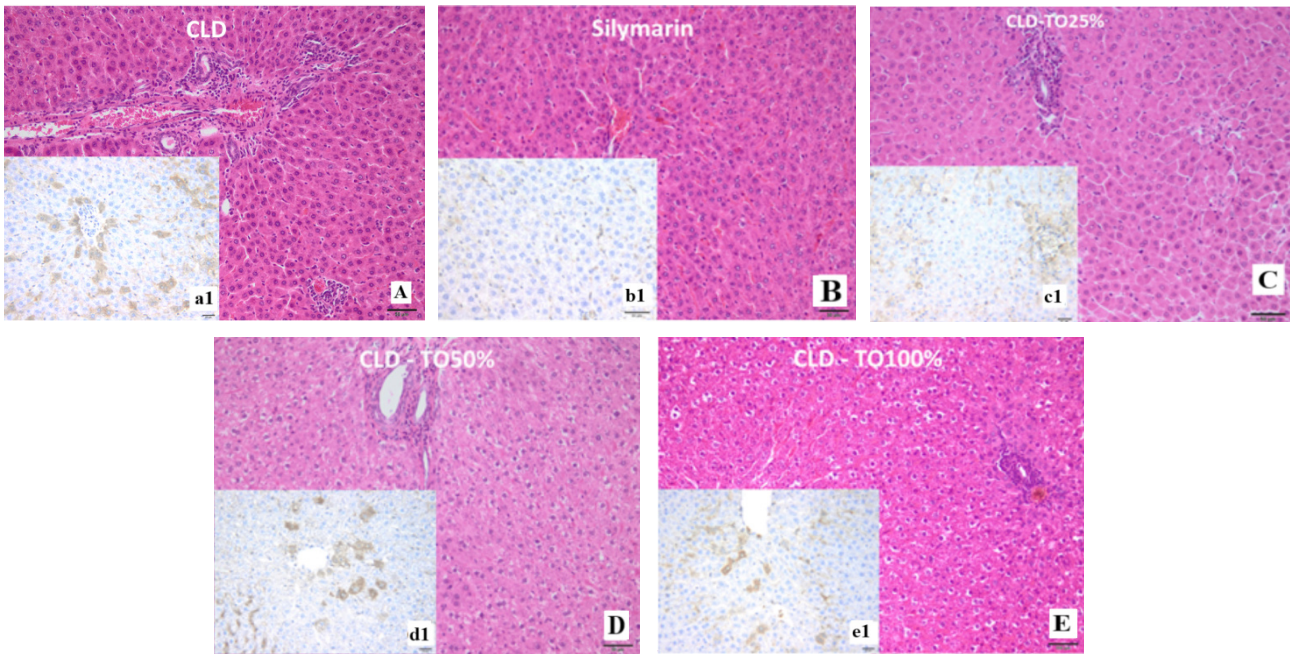

Fig. 1 – Photomicrographs of the liver tissues from the control and experimental animals. H&E stain: A. CLD; B. Silymarin; C. CLF-TO25%; D. CLF-TO50%; E. CLF-TO100%; Bar=50 µm (A, B) and 20 µm (C, D, E). 3-nitrotyrosine immunoexpression: a1. CLD; b1. Silymarin; c1. CLF-TO25%; d1. CLF-TO50%; e1. CLF-TO100%; Bar= 20 µm (a,b,c,d,e). CLF-TO100% = chronic liver failure and T. officinale 200 mg/day; CLF-TO50% = chronic liver failure and T. officinale 100 mg/d; CLF-TO25% = chronic liver failure and T. officinale 50 mg/day; Silymarin = chronic liver failure and silymarin 200 mg/kg b.w./day; CLF = chronic liver failure.

Discussions

The study results showed that a seven day treatment with TO root extract significantly attenuated the liver injuries and 3NT expression in rats with HSA-induced CLF.

Previous studies showed that HSA administration in rats triggered an immune liver injury and fibrosis (Pfingstgraf et al., 2021; Pfingstgraf et al., 2020; Wu et al., 2009). CLF diagnostic serum markers target the consequences or the mechanism of the liver injury (Pfingstgraf et al., 2020; Bajaj et al., 2018; Sarin et al., 2019). In the present study, the hepatoprotective effect of the TO root extract was evaluated by analyzing the liver biopsy.

Continuous liver injury due to chronic inflammation and/or oxidative stress leads to continuous stimulation of the HSC, HSC proliferation and dedifferentiation into myofibroblasts producing excessive extracellular matrix (ECM), and liver fibrosis (Mehta et al., 2019). Moreover, in CLD, regeneration is severely impaired due to the chronic pathological inflammation, chronic hepatocyte loss, reduced hepatocyte proliferation, and decreased matrix remodeling (Gual et al., 2017). Therefore, hepatic fibrosis associated with CLD is used as a severity and progression index (Gual et al., 2017). There is high need to study the liver structural and molecular changes in order to better understand the mechanisms that control the pathogenesis of liver cell death and liver fibrosis, its progression toward cirrhosis, CLF or hepatocarcinoma (Ehlken et al., 2011).

Because liver fibrosis represents a key characteristic of CLD progression, liver biopsy could serve as diagnostic and/or prognostic criteria. It is used in order to confirm chronic injury in known CLD, its stage and the CLF. Usually, the interpretation of H&E liver samples can help in the histological distinction between old and recent liver injury. In CLD, inflammation and fibrosis patterns are relevant not just for progression evaluation, but also for the etiological diagnosis (van Leeuwen et al., 2018). The liver biopsy of animals with HAS-induced CLF had extended necro-inflammatory changes, associated with fibrosis, and important intrahepatic bile duct hyperplasia (Pfingstgraf et al., 2021). The repair process causes hepatic remodeling, due to fibrosis, and the consequence of such structural changes is liver dysfunction (Kubes et al., 2018; Hfaiedh et al., 2016; Karakuş et al., 2017) as we also previously found (Pfingstgraf et al., 2020). In CLF animals, intralobular inflammation was significant, mainly periportal, with portal space distension, lymphocyte and macrophage infiltration, supporting the autoimmune mechanism of the liver injury induced by HAS.

It was already mentioned that in a healthy liver there is a hypoimmune response, and in CLD and CLF there is a significant cellular inflammatory response with important white blood cell infiltration and extended tissue damage. ROS have been proposed to be involved in such changes (Parola & Pinzani, 2019). The 3-NT immunostaining score of the CLF animals confirms a significant ROS and NO production in the injured liver of the HAS-induced liver injury.

Medicinal plants are a rich source of bioactive compounds such as polyphenols with important antioxidant properties. *T. officinale* leaves and flowers extracts are rich in flavonoids and coumarins, but also some hydroxycinnamic acid derivatives (Pfingstgraf et al., 2021). In phytotherapy, TO is frequently empirically used in order to prevent or treat several chronic liver diseases (Gerbino et al., 2018). TO root extract DPPH and FRAP tests indicated a strong *in vitro* antioxidative activity (Pfingstgraf et al., 2021; Hu & Kitts, 2003).

TO treatment reduced liver screening test alteration in experimental CLF and acute on chronic liver failure, and the effect was dose dependent, with the higher concentration having the stronger inhibitory effect (Pfingstgraf et al., 2021; Pfingstgraf et al., 2020). Previously, we showed that in acute on chronic liver failure, pretreatment with TO hepatoprotective activity consisted of reducing liver necro-inflammatory changes and 3NT expression, with no significant influence on liver fibrosis (Pfingstgraf et al., 2021).

In CLF, NO released by injured liver cells can have a dual effect by promoting HSC apoptosis and increase liver damage. In CLF animals, TO treatment reduced liver 3NT expression, suggesting that TO root extract may reduce liver inflammation by decreasing nitrosative stress.

Silymarin is a frequently used hepatoprotector due to its antioxidant and anti-inflammatory effects (Owatari et al., 2018). In experimental CLF, silymarin reduced intralobular necrosis, fibrosis and HAI to a greater extent than TO.

Conclusions

1. Taken together, we can conclude that in CLF, the hepatoprotective effect of an ethanolic *T. officinale* root extract consists of a reduction in necro-inflammatory injuries and 3-NT expression, without an important effect on liver fibrosis.

2. Further research is needed in order to confirm the beneficial effects of *T. officinale* root extract supplementation in CLD.

Conflict of interest

There is no conflict of interests.

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