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Research Article

ANTI-OXIDATIVE EFFECTS OF *GYPSOPHILA PERFOLIATA* ON D-GALACTOSE INDUCED AGING IN RAT

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ABSTRACT

D-Galactose (D-GAL), a monosaccharide, induces the oxidative stress and results in the mimicked aging model in rats. *Gypsophila perfoliata* belongs to Caryophyllaceae family and a perennial plant. The content of this plant is rich in saponarin and Phenolics, therefore scientist has been focused on its possible antioxidative effects. In this study, we studied the antioxidant effect of *Gypsophila perfoliata* on aging mimicked rats induced by D-GAL. For this, Wistar-albino rats (n=8 in each group of 4) have been applied D-GAL (150 mg/kg/day) subcutaneously (s.c.) and Gypsophila extract (100 mg/kg/day) via gavage for 12 weeks. After this period, the rats were sacrificed and their blood serum and brains used to determine Glutathione (GSH) and Malondialdehyde (MDA). The data were analyzed by use of the SPSS software package (20th version) One-way ANOVAs followed by post-hoc Bonferroni tests were used, and $p< 0.05$ was considered as significantly different value. When the data of examined groups compared to the control, we observed a significant increase in the MDA while significant decrease in the GSH levels. However, when we compared the Gypsophila group with D-GAL, the levels of both the GSH and MDA varied significantly. Our findings showed that the extract of Gypsophila could be used in reducing the oxidative stress during the aging.
Keywords: Aging, D-galactose, *Gypsophila perfoliata*, lipid peroxidation.

1. INTRODUCTION

Aging is an inevitable natural process and is one of the causes or factors of most degenerative diseases. Many reasons are known to affect the aging process in the organism such as lifestyle, diet, physical activity and heredity [1]. It has been reported that the incidence of chronic diseases such as cardiovascular diseases, Alzheimer's disease, and diabetes has increased with aging [2].

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Oxidative stress is an imbalance when free radicals in the tissue can not be cleaned by an adequate amount of antioxidants. Many studies have shown that aging is related to accumulation of free radicals [3]. Excessive production of free radicals leads to lipid peroxidation (LPO) resulting in damage to DNA, mitochondria and cell membrane structure and cell death [4]. The living organism has contained a variety of antioxidants, some of the enzymatic, such as superoxide dismutase, catalase and glutathione peroxidase, and other non-enzymatic, such as glutathione (GSH) that serve to compensate the effect of the reactive oxygen species. In addition to the endogenous system, antioxidant status can be ameliorated by exogenous antioxidant food supplementation. Among the nutritional factors which promote antioxidant status, the beneficial role of flavonoids has been established in animals and in humans [5,6].

D-Galactose (D-GAL) is a monosaccharide and known as milk sugar. Even though D-GAL normally metabolized by the enzyme β galactosidase, high doses induce accumulation of galactitol and cause osmotic and oxidative stress [7]. Moreover D-GAL able to react with a free amino group of amino acids and peptides, then product by advanced glycation end products (AGEs) [8]. Many studies have shown that D-GAL increases oxidative stress by increasing lipid peroxidation levels [9,10].

Gysophila species widely distributed in Eurasia and used as a pharmaceutical [11]. Some of these species have long been used as emulgators on food industry in Turkey. It was reported that the saponins are found in the different parts of plants in different doses *Gysophila perfoliata* has high content of saponin and the saponin amount has been found to be 15 to 19 [12,13]. Previous research has shown that *Gypsophila* species exhibit a variety of pharmacological activities including antioxidant, hypoglycemic, hepatoprotective [6, 14-16].

Considerable evidence suggests that oxidative stress as a cause of aging is one of the main drivers of these processes [17,18]. The aim of this study was to examine the antioxidative effect of *Gysophila perfoliata* by using D-GAL-induced aging model in rat.

2. STUDIES

2.1. Plant Extraction

Gysophila perfoliata was collected from Afyonkarahisar, Turkey in July 2018. The aerial parts were cut into pieces and dried for 10 days at room temperature (22±2°C). D-GAL and other chemicals were obtained from Sigma-Aldrich, USA.

Then, these samples were powdered with a laboratory mill. To prepare methanol extract, dried samples (20 g in 400 mL solvent) were stirred overnight (24 h, 250 rpm in a shaker (Lab Companion SI-300 Benchtop Shaker) at RT. After filtration, the extracts were concentrated using a rotary evaporator under vacuum at 40 °C. The extracts were completely dried at 40 °C in the oven (Memmert- UNB 400) and they were stored at +4 °C until further analysis. The extraction yield was 8.2%.

2.2. Animals and Treatment

Wistar Albino rats aged 3 months and weighing 200-240 g were employed in this study. The rats were housed in temperature and light controlled rooms (12 h dark-light cycles, 22^{0} C ± 2 and humidity 60% ± 5). All animals were free access to water and pellet food and experiments were performed according to the national laws and guidelines. The protocol used in this study was approved by the Committee on the Ethics of Animal Experiments of Üsküdar University, İstanbul, Turkey (No. 2018-22).

32 rats randomly divided into four equals groups (n=8):

Control: Rats were subjected to once daily with normal saline by subcutaneous injection for 12 weeks.
D-GAL: Rats were subjected to once-daily D-GAL (150 mg/kg) by subcutaneous injection for 12 weeks.
GYP: Rats were subjected to once-daily GYP (100 mg/kg) by oral gavage for 12 weeks.
D-GAL + GYP: Rats were subjected to once-daily D-GAL (150 mg/kg) by subcutaneous injection and GYP (100 mg/kg) by oral gavage for 12 weeks.

After 12 weeks' period, the rats were anesthetized by the withdrawal of blood via cardiac puncture under anesthesia by intraperitoneal injection of ketamine HCl (80 mg/kg) and xylazine (10 mg/kg) and were then sacrificed. Brains were removed, except for cerebellum, and soaked in sterile NaCl (0.9 %). The tissue samples were then stored at - 80℃. Brain tissue samples were then gently washed and homogenized in chilled phosphate buffer (pH 7.4), and homogenates were centrifuged at 800 × g for 5 min at 4°C to remove nuclear residues. The supernatant was then centrifuged at 10.500 × g for 20 min at 4°C to obtain the post-mitochondrial supernatant used for analyzing GSH and MDA levels.

2.3. Determination of GSH Levels

The level of GSH in homogenized brain tissue samples was measured by using 5,5′- dithiobis (2-nitrobenzoic acid) on a spectrophotometer at 412 nm wavelength by following the methods of Beutler et al. and Coban et al. [7,19].

2.4. Determination of MDA Levels

MDA levels in tissue homogenates were estimated based on the analysis of LPO levels. LPO was estimated by measuring the level of MDA by the thiobarbituric acid test as explained by Coban et al. and Ohkawa et al. [7,20].

2.5. Statistical Analysis

Experimental results are presented as mean ± SD. Data were analyzed using the SPSS software package (20^{th} version, IBM, New York, USA). One-way ANOVAs followed by post-hoc Bonferroni tests were used for statistical analysis, and $p< 0.05$ values were considered as significantly different.

3. CONCLUSION

As seen in Figure 1, serum GSH levels in both D-GAL and D-GAL+GYP groups decrease significantly when compared to the Control ($^{*}p<0.001$, ($^{\#}p=0.001$).

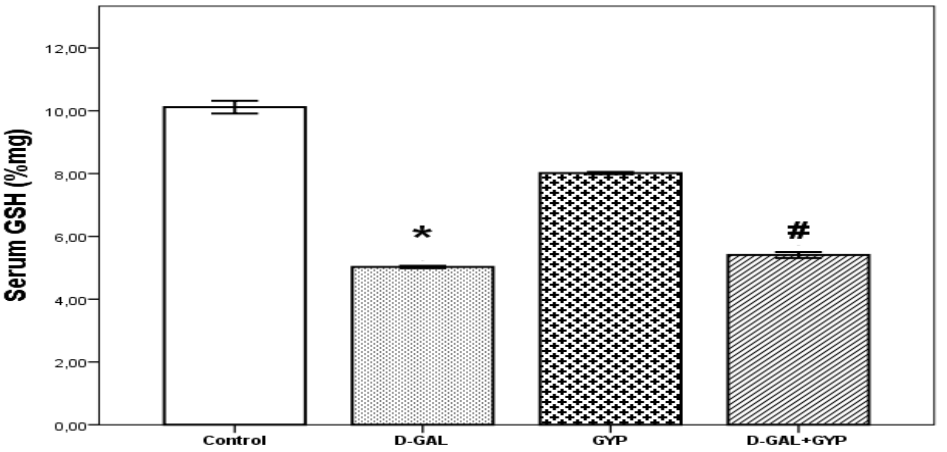

Figure 1. Comparison of Serum GSH levels of the four groups

When we look at Figure 2, serum MDA levels in both D-GAL and D-GAL+GYP groups increased significantly as compared to Control ($^{*}p=0.001$, $^{\#}p=0.035$). On the other hand, the MDA levels were decreased in both GYP and D-GAL+GYP when compared to D-GAL group ($^{\#}p=0.008$, $^{*}p<0.001$).

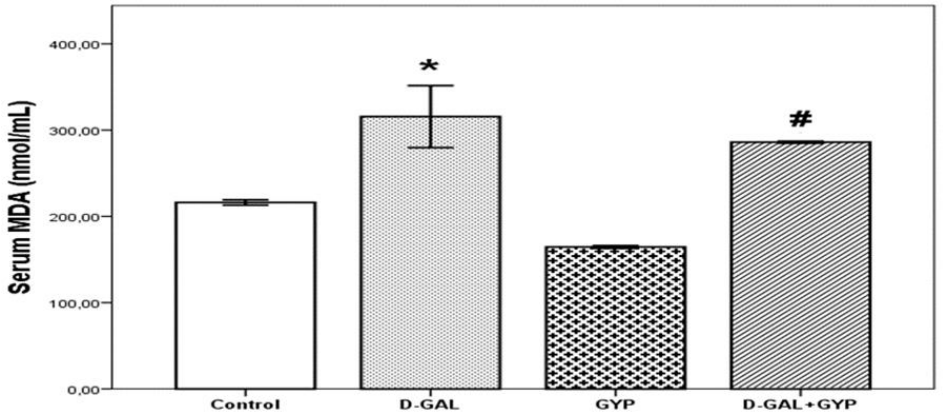

Figure 2. Comparison of Serum MDA levels of the four groups

In Figure 3, brain GSH levels and comparisons are summarized. The results showed that although the GSH levels decreased in comparison to the control, it was not statistically significant ($^{*}p=0.520$). On the other hand, these levels in GYP group increased compared to the control, their increase was found not to be significant ($^{*}p=0.120$). In the D-GAL+GYP group the GSH level increased significantly when compared to both the control and D-GAL groups ($^{\#}p=0.005$, $^{\#}p<0.001$).

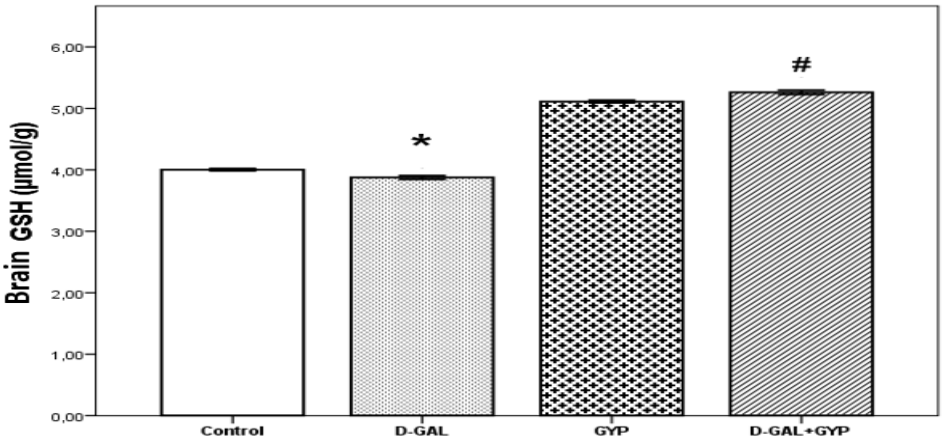

Figure 3. Comparison of Brain GSH levels of the four groups

Figure 4 shows the results of the brain MDA levels of the groups examined. According to these results, there were some increase and decrease in the MDA levels but these fluctuations were not statistically significant when they compared to control. The p values were between 0.08 and 0.150. However, when D-GAL MDA levels compared to D-GAL+GYP, there was a significant decrease ($^{\#}p=0.006$).

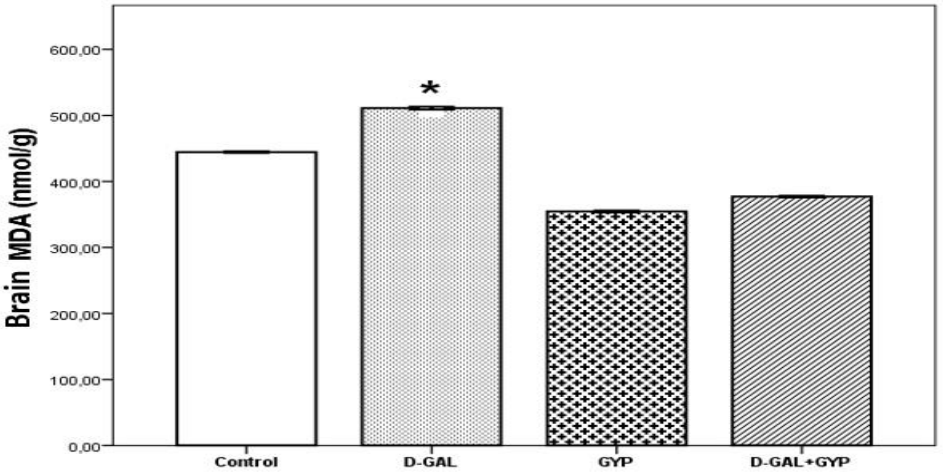

Figure 4. Comparison of Brain MDA levels of the four groups

The brain is one of the most sensitive organs to oxidative damage due to its high metabolic rate, high lipid content and limited antioxidant mechanism [21]. As it is well-known, the oxidative stress one of the main reasons for neurodegenerative diseases by resulting the aging. Nowadays there are a number of publications showing the anti-aging effect of the plants [22].

In the literature, it was reported that LPO levels had increased after D-GAL administration and concluded it was due to decrease of antioxidant systems and resulted with DNA damages

[23,24]. In normal condition, there is a balance between free radical formation and antioxidant status. This stability starts changing during the aging processes.

Gypsophila perfoliata is reported to be rich in saponarin which reduces MDA and its related free radical products [25,26]. Additionally, it was also reported that it has a potent antioxidant property [27,28]. Besides its antioxidant properties, it was reported to be an anti-diabetic agent that due to its α-glucosidase contents [29].

In this study, we studied *Gypsophila perfoliata's* anti-oxidant effects on serum and brain tissues of the aging modeled rats. For this, we administrated D-GAL, a chemical used to develop and mimic the aging behaviors of human beings, to the rats to perform the aging model by following the method described by Shwe et al. [30].

We observed the antioxidant effects of *Gypsophila perfoliata*'s on both serum and brain tissues as similarly reported by literature (Figure 1-4). It decreased the serum GSH levels and decreased LPO end products, the MDA, as it's content saponarin does (Figure 1-2).

Zheleva-Dimitrova et. al. showed that *Gypsophila perfoliata* is rich in antioxidants because of its phenolics and flavonoids content and they proposed that its antioxidant activities might be because of these contents [16]. Our findings on both the MDA and the GSH levels were similar to that of the literature mentioned comparing to control group data to GYP group's (Figure 1-2).

Vitcheva et al. and Bai et al. declared the liver protective effect of saponarin in the *Gypsophila* species [14,30]. They used pure saponarin, 80 mg/kg/day, in their studies for 3 days [13,28]. We used *Gypsophila* extract, 100 mg/kg/day, and gave to the animals for 12 weeks. Although we studied different tissues, we got similar results from point of its antioxidative and protective effects on brain. As seen in Figures 3-4, the extract used decreased brain MDA and increased GSH levels.

Literature survey showed that there are no sufficient reports on the protective effect of *Gypsophila* species' on brain and aging. Similarly, the studies on neurodegenerative protective effect of *Gypsophila* are also limited. Zheleva-Dimitrova et al. conducted a study on Gypsophila species' inhibitory effect on acetylcholinesterase and found that *G. perfoliata* extract was the most effective one [15,16]. As it is known, this enzyme plays a crucial role in Alzheimer's disease (AD) and thought its inhibition one of the treatment choice [24].

As a conclusion, it is thought that *Gypsophila perfoliata* is one of the important plants to be used against neurodegenerative diseases like AD due to its features explained above. Additionally, these explanations also showed the importance of this study.

Conflict of interest

The authors declare no conflicts of interest.

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