NATIA BOKUCHAVA [1], TAMAZ MURTAZASHVILI [1], NODAR MITAGVARIA [2], KOBA SIVSIVADZE [1], MARIAM TATANASHVILI [1]

EFFECTS OF GRAPEVINE SHOOTS EXTRACTS ON THE DEGREE OF OXIDATIVE STRESS IN LABORATORY RATS

[1] TSMU, Department of Pharmaceutical and Toxicological Chemistry, Tbilisi, Georgia;
[2] Ivane Beritashvili Center of Experimental Biomedicine, Laboratory of Cerebral Blood Flow and Metabolism, Tbilisi, Georgia

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ნათია ბოკუჩავა [1], თამაზ მურთაზაშვილი [1], ნოდარ მითაგვარია [2], კობა სივსივაძე [1], მარიამ ტატანაშვილი [1]

ვაზის ყლორტებიდან მიღებული ექსტრაქტის გავლენა ოქსიდაციური სტრესის ხარისხზე ლაბორატორიულ ვირთაგვებში

[1] თსსუ, ფარმაცევტული და ტოქსიკოლოგიური ქიმიის დეპარტამენტი, საქართველო; [2] ივანე ბერიტაშვილის სახელობის ექსპერიმენტული ბიომედიცინის ცენტრი, ცერებრალური სისხლის მიმოქცევისა და მეტაბოლიზმის ლაბორატორია, თბილისი, საქართველო

რეზიუმე

ოქსიდაციური სტრესი მნიშვნელოვან როლს ასრულებს სხვადასხვა დაავადების განვითარების პროცესში. ROS უაღრესად აგრესიული ნაერთებია, რომლებიც წარმოიქმნებიან ორგანიზმის მეტაბოლური რეაქციების დროს. მათ შეუძლიათ რეაქციაში შევიდნენ და დააზიანონ უჯრედები, ლიპიდები, ცილები და ა.შ. ყურძნის გადამუშავების ნარჩენი პროდუქტები, როგორიცაა ყლორტები, ლერწამი, ჩენჩო და ა.შ, უხვად მოიპოვება ღვინის მრეწველობაში. ინტერესი მათ მიმართ იზრდება, რადგან ნარჩენ პროდუქტებად აღქმის ნაცვლად, მათი გამოყენება შესაძლებელია იაფი, ფართოდ ხელმისაწვდომი ბუნებრივი ბიოლოგიურად აქტიური ნაერთების წყაროდ ფარმაცევტულ და კოსმეტიკურ ინდუსტრიაში.

აღნიშნული კვლევის მიზანი იყო ნარჩენი პროდუქტის - ვაზის ყლორტების პოლიფენოლების წყაროდ გამოყენება და ლაბორატორიულ ვირთაგვებში ოქსიდაციური სტრესის შემცირებაზე მისი ზემოქმედების შეფასება. საფერავის ყლორტებიდან მიღებული ექსტრაქტი ვირთაგვების ექსპერიმენტულ ჯგუფებს წყლის ნაცვლად მიეცათ კვლევის განმავლობაში. სითბური სტრესის გამოწვევა მოხდა ვირთაგვების სითბურ კამერაში მოთავსებით. ექსპერიმენტის დასასრულს, აღებული იქნა სისხლის ნიმუშები და ოქსიდაციური სტრესის სტატუსი გაანალიზდა D-ROM და PAT ტესტების გამოყენებით FRAS-5-ზე. მიღებულმა შედეგებმა ნათლად აჩვენა ლაბორატორიულ ვირთაგვებში ოქსიდაციური სტრესის შემცირების მიზნით საფერავის ყლორტების ექსტრაქტის გამოყენების შესაძლებლობა. ასევე აღსანიშნავია, რომ ექსპერიმენტულმა ჯგუფმა, რომელსაც ექსტრაქტი მიეცა პრევენციულად და შემდეგ მოხდა მათზე სითბური სტრესის ზემოქმედება, გამოავლინეს თავისუფალი რადიკალების წინააღმდეგ ბრძოლის უკეთესი უნარი. შესაბამისად, ყლორტებიდან მიღებულმა ექსტრაქტმა გამოავლინა ოქსიდაციური სტრესის შემცირების ძლიერი უნარი და მათი გამოყენება შესაძლებელია როგორც სამკურნალო, ასევე პრევენციული საშუალების სახით.

INTRODUCTION. An imbalance between the formation and accumulation of oxygen reactive species (ROS) in cells and tissues, as well as the ability of biological system to detoxify these reactive products, induces oxidative stress. ROS are routinely produced as by-products of oxygen metabolism and can play, and do, a variety of physiological roles (e.g., cell signalling). Despite this, environmental stressors (including pollutants, UV, ionizing radiations and heavy metals) and xenobiotics (such as antiblastic agents) both contribute to a significant increase in ROS generation, resulting in an imbalance that leads to cell and tissue damage (oxidative stress) [9]. The development of numerous metabolic, chronic diseases, or cancers has been linked to oxidative stress [13,8].

Although the concept of oxygen free radicals has been in use for more than 50 years, it was only in the last two decades that their involvement in disease development was established, and thus the positive outcomes of antioxidants were thoroughly explored [2].

Oxidative stress plays an important part in a number of disorders. ROS are extremely harmful compounds that are produced during metabolic reactions in the body and can interact with and damage cellular molecules including lipids, proteins, as well as other molecules. Because of the similarities in responses found following heat stress and those happening in the state of oxidative stress, heat stress is assumed to be an environmental factor for increasing reactive oxygen species (ROS) generation [7].

Various antioxidants, such as vitamin E, flavonoids, and polyphenols, have been studied in recent years for their actual or alleged anti-oxidant properties. Phenolic compounds' antioxidant activity appears to be linked to their molecular structure, specifically the presence and quantity of hydroxyl groups, as well as conjugation and resonance effects [6]. The quantity of hydroxyl groups has a significant impact on numerous antioxidant pathways, including radical scavenging and metal ion chelation [5]. The ability of polyphenols to scavenge a wide range of reactive oxygen species is linked to their antioxidant activity. Indeed, suppressing ROS synthesis by inhibiting enzymes involved in their formation, scavenging ROS, or upregulating or protecting antioxidant defences are all mechanisms involved in polyphenols' antioxidant activity [1]. Polyphenols may inhibit the catalytic activity of enzymes engaged in the production of reactive oxygen species. Polyphenols protect against oxidative damage via a variety of methods [11]. By reducing hydrogen peroxidase and generating the extremely reactive hydroxyl radical, ROS has been shown to increase free metal ions. Due to their ability to chelate metal ions (copper, iron, etc.) and free radicals, polyphenols with lower redox potentials can thermodynamically reduce highly oxidizing free radicals [12].

By-products from grape processing, such as shoots, canes, and pomace, are abundant in the wine industry. The interest to these goods is increasing because they can be exploited as a low-cost, widely available natural resource for the recovery of a wide range of bioactive compounds for pharmaceutical and cosmetic purposes rather than being wasted [4,10]. Because of their well-documented biological activity, polyphenolic compounds are the most significant bioactive component obtained from vineyard/wine by-products. Polyphenols are natural products that have phenolic structural properties. This group of natural goods is extremely diverse, with various sub-groups of phenolic components. Polyphenols have been identified in over 8.000 different varieties, with carbon atoms ranging from C6 to C30. The presence of significant multiples of phenol structural units distinguishes polyphenols. The quantity and features of these phenol structures are responsible for the class's distinct physical, chemical, and biological properties [3].

The aim of this study was to use waste product – vine shoots as a source of polyphenols and evaluate its impact on reduction of oxidative stress in laboratory rats.

EXPERIMENTAL PART

Materials and method. Experiments were carried out on the bases of Tbilisi State Medical University, department of Pharmaceutical and Toxicological Chemistry and Ivane Beritashvili Center of Experimental Biomedicine, Laboratory of Cerebral Blood Flow and Metabolism.

Saperavi vine shoots were collected in Kakheti Region in June 2021. Shoots were shredded and placed into flask. Extraction was done on ultrasonic bath with 50% ethanol (extraction time – 30min; ratio - 1:10). Derived extract (Drug X) was given to experimental groups of rats during research instead of water.

Adult male laboratory rats (n=18, weight 250-350 g.) were maintained for a week on a 12/12 light/dark cycle and *ad libitum* access to food and water. Rats were divided into following groups:

- **Group 1**: Control group (6 rats) – WBH – 1 hour on 43°C for 4 days. Then 8 days on room temperature with unlimited access to water;
- **Group 2**: (6 rats) WBH – 1 hour on 43°C for 4 days. Then 8 days on room temperature with unlimited access to "Drug X"
- **Group 3**: (6 rats) 8 days unlimited access to "Drug X". Then WBH – 1 hour on 43°C for 4 days. (Picture 1)

The rats were placed in a heat chamber with a regulated temperature (41±0.5°C) for 1 hour to induce heat stress.

Picture 1 – *control and experimental groups of rats.*

FRAS-5. "FRAS-5 is a photometric analytical system dedicated solely to the global assessment of oxidative stress in biological systems by enabling the measurement of pro-oxidant status in plasma samples by means of d-ROMs fast test and of anti-oxidant status in plasma and saliva samples by means of PAT test (picture 2). The principle adopted is the absorbance measurement of a sample solution in a cuvette through a monochromatic light beam; once the absorbance value is obtained, the instrument automatically provides conversion into the appropriate measurement units by using proprietary software.

d-ROMs fast test is a photometric test that allows to assess the pro-oxidant status in a biological sample, by measuring the concentration of hydroperoxides (ROOH)." [14].

The d-ROMs test uses the principle of Fenton's reaction: by mixing a biological sample with an acidic buffer (Reagent R1), the transition metal ion (iron or copper) formed catalyzes the hydroperoxides breakdown, generating new radical species, such as hydroperoxyl (ROO*) and alkoxyl (RO*) radicals.

By adding to this sample, a chromogen (N, N-diethyl-paraphenylenediamine, Reagent R2) that has the ability to donate an electron and turn its color when oxidized by the free radicals, it becomes possible to quantify the amount of hydroperoxides available in the sample by photometric reading using the dedicated analytical device, "FRAS5" [15].

PAT test is an automated test assessing the antioxidant potential of plasma by measuring its ferric reducing ability. Ferric to ferrous ion reduction at low pH causes a color change that can be photometrical assessed using the integrated analytical device FRAS5.

In the PAT test, a small amount of plasma (10 µl) is added to a colored solution, obtained by mixing a source of ferric ions (Reagent R2 – $FeCl_3$ ferric chloride) with a chromogen (Reagent R1 – chromogenic mixture containing thiocyanate). After only 1 minute of reading time at 37C, the solution will change the color and the intensity of this chromatic change will be directly proportional with the plasma ability to reduce the ferric ions to ferrous ions. By photometrically assessing the intensity of discoloration, the amount of reduced ferric ions can be adequately evaluated, thus allowing an effective measurement of reducing ability or antioxidant capacity of tested blood plasma" [16].

Redox OB Fast enables the assessment of oxidative stress through redox balance analysis. In every organism there is delicate balance, called redox, between oxidizing and antioxidizing molecules. Redox OB Fast makes it possible to determine both the pro-oxidant power, using d-ROMs Fats method, and the antioxidant power, using the PAT method, allowing a complete analysis of the redox balance" [17].

Picture 2 – *FRAS5 – Free Radical Analytical System.*

OBRI Index *REFERENCES VALUES*	
0,8 - 1,2	*Normal*
1,3 - 1,7	*High*
1,8 - 2,2	*Very high*
>2,2	*Extremely high*

OBRI Index range

d-ROMs test REFERENCE VALUES	
250-300	Normal range
300-320	Border condition
321-340	Low level of oxidative stress
341-400	Middle level of oxidative stress
401-500	High level of oxidative stress
>500	Very high level of oxidative stress
Unit of measurement U. Carr 1 U. Carr = 0.08 mg H2O2/dl	

d-ROMs test range

PAT test REFERENCE VALUES	
>2800	Very high value
2200– 2800	Normal value
2200– 2000	Border line low range
2000– 1800	Slight deficency status
< 1800	Deficiency status
Unit of measurement U. Cor 1 U. Cor = 1.4 μmol/L of ascorbic aci	

PAT test range

Collecting blood samples. Experimental animals received anesthesia with 1ml Chloral Hydrate 4%/100g. Blood samples were collected from superior vena cava (picture 3) and centrifuged using integrated in FRAS-5 centrifuge to separate the plasma. Oxidative stress status of each animal was analyzed using FRAS-5 kits and the results are provided in the table 1.

Picture 3 – *collecting of blood samples*

RESULTS AND DISCUSSION. Oxidative stress status was analyzed using d-ROMs and PAT tests on FRAS-5 for each experimental animal in all 3 groups. Results demonstrated, that free radical's total content was significantly high in control group, then in groups, which received grapevine shoots extract. It is noteworthy, that group 3, who primarily received treatment with "Drug X" for 8 days and only afterwards was put in the chamber with high temperature, demonstrated better results in coping with oxidative stress and developed much less free radicals, then first 2 groups.

Table 1 – *Blood sample analysing results*

	Rat №	d-ROMs FAST UCarr	PAT U Cor.	OBRI
Group №1 Control	1	332	2956	1.0
	2	385	2516	1.3
	3	655	2842	2.0
	4	315	2384	1.2
	5	333	2555	1.2
	6	365	2516	1.3
Group №2	1	394	2430	1.4
	2	248	2384	0.9
	3	272	2939	0.8
	4	412	2385	1.2
	5	415	2381	1.1
	6	410	3302	1.1

Group №3	1	306	2475	1.1
	2	359	2618	1.2
	3	332	2618	1.1
	4	339	2865	1.0
	5	255	2627	1.0
	6	250	2842	0.8

CONCLUSION. Free radicals and oxidative stress – both have damaging effect on human health. Numerous studies worldwide have shown that free radicals play a significant role in the initiation and progression of a variety of diseases, from cardiovascular disease to cancer. Antioxidants have received much interest from the scientific research group, not only because of their value in disease prevention and/or therapy, but also because of the widespread assumption that they have no serious negative effects.

Big amount of waste products is gathered during winemaking in Georgia, which definitely can be used as a source of biologically active ingredients. The aim of this study was to show Saperavi shoots extract's ability to reduce oxidative stress. During the experiment, 2 groups of laboratory rats received treatment Saperavi shoots extract and 1 group was used as a control. Oxidative stress status was analyzed using d-ROMs and PAT tests on FRAS-5 for each experimental animal in all 3 groups. Results demonstrated that free radicals total content was significantly high in control group, then in groups, which received grapevine shoots extract. The most interesting results were received in group 3, which primarily received treatment with "Drug X" for 8 days and only afterwards was put in the chamber with high temperature. This group demonstrated better results in coping with oxidative stress and developed much less free radicals, then first 2 groups. Received results established possibility of using Saperavi shoots extract for reduction of oxidative stress in laboratory rats. It is also noteworthy, that experimental group, which received preliminary treatment and afterwards was affected by heat stress, demonstrated better ability to fight against free radicals. Based on the study results conclusion can be done, that Georgian Saperavi shoots can be used as a source of polyphenols, which will reduce amount of winemaking waste products. Accordingly, derived from shoots extract, demonstrates strong ability to reduce oxidative stress and can be used as treating, as well as preventive agent.

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[1] TSMU, Department of Pharmaceutical and Toxicological Chemistry, Tbilisi, Georgia; [2] Ivane Beritashvili Center of Experimental Biomedicine, Laboratory of Cerebral Blood Flow and Metabolism, Tbilisi, Georgia

SUMMARY

Oxidative stress plays an important part in a number of disorders. ROS are extremely harmful compounds that are produced during metabolic reactions in the body and can interact with and damage cellular molecules including lipids, proteins, as well as other molecules. By-products from grape processing, such as shoots, canes, and pomace, are abundant in the wine industry. The interest to these goods is increasing because they can be exploited as a low-cost, widely available natural resource for the recovery of a wide range of bioactive compounds for pharmaceutical and cosmetic purposes rather than being wasted.

The aim of this study was to use waste product – grapevine shoots as a source of polyphenols and evaluate its impact on reduction of oxidative stress in laboratory rats. Extract from Saperavi grapevine shoots was given to experimental groups of rats during research instead of water. Heat stress was initiated by placing the rats in a heat chamber. At the end of experiment, blood samples were collected and the oxidative stress status was analyzed using d-ROMs and PAT tests on FRAS-5. Received results established substantial possibility of using Saperavi shoots extract for reduction of oxidative stress in laboratory rats. It is also noteworthy, that experimental group, which received preliminary treatment and afterwards was affected by heat stress, demonstrated better ability to fight against free radicals. Accordingly, derived from shoots extract, demonstrates strong ability to reduce oxidative stress and can be used as treating, as well as preventive agent.

Keywords: Polyphenols, Oxidative stress, FRAS-5, Vine shoots