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CARDIOPROTECTIVE EFFECTS OF LADY'S BEDSTRAW EXTRACT: FOCUS ON OXIDATIVE STRESS

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Abstract: Beside the widespread traditional use of Lady's Bedstraw in the treatment of numerous diseases and conditions, it's effects on heart function and redox status has still not been fully clarified. The aim of our study was to examine the effects of methanol extract of Lady's Bedstraw on oxidative stress parameters during on ischemia-reperfusion (I/R) injury in isolated rat heart**.** Our results demonstrated that treatment with methanol extract of Lady's Bedstraw preserved diminished production of many prooxidants. Promising potential of Lady's Bedstraw in the present study in a model of pharmacological preconditioning may be a starting point for future researches.

Keywords: *Lady's Bedstraw*; ischemia-reperfusion injury; oxidative stress; rat heart

Introduction

Acute myocardial infarction (AMI) continues to be a prominent cause of hospital admissions and mortality worldwide. The current therapeutic focus revolves around promptly restoring blood flow through thrombolytic therapy or primary percutaneous coronary artery angioplasty, emphasizing its paramount importance in mitigating the extent of myocardial infarction (Xia et al. 2016).

However, the reestablishment of blood flow to oxygen-starved myocardium during reperfusion paradoxically intensifies tissue injury induced by hypoxic stress. This phenomenon, recognized as ischemia/reperfusion (I/R) injury, has been a subject of extensive research for over decades.Among the numerous

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mechanisms contributing to I/R injury, oxidative/nitrosative stress, calcium overload, and a pronounced inflammatory response present the major contributors to myocardial damage (Kalogeris et al. 2016). Initial investigations primarily focused on ischemic preconditioning as a strategy for myocardial protection. This approach involves subjecting the heart to brief episodes of I/R before inducing myocardial infarction through prolonged ischemia. Conversely, certain non-ischemic, non-hypoxic stimuli, such as the application of pharmacological agents prior to ischemia, have been explored to mimic the signaling induced by ischemic preconditioning. This exploration aims to alleviate the detrimental effects of I/R injury, providing an alternative avenue for myocardial protection (Li et al. 2015).

Groving evidence suggest that medicinal plants, enriched with polyphenols, harbor the potential to mitigate the detrimental effects of acute myocardial infarction, thereby validating their traditional use in cardiovascular disease prevention. The inhibition of reactive oxygen species (ROS) overproduction through the antioxidant properties found in plant extracts emerges as a significant target for cardioprotection (Bartekova et al. 2010).

Lady's Bedstraw., plant from the Rubiaceae family, has been used in traditional medcicine for the treatment and prevention of various conditions (Lakic et al. 2010). Phytochemical investigation showed that this plant has bioactive compounds such as phenols, iridoid glycosides, anthraquinones, triterpenes, and small quantities of tannins, saponins, and essential oils (Lakic et al. 2010). Although phytochemical investigations have unveiled the plant's chemical composition and confirmed its antioxidant activity in vitro, its biological effects, particularly on cardiac function and oxidative stress, remain partially understood (Vlase et al. 2014).

Considering the presented data, our study aims to evaluate the effects of methanol extract of Lady's Bedstraw on oxidative stress parameters during on ischemia-reperfusion (I/R) injury in isolated rat heart

Materials and methods

Plant material and extract preparation

The whole plant Lady's Bedstraw was collected in the village Dobroselica, on the southern cliff of the Mt Zlatibor. Voucher specimens are deposited in Herbarium of the Institute of Botany and Botanical garden "Jevremovac". The collected material was dried under the shade and powdered (sieve 0.75). Methanol extract was prepared extracting 100 g of aerial part of plant with 500 ml

of methanol by heat reflux extraction, at temeperature of 90°C, in a duration of 2 h (Hijazi et al. 2015). The mixture was filtered through filter paper (Whatman, No.1). Dry extract was obtained by evaporation under reduced pressure (RV05 basic IKA, Germany). The residue (17.07 g) was stored in a dark glass bottle at +4°C for further processing. In order to feed the animals, Lady's Bedstraw extract was daily dissolved in the water just before administered to experimental animals.

Animals and experimental design

Twenty Wistar albino rats (males, 8 weeks old, body weight, 200 ± 50 g) were included in the study. The animals consumed commercial rat food (20% protein rat food, Veterinary institute Subotica, Serbia) ad libitum and were housed at temperature of 22 ± 2 °C, with 12 h of automatic illumination daily. The rats were divided into two groups: 1.Control group – rats that drank only tap water; 2. Lady's Bedstraw group – rats that drank tap water containing 500 mg/kg of methanol extract for 28 days.

A day after accomplishing 28-day drinking protocol after ashort-term ket amine/xylazine-induced narcosis, rats were sacrificed by decapitation. The chest was then opened via midline thoracotomy. The hearts were immediately removed and immersed in cold saline and were then attached on a cannula of the *Langendorff* perfusion apparatus to provide retrograde perfusion under constant coronary perfusion pressure of 70 cmH_2O. Following the establishment of heart perfusion, the hearts were stabilised within 30 min. In both groups, after stabilisation period, hearts were subjected to global ischemia (perfusion was totally stopped) for 20 min, followed by 30 min of reperfusion. In the period of reperfusion (30 min), all cardiodynamic parameters and CF were measured in intervals of 5 min (RP1–RP7).

Biochemical analysis in coronary venous effluent and heart tissue

Coronary venous effluent was collected in point of stabilisation (S), in first point of reperfusion (R1) and on every 5 min in period of reperfusion (R1–R7) The following oxidative stress parameters were determined spectrophotometrically (Shimadzu UV 1800, Japan) using collected samples of the coronary venous effluent: the index of lipid peroxidation, measured as thiobarbituric acid-reactive substances (TBARS), nitrite (NO_2-), levels of superoxide anion radical (O_2-) and hydrogen peroxide (H_2O_2).

After accomplishing experiments, hearts from all animals were frozen at -80 °C, and then a 0.5 section of each tissue was homogenised in 5 ml phosphate

buffer pH 7.4 using an electrical homogeniser, on ice. Then tissue homogenates were centrifuged at 1200 xg for 20 min at 4 °C. The resulting supernatants were isolated and stored at -80°C until determination of biochemical parameters. Index of lipid peroxidation as well as parameters of antioxidant defence system such as reduced glutathione (GSH), catalase (CAT), and superoxide dismutase (SOD) were determined in heart tissue (Bradic J, 2019)

Results and discussion

Biochemical analysis

The index of lipid peroxidation in coronary venous effluent did not differ significantly in the experimental group, while in control group there was a rise in the first minute of reperfusion and at the end of recovery period compared to the values in stabilisation. There was no change in lipid peroxidation in both control and experimental hearts. In the control group, there was an increase in NO_2- production at the first and at the last point of recovery period in comparison to stabilisation. In the group treated with Lady's Bedstraw, higher generation of NO_2- was noticed in the first minute of reperfusion when compared to stabilisation and the end of reperfusion At the end of reperfusion a significantly increased level of O_2- was observed in the control group in comparison to the level before ischemia. On the contrary in Lady's Bedstraw group significantly diminished production of this pro-oxidant was noticed at the end of recovery period compared to stabilisation. The level of H_2O_2 did not vary significantly within the experimental group, while in the control group, enhance in production of H_2O_2 was found at the last point reperfusion in comparison to values before ischemia. The activities of SOD and CAT were significantly higher in the group treated with Lady's Bedstraw in comparison to the control group, while the level of GSH was similar in both observed groups. The analysis of pro-oxidants in the coronary venous effluent provides insights into oxidative stress within the endocardium of the left ventricle and the endothelium of the coronary circulation. Our findings indicated that I/R injury was associated with increased oxidative stress, evidenced by significantly elevated TBARS, NO_2-, O_2^- and H_2O_2 levels in the coronary venous effluent at the end of reperfusion compared to stabilization. However, pretreatment with Lady's Bedstraw led to a reduction in O_2^- levels. Additionally, Lady's Bedstraw extract prevented ischemia-induced lipid peroxidation in heart tissue, thereby preserving membrane integrity.

Table 1. Percent difference in values of oxidative stress parameters between control and Lady's Bedstraw group

Lady's Bedstraw vs control	(%) increase in Stabilisation	(%) increase in RP1	(%) increase in RP7
TBARS	-18.39%	-14.63%	-28.36%
NO_2-	-5.07%	+3.24%	-4.12%
O_2^-	-12.49%	-12.78%	-32.06%
H_2O_2	-5.39%	-11.46%	-22.05%

Concerning the components of the antioxidant defense system, myocardial SOD and CAT activity were higher in the Lady's Bedstraw group, while the level of GSH remained similar between groups. The increased CAT activity might explain the absence of a rise in H_2O_2 production in the Lady's Bedstraw pretreated group, as this enzyme facilitates the decomposition of hydrogen peroxide to water and oxygen. Moreover, the heightened activity of SOD, a key element in antioxidant defense against O_2, aligns with the observed decline in O_2^- levels. It could be hypothesized that the unaltered coronary vasodilatory response, coupled with a decrease in O_2^-, might result from the interplay between increased NO (responsible for the regulation of coronary flow) and the overproduced O_2^-, leading to the generation of toxic peroxynitrite.

Polyphenols, particularly flavonoids, are renowned for their robust antioxidant potential, enabling them to scavenge suddenly generated ROS during the restoration of blood flow. Furthermore, polyphenols activate the endogenous antioxidant defense system, particularly SOD and CAT, leading to a reduction in oxidative stress-induced tissue damage (Mattera et al. 2017). Consequently, the structural and functional integrity of cardiomyocytes is preserved. Moreover, it has been demonstrated that the mechanisms through which polyphenols protect the myocardium from oxidative damage involve the preservation of mitochondrial function, inhibition of xanthine oxidase and nicotinamide adenine dinucleotide phosphate-oxidase (NADPH) oxidase, as well as the chelation of iron ions, which catalyze several free radical-generating reactions (Debnath et al. 2014). The impact on transcription-mediated signaling is responsible for the long-lasting antioxidative effects of these natural molecules (Mattera et al. 2017).

Conclusion

Lady's Bedstraw tended to modulate the activity of myocardial antioxidant enzymes and decrease the generation of pro-oxidants, thus mitigating oxidative stress-induced heart dysfunction.

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