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Taraxacum Officinale Roots Acts As A Powerful Antioxidant

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ORIGINAL PAPER

Abstract

Introduction. Taraxacum officinale roots are high in phenolic and terpene compounds, sesquiterpene lactones, fructosans, and inulin, all of which have significant antioxidant properties when used alone or in combination. **Aim.** To investigate the antioxidant properties of Taraxacum officinale. **Material and methods.** The antioxidant activity of Taraxacum officinale ethanolic and dimethyl sulfoxide extracts on red blood cells superoxide dismutase and catalase activity in healthy people was investigated. **Results.** The current study's findings revealed that plant extracts have a high antioxidant potential. TOR extracts in DMSO and 50% ethanol significantly increased superoxide dismutase activity, while 25% ethanolic extracts increased catalase activity. **Conclusion.** Taraxacum officinale roots have a high antioxidant activity that varies depending on the extractant and alcohol concentration. More research is required to determine the underlying mechanisms of this activity.

Keywords: *Taraxacum* officinale roots, red blood cells, SOD, CAT, antioxidants.

I. INTRODUCTION

Reactive oxygen species (ROS) are a class of unstable molecules that contain oxygen and react with other molecules in cells, such as superoxide anion (O2), hydrogen peroxide (H2O2), and hydroxyl radical (OH). ROS are free radicals, which can damage proteins and lipids of membranes, DNA, RNA, also may cause cell death [1, 2].

The antioxidant compounds can prevent or reduce the harmful effects of ROS. Enzymatic antioxidants such as catalase (CAT), superoxide dismutase (SOD), glutathione peroxidase (GPOx), glutathione S-transferase (GST), glutathione reductase (GR), and others, and non-enzymatic antioxidants such as fat soluble vitamin E (-tocopherol), water soluble vitamin C (ascorbic acid), glutathione (GSH), polyphenol

Oxidative stress (OS) is defined by increased production and high levels of ROS and O2 that far outnumber the cell's defense system. As a result, OS causes cell and tissue damage and plays a role in the pathogenesis and progression of many diseases [4,5]. O2 is a byproduct of O2 metabolism that, if not controlled, causes a variety of cell damage [6].

SOD is an important antioxidant because it converts superoxide (O2) anion into oxygen and hydrogen peroxide in a redox reaction: H2O2, which is also a damaging molecule, is degraded by several enzymes, including CAT, found in cell organelles, peroxisomes: $2H_2O_2 \rightarrow 2H_2O + O_2$.

The antioxidant system's activity is influenced by a variety of internal and external factors, including natural substances. Plants are still used in the discovery and development of drugs and dietary supplements. Taraxacum officinale F. H. Wigg (TO) is a natural medicinal plant that can be found all over the world. TO roots (TOR) are a good coffee substitute and a folk remedy for liver and gallbladder diseases, digestive disorders, and lowering body weight, blood pressure, and cholesterol [7]. TOR are high in microelements, vitamins, and proteins, as well as beta-carotene and polyphenolic compounds (chicoric, 4-caffeoylquinic, chlorogenic, caffeic, p-coumaric, ferulic acids, and their derivatives) [8].

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TO extracts contain significant amounts of antioxidants [9]. The antioxidant activity of TO and total phenolic compounds were found to have a strong positive correlation. TO's antioxidant activity is affected by several factors, including plant part, solvent used, and extraction time [10].

The purpose of this study was to see how DMSO and ethanolic TOR extracts affected red blood cell (RBC) CAT and SOD activity in healthy people. There are currently no concurrent studies that compare the effects of TOR ethanolic and DMSO extracts on enzymatic system activity.

II. MATERIAL AND METHODS

The fresh middle-sized TO roots were harvested from a natural habitat in the Republic of Moldova. After cleaning and weighing, the roots were dried under laboratory conditions at room temperature for 2 weeks. Dried roots were grinded (Scarlett Coffee grinder, SC-4145) to a fine powder and samples were soaked in ethanol (Luxfarmol, MD) of 80%, 50%, 40%, 25% 20% and 10% (100 mL of each solution). In addition, the powder of roots was soaked in 100 mL of dimethyl sulfoxide (DMSO) of 0.1% (Merck, DE). 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 for 24 hours. The extracts were filtered Whatman No.1 (WHA10010155, Merck, DE) and stored at $+4^0$C. Extracts' aliquots (1.5 mL) were centrifuged (MPW 370, 5 min, 5000 rpm). The absence of stratification or sedimentation confirmed the samples purity. All further assays were made in triplicate in 24-wells microplates.

The influence of TOR extracts on RBC's enzymatic system was evaluated in accordance with Ryzhikov S.L. *et al.* (2011) [11] modified by us (12). Healthy persons' blood was diluted 1:4 v/v with DMEM (Dulbecco medium), mixed up with gentamicin (100µg/mL), heparin (2.5 un/mL) and L-glutamine (0.6 mg/mL). The amount of 0.9 mL of diluted blood in all tested wells was supplemented with 0.1 mL of TOR ethanolic or DMSO extracts. The alcohol or DMSO solution substituted the TOR extracts in case of control wells. After 24 hours of incubation (Memmert lab incubator I, DE) at 37^0C, 3.5% CO_2 humidified atmosphere, microplates were centrifuged (5 min, 1500 rpm). The obtained RBC mass was used for further SOD activity assessments according to Gudumac *et al.* method [12, 13]. SOD activity was determined by measuring the inhibition in photoreduction of nitroblue tetrazolium (NBT) to colored formazan (Fig.1). The color change of the solution was measured spectrophotometrically at 540 nm (BioTek, USA).

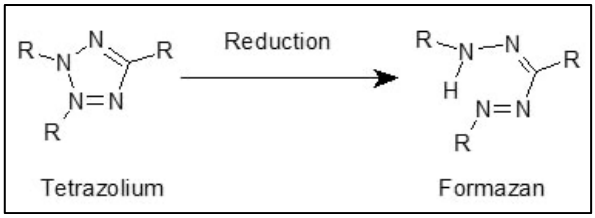

Figure 1. Photoreduction of nitroblue tetrazolium to formazan.

One unit (U) of SOD activity was defined as the amount of enzyme causing 50% inhibition of photochemical reduction of NBT. Enzyme activity was reported per 1 g of protein.

The influence of TOR extracts on RBC's CAT was evaluated in accordance with the method described by Gudumac V. *et al.* (2012) [12, 13]. The assay is based on the property of the enzyme to catalyze the breakdown of hydrogen peroxide to H_2O and O_2. H_2O_2 forms a yellow complex with ammonium tetrathiomolybdate, which loses its color when hydrogen peroxide is cleaved. The degree of discoloration of the solution corresponds to the catalase activity measured spectrophotometrically at 410 nm (BioTek, USA). In case of control, H_2O_2 was replaced with distillated water. The results were expressed as µM/g.Hb. All experiments were made in triplicate in 24-wells microplates.

The statistical analysis included calculation of mean and standard deviation (M±SD), Mann-Whitney *U* test (control *vs* experimental groups) and Spearman (r_s) correlation (ethanol concentration *vs* enzymatic activity in tested samples). The p-values equal or less than 0.05 were considered statistically significant.

The present study was approved by the Research Ethics Committee of the "Nicolae Testemitanu" State University of Medicine and Pharmacy (decision nr. 81 of 19.09.2020). All assays were performed in accordance with the ethical standards as laid down in the 1964 Declaration of Helsinki.

III. RESULTS AND DISCUSSIONS

A. Results

Our results showed different influence of individual ethanolic extracts on SOD in healthy persons RBC. Only the 40% TOR ethanolic extract determined statistical significant decrease of SOD activity below the control values by 9% (26.25±0.68, p=0.05), while the rest of the tested alcoholic extracts increased statistical significant the activity of the enzyme (p=0.05 in all cases). Thus, SOD activity was enhanced by 3.5% in ethanolic extracts of 25% (31.4±0.48), by 10.1% in

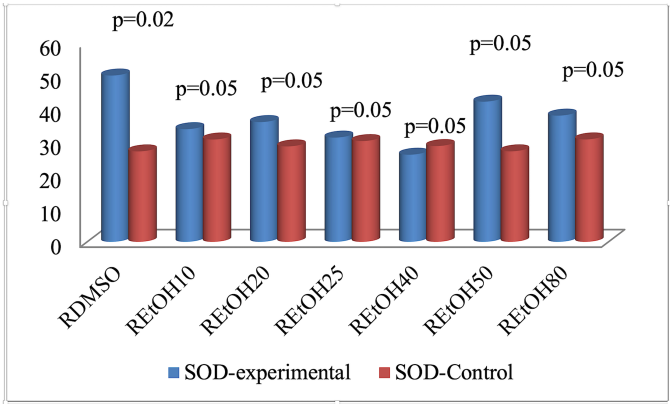

Figure 2. Influence of TOR on RBC's SOD level (U/g.Hb). **Note:** RDMSO – roots extract in DMSO; REtOH – roots extract in ethanol of different concentration (10-80%). p – statistical significance of differences compared to control.

In RBC, only under the influence of 25% extracts of

TOR, the activity of CAT was statistically significant increased by 47.5% (56.12±3.26 µM/g.Hb, p=0.05) compared to the control. The rest of the TOR ethanolic extracts lowered CAT activity in comparison with the control samples – 20% extracts of TOR by 13.3% (48.04±0.73, p=0.05), 40% extracts by 7.3% (41.72±0.16, p=0.51), 50% extracts by 15.6% (41.88±11.67, p=0.51), 80% extracts by 11.4% (41.71±2.49, p=0.51) and 10% extracts by 21.7% (55.06±4.52, p=0.13). The DMSO extract of TOR also diminished CAT activity in comparison with the control sample by 42.3% (26.17±9.84, p=0.02). The results were presented in Fig. 3.The Spearman correlation reported a strong, negative and statistically significant association between ethanol's concentration of TO roots extracts and CAT activity in the RBC of healthy persons (r_s=-0.73; p=0.001).

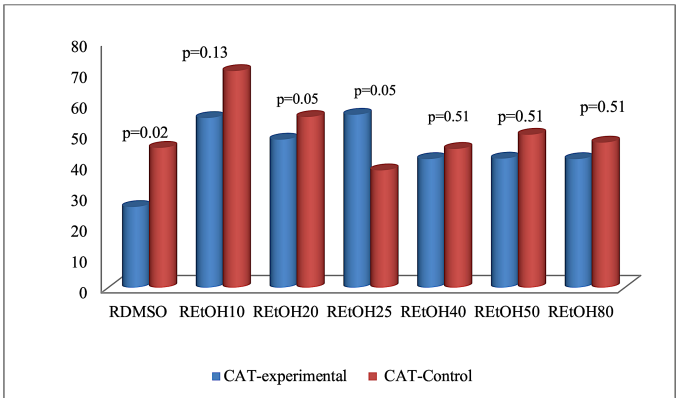

Figure. 3 Influence of TOR on RBC's CAT level (µMol/g.Hb). **Note:** RDMSO – roots extract in DMSO; REtOH – roots extract in ethanol of different concentration (10-80%); p – statistical significance of differences compared to control.

B. Discussion.

Taraxacum species and subspecies have shown a variety of promising activities due to phytochemicals such as phenolic acids, flavonoids, terpenes, oligo- and polysaccharides, alkaloids, peptides, and other compounds. TO roots have been used in herbal medicine to help and enhance immune systems, as a liver tonic, for urinary tract infections, and pneumonia, as well as for antimicrobial and anti-inflammatory properties [14]. Serum ASAT (aspartate aminotransferase), ALAT (alanine aminotransferase), ALP (alkaline phosphatase), LDH (lactate dehydrogenase), and MDA (malondialdehyde) levels were significantly reduced by TOR extract. TOR also increased the activity of the hepatic antioxidant enzymes CAT, GPOx, GST, GR, and GSH while decreasing the activity of the hepatic antioxidant enzyme SOD [15,16]. TOR ethanolic extract increased MT (metallothionein), GFAP (glial fibrillary acidic protein), and -SMA (-smooth muscle actin) expression in chronic hepatitis C virus infection, according to Zakaria et al. (2010) [17]. The biological activities of TOR are based on its chemical composition, which includes polyphenols and flavonoids, terpene and sesquiterpene lactones, fructosans and inulin, microelements and vitamins, all of which play an important role alone or in combination [6, 7]. Individual compound content in TOR varies depending on many factors, including environmental conditions and harvesting time, location, and storage method [10]. Popescu et al. (2010) demonstrated that the amount of bioactive compounds varies depending on the part of the TO: flowers and leaves contained more polyphenols than roots and stems [18]. Furthermore, the authors concluded that the action of TO on cell division is dependent on the type of extractant used. The authors described for the first time the modulatory action of TO aqueous extracts on cell division, whether inhibitory or stimulatory. Hagymasi et al. (2000) demonstrated that root and folium extracts can stimulate NADPH-cytochrome P-450 reductase activity even in the absence of NADPH. The same authors reported that the polyphenol content of leaf extract is approximately three times that of radix extract and that it is more effective [19].

Numerous data presented in the specialized literature in recent years revealed that the biological action of TO, both in normal and pathological conditions, is dependent on the part of the plant and the type of solvent used to prepare the extracts [20-22]. Fulga et al. (2021) described the significant antioxidant activity of TO extracts as evidenced by changes in the activity/content of GSH, GSSG, GPOx, GST, and GR on healthy people's RBCs [10, 23]. The results of our previously reported study show that roots ethanolic extracts of 25% had the greatest influence on RBC catalase activity, which corresponds to the literature data [23]. According to Grauso et al. (2019), the highest CAT activity was determined in the same alcoholic extraction of 25% [24]. Sumanth et al. (2006) discovered that TOR extract increased the activities of SOD, CAT, GSH, and GPOx while decreasing lipid peroxidation in liver disorders [25]. Park et al. (2007) discovered that root extract has a protective effect against liver injury caused by carbon tetrachloride (CCl4) in Sprague-Dawley rats. The serum ALAT and ASAT activity were reduced by the TOR supplement. GPOx, GR, and SOD levels increased in a dose-dependent manner, but cytochrome P450 2E1 mRNA and protein expression levels decreased significantly in the TO-treated group [26].

Jung et al. (2015) compared the antioxidant (CAT, DPPH scavenging activity, lipid peroxidation inhibitory activity) and antitumor (HepG2) activities of TO aerial parts, roots, and mixed extracts (aerial parts + roots) [27]. The results show that a small addition of roots to leaves (9:1) improved all activities. Wojtowicz et al. (2007) previously described the high activity of TOR in 80% methanol extract [28]. In another recent study, we demonstrated that the type of extractant, its concentration, and plant part are all requirements that should be considered when evaluating TO activity. The extract of roots made in 80% ethanol had the greatest effect on glial tumors [29].

On the 5th of May 2021, the Committee on Herbal Medicinal Products (HMPC) stated in the Assessment report on TO radix (EMA/HMPC/475725/2020) that ethanolic extracts of dandelion root caused a dose-dependent inhibition of ADP-induced human platelet aggregation, with a maximal inhibition of 85% extract with a concentration of 0.04 g dried root/mL of human platelet-rich plasma (PRP). Arachidonate and collagen had no effect on platelet aggregation. At a concentration equivalent to 0.04 g crude material/mL PRP, a high molecular

weight fraction (Mr>10,000) enriched in low-molecular polysaccharides inhibited platelet aggregation by 91%, while a lower one (Mr10,000) containing triterpenes and steroids inhibited it by 80%. The commission's monographs recommended taking TO three times a day as a 1:1 herbal or liquid extract in 25% alcohol, which is consistent with our findings, which show that high CAT activity is precisely determined when this concentration of ethanol is used for extraction [24].

We did not determine the composition of the extracts or the ratio of the substances in the individual extracts, so this study has limitations. Furthermore, we are unable to draw any conclusions about the specific TOR compounds or components that exhibit activity or are responsible for the effect of ethanolic and DMSO extracts on SOD and CAT activity. New research is needed in these areas to determine the mechanisms that underpin the possibility of using TOR-derived compounds as drugs.

IV. CONCLUSION

The results of the current study, which looked at the effects of various TOR ethanolic and DMSO extracts on the RBCs, SOD, and CAT activities of healthy people, revealed that plant extracts have a high antioxidant potential. TOR extracts in DMSO and 50% ethanol significantly increased SOD activity, while 25% ethanol extracts increased CAT activity. The current study confirms our and other researchers' findings that the effects of TO vary depending on the type of extractant and alcohol concentration. More research is needed to determine the underlying mechanisms of TO's established actions, which highlight the plant's potential as a tool for the prevention and/or treatment of diseases associated with oxidative stress.

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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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