

The influence of the methanol extract of *Galium verum* on cardiac oxidative damage in hypertensive rats in a model of global ischemia

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Abstract: *Galium verum* (*G. verum*, Lady's bedstraw) is a perennial herbaceous plant that has been used for centuries as a sedative, an anticancer agent, in the treatment of gout, epilepsy, as antioxidant. Previous studies confirmed cardioprotective properties of this plant species extract in animal models of heart dysfunction, however, the impact of *G. verum* consumption on cardiac redox state in a condition of global ischemia has not been fully clarified. Therefore, our goal was to examine the effect of *G. verum* methanol extract on cardiac redox state in spontaneously hypertensive rats in the model of global ischemia. The study involved 20 Wistar kyoto spontaneously hypertensive rats, divided into a control (CTRL) and an experimental group (GVE). CTRL group included untreated rats, while the GVE group included rats that received 100 mg/kg of the methanol extract of *G. verum* for 14 days. After the treatment protocol, animals were sacrificed, and the hearts of all rats were isolated and subjected to 20-minute ischemia followed by a 30-minute reperfusion period. After accomplishment of the experimental protocol (*ex vivo* ischemia-reperfusion injury), heart tissue samples were used to determine the markers of cardiac oxidative stress such as superoxide dismutase (SOD), catalase (CAT), reduced glutathione (GSH) and index of lipid peroxidation (TBARS). The results have shown that methanol extract of *G. verum* increased the level of GSH and the activity of SOD and CAT in the experimental group, while reduced TBARS levels compared to the CTRL group. It might be concluded that treatment with *G. verum* extract can attenuate oxidative damage resulting from ischemia-reperfusion injury in the hearts of spontaneously hypertensive rats.

Keywords: *Galium verum*, cardiac ischemia-reperfusion injury, oxidative stress, rats

1. Introduction

Galium verum (*G. verum*, Lady's bedstraw) is a perennial herbaceous plant that has been used for centuries as a sedative, an anticancer agent, in the treatment of gout, epilepsy, as antioxidant. Previous studies confirmed cardioprotective properties of this plant species extract in animal models of heart dysfunction. The two most important groups of biomolecules found in the extract of *G. verum* which are responsible for antioxidant activity include phenolic acids and flavonoids. The beneficial effect of *G. verum* extract on the heart is reflected in the ability of this plant to prevent structural damage of the heart after ischemia, to reduce coronary circulation disorders, and to preserve the contractility and systolic and diastolic function of the heart. However, the impact of *G. verum* consumption on cardiac redox state in a condition of global ischemia has not been fully clarified [1, 2]. Therefore, our goal was to examine the effect of *G. verum* methanol extract on cardiac redox state in spontaneously hypertensive rats in the model of global ischemia.

2. Material and methods

This investigation was carried out in the Center for Preclinical and Functional investigations, Faculty of Medical Sciences, University of Kragujevac, Serbia. The study design was approved by the Ethical Committee for the welfare of experimental animals of the Faculty of Medical Sciences. All experiments were performed according to the EU Directive for the welfare of laboratory animals (86/609/EEC) and principles of Good Laboratory Practice (GLP).

2.1 Plant material and extract preparation

The methanol extract of *G. verum* was prepared by extracting the aerial parts of the plant with methanol as solvent by heat reflux extraction method. The dry extract was obtained by evaporation under reduced pressure. Before administration to animals, dry extract was *ex tempore* dissolved in the tap water [1].

2.2 Animal treatment

This study involved 20 spontaneously hypertensive Wistar kyoto rats randomly divided into two groups: CTRL group - control untreated rats and GVE group - rats that received *G. verum* methanol extract for two weeks once daily per os (100 mg/kg). After the accomplishment of two-week treatment, animals were sacrificed, and hearts were isolated and perfused retrogradely on Langendorff apparatus. After achieving stable rhythm, hearts were subjected to 20-minute ischemia (induced by blockage of coronary flow) and 30-minute reperfusion. At the end of *ex vivo* ischemia-reperfusion injury, hearts were collected for spectrophotometric determination of cardiac oxidative stress markers: index of lipid peroxidation, measured as thiobarbituric acid-reactive substances (TBARS), reduced glutathione (GSH), catalase (CAT) and superoxide dismutase (SOD) [3].

3. Results and Discussion

Rats treated with a methanol extract of *G. verum* had significantly lower levels of TBARS compared to the control group. Additionally, the activity of SOD and CAT as well as the level of GSH were markedly increased in rats that received GVE for two weeks (Table 1).

Table 1. The effects of GVE on oxidative stress markers in heart tissue samples

Group	SOD (U/g tissue)	CAT (U/g tissue)	GSH (nmol/g tissue)	TBARS (μ mol/g tissue)
CTRL	12.2 \pm 1,1	13.01 \pm 2.2	76121 \pm 234	2.15 \pm 0.05
GVE	16,3 \pm 0.9*	18.5 \pm 1.6*	96443 \pm 170	1.83 \pm 0.12*

p<0.05 statistical significance at the level p<0.05 compared to CTRL group.

Hypertension, as one of the most important risk factors for cardiovascular diseases, can worsen the ischemia-reperfusion outcome by leading to a significant mechanical load and stress of the myocardium. Oxidative stress is recognized as one of the significant factors that contributes to ischemia-reperfusion damage of the myocardium [4]. Exposure of the isolated heart to ischemia-reperfusion causes increased generation of all cardiac prooxidants. The main source is the mitochondrial respiratory chain and the activation of xanthine oxidase, which results in increased production of reactive oxygen species such as O_2^- and H_2O_2 . Previous research revealed that *G. verum* has the potential to preserve the structure and function of the heart in conditions of ischemia. Additionally, *G. verum* extract may prevent ischemia-induced lipid peroxidation by ensuring adequate cell membrane integrity [1, 2]. In this research, all investigated parameters of antioxidant protection were increased in the group treated with *G. verum* extract compared to the control group. Polyphenols have been recognized as the main constituents of *G. verum* extract responsible for the increase in SOD and CAT, which activate endogenous antioxidant mechanisms, reducing tissue damage caused by oxidative stress [1]. The current results are in line with the previous investigation that revealed the antioxidant capacity of *G. verum* extract in terms of alleviation of cardiac redox damage after 4-week treatment. The current findings indicate that a two-week consumption of *G. verum* methanol extract was sufficient to achieve protective effects on redox signaling in heart tissue. Alleviation of cardiac oxidative damage can contribute to functional recovery of the heart exposed to ischemia [5].

4. Conclusions

It can be concluded that treatment with *G. verum* extract can alleviate oxidative damage that occurs as a result of cardiac ischemia-reperfusion injury in spontaneously hypertensive rats via promotion of antioxidant defense capacity. Further studies are required in order to reveal the role of this extract as a potential antioxidant dietary supplement in various oxidative stress-related pathologies.

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