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Research Article

Wogonin Hampers Dexamethasone-Induced Oxidative Imbalance in Sprague Dawely Rats

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ABSTRACT

Corticosteroids are frequently used for their powerful anti-inflammatory activity in the management of various chronic inflammatory diseases. They are also used because of their potent immunosuppressive power in the manipulation of adverse effects associated with chemotherapeutic treatments. However, their long-term usage is associated with several side effects. The majority of these side effects are due to the increased oxidative stress associated with their administration. Dexamethasone (DEX) is one of the most commonly used glucocorticoids either as an anti-inflammatory and/or immunosuppressive agent. Wogonin, a mono-flavonoid present in the root of *Scutellaria baicalensis Georgi*, has attracted considerable attention in recent years. Wogonin has a well-documented antioxidant activity that is mainly responsible for its multiple pharmacological activities. The current study aimed at investigating the possible protective activity of wogonin against DEX-induced oxidative stress in Sprague Dawley rats. The results showed that wogonin co-treatment successfully counteracted DEX-induced oxidative stress. Co-administration of wogonin doses of 30 and 60 mg/kg/day significantly improved superoxide dismutase (SOD) activity by 1.7 ($P<0.001$) and 3.9 folds ($P<0.001$) and significantly improved catalase (CAT) activity by 1.9 and 2.2 folds ($P<0.05$) as compared to DEX group. Moreover, co-treatment with wogonin (30 mg/kg/day) improved reduced glutathione (GSH) level by 1.5 folds, while the higher dose significantly improved GSH level by 2.3 folds ($P<0.05$) as compared to DEX group. Also, co-treatment with wogonin improved serum malondialdehyde MDA level by 11.8% and 30% compared to the DEX group. In conclusion, wogonin displayed a promising protective antioxidant effect against DEX-induced oxidative stress.

Keywords: *wogonin; flavonoids; oxidative stress; corticosteroids; rat.*

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1. INTRODUCTION

Oxidative stress plays an important role in the pathogenesis of multiple diseases including obesity-associated metabolic syndrome **[1]**, cancer **[2, 3]**, neurodegenerative disease **[4, 5]**, diabetes **[6]**, renal diseases **[7]** and osteoporosis **[8]**. Oxidative stress results from an imbalance between the generation of reactive oxygen

species and/or reactive nitrogen species **[9]**. Internal or natural defense mechanisms include either antioxidant (tocopherols, ascorbic acid, and glutathione) or antioxidant enzymes such as catalase (CAT), glutathione peroxidase (GPX) and superoxide dismutase (SOD) **[10, 11]**. The consequences of oxidative stress are multiple including lipid peroxidation **[12]** and DNA damage **[13]**. This, in turn, causes the loss of cell viability either *via* necrotic or apoptotic pathways **[14, 15]**.

Glucocorticoids are one of the most commonly used agents for the management of many chronic diseases **[16]**. They have a strong anti-inflammatory **[17]** and immunosuppressive activity **[18]**. These activities enhanced their choice for treating and managing inflammatory and immune-related disorders **[19]**. They are used as an adjuvant with chemotherapeutic agents **[20]** to manage their undesirable adverse effects such as pain and inflammation **[21]**. Unfortunately, they increase oxidative stress which in turn is associated with many side effects **[22]**. Dexamethasone (DEX), a glucocorticoid, has been reported to induce oxidative stress in many *in vivo* and *in vitro* studies **[23, 24]**.

Modulation of oxidative stress is a key therapeutic target in various diseases **[4, 25]**. Antioxidants are widely used in the management of various diseases as inflammatory conditions **[26]** and diabetes **[27]**. Wogonin (5,7-dihydroxy-8-methoxy flavone) is a naturally existing mono-flavonoid in *Scutellaria Baicalensis Georgi* root **[28]**. Wogonin showed numerous pharmacological activities such as anti-inflammatory **[29]**, antitumor **[30]**, neuroprotective **[31]** and anti-diabetic activities **[32]**. Wogonin also has been reported to have a potential antioxidant activity **[33, 34]**. Accordingly, the current study aimed at investigating the potential protective activity of wogonin against DEX-induced oxidative stress *in vivo* in Sprague Dawley rats. The systemic oxidative imbalance was investigated by assessing several oxidative stress markers and antioxidant enzymes.

2. METHODS

2.1. Experimental Animals

A total of 40 male Sprague Dawley rats were obtained from the animal facility of Misr University for Science and Technology, 6th of October, Egypt. Animals were allowed a period of 2 weeks for adaptation with the environmental conditions of the animal facility at the Faculty of Pharmacy Ain Shams University. Throughout the study, the animals had free access to water and standard food pellets. The study protocol was approved by the ethical committee of the Faculty of Pharmacy Ain Shams University (ENREC-ASU.2019-95).

2.2. Animal Treatment Protocol and Samples Collection

The animals were randomly divided into four groups (10 rats per group), the first group was used as control while groups from 2-4 were treated with dexamethasone, intramuscularly (I.M.) once weekly for 5 weeks. Groups 3 and 4 were treated with wogonin (purity HPLC 99%, SHAANXI XINHENG BIOTECH CO., LTD, China) intraperitoneally at doses 30 mg/kg and 60 mg/kg/day. Wogonin has dissolved in a mixture of saline and TWEEN 80 ratio 9:1 **[35]**. The doses were selected based on previously published data **[36]**. At the end of the week, 5 animals were euthanized and blood samples were collected from the retro-orbital sinus. Blood samples were allowed to clot then centrifuged at 15 min at 2500 rpm at 4 °C. Then the isolated serum samples were kept at -20 °C freezer for subsequent evaluation of following oxidative stress markers.

2.3. Assessment of Serum Superoxide Dismutase (SOD) Activity

SOD activity was assessed in serum specimens isolated from the different groups. The principle of SOD activity assay relies on the ability of this enzyme to inhibit the phenazine methosulphate-mediated reduction of nitroblue-tetrazolium dye. Absorbance was measured at 560 nm and superoxide dismutase activity was expressed in the unit (U)/mL **[37]**.

2.4. Determination of Catalase (CAT) Activity

CAT activity (U/mL) was assessed in the isolated serum samples from the different groups using a catalase assay kit **[38]** purchased from Biodiagnostics (Giza, Egypt) following the manufacturer's recommendations. Absorbance was measured at 510 nm where there was an inverse relationship between catalase activity and released chromophore's color intensity.

2.5. Determination of Reduced Glutathione (GSH) Serum Level

Evaluation of the serum level of GSH (mg/dL) was based on the reduction of 2-nitrobenzoic acid with GSH to produce a yellow compound. The yield chromogen is directly proportional to GSH concentration and its absorbance was measured at 405 nm **[39]**.

2.6. Assessment of Serum Lipid Peroxides Level

Lipid peroxidation was estimated colorimetrically using the thiobarbituric acid reactive method **[40]**. Absorbance was measured at 534 nm. Malondialdehyde (MDA) serum levels were expressed as (nmol/mL).

2.7. Statistical Analysis

Data are presented as the mean ± SD. Comparisons were done via a one-way analysis of variance followed by Tukey– Kramer's post hoc test. Statistical significance was accepted at $P<0.05$. GraphPad Prism software, version 5.00 (GraphPad Software, La Jolla, CA) was used for statistical analysis and plotting the graphs.

3. RESULTS

3.1. Effect of Wogonin on Serum SOD Activity

Treatment of rats with the corticosteroid DEX significantly reduced SOD activity by 63.3% ($P<0.001$) vs control. However, co-administration of wogonin doses of 30 and 60 mg/kg/day significantly improved SOD activity by 1.7 ($P<0.001$) and 3.9 folds ($P<0.001$) as compared to DEX group **(Fig. 1)**.

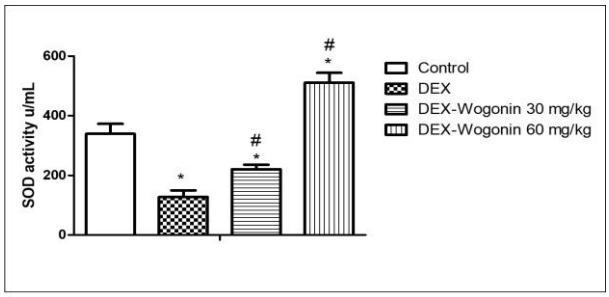

Fig. 1. Effect of wogonin co-treatment on dexamethasone-induced alterations in SOD activity. Data are means ± SD. (n=10). * indicates significant difference vs control at $p<0.05$; # indicates significant difference vs DEX at $p<0.05$.

3.2. Effect of Wogonin on Serum CAT Activity

Assessment of serum CAT activity showed that DEX treatment reduced CAT activity by 39.6% vs control. However, co-administration of wogonin doses of 30 and 60 mg/kg/day significantly improved CAT activity by 1.9 and 2.2 folds ($P<0.05$) as compared to DEX group **(Fig. 2)**

3.3. Effect of Wogonin on Serum GSH Levels

Treatment of rats with the corticosteroid DEX significantly reduced GSH level by 54.7% ($P<0.05$) vs control. Furthermore, co-treatment of wogonin doses of 30 mg/kg/day improved GSH level by 1.5 folds, while the higher dose 60 mg/kg/day significantly improved GSH level by 2.3 folds ($P<0.05$) as compared to DEX group **(Fig. 3)**.

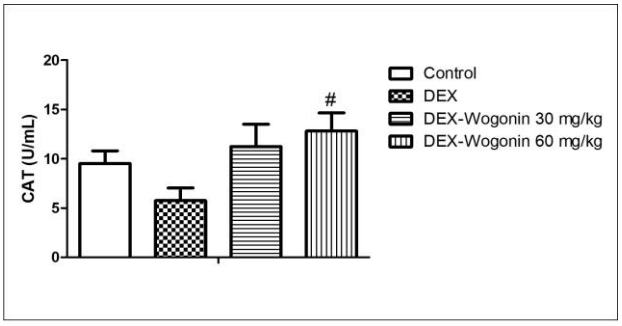

Fig. 2. Effect of wogonin co-treatment on dexamethasone-induced alterations in catalase (CAT) activity. Data are means ± SD. (n=10). # indicates significant difference vs DEX at p<0.05.

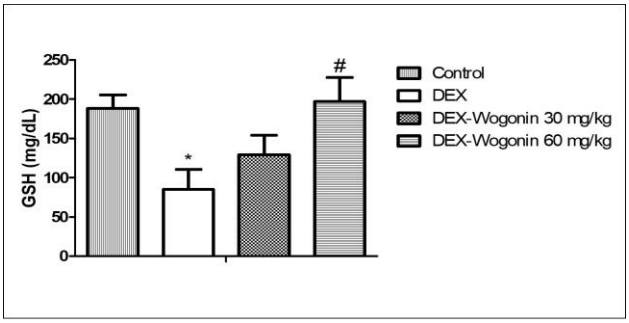

Fig. 3. Effect of wogonin co-treatment on dexamethasone-induced alterations in GSH activity. Data are means ± SD. (n=10). * indicates significant difference vs control at p<0.05; # indicates significant difference vs DEX at p<0.05.

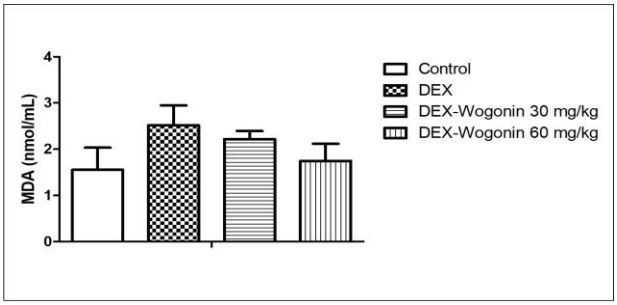

Fig. 4. Effect of wogonin co-treatment on dexamethasone-induced alterations serum lipid peroxides (MDA). Data are means ± SD. (n=10).

3.4. Effect of Wogonin on Serum Lipid Peroxides

Evaluation of lipid peroxide levels in the serum indicated that rats treated with DEX showed an increased serum MDA level by 1.6 folds as compared to control. However, co-treatment of wogonin doses of 30 and 60 mg/kg/day improved serum MDA level by 11.8% and 30% compared to the DEX group **(Fig. 4)**.

4. DISCUSSION

Dexamethasone (DEX) long-term usage is associated with adverse effects involving most major organ systems **[22]**. These adverse effects include osteoporosis **[40]**, gastritis **[42]**, hypertension **[43]**, lipid disorders **[44]**, neuropsychiatric **[45]**, dermatological changes **[46]** and ocular hypertension **[47]**. The aforementioned adverse effects are mainly caused by increased oxidative stress **[22, 23]**.

Imbalance in the oxidation status which is usually due to overproduction of reactive oxygen species (ROS) or defective in ROS defense capacities leads to subsequent damage at the cellular level which ultimately triggers secondary tissue injury in the affected target organs. The activities of the antioxidant enzymes including SOD, and CAT among the well-known markers that indicate the status of the endogenous defense systems against ROS. The level of reduced glutathione is an additional valid marker to predict the oxidative status **[48]**. Glutathione is capable of existing in different redox species. Therefore, it is implicated in the physiological functions that involve regulation of the thiol-redox status. GSH is the reduced form of glutathione that is capable of neutralizing ROS and protecting normal cells against ROS mediated-damage **[49]**. The activity of SOD is essential to neutralize the superoxide radicals by transforming them into hydrogen peroxide. Catalase enzyme works on the generated hydrogen peroxide to produce water **[50]**. Lipid peroxidation and tissue injury **[50]** is the outcome of oxidative imbalance in case of reduced activity or levels of the inherent antioxidative defense systems.

Herein, rat's challenge with dexamethasone resulted in abolished SOD and CAT activities as well as a marked depletion of GSH. Moreover, a slight increase in serum lipid peroxides was observed in dexamethasone-treated rats. Concurrent treatment of dexamethasone challenged rats with wogonin at either 30 mg/kg or 60 mg/kg doses improved the antioxidant defenses SOD, CAT, and GSH and alleviated dexamethasone-induced systemic oxidative imbalance. Our data are in line with previously published data which indicated that wogonin exhibits strong antioxidant activity in several *in vitro* and *in vivo* models **[33, 34]**. It was previously reported that wogonin protects against cadmium-triggered oxidative renal injury **[52]**. Studies also showed that the antioxidant activity of wogonin contributed to its protective effect against diabetes cardiomyopathy by enhancing CAT and SOD activities **[53]**. Moreover, previous work supported that wogonin enhanced both SOD and GSH serum levels and reduced the MDA level in a model of nonalcoholic fatty liver **[54]**.

In conclusion, the current study showed that treatment with wogonin hampers the systemic oxidative imbalance induced by long term treatment with glucocorticoids like dexamethasone. Therefore, co-treatment with wogonin is anticipated to alleviate corticosteroid -mediated adverse reactions and complications. Studies are in progress in our laboratories to verify the effect of wogonin against dexamethasone target specific adverse effects.

Declarations

Ethics Approval and Consent to Participate

The animal treatment protocol was approved by the research ethics committee of the Faculty of Pharmacy, Ain Shams University.

Consent to Publish

All the authors approved the final manuscript and agreed on the publishing of the submitted work in the APS journal.

Availability of Data and Materials

All data generated or analyzed during this study are included in this published article in the main manuscript.

Competing Interests

The authors declare that there is no conflict of interest.

Funding Statement

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