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THE RED BLOOD CELLS CATALASE ACTIVITY IS INFLUENCED BY TARAXACUM OFFICINALE

Abstract. Natural antioxidants protect cells against oxidative stress, which is directly involved in ageing processes and in the pathogenesis of cardiovascular, neurodegenerative and neoplastic diseases. Taraxacum officinale (TO) due to its rich content of biologically active ingredients has been commonly used in traditional medicine. It contains a wide spectrum of compounds with antioxidant activity. The content depends on parts of this plant. The roots are rich in phenolic and terpene compounds, sesquiterpene lactones, fructosans and inulin, while leaves are rich in substances belonging to flavonoids, phenolic acids, coumarins and vitamins, especially vitamin A. The literature results prove that antioxidant activity of TO depends on several factors including the plant part, the solvent used, as well as the duration of extraction. The aim of this study was to evaluate and to compare the action of alcoholic extracts of roots and leaves of TO on RBC’s catalase activity. Material and methods. Raw Taraxacum officinale plant material consisted of dried leaves and roots were harvested from a natural site. The extracts for analysis were prepared using 20, 25, 40, 50 and 80% (v/v) ethanol mixtures used as solvents. The catalase activity was established by using RBC of healthy persons. Conclusions. Phytotherapeutic herbs and plants continue to play an important role in the discovery and development of drugs. Leaves and roots of dandelion represent a rich source of bioactive compounds for potential exploitation in nutraceuticals and pharmacological sectors. Taraxacum officinale has a high ability to act as an antioxidant. The highest influence on RBC’s catalase activity was reported in case of roots ethanolic extracts of 25%. These actions are realized due to multiple substances, whose content probably depends of ethanol’s concentration. Additional studies are needed to characterize biological activities of these extracts. Keywords: Taraxacum officinale, dandelion, oxidative stress, catalase activity.

Introduction. Reactive oxygen species are produced by cells as a result of normal metabolism. Molecules containing one or more unpaired electrons and thus giving reactivity to the molecule are called free radicals. There are three major reactive oxygen species, with a very important physiological significance: superoxide anion ($\text{O}_2^-$), hydroxyl radical (•OH) and hydrogen peroxide (H$_2$O$_2$).

Hydrogen peroxide is a harmful product of usually normal, metabolic processes. It is also produced by xanthine oxidase, amino acid oxidase, NAD(P)H oxidase and by consumption of molecular oxygen in metabolic reactions in
peroxisomes. In a succession of reactions (Haber–Weiss and Fenton), H₂O₂ can breakdown to OH⁻ in the presence of transmission metals like Fe²⁺ or Cu²⁺. It easily diffuses across the plasma membranes of cells. The redox properties of hydrogen peroxide depend of pH. In acidic solutions, it is a powerful oxidizer, and in basic it can reduce multiple inorganic ions. It represents one of the agents by which oxidative stress can induce proteins, lipids membranes and DNA damages.

The cells developed a series of antioxidant agents which protect against harmful effects of H₂O₂. Glutathione (GSH) donates its electron to H₂O₂ to reduce it into H₂O and O₂. The superoxide dismutase by a redox reaction converts superoxide into oxygen and hydrogen peroxide. The last, normally is decomposed in water and oxygen by an enzyme, called catalase, found in cells organelles, peroxisomes. This enzyme represents a tetramer of four polypeptide chains, with four iron-containing heme groups that allow the enzyme to react with the hydrogen peroxide. By means of a peroxidation reaction the catalase can eliminates the poisonous hydrogen peroxide in the process of oxidizing other substances, including alcohols, phenols, formic acid and formaldehyde. The optimal pH for catalase activity is about 7 and the rate of reaction does not change too much between 6,8 and 7,5.

People always tried to find in the nature components that can solve cruel health problems. The evaluation of the antioxidant activity of plants has been an important issue taking into account its importance on human health. Taraxacum officinale (TO), popularly known as dandelion, demonstrated its effectiveness in treatment of liver disorders, inflammations, as well as exhibited promising antibacterial, antiviral, antiparasitic, antifungal and cytotoxic activities.

Taraxacum officinale is an excellent source of terpene derivatives (taraxerol, taraxasterol and the glycoside taraxacoside), as well as sterols (β-sitosterol, stigmasterol), rubber, tannins, fatty acids, levulose, caffeic acid, ψ-hydroxyphenylacetic acid, asparagine, tyrosine, carotenoids, phytosterol, β-amirin, flavonoids, citric acid, amino acids, saponins and inulin. This plant is also rich in minerals such as iron, copper, potassium, contains a lot of vitamins A, C B1, PP and D. Extracts from TO exhibit a significant antioxidant activity, which seems to be
related to the presence of phenolic compounds. However, in plasma, the best protective effect against \( \text{H}_2\text{O}_2/\text{Fe} \) oxidation was reported in the presence of enriched fractions of SL amino acid adducts (flavonoids, sesquiterpene lactones) and inositol 4-hydroxyphenylacetate esters. Because TO contain many components, it is difficult to assimilate their mechanisms of action, since these are generally ill-understood. Nowadays, there is no exhaustive data which can describe the TO content and action regarding to different types of extractants and their concentration, as well to compare roots and leaves action on catalase activity.

**Aim of the study.** To explore the impact of different *Taraxacum officinale* ethanolic extracts with different bioactive ingredients on red blood cells (RBC) catalase activity.

**Materials and methods.** Leaves and roots of *Taraxacum officinale* F. H. Wigg were harvested from a natural habitat (May 2017). Two weeks of desiccation took place in the lab conditions, at room temperature. Dried leaves and roots were ground manually to a fine powder and five series (20, 25, 40, 50 and 80%) of ethanolic extracts were made. The ratio of biomass-to-solvent was 10:1 (expressed in mg/ml). The extraction of active components was done in recipients of 100 ml during 24 hours, at room temperature. The extracts were filtered (Whatman No.1) and stored at +4\(^\circ\)C. Extracts’ aliquots (1.5ml) were centrifuged (MPW 370, 5 min, 5000 rpm) and supernatants were used for further assays.

The RBC’s catalase was obtained in accordance with method purposed by Ryzhikov et al. (2011) (1). Healthy persons’ blood was diluted 1:4 v/v with DMEM (Dulbecco medium), mixed up with gentamicin (100\(\mu\)g/ml), heparin (2.5 un/ml) and L-glutamine (0.6 mg/ml). An amount of 0.9 ml of diluted blood was supplanted with 0.1 ml of TO ethanol extracts in all tested wells. The TO extracts substituted with equivalent quantity of ethanol in NaCl (0.9%) isotonic solution represented the control group. The 24 hours of microplates incubation (37\(^\circ\)C, 3.5% CO2 humidified atmosphere) was continued with centrifugation (5 min, 1500 rpm). The obtained RBC mass was used for further catalase activity assessments, by method published by Gudumac et al. (2012) (2). This assay is based on enzyme’s property to catalyze cleavage of hydrogen peroxide to \( \text{H}_2\text{O} \) and \( \text{O}_2 \). The \( \text{H}_2\text{O}_2 \) forms with
ammonium tetrathiomolybdate a yellow complex, which during hydrogen peroxide cleavage lose its color. The grade of fading corresponds to catalyse activity, measured spectrophotometrically at 410 nm. In case of control, H₂O₂ was replaced with distillated water. The results were expressed as µM/g.Hb. All experiments were made in triplicate in 24-wells microplates.

The statistics (GraphPad Prism 8.0) included calculation of mean and standard deviation (M±SD), the percentage of difference between experimental group and control, Mann-Whitney U test (control vs experimental groups, leaves vs roots extracts) and Spearman (rₛ) correlation (ethanol concentration vs catalase activity in tested samples). The significance level was fixed at p ≤0.05 for all statistical analyses. The Research Ethics Committee of the “Nicolae Testemitanu” State University of Medicine and Pharmacy approved the present study (nr.81 of 19.09.2020).

**Results.** The catalase activity was different in diverse ethanolic extracts of TO. This enzyme activity was evaluated with 23,8±2,3*, in case of leaves ethanol of 20%, which was lower with 47,2% than control. In case of 25% of extractant concentration, catalase activity was recorded with 47,6±0,4, result which was a little bit higher (5,7%) than control, but not statistically significant (p=0,51). Appropriated results were obtained and in case of TO extracts with 40% (43±2; 2,2%, p=0,51) and 50% of ethanol (43,6±1,1; 7,3%, p=0,51). In case of ethanol of 80%, obtained results regain statistical credibility (43,8±1,2*; p=0,05), which was with 15,2% higher than control. Results marked with asterix confirmed a statistically significant difference of control and tested group. The catalase activity in case of leaves extracts recorded a negative, low and not statistically significant correlation to alcohol concentration (rₛ=-0,33; p=0,11).

In case of roots extracts of 20%, catalase was measured as 48,04±0,73 (p=0,05), data which were lower with 13,3% than control. This enzyme activity grew up (56,12±3,26, p=0,05) in case of 25% of ethanol concentration, results which were with 47,5% higher than control. By growing the ethanol’s concentration, the catalase activity get lower in comparison to controls: in ethanol of 40% enzyme activity was reported as 41,72±0,16, results lower with 7,3% than control (p=0,51), in ethanol of
50% as 41.88±11.67, data lower as control with 15.5% (p=0.51), in ethanol of 80% - 41.71±2.49, which means 11.4% less than control (p=0.51). The Spearman correlation reported a strong, negative and statistically significant association between ethanol’s concentration of roots extracts and catalase activity ($r_s=-0.73; p=0.001$).

By comparing the results, leaves vs roots have been established: catalase activity in leaves extracts, made with ethanol of 20% is lower than roots made with the same extractant with 24.3% (p=0.05), leaves extracted with 25% ethanol exhibited a lower activity than roots with 8.5% (p=0.05), equal in ethanol’s of 40% (1.3%, p=0.51), 50% (1.8%, p=0.51) and 80% (2.1%, p=0.28). These results were plotted in Fig.1.

Fig. 1. Catalase activity: leaves vs roots ethanolic extracts (20-80 represents leaves (LEtOH) and roots (REtOH) ethanolic extracts concentration)

Discussion. TO bioactive compounds have been widely studied as natural phytochemical drugs due to promising antioxidant properties compared with synthetic antioxidants. Bioactive compounds have been investigated for the
beneficial effects (anti-oxidant, anti-tumor, anti-inflammatory, anti-diabetic…) on human health. It is traditionally used in the form of infusions or decoctions as aperitive, laxative, stimulant and mild diuretic. The TO fascinates us with its chemical content. Shutz et al. (2005) evaluated the content of flavonoids and phenolic acids by high-performance liquid chromatography (3). About 43 compounds, without microelements and vitamins, were detected and the most abundant was chicoric acid, that is a dicaffeoyltartaric acid.

The content of TO is different and depends on many factors, related to climate, type of soil and plant’s part. As example, free 4-dymethil sterol esters were found to be maximal during winter and their quantity correlates negatively with temperature and sunshine (4). Opposite, sitosterol and cycloartenol esters correlate positively with sunshine and temperature. The time of harvesting importance was demonstrated and by Rodrigues-Ortega et al. (2013) experiment where chloroform and hexane extracts of 2 and 5 months old TO were used to estimate the antiviral activity (5). The chemical composition of leaves of 5 months was more complex than leaves of 2 months. Plus, only chloroform extracts of 5 months old leaves inhibited the yellow fever virus replication in a dose dependent manner, which was evaluated by researches as 8 times more effective as leaves of 2 months, chloroform extracted too.

The content of TO parts seems to be different. The hydroxycinnamic acids, as chicoric, monocaffeyltartaric and chlorogenic were found in whole plant, while coumarins, cichoriin and aesculin were found mainly in leaves (6). Mir et al. (2013) reported interesting data of qualitative and quantitative analysis of TO in aqueous and methanol extracts of roots, stems and flowers (7). Authors found that flavonoids content is higher in flowers’ extracts, while saponins, phenols and alkaloids were highly concentrated in stems and roots. By Popescu et al. (2010) flowers and leaves have higher amount of polyphenols, compared to stems and roots (8).

The investigation, identification and quantification of the bioactive compounds of TO extracts are related mainly to phenolic compounds, which play an important role in plants defense reactions against biotic and abiotic stresses, as well as in nutrition and welfare. But TO contains a wide spectrum of bioactive components
whose ratio and summary effects on different biochemical processes will be debated by many experiments.

The type of extractant seems to be very important. In accordance to Hu et al. (2003), which investigated chemical and bioactive properties of TO flowers in water and ethyl acetate fractions, the content and type of activity is different (9). Both types of extracts exhibited free radical scavenging activities and reduced the breakage of DNA strands induced by free radicals. Plus, both types of extracts reduced oxidation of structured phosphatidylcholine liposome by peroxyl radical, but ethyl acetate extract had greater affinity to scavenge peroxyl radical than water extracts. Moreover, the concentration of extracts seems to be important too. The same authors reported that this extracts has higher prooxidant activity at low concentration, thus indicating that the reducing power of TO flowers had resulted in generation of reactive copper ions and high concentrations, especially of ethyl acetate did not promote oxidation in the presence of copper, suggesting that the free radicals scavenging activity was sufficient to minimize the oxidative potential of metal ions, associated with prooxidant activity.

The Mir et al. (2013) demonstrated how can vary content due to type of extractant: the phenols and steroids in stems and roots represents 21-22% in water extract and 18% in methanol. The same components in flowers water extract measured 19% and 16% in methanol (7).

Recently, Khan et al. (2019) evaluated the total phenolic content and found the maximal value (692 mg/g gallic acid equivalent) in hydro-alcoholic extracts (50:50), in comparison to aqueous extracts (42 mg/g (10).

In Oseni and Yussif (2012) experiment, the TO leaf ethanolic and water extracts exhibited antibacterial activity (11). These results revealed that ethanolic extracts kills germs better than water extracts, plus antibacterial activity correlated positively with alcohol concentration.

The Triticum bioassay performed by Popescu et al. (2010) revealed concentration dependent effects of TO aqueous extracts on cell division (8).

The Sigstedt et al. (2008) evaluated the influence of TO on tumors by using aqueous extracts of leaves, roots and flowers (12). Their results showed that TO
leaves extract blocks the growth of MCF-7/AZ cancerous cells, while flowers and roots extracts had no effect on cells growth, but roots extracts in turn were able to block tumor invasion.

Esatbeyoglu et al. (2017) supplemented the TO heterogenic activity by demonstrating that leaves extracts activate the transcription of nuclear related factor 2 in human hepatocytes more than roots extracts (13).

Such kind of data generates assumption that content and ratio of bioactive components, as well the action in case of oxidative stress in our experiment should be different. And it was proved. In our experiment, the catalase activity was influenced also by ethanol concentration, in both cases, roots and leaves. We reported before that other biochemical pathways of oxidative stress are influenced by TO in ethanol concentration dependent manner (14–16).

Commission monographs recommended the use of TO three times daily as herb or liquid extract 1:1 in 25% alcohol, which corresponds in fact with our results, where highest activity of catalase was determined exactly in this ethanol’s concentration (6).

*Taraxacum officinale* is not a single example which describes how important is the type of solvent, the literature abounds in fresh data which underlines the importance of extractant type and its concentration (17–19).

Conclusions. Phytotherapeutic herbs and plants continue to play an important role in the discovery and development of drugs. Leaves and roots of dandelion represent a rich source of bioactive compounds for potential exploitation in nutraceuticals and pharmacological sectors. *Taraxacum officinale* has a high ability to act as an antioxidant. The highest influence on RBC’s catalase activity was reported in case of roots ethanolic extracts of 25%. These actions are realized due to multiple substances, whose content probably depends of ethanol’s concentration. Additional studies are needed to characterize biological activities of these extracts.

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Conflict of interests. The authors declare that there is no conflict of interests regarding the publication of this paper.
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