DOI: 10.46793/SBT29.77KM

ASSESSMENT OF *GALIUM VERUM* EXTRACT SUPPLEMENTATION ON REDOX HOMEOSTASIS IN PSORIATIC RAT MODEL

Katarina Mihajlovic[1,2], Vladimir Jakovljevic[1,2,3], Jovana Bradic[1,2], Maja Savic[1,2], Marina Nikolic[1,2], Jasmina Sretenovic[1,2]

Abstract

Psoriasis lacks comprehensive data on its systemic oxidative impact. This study explores *Galium verum* extract's influence on systemic oxidative state in psoriatic rats. Twenty-four male W.albino rats were divided into control (CTRL), psoriasis (PSORI) and psoriasis treated with *G.verum* extract (PSORI+GV) groups. Blood samples analyzed for redox state biomarkers revealed increased TBARS levels in PSORI *vs* CTRL and PSORI+GV. PSORI exhibited significantly lower nitrite levels compared to CTRL and PSORI+GV, with elevated O_2^- and H_2O_2 levels. GSH and SOD values were reduced in PSORI and PSORI+GV, while catalase activity increased. *G.verum* extract positively modulated psoriatic rat redox state.

Keywords: psoriasis, redox homeostasis, *Galium verum* extract

Introduction

Psoriasis, a chronic inflammatory skin disorder characterized by dysregulated immune responses and aberrant keratinocyte proliferation, poses significant challenges in understanding its underlying mechanisms. Psoriasis is characterized by sharply demarcated erythematous skin lesions adorned with silver hyperkeratotic plaques, often accompanied by systemic manifestations. Its prevalence varies across diverse populations and geographical regions globally. Estimates indicate that approximately 2-3% of the world's population

[1]University of Kragujevac, Faculty of Medical Sciences, Svetozara Markovića 69, Kragujevac, Serbia (katarina.mihajlovic@fmn.kg.ac.rs; drvladakgbg@yahoo.com; jovanabradickg@gmail.com; maja.jovanovic.2008.38@gmail.com; marina.rankovic.95@gmail.com; drj.sretenovic@gmail.com)

[2]Center of Excellences for Redox Balance Research in Cardiovascular and Metabolic Disorders, Svetozara Markovića 69, Kragujevac, Serbia

[3]1st Moscow State Medical University IM Sechenov, Moscow, Russia

is afflicted by psoriasis. Nevertheless, prevalence rates can escalate in specific regions, ethnicities, and age cohorts (Medovic et al., 2022).

Among the various factors implicated, oxidative stress stands out for its role in perpetuating inflammation and tissue damage in psoriatic lesions. Oxidative stress, arising from an imbalance between reactive oxygen species (ROS) or reactive nitrogen species (RNS) production and antioxidant defense mechanisms, plays a critical role in the pathogenesis of psoriasis. This imbalance may arise from reduced activity of antioxidative enzymes, including catalase (CAT), superoxide dismutase (SOD), and glutathione peroxidase (GPx), or a decrease in the level of scavenging antioxidants, both endogenous (e.g. reduced glutathione (GSH)) and exogenous (such as vitamin C, vitamin E, and carotenoids). At low concentrations, ROS/RNS serve as signaling molecules in the regulatory cascades of various biological processes. However, excess ROS/RNS and consequent oxidative stress can induce oxidation of cellular structures, including deoxyribonucleic acid (DNA), lipids, and proteins, ultimately leading to cell death. The skin, being constantly exposed to environmental stressors, is particularly vulnerable to oxidative damage. In psoriasis, this delicate balance is disrupted, leading to sustained oxidative stress within the cutaneous microenvironment. So the redox imbalance in psoriasis has been reported in skin cells and, systemically, in plasma and blood cells as well (Ambrozewicz et al., 2018).

Numerous treatments, including topical medications with corticosteroids, retinoids, vitamin D_3 analogues, anthralin, and systemic medications with diverse mechanisms of action, are used for psoriasis. However, most therapies are unsuitable for long-term use due to serious side effects and high costs. Commonly used drugs like methotrexate, acitretin, cyclosporine, dexamethasone, and salicylic acid have limited efficacy and pose challenges such as low absorption, inconsistent release, and adverse reactions (Lai et al., 2018).

Throughout history, traditional medicine has utilized medicinal plants for therapeutic purposes. A multitude of plant species possess inherent anti-inflammatory and antioxidant attributes, among which *Galium verum* (GV) stands notable (Goncalves et al., 2023). *Galium verum* L., a perennial herbaceous plant from the *Rubiaceae* family, boasts a rich historical legacy of employment in treating and preventing various ailments. This plant species has yielded diverse classes of bioactive compounds, including phenols, triterpenes, iridoid glycosides, and anthraquinones, alongside trace amounts of saponins, tannins, and essential oils. *Galium verum* extract, derived from the plant *Galium verum* L.,

has attracted attention for its antioxidant properties and potential therapeutic effects in inflammatory conditions. Its significance in traditional medicine dates back to ancient times, esteemed for its multifaceted therapeutic properties such as sedative, spasmolytic, anticancer, and cardioprotective effects (Turcov et al., 2022). Studies have also demonstrated its ability to scavenge free radicals, reduce oxidative damage, and modulate inflammatory pathways (Bradic et al., 2020). However, as far as we know based on the available literature, there have been no investigations of psoriasis in an animal model with the therapeutic application of *Galium verum* extract. Therefore, this study aims to elucidate the impact of *Galium verum* extract administration on systemic redox balance in psoriasis-induced rats.

Material and methods

Experimental animals

A total of 24 adult male Wistar albino rats, aged 12 weeks with body weight (BW) of 250-300 g were included in the study. The animals were randomly divided into three groups (n=8 in each group) and were housed in separate clean plastic cages under controlled standard environmental conditions of temperature (22 ± 1 °C) and light (12/12 h light/dark cycle). Food and water were provided ad libitum throughout the treatment.

Treatment protocol and experimental design

After a week of adaptation to the controlled conditions, the rats were divided into three equal groups: control (CTRL), psoriatic (PSORI), and psoriatic rats treated with *Galium verum* extract (PSORI+GV). CTRL group were healthy rats, while the PSORI group consisted of rats in which psoriasis was induced according to the protocol described below. The PSORI+GV group consisted of rats with induced psoriasis that were treated with daily oral administration of methanol extract of *Galium verum* at a dose of 250 mg/kg BW for a total of 4 weeks. The methanol extract of *Galium verum* was prepared according to the protocol for extract preparation described in the literature (Bradic et al., 2020).

Induction of psoriasis

Psoriasis was induced using 5% imiquimod cream, an agonist of toll-like receptors (TLR) 7/8 of macrophages, monocytes, and dendritic cells. TLR 7/8 is

known for its potent immunoactivating properties and induction of apoptosis. The cream was applied topically on the shaved back skin of the rats (3 cm x 2.5 cm) once daily for seven consecutive days. Imiquimod cream was selected for psoriasis induction due to its ability to locally induce psoriatic dermatitis, resembling the morphological and clinical characteristics of psoriasis. Despite its clinical indications for other conditions, imiquimod has been observed to induce worsening of psoriasis and the development of new lesions even in well-controlled patients. This model offers a straightforward approach for psoriasis induction, making it valuable for preclinical studies aimed at investigating pathophysiological and cellular mechanisms underlying psoriasis (Chatterjee and Sur, 2024).

Sample collection

After completion of the experimental protocol, the rats were sacrificed, and blood samples were collected. Plasma and erythrocytes were isolated from the blood samples for the determination of oxidative stress parameters using spectrophotometric method (Shimadzu UV 1800, Japan).

Determination of oxidative stress biomarkers

Determination of the index of lipid peroxidation (measured as TBARS)

The concentration of lipid peroxidation in the plasma was assessed by measuring TBARS using 1% thiobarbituric acid in 0.05 NaOH, incubated with 0.8 mL of plasma samples at 100 °C for 15 min and measured at 530 nm (Ohkawa et al., 1979).

Hydrogen peroxide (H_2O_2) determination

The assessment of H_2O_2 was performed using the oxidation of phenol red with H_2O_2 in a reaction catalyzed by horseradish peroxidase (HRPO). A 200 µl plasma sample was precipitated with 800 µl of phenol red solution with 10 µl of (1:20) HRPO added. An adequate volume of Krebs–Henseleit solution was used as a blank. The degree of H_2O_2 was measured at 610 nm (Pick and Keisari, 1980).

Superoxide anion radical (O_2^-) determination

The degree of O_2^- was measured by the reaction of nitro blue tetrazolium in 2-amino-2-(hydroxymethyl)-1,3-propanediol with plasma samples at 530 nm. Distilled water was used as a blank probe (Auclair and Voisin, 1985).

Nitrite (NO_2^-) determination

The level of NO_2^- was determined as an index of nitric oxide production. A 0.5 mL plasma sample was precipitated with 200 µl of 30% sulfosalicylic acid,

vortexed, and then centrifuged at 3000g. The resulting supernatant was mixed with an equal volume of Griess reagent, incubated for 10 min in the dark, and measured at 550 nm. The NO_2^-concentrations were calculated using sodium nitrite as the standard (Green et al., 1982).

Determination of CAT activity

The activity of CAT was estimated according to Aebi (1984). Lysates of erythrocytes were diluted in distilled water and treated with chloroform-ethanol to eliminate hemoglobin. Then, 50 µl of CAT buffer, 100 µl of sample, and 1 mL of 10 mM H_2O_2 were added to the samples. Measurement was performed at 360 nm.

Determination of SOD activity

SOD activity was determined by the epinephrine method of Misra and Fridovich (1972). A total of 100 µl lysate of erythrocytes and 1 mL of carbonate buffer were mixed, then 100 µl of epinephrine was added and detected at 470 nm.

Reduced glutathione (GSH) determination

The level of GSH was assessed based on GSH oxidation with 5,5-dithio-bis-6,2-nitrobenzoic acid using the method of Beutler (1975). Measurement was made at 412 nm.

Statistical analysis

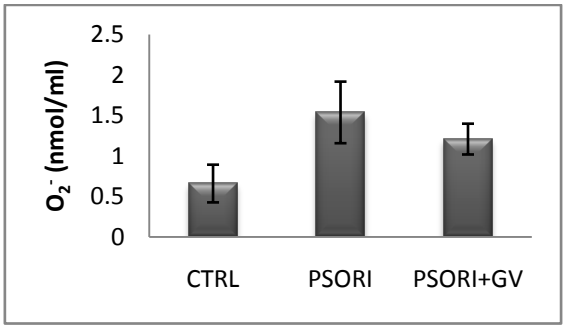
Data are presented as the mean ± SD. The effects on oxidative parameters and antioxidant status between groups were analysed by one-way ANOVA (Scheffe's F test) and the nonparametric analogue test of ANOVA (Kruskall–Wallis test). Differences were considered to be significant at $p< 0.05$. Statistical analysis was performed with SPSS version 24.0 statistical package (IBM SPSS Statistics 24).

Results and discussion

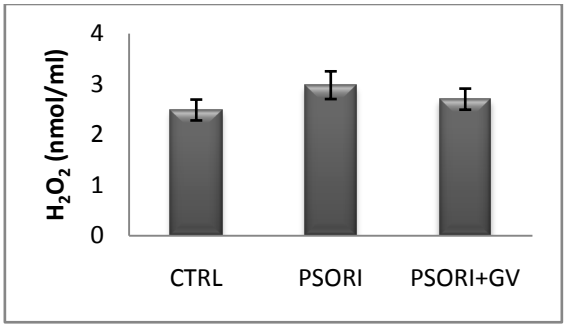
The effects of *Galium verum* extract on pro-oxidative parameters (TBARS, H_2O_2, O_2^-,NO_2^-) in plasma

Our results showed that the concentrations of TBARS, H_2O_2 and O_2^- were significantly elevated in PSORI group compared to CTRL and PSORI+GV as well. On the other hand, the level of nitrites in plasma were decreased in rats with psoriasis in comparison with control and PSORI+GV group (Graphs 1-4). Since this is the first such research and there are no investigations with psoriatic animal model and treatment with *Galium verum* extract, we can only interpret

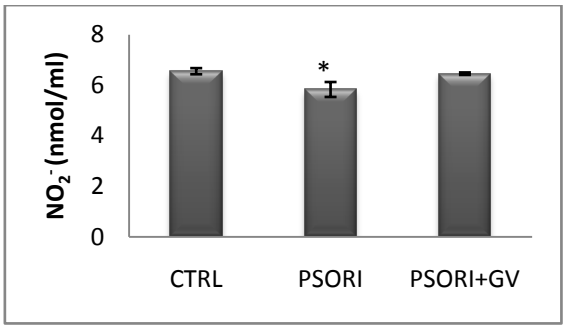
our results in the light of the application of this plant extract on models of other diseases and analysis of oxidative stress. Two such studies referred to the evaluation of effects of *Galium verum* extract on the functional recovery and redox equilibrium of isolated rat heart after ischemia. The levels of nitrites and superoxide anion radical observed in our investigation align with the findings of Bradic et al. (2019, 2020), who delineated a similar pattern in pro-oxidative parameters within cardiac tissue subsequent to ischemia-reperfusion injury. This concurrence points to a presumed cardioprotective effect of *Galium verum* extract, which acts by modulating the redox balance and mitigating the generation of free radicals, similar to our model of psoriasis in rats where this extract positively modulates systemic redox homeostasis.



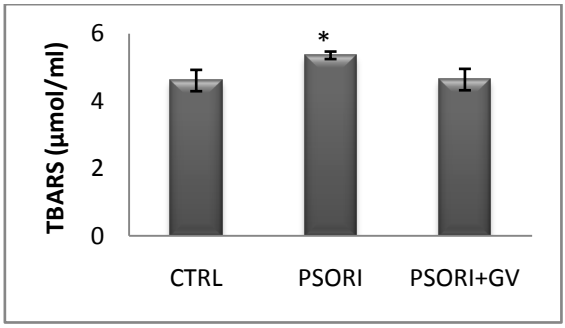
Graph 1. The level of O_2^- in plasma



Graph 2. The level of H_2O_2 in plasma



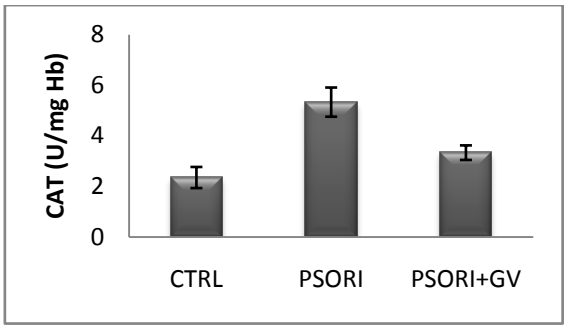
Graph 3. The level of NO_2^- in plasma



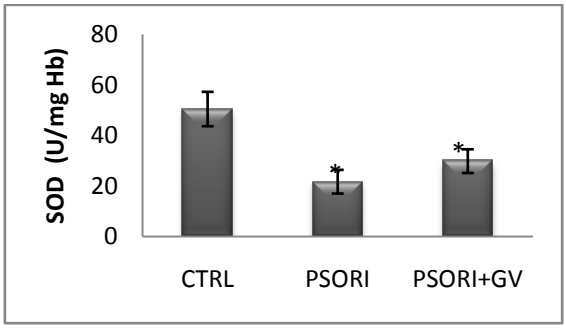
Graph 4. The level of TBARS in plasma

The effects of *Galium verum* extract on antioxidative parameters (CAT, SOD, GSH) in erythrocytes

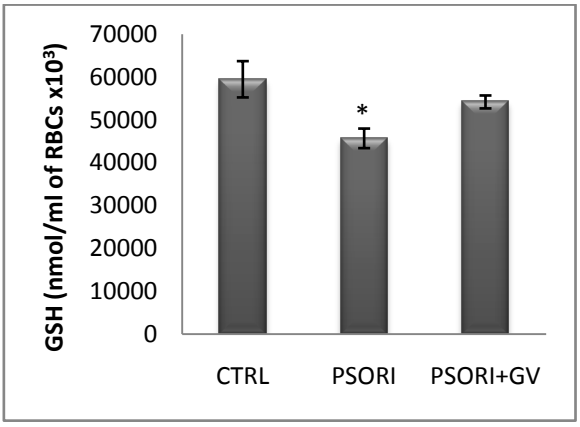
In our study the activity of antioxidative enzyme catalase was increased in both rats with psoriasis and those that was treated with *Galium verum* extract (Graph 5). The level of reduced GSH as well as the activity of SOD enzyme were decreased in PSORI and PSORI+GV groups compared to CTRL group (Graphs 6, 7). The dynamics of antioxidant defense enzyme activity correspond to the observed trends in these parameters within the myocardial tissue of rats subjected to ischemia/reperfusion injury, yet diverge from the values documented in the myocardial tissue of spontaneously hypertensive rats under identical ischemic injury conditions (Bradic et al., 2019, 2020).



Graph 5. The activity of CAT in erythrocytes



Graph 6. The activity of SOD in erythrocytes



Graph 7. The level of GSH in erythrocytes

Conclusion

This study aimed to provide a comprehensive overview of the intricate interaction between oxidative stress and the pathogenesis of psoriasis, while also investigating the therapeutic potential role of *Galium verum* extract in alleviating oxidative stress and ameliorating the clinical manifestations of psoriasis. Although this is the pioneering investigation in employing a psoriatic animal model treated with *Galium verum* extract, our findings suggest potential therapeutic implications as it shows a beneficial effect on redox balance modulation in psoriasis. Moreover, the potential synergistic effects of *Galium verum* extract with conventional treatments and its safety profile warrant further investigation.

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