ISSN: 1220-3173; E-ISSN: 2457-7618

# EFFECT OF *TARAXACUM OFFICINALE L.* (DANDELION) ROOT EXTRACT IN EXPERIMENTAL CHRONIC LIVER FAILURE

## EFECTUL EXTRACTULUI DE RĂDĂCINI DE *TARAXACUM OFFICINALE L.* (PĂPĂDIE) ÎN INSUFICIENȚA HEPATICĂ EXPERIMENTALĂ

Iulia Olimpia PFINGSTGRAF[1),*)], M. TAULESCU[2),*)], R. ORĂȘAN[1)], Raluca Maria POP[1)], L. VLASE[1)], Corina TOMA[2)], Alina Elena PÂRVU[1)]

**ABSTRACT | REZUMAT**

The study was conducted to evaluate the hepatoprotective effect of the ethanolic extract of *Taraxacum officinale* roots on experimental chronic liver failure (CLF). *T. officinale* (TO) roots extract total phenolics content, polyphenols, phytosterols, and antioxidant activity (DPPH, FRAP) were determined. CLF was induced in rats by human serum albumin.

Six groups were used: control, CLF, CLF-silymarin, and CLF-TO administer in three doses (200mg/d, 100mg/d, 50mg/d). Sylimarin and *T. officinale* were administered orally for seven days after CLF induction.

The effect was evaluated by measuring liver screening parameters (aspartate aminotransferase, alanine aminotransferase, alkaline phosphatase, gamma glutamate transferase, total bilirubin, cholesterol, triglycerides, total proteins), renal parameters (creatinine, urea), and oxidative stress markers (total oxidative status, total antioxidant reactivity, oxidative stress index, malondialdehyde, total thiols, total nitrites and nitrates, 3-nitrotyrosine).

*T. officinale* has an important phenolic and phytosterols content, and a significant antioxidant activity.

CLF was associated with elevation of the liver injury parameters, cholesterol and total proteins reduction, triglyceride increase, renal dysfunction and oxidative stress. *T. officinale* reduced liver injury tests, triglyceride, renal tests and oxidative stress tests.

In conclusion, experimental CLF ethanol *T. officinale* roots extract has hepatoprotective effect and reduces renal dysfunction. These effects were correlated with the antioxidant activity and systemic oxidative stress reduction.

**Keywords**: Chronic liver failure, Hepatoprotective, Oxidative stress, *Taraxacum officinale*, 3-nitrotyrosine

Studiul a fost realizat pentru a evalua efectul hepatoprotector al extractului etanolic din rădăcini de *Taraxacum officinale* asupra insuficienței hepatice cronice experimentale (CLF). Au fost determinați polifenolii, fitosterolii și activitatea antioxidantă (DPPH, FRAP) ale extractului etanolic de rădăcini de *T. officinale* (TO). CLF a fost indusă la șobolani cu albumină serică umană.

Au fost utilizate șase grupuri: martor, CLF, CLF-silimarină și CLF-TO cu TO administrat în trei doze (200 mg/zi, 100 mg/zi, 50 mg/zi). Silimarina și *T. officinale* au fost administrate pe cale orală timp de șapte zile după inducerea CLF.

Efectul a fost evaluat prin efectuarea testelor de screening hepatic (aspartataminotransferaza, alaninaminotransferaza, fosfataza alcalină, gamma-glutamiltransferaza, bilirubina totală, colesterolul, trigliceridele, proteinele totale), testele renale (creatinină, uree) și markerii de stres oxidativ (statusul oxidativ total, reactivitatea antioxidantă totală, indicele de stres oxidativ, malondialdehida, tiolii totali, nitriți și nitrați totali, 3-nitrotirozina).

*T. officinale* are un conținut important de compuși fenolici și fitosteroli și o activitate antioxidantă semnificativă. CLF a fost asociată cu o creștere a parametrilor hepatici, reducerea colesterolului și a proteinelor totale, creșterea trigliceridelor, disfuncție renală și stres oxidativ. *T. officinale* a redus testele hepatice, trigliceridele, testele renale și testele de stres oxidativ.

În concluzie, în CLF experimentală extractul etanolic de rădăcini de *T. officinale* are efect hepatoprotector și reduce consecințele renale, iar aceste efecte au fost corelate cu activitatea antioxidantă și reducerea stresului oxidativ sistemic.

**Cuvinte cheie**: insuficiență hepatică cronică, hepatoprotecție, stres oxidativ, *Taraxacum officinale*, 3-nitrotirozina

1) Iuliu Hațieganu University of Medicine and Pharmacy Cluj-Napoca, Romania
2) University of Agricultural Sciences and Veterinary Medicine Faculty of Veterinary Medicine of Cluj-Napoca, Romania
*) *Corresponding author: cheta.iulia@gmail.com, marian.taulescu@usamvcluj.ro*

Liver is a critical organ because more than 500 physiological vital functions have been identified with the liver. Some of these are proteins, lipids and carbohydrates metabolism, production of bile, clearance of bilirubin, drugs and other poisonous substances, im-

mune system support, blood volume regulation. In the same time, liver is sensitive to many types of injuries, but it also has the property to repair itself. Today, chronic inflammatory diseases have been recognized as the most important cause of death in the world and chronic liver disease are a major health issue due to the high mortality and morbidity worldwide. Moreover, the incidence of CLD continues to rise due to the increase in obesity and excess alcohol consumption related liver disease.

In CLD, regardless of the etiology, the pathophysiology includes a persistent tissue injury with cellular death, followed by a chronic inflammation, activation of hepatic stellate cells, liver fibrosis, chronic liver failure (CLF), and finally cirrhosis with a high risk of hepatocellular cancer (7). During the chronic liver injury oxidative stress products are also generated and stimulate the chronic release of proinflammatory cytokines and chemokines. Consequently, in CLF the novel therapeutic targets are inflammation-driven fibrosis and stimulation of the protective immune responses. Because no effective therapies to reverse advanced liver fibrosis were found, the search of new medical therapies for CLF continues. An accumulating body of evidence suggests that plants polyphenols are health promoting phytochemicals that have multiple biological mechanisms of action, and can play a pivotal role in the management of inflammatory diseases. They are classified as flavonoids and non-flavonoids, based on their chemical structure.

Polyphenols are an integral part of the human diet, and many studies have demonstrated that polyphenols intake reduced the risk of chronic diseases, such as cardiovascular, neurodegenerative, and gastrointestinal diseases, diabetes, and cancers. These effects are the consequence of their antioxidant, anti-inflammatory, antiapoptotic, antiviral, antiallergic, antiplatelet, and anti-proliferative activities (2).

*Taraxacum officinale* (dandelion) is a perennial herbaceous plant from the Asteraceae family, rich in polyphenols with anti-inflammatory and antioxidative effects. Moreover, dandelion is a very rich source of vitamins and minerals such as Fe, Mg, Cu, and Zn. It can be found in Eurasia and other areas of the world. In traditional and modern medicine *T. officinale* (TO) have been used as diuretic, immune system stimulant, against gallbladder and hepatic disorders. These empirical effects have not been completely validated by scientific studies and more scientific evidence is needed to support the recommendation as a natural therapeutic product (4).

Considering the wide variety of therapeutic benefits which have been reported for *T. officinale*, the current study aimed to analyze the hepatoprotective and antioxidant effects of *T. officinale* root extract in an experimental CLF.

## MATERIALS AND METHODS

### Plant Material

Fresh *T. Officinale* F.H. Wigg. roots (TO) were harvested in June 2020 from the Alexandru Borza Botanical Garden "Babes-Bolyai" University of Cluj-Napoca, Romania and deposited in "Alexandru Borza" Botanical Garden Herbarium (Voucher CL:669002). The roots were dried in a shaded place, grounded and extracted in 70% ethanol (2/20, w/v) using the Ultra-Turrax extraction (UTE) performed in two steps as previously described (4). For further determinations, the lyophilized extract was dissolved in EtOH 70% (10 mg/mL). All assays were performed in triplicate.

### Determination of total phenolic content

The total phenolic content (TPC) of the TO extract was determined by Folin-Ciocâlteu spectrophotometric method as previously described, with gallic acid (GAE) as a reference standard (5).

### Identification and Quantification of Polyphenolic Compounds by HPLC-DAD-ESI MS

The phenolic compounds of the TO extract were determined by HPLC-DAD-ESI MS as previously described (6), using an Eclipse column, XDB C18 (4.6 × 150 mm i.d., 5 µm) (Agilent Technologies, U.S.A.) with a gradient elution of two mobile phases at a flow of 0.5 mL/min.

### Identification and Quantification of Phytosterols by LC-MS/MS

TO extract phytosterols content was determined by using a Zorbax SB-C18 (100 mm × 3.0 mm i.d., 5 µm) column (Agilent Technologies) with a mixture of methanol: acetonitrile (10:90, v/v) and isocratic elution, at 45 °C with a flow rate of 1 mL/ min as previously described, and the results were expressed as milligrams phytosterols/g of dry plant material (mg/g d.w.) (5).

### DPPH radical scavenging activity

The free radical scavenging activity of TO roots extract was measured using a method previously described (7). The assay was repeated in triplicate and the results were expressed as antioxidant activity (AOA) and also Trolox® equivalents (TE) per gram of dry plant material (mg TE/g d.w.).

### FRAP Assay

Using the FRAP (ferric reducing antioxidant power) assay was measured the reduction capacity of the TO extract as previously described (4). The experiment was repeated in triplicate, and results were expressed as Trolox® equivalents (TE) per gram of dry plant material (mg TE/g d.w.).

**Animals and experimental design**

Male Wistar rats (220-250g) obtained from the Animal Facility of Iuliu Haţieganu University of Medicine and Pharmacy, Cluj-Napoca, were used in the current study.

Animals were kept under standard conditions of temperature, humidity, ventilation, and lighting (light/dark: 13 h/11 h).

Rats were under standard diet with pellets including all alimentary elements. Food and water were available *ad libitum*. Animals were randomly divided into 6 groups (n = 5): Control, chronic liver disease (CLF), CLF with Silymarin treatment (CLF-SYL), and CLF with TO treatment in three doses (1ml/day): CLF-TO200 (200mg d.w.), CLF–TO100 (100mg d.w.), CLF–TO50 (50mg d.w.). All the procedures performed on laboratory animals, comply with the Directive 2010 /63/EU, and Romanian national law 43/2014 for animal protection used for scientific purposes. The project was approved by the Veterinary Sanitary Direction and Food Safety Cluj-Napoca (no. 19/ 13.12.2016).

The CLF rat model was induced by human serum albumin (HSA), as previously described (8).

After CLF induction the treatments were administrated *per os* (*p.o.*) by gavage for 7 days: the Control and CLF groups were treated with physiological saline (1ml/day), the CLF-TO200, CLF–TO100, CLF–TO50 groups were treated with 1 mL/kg body weight (b.w.) of the corresponding TO dilution, and those from the CLF-SYL group with silymarin (200 mg/kg b.w./day). After completing the treatments, general anesthetized was induced with ketamine (60 mg/kg b.w.) and xylazine (15 mg/kg b.w.), blood was withdrawn by retro-orbital puncture, serum was separated and stored at −80◦C until use, and animals were killed by cervical dislocation. The experiments were performed in triplicate.

**Biochemical serum analysis**

Liver injury was assessed by measuring serum aspartate aminotransferase (AST), alanine aminotransferase (ALT), total bilirubin (TB), alkaline phosphatase (ALP) and gamma glutamate transferase (GGT).

Liver failure was evaluated with serum total proteins (TP), cholesterol (CST) and tryglicerides (TG). Renal function was appreciated by measuring serum creatinine (CR), and urea. Oxidative stress was evaluated with serum total antioxidant reactivity (TAR), total oxidative status (TOS), oxidative stress index (OSI), malondialdehyde (MDA), total thiols (SH), total nitrites and nitrates (NOx), and 3-nitrotyrosine (3NT) as previously described (9, 10, 11).

**Statistical analysis**

All results were expressed as mean ± standard deviation (SD) whenever data were normally distributed. Comparisons between the different experimental groups were performed using the ANOVA test and the *post hoc* Bonferrony-Holm test.

The correlations analysis was performed with the Pearson test. Values of $p<0.05$ were considered statistically significant. The analysis was performed using IBM SPSS Statistics, version 20 (SPSS Inc. Chicago, IL, USA).

## RESULTS

**Phytochemical analysis**

The TO extract had a high TPC (33.1±2.04 mg GAE/g d.w.), and polyphenols analysis by HPLC-DAD-ESI MS found significant concentrations of hydroxybenzoic and hydroxycinnamic acids (Table 1).

Phytosterols analysis identified beta-sitosterol (32.03±1.47 µg/ml extract, corresponding to 71.74± 3.48 µg/g d.w. TO), stigmasterol (3.619±0.19 µg/ml,

**Table 1**

**Identification and quantification of *T. officinale* root extract polyphenols from hydroxybenzoic and hydroxycinnamic acids groups**

| Retention time $R_t$ (min) | UV $\lambda_{max}$ (nm) | $[M+H]^-$ (m/z) | Tentative identification | Subclass | Concentration* (mg CA/g d.w.) |
|---|---|---|---|---|---|
| 3.27 | 270 | **138** | Hydroxybenzoic acid | Hydroxybenzoic acid | 2,58 |
| 11.64 | 321 | **355** | 3-Caffeoylquinic acid (Neochlorogenic acid) | Hydroxycinnamic acid | 0,84 |
| 12.07 | 321 | **355** | 5-Caffeoylquinic acid (Chlorogenic acid) | Hydroxycinnamic acid | 0,89 |
| 13.45 | 320 | **181** | Caffeic acid | Hydroxycinnamic acid | 1,14 |
| 14.14 | 322 | **475** | Chicoric acid | Hydroxycinnamic acid | 1,70 |
| 17.20 | 322 | **195** | Ferulic acid | Hydroxycinnamic acid | 0,39 |
| | | | **Total** | | **7.56** |

* ***mg CA/g d.w. -*** *chlorogenic acid equiv. mg/g dry weight*

**Table 2**

**Liver screening tests**

| | ALT (U/l) | AST (U/l) | GGT (U/l) | ALP U/l) | TB (mg/dl) |
|---|---|---|---|---|---|
| CLF-TO200 | 74.22 ± 7.85 | 73.88 ± 5.10 | 61.76 ± 7.21 | 334.74 ± 17.36 | 1.34 ± 0.19 |
| CLF-TO100 | 59.95 ± 9.08 | 61.88 ± 8.22 | 44.62 ± 3.81 | 328.06 ± 21.95 | 1.68 ± 0.42 |
| CLF-TO50 | 60.08 ± 5.38 | 62.93 ± 8.70 | 51.22 ± 5.52 | 292.65 ± 10.99 | 1.22 ± 0.19 |
| CLF-SYL | 71.78 ± 3.70 | 74.45 ± 5.56 | 61.72 ± 3.46 | 319.77 ± 51.29 | 1.45 ± 0.48 |
| CLF | 98.59 ± 5.53 | 98.68 ± 8.59 | 67.07 ± 5.34 | 285.69 ± 18.02 | 2.41 ± 0.86 |
| CONTROL | 47.55 ± 6.09 | 51.04 ± 4.32 | 44.31 ± 4.59 | 263.75 ± 15.20 | 1.01 ± 0.11 |

*CLF-TO200 = chronic liver failure and T. officinale 200mg/day; CLF-TO100 = chronic liver failure and T. officinale 100mg/d; CLF-TO50 = chronic liver failure and T. officinale 50mg/day; CLF-SYL = chronic liver failure and sylimarin 200 mg/kg b.w./day; CLF = chronic liver failure; CONTROL = no treatment; ALT = alanine aminotransferase; AST = aspartate aminotransferase; GGT = gamma glutamate transferase; ALP = alkaline phosphatase; TB = total bilirubin (TB)*

**Table 3**

**Liver metabolic test and renal tests**

| | CST (mg/dL) | TG (mg/dL) | TP (g/dL) | CR (mg/dL) | UREA (mg/dL) |
|---|---|---|---|---|---|
| CLF-TO200 | 77.48 ± 15.38 | 289.62 ± 64.92 | 6.01 ± 0.76 | 0.62 ± 0.29 | 75.30 ± 26.18 |
| CLF-TO100 | 68.50 ± 17.18 | 279.67 ± 29.05 | 5.82 ± 1.38 | 0.62 ± 0.10 | 70.22 ± 48.70 |
| CLF-TO50 | 73.16 ± 20.64 | 288.67 ± 62.36 | 5.77 ± 1.26 | 0.60 ± 0.09 | 73.70 ± 16.40 |
| CLF-SYL | 79.02 ± 35.34 | 179.71 ± 50.55 | 5.70 ± 1.01 | 0.60 ± 0.15 | 82.52 ± 60.41 |
| CLF | 75.79 ± 9.92 | 315.61 ± 54.87 | 5.80 ± 0.82 | 0.94 ± 0.26 | 142.09 ± 130.13 |
| CONTRO | 95.77 ± 7.37 | 207.33 ± 70.77 | 6.54 ± 0.83 | 0.57 ± 0.04 | 40.48 ± 1.78 |

*CLF-TO200 = chronic liver failure and T. officinale 200mg/day; CLF-TO100 = chronic liver failure and T. officinale 100mg/d; CLF-TO50 = chronic liver failure and T. officinale 50mg/day; CLF-SYL = chronic liver failure and sylimarin 200 mg/kg b.w./day; CLF = chronic liver failure; CONTROL = no treatment; CST = cholesterol; TG = tryglicerides; TP = total proteins; CR = creatinine; BUN = blood urea nitrogen*

corresponding to 8.106±0.43 µg/g d.w.TO), and campesterol (1.301±0.04 µg/ml, corresponding to 2.914 ±0.11 µg/g d.w. TO) in the TO extract.

**Antioxidant Activity**

The DPPH test antioxidant activity was 179.74 mg TE /g d.w. (AOA = 129.3%), and FRAP test result was 642.3 mg TE/g d.w.. DPPH, FRAP, and TPC tests results were significantly correlated (r = 0.8, p<0.01).

**Biochemical serum analysis**

The hepatic injury was evaluated by performing the liver screening tests (Table 2). CLF induction by HAS caused a significant increase of AST, ALT, TB, ALP, and GGT (p<0.001). Administration of TO at three different dose levels in CLF animals for a week was not dose-dependent, reduced AST and ALT (p< 0.001), TB and GGT (p<0.01), and had no important effect on ALP (p>0.05). Furthermore, SYL administration had a smaller inhibitory effect on AST, ALT, and TB (p<0.01), and no effect on GGT and ALP (p>0.05) (Table 2).

In CLF animals metabolic tests showed a decrease of the CST (p<0.01) and TP (p<0.01), and an increase of the TG (p<0.001). TO extract treatment had no significant effect on CST (p>0.05) and TP (p>0.05), but reduced TG (p<0.05). SYL had no important effect on CST and TP too (p>0.05), but reduced TG (p< 0.001) more than TO (Table 3). There was a significant correlation between the liver tests and TG changes (r = 0.8, p<0.01) in TO treated animals.

CLF induction by HAS administration was associated with renal dysfunction with high serum CR (p< 0.01) and urea (p<0.001). The treatment of CLF rats with TO improved renal function, reducing serum creatinine (p<0.01) and urea (p<0.001), with no dose-dependent difference. SYL treatment had a smaller inhibitory effect on creatinine (p<0.05) and urea (p< 0.01) (Table 3). There was a significant correlation between the renal and liver tests (r = 0.6-0.8, p<0.01) in TO treated animals.

The systemic oxidative stress analysis showed that in CLF animals serum TOS and OSI were elevated (p<0.001), and the treatment with TO and SYL reduced them (p<0.01) (Table 4). TAR was not influenced by CLF induction and by the treatment with TO or SYL (p>0.05). The MDA level was increased by CLF (p<0.001), and both TO and SYL treatments reduced

**Table 4**

**Oxidative stress tests**

| | TAR (µmol TE Eqv./L) | TOS (µmol H2O2 Eqv./L) | OSI | MDA (nmol/L) | SH (µmol/L) | NOx (µmol/L) | 3NT (nmol/L) |
|---|---|---|---|---|---|---|---|
| CLF-TO200 | 0.91 ± 0.05 | 22.00 ± 2.70 | 35.08 ± 2.96 | 2.89 ± 0.12 | 0.58 ± 0.11 | 21.05 ± 5.08 | 865.59 ± 87.17 |
| CLF-TO100 | 0.91 ± 0.02 | 25.83 ± 3.57 | 28.44 ± 3.92 | 2.36 ± 1.16 | 0.62 ± 0.07 | 24.20 ± 1.74 | 843.54 ± 80.49 |
| CLF-TO50 | 0.91 ± 0.05 | 24.85 ± 3.05 | 27.24 ± 3.37 | 3.05 ± 0.17 | 0.55 ± 0.09 | 23.70 ± 4.80 | 790.03 ± 79.44 |
| CLF-SYL | 0.91 ± 0.02 | 27.37 ± 4.55 | 30.01 ± 4.96 | 3.03 ± 0.41 | 0.65 ± 0.10 | 24.94 ± 6.82 | 977.91 ± 38.42 |
| CLF | 0.91 ± 0.04 | 35.06 ± 2.50 | 43.92 ± 14.84 | 5.15 ± 0.68 | 0.50 ± 0.09 | 31.43 ± 2.85 | 1046.40 ± 96.35 |
| CONTROL | 0.91 ± 0.05 | 21.10 ± 1.72 | 23.21 ± 1.05 | 3.37 ± 0.30 | 0.59 ± 0.12 | 19.90 ± 1.99 | 480.45 ± 86.62 |

*CLF-TO200 = chronic liver failure and T. officinale 200mg/day; CLF-TO100 = chronic liver failure and T. officinale 100mg/d; CLF-TO50 = chronic liver failure and T. officinale 50mg/day; CLF-SYL = chronic liver failure and sylimarin 200 mg/kg b.w./day; CLF = chronic liver failure; CONTROL = no treatment; TAR = total antioxidant reactivity; TOS = total oxidative status; OSI = oxidative stress index; MDA = malondialdehyde; SH = total thiols (SH); NOx = total nitrites and nitrates; 3NT = 3-nitrotyrosine*

MDA ($p<0.001$) (Table 4). The SH was reduced by CLF induction (<0.05), and TO and SYL increased SH ($p< 0.01$). In CLF animals NOx and 3NT were elevated ($p<0.001$) and correlated ® = 0.9). The TO and SYL treatments lowered NOx and 3NT ($p<0.01$).

TO effects on the oxidative stress parameters was not dose-dependent (Table 4). There were good correlations between the oxidative stress tests and liver and renal tests ($r = 0.7$-$0.9$, $p<0.01$).

## DISCUSSION

In the current study, we showed that a seven days treatment with ethanol extract of TO roots had hepatoprotective activity and reduced systemic oxidative stress in HAS-induced rats CLF. These effects were correlated to the phytochemical profile.

It is generally accepted that medicinal plants are a rich source of bioactive compounds with antioxidant properties, and provide many beneficial applications in medicine, nutrition, and cosmetics.

The quality of plant products depends on the environmental factors, cultivation, harvest, postharvest, and storage methods (12). Lately, the phytochemical composition of TO extracts had been reported by many studies. TPC of the ethanol TO root extract obtained in this study was higher than that reported by other studies in TO flowers extracts and lower than in TO roots and vegetative parts extracts (13, 14). Such differences are accepted because extracts composition depends also on which part of the plant has been used (whole plant, roots, stem, leaves, flowers), on the extraction protocol and solvent (ethanol, acetone, water, or methanol) (3).

Previous phytochemical analysis of TO root powder found a rich hydroxycinnamic acids composition, and hydroxybenzoic acids derivatives and flavonoids were in lower concentrations. In this study, the phytochemical composition of the ethanol extract of TO roots had similar results, with a higher hydroxycinnamic acids concentration and lower hydroxybenzoic acids content. From the hydroxycinnamic acids, in our extract chicoric, caffeic, chlorogenic, and neochlorogenic acids were the most abundant, and feluric acid was in a smaller amount. In Kenny et al. study chlorogenic and caffeic acids had a in higher concentration (14). Many of these compounds have anti-inflammatory and antioxidant properties (15).

Some phytosterols were also measured in our ethanol extract of TO roots. These substances have anti-inflammatory and antioxidative activity, and reduce LDL-cholesterol. In oxidative stress conditions phytosterols oxidize forming oxysterols (16).

Campesterol has the highest oxidation rate, than sitosterol follow, and stigmasterol has the lowest oxidation rate. In the same plant extract the antioxidant compounds can protect phytosterols from oxidation.

In our extract beta-sitosterol was found in high amount, but stigmasterol and campesterol had a smaller concentration. Overall, these data suggest that in oxidative stress the anti-inflammatory and antioxidant activity of our ethanol extract of TO roots depends of the ratio between the TPC and phytosterols content.

The chemical composition and the pharmacological effects of plant extracts are correlated. That makes TO extracts from different plant parts to have different activities (12).

TO is an ethnomedicinal plant used from ancient times as choleretic, laxative, hepatoprotective, anti-inflammatory and antioxidant. The mechanisms of TO effects are still incompletely known and the use of different types of TO products is based on empirical findings. Gerbino et al. showed for the first time that acute exposure to an ethanolic dandelion root extract induced a dose-dependent and reversible Ca2+ increase in cells, process that seem to directly stimulate phospholipase C activity and signal transduction. Because Ca2+ signaling is involved in inflammation and oxidative stress, TO–Ca2+ interplay was pro-

posed as a starting point for the therapeutic use of TO extracts (3). These data encouraged us to continue by testing the antioxidant and hepatoprotective effect of TO root extract.

The antioxidant activity of out TO roots extract measured by DPPH and FRAP tests was in agreement with other studies, showing a strong antioxidant effect (14). From the results, we also observed a significant correlation between the TPC and the antioxidant activity of our extract, in both the DPPH and FRAP assays, which suggested that the phenolics had a significant contribution to the antioxidant activity.

For the hepatoprotective effect an experimental CLF model of immune liver injury and fibrosis induced by albumin in rats was used (8). TO roots extract was evaluated by measuring liver enzymes AST, ALT, ALP, and GGT, and TB. In CLF rats liver screening tests indicated hepatocytes injury. The treatment with our TO roots extract had hepatoprotective activity by reducing liver injury markers. These results were in agreement with other studies that tested the hepatoprotective effects of TO against hepatotoxicity induced by carbon tetrachloride or acetaminophen (17).

Sylimarin, a hepatoprotector drug with antioxidant and anti-inflammatory activity, caused a smaller improvement of the liver injury parameters.

In CLF liver metabolic functions were also disturbed. Serum TP and CST were decreased, and TG increased. Proteins synthesis reduction may trigger complications like coagulopathy, hemodynamic instability, jaundice, hepatic encephalopathy, hepatorenal syndrome and sepsis. There is a complex relationship between serum lipids and CLF. The evidences suggested that lipoprotein dysfunction may trigger bacterial malnutrition, infections, hematological complications, and adrenal dysfunction. In chronic liver diseases hypocholesterolemia may be a marker of liver injury, and represents an independent negative predictor of survival (18). The treatment with our TO roots extract in CLF animals had no effect on serum CST and TP, but reduced TG more than did SYL.

In CLF extrahepatic organs dysfunction may associate (19). The kidneys are frequently affected organs, and renal dysfunction may range from acute kidney injury to chronic kidney failure. Two mechanisms of secondary renal dysfunctions can be associated. One is the hepato-renal syndrome, a reduction of kidney function caused by renal hypoperfusion without parenchymal damage. The other one is the non-hepatorenal syndrome, induced by a renal insult such as inflammatory tubular injury in sepsis, bile acid nephropathy, and drug-induced tubular damage. In our study CLF animals had high serum creatinine and urea, and both TO and SYL treatments reduced creatinine and urea, TO having a better inhibitory effect.

Oxidative stress is the result of the imbalance between reactive oxygen species (ROS) production and antioxidant mechanisms scavenging capacity. It the liver, excessive mitochondrial ROS production interferes with the normal cell function. Hepatic stellate cells will be activated and an excess of extracellular matrix will be produced in the liver, leading to structural and functional changes. Moderate ROS release may regulate inflammatory mediators, and high levels of ROS activate hepatocytes necrosis and apoptosis leading to liver injury and CLF (20). The freely diffusible NO reacts with superoxide and form peroxynitrite, which causes further 3-nitrotyrosine residues formation on several proteins. These ROS and RNS damage mtDNA, proteins, and lipids, increasing further ROS production and closing a vicious cycle. In HAS-induced CLF liver immune injury associated an important systemic oxidative stress, with high TOS and OSI, along with increased production of MDA, NOx and 3NT, and decrease SH. The treatment with ethanol TO roots extract reduced oxidative stress, and the effect was comparable to SYL effect. There are evidences that chicoric, caffeic, chlorogenic, and ferulic acids have antioxidant activity. Because the best represented phenolics from our TO extract were chicoric, caffeic and chlorogenic acids, we attributed the oxidative stress reduction activity of to these phytochemicals. Furthermore, oxidative stress markers correlation to the liver injury and metabolic tests suggested that in HAS-induced CLF TO roots extract hepatoprotective effect depends on oxidative stress reduction.

In conclusion, this study reports that the ethanol extract of TO roots has hepatoprotective effect on HAS-induced CLF, and this is correlated with antioxidant activity and the phytochemical composition.

These findings suggest that TO root extract may be promising natural pharmaceutical tool. Further studies are required to fully elucidate the beneficial effects of TO root extract supplementation in CLD.

## ACKNOWLEDGMENTS

The authors would like to thank the Iuliu Hatieganu University of Medicine and Pharmacy, Cluj-Napoca, Romania for the financial support.

## REFERENCES

1. *Andreicut A.D., Pârvu A.E., Mot A.C., Pârvu M., Fischer Fodor E., Cătoi A.F., Feldrihan V., Cecan M., Irimie A.,* (2018), Phytochemical analysis of anti-inflammatory and antioxidant effects of *Mahonia aquifolium* flower and fruit extracts. Oxid Med Cell Longev, 2018:1-12
2. *Bajaj J.S., Moreau R., Kamath P.S., Vargas H.E., Arroyo V., Reddy K.R, Szabo G, Tandon P, Olson J., Karvellas C., Gustot T., Lai J.C., Wong F.,* (2018), Acute-on-chronic liver failure: getting ready for

prime time ? Hepatology, 68:1621-1638
3. *Balea Ş.S., Pârvu A.E., Pop N., Marín F.Z., Pârvu M.*, (2018), Polyphenolic compounds, antioxidant, and cardioprotective effects of pomace extracts from Fetească Neagră cultivar. Oxid Med Cell Longev. 2018:8194721
4. *Dias M.I., Barros L., Alves R.C., Oliveira M.B.P.P., Santos-Buelga C., Ferreira I.C.F.R.*, (2014), Nutritional composition, antioxidant activity and phenolic compounds of wild *Taraxacum* sect. Ruderalia. Food Res Int, 56:266-271
5. *Dreanca A., Sarosi C., Parvu A.E., Blidaru M., Enacrachi G., Purdoiu R., Nagy A., Sevastre B., Oros N.A., Marcus I.*, (2020), Systemic and local biocompatibility assessment of graphene composite dental materials in experimental mandibular bone defect. Materials (Basel), 13(11):1-17
6. *Farzaei M.H., Abdollahi M., Rahimi R.*, (2015), Role of dietary polyphenols in the management of peptic ulcer. World J Gastroenterol, 21(21):6499-6517
7. *Furman D., Campisi J., Verdin E., Carrera-Bastos P., Targ S., Franceschi C., Ferrucci L., Gilroy D.W., Fasano A., Miller G.W., Miller A.H., Mantovani A., Weyand C.M., Barzilai N., Goronzy J.J., Rando T.A., Effros R.B., Lucia A., Kleinstreuer N., Slavich G.M.*, (2019), Chronic inflammation in the etiology of disease across the life span. Nat Med, 25(12): 1822-1832
8. *Gerbino A., Russo D., Colella M., Procino G., Svelto M., Milella L., Carmosino M.*, (2018), Dandelion root extract induces intracellular Ca2+ increases in HEK293 cells. Int J Mol Sci, 19(4):1112
9. *Hamza A.A., Mohamed M.G., Lashin F.M., Amin A.*, (2020), Dandelion prevents liver fibrosis, inflammatory response, and oxidative stress in rats. J Basic Appl Zool, 2020:9
10. *Kenny O., J Smyth T., M Hewage C., P Brunton N.*, (2014), Antioxidant properties and quantitative UPLC-MS/MS analysis of phenolic compounds in dandelion (*Taraxacum officinale*) root extracts. Free Radicals Antioxidants, 4(1):55-61
11. *Luangmonkong T., Suriguga S., Mutsaers H.A.M., Groothuis G.M.M., Olinga P., Boersema M.*, (2018), Targeting oxidative stress for the treatment of liver fibrosis. Rev Physiol Biochem Pharmacol, 175:71-102
12. *Massaad C., Iuliano L., Lizard G.,* (2017), Oxysterols and phytosterols in human health. Chem Phys Lipids, 207:49-50
13. *Miłek M., Marcinčáková D., Legáth J.*, (2019), Polyphenols content, antioxidant activity, and cytotoxicity assessment of *Taraxacum officinale* extracts prepared through the micelle-mediated extraction method. Molecules, 24(6):1-14
14. *Pârvu M., Moţ C.A., Pârvu A.E., Mircea C., Stoeber L., Roşca-Casian O., Ţigu A.B.,* (2019), *Allium sativum* extract chemical composition, antioxidant activity and antifungal effect against *Meyerozyma guilliermondii* and *Rhodotorula mucilaginosa* causing onychomycosis. Molecules, 24(21):1–16
15. *Pop R.M., Puia I.C., Puia A., Chedea V.S., Leopold N., Bocsan I.C., Buzoianu A.D.*, (2018), Characterization of *Trametes versicolor*: Medicinal mushroom with important health benefits. Not Bot Horti Agrobot Cluj-Napoca, 46(2):343-349
16. *Privitera G., Spadaro L., Marchisello S., Fede G., Purrello F.*, (2018), Abnormalities of lipoprotein levels in liver cirrhosis:clinical relevance. Dig Dis Sci, 63(1):16-26
17. *Rusu M.E., Gheldiu A.M., Mocan A., Moldovan C., Popa D.S., Tomuta I., Vlase L.*, (2018), Process optimization for improved phenolic compounds recovery from walnut (*Juglans regia* L.) Septum: Phytochemical profile and biological activities. Molecules, 23(11):2814
18. *Sharifi-Rad M., Roberts T.H., Matthews K.R., Bezerra C.F., Morais-Braga M.F.B., Coutinho H.D. M., Sharopov F., Salehi B., Yousaf Z., Sharifi-Rad M., Del Mar Contreras M., Varoni E.M., Verma D.R., Iriti M., Sharifi-Rad J.*, (2018), Ethnobotany of the genus *Taraxacum*—Phytochemicals and antimicrobial activity. Phyther Res, 32(11):2131-2145
19. *Toiu A., Mocan A., Vlase L., Pârvu A.E., Vodnar D.C., Gheldiu A.M., Moldovan C., Oniga I.*, (2018), Phytochemical composition, antioxidant, antimicrobial and in vivo anti-inflammatory activity of traditionally used Romanian *Ajuga laxmannii* (Murray) Benth. ("nobleman's beard" - barba împăratului). Front Pharmacol, 9:7
20. *Wu Y.L., Lian L.H., Jiang Y.Z., Nan J.X.*, (2009), Hepatoprotective effects of salidroside on fulminant hepatic failure induced by D-galactosamine and lipopolysaccharide in mice. J Pharm Pharmacol, 61(10):1375-1382.