



## Research Article

# The effect of hesperidin on neuron-specific enolase and oxidative damage in the pre-eclampsia model

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## ABSTRACT

This study evaluates the protective effect of Hesperidin (HES), flavanoglycone, during pre-eclampsia developed pregnancy. The pregnant rats were randomly divided into five groups as Control, L-NAME (25 mg/kg), HES (100 mg/kg), L-NAME-HES and L-NAME P-HES. After the 13<sup>th</sup> day of pregnancy, the chemicals were administrated until the 20<sup>th</sup> day. 20 days after the offspring's birth, they were separated from their mothers and all rats were sacrificed after 6 weeks' time. Serum samples were analyzed for levels of Glutathione (GSH), lipid peroxidation (LPO), and neuron-specific enolase (NSE) activity. Urine samples were analyzed for the proteinuria. While Proteinuria and LPO level increasing, GSH levels decreased in the L-NAME group when compared to the Control. As we compared L-NAME group with the L-NAME-HES and L-NAME P-HES groups, the GSH levels increased significantly while LPO levels in the HES, L-NAME-HES, L-NAME P-HES groups were decreasing. The NSE activity significantly increased in the L-NAME group compared to the Control. In contrast to the L-NAME group, its activity decreased in HES, L-NAME-HES and L-NAME P-HES groups significantly. Briefly, it can be said that HES has a significant effect on both the LPO levels and the NSE activity in the case of pre-eclampsia.

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## INTRODUCTION

Pre-eclampsia is a multi-system disease characterized by new-onset hypertension and both proteinuria and end-organ dysfunction throughout the last half of pregnancy, with and approximately 5%-7% morbidity. The disease activates maternal hypertension, proteinuria, fetal distress,

intrauterine growth restriction, premature birth, increased perinatal mortality, and extra clinical symptoms as reported by Magee et al. and Zhu et al. [1,2].

Acute implementation of NG-nitro-L-arginine methyl ester (L-NAME), an inhibitor of nitric oxide synthesis, in rats induces hypertension, which further causes symptoms

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of hypertensive disorders in pregnancy [2,3]. Gestational hypertensive disorders increased the vulnerability to multiple neurodevelopmental disorders such as schizophrenia, depression, cognitive impairment, and even raised lifetime stroke risks [4]. Neuron-specific enolase (NSE) is a glycolytic, a member of the enolase family, enzyme and composed of the  $\gamma\gamma$  homodimer and the  $\alpha\gamma$  heterodimer [5]. As NSE is present in neurons and not in glial cells, it is claimed as a potential determinant in the development of vascular events and subclinical brain damage among asymptomatic hypertensive patients [6]. NSE is a critical molecule for maintenance of the irritability of the neuronal membrane. Under normal conditions, NSE is present as a trace amount in body fluids. Once the neuronal cell damage, it is immediately released into body fluids and its amount begins increasing [7-9].

Therefore, NSE level is used a useful diagnostic agent for neurologic diseases like intracerebral hemorrhage, traumatic brain injury, ischemic stroke, Guillain-Barre syndrome, epilepsy, delirium, Alzheimer disease, and Creutzfeldt-Jacob disease [8,10]. It was observed that NSE was clearly high in the neonatal period in baby rats with autism spectrum disorder (ASD) modeled [11]. Flavonoids are phenolic compounds and present especially in fruits and vegetables. It is well known that they are mainly potent antioxidants [12]. Hesperidin (HES, 30,5,9-dihydroxy-40-methoxy-7-orutinosyl flavanone, a biologically active flavonoglycone) is one of these compounds mentioned and having antihypertensive, antioxidative, insulin-sensitizing, lipid-lowering, and anti-inflammatory properties [13-14], sweet citrus fruits are the best source for HES. The objectives of this study were to investigate the effects of HES on NSE levels and oxidative stress markers during pre-eclampsia.

## MATERIALS AND METHODS

### Chemicals

All chemicals were purchased from Sigma Chemical Co (St. Louis, MO).

### Animals

The animals were housed in cages in an environment-controlled room (room temperature,  $22 \pm 2^\circ\text{C}$ ; relative humidity, light/dark cycle, 12h/12h) with free access to food and water.

### Experimental Design

Female rats, weighing 250-300 g, were obtained from the animal laboratory of Üsküdar University and coupled with males. After coupling was established by the visualization of spermatozoa in a vaginal smear, the pregnant rats were randomized to the Control (C, n=8), L-NAME (n=8) and HES (n=8) groups. On the 13<sup>th</sup> day of gestation, the maternal rats in the L-NAME groups were applied by 25 mg of L-NAME per kg (Sigma-Aldrich) by following the method of Zhu et al. (2017) [2]. At the 20<sup>th</sup> day, the pregnant

mothers were taken to the metabolic cage and their urines were collected for 24 hours and evaluated for proteinuria in them. After the offspring were born, as they were separated from their mothers at the 20<sup>th</sup> day and all rats were sacrificed after 6 weeks by taking blood via cardiac puncture under ketamine HCl [80 mg/kg intraperitoneal (i.p.)] and xylazine (10 mg/kg i.p.). The chemicals were administered to experimental animals by oral gavage at the 13<sup>th</sup> day of pregnancy and continued for 7 days.

In this study, 5 groups of rats were managed as follows:

Group 1: Control (0.9 % saline)

Group 2: L-NAME (Pre-eclampsia with L-NAME generated 25 mg/kg/body weight)

Group 3: HES (Hesperidin 100 mg/kg/body weight)

Group 4: L-NAME + HES (25 mg/kg, 100 mg/kg, respectively)

Group 5: L-NAME + P-HES (Hesperidin was started on the 7<sup>th</sup> day of pregnancy (P-HES: Pregnancy-Hesperidin).

### Biochemical Parameters

Proteins in 24-h urine collected from each animal of the different groups were determined by auto-analyzer (Abbott Architect C8000). GSH levels in serum were determined by employing 5,5'-dithiobis-(2-nitrobenzoate) (DTNB) at 412 nm spectrophotometrically [15]. LPO was determined by measuring malondialdehyde (MDA) levels in serum. For this, the method of Ohkawa *et al.* was followed [16]. Serum NSE (2-phospho-D-glycerate hydrolase) activities were measured by employing a commercial ELISA kit using the Sandwich-ELISA method by following the method of Sorensen et.al [17].

### Statistical Analysis

The obtained data were analyzed using the SPSS software package (20<sup>th</sup> version, IBM, New York, USA). One-way ANOVA followed by post-hoc Bonferroni tests were used for statistical analysis, and  $p < 0.05$  was considered as statistically significant value.

## RESULTS AND DISCUSSION

### Effects of L-Name and Hes on Proteinuria, GSH and LPO Levels

The effects of L-NAME on Proteinuria, GSH and LPO levels were shown in Figure 1 (a-c). The data were presented as mean  $\pm$  SD.

As seen in Figure 1 (a-c), Proteinuria levels increased significantly in L-NAME group compared to Control group ( $p=0.001$ ) while these levels significantly decreasing in L-NAME-HES and L-NAME P-HES groups compared to L-NAME group ( $*p=0.001$ ). Therefore, the supplement of hesperidin reduced the proteinuria effectively in the L-NAME-induced pre-eclampsia rat model. GSH levels decreased significantly in the L-NAME group compared to the Control group. Similarly, it declined significantly in the HES group as well ( $p=0.001$ ). Compared to the

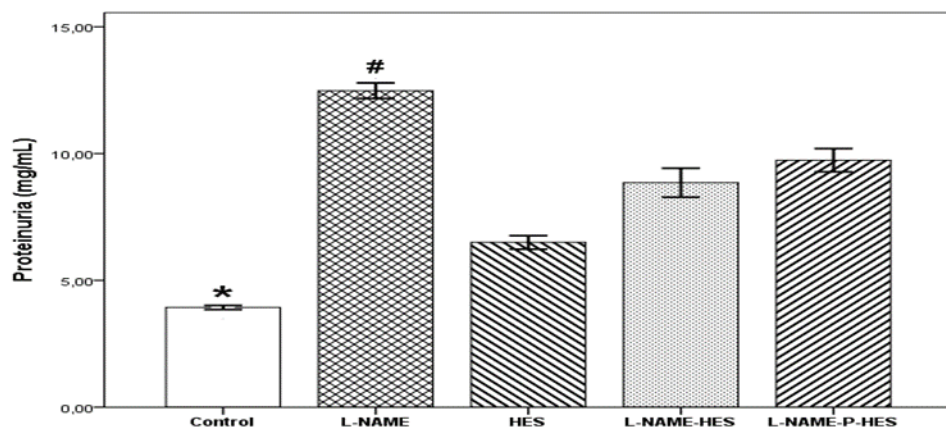


Figure 1a. Proteinuria levels of the five groups.

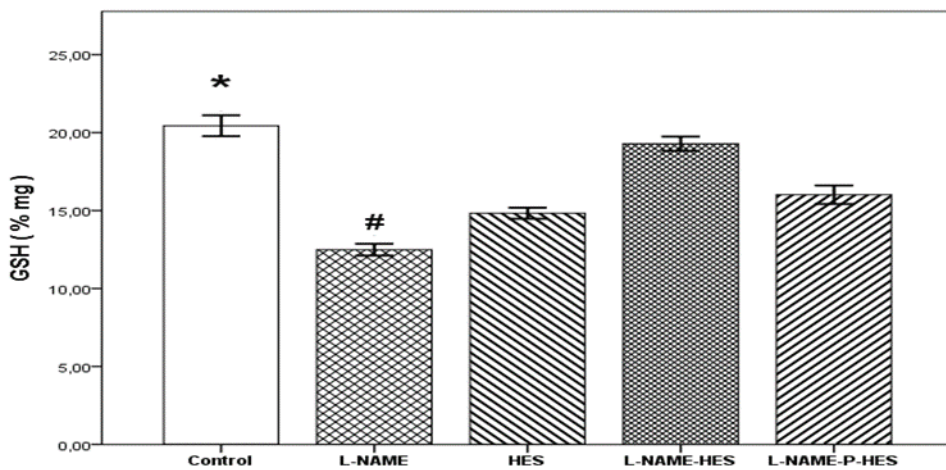


Figure 1b. GSH levels of the five groups.

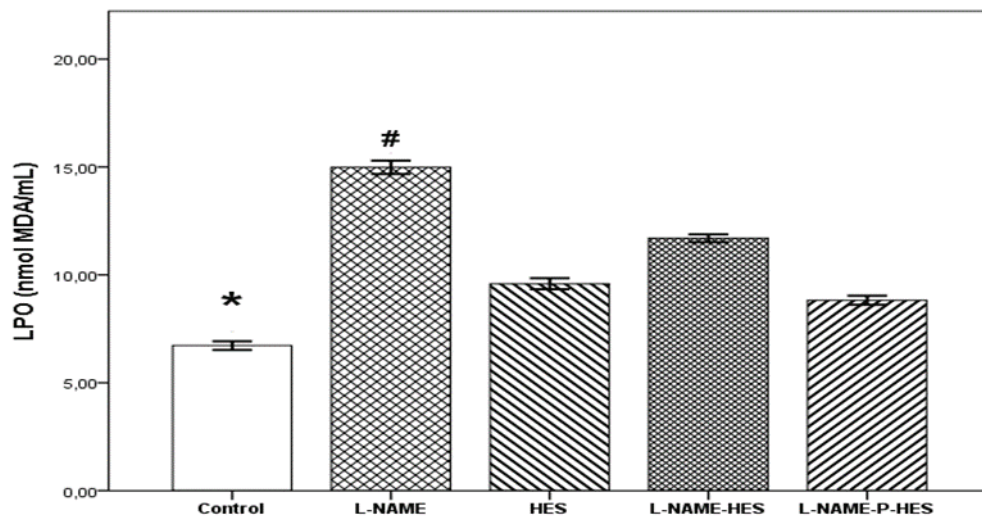
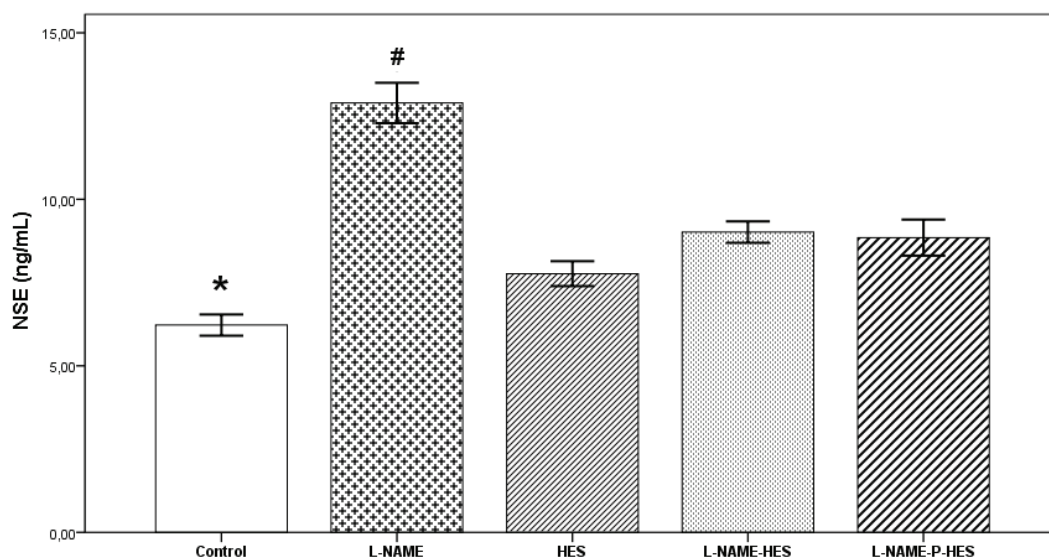


Figure 1c. LPO levels of the five groups.



**Figure 2.** Serum NSE activities of the five groups.

L-NAME group, GSH levels have significantly increased in the L-NAME-HES and L-NAME P-HES groups ( $*p=0.001$ ). While the LPO levels increased significantly in the L-NAME group when compared to the Control group ( $*p=0.001$ ), these values in the HES, L-NAME-HES, L-NAME P-HES groups decreased significantly compared to L-NAME group ( $*p=0.001$ ). These data indicated downregulation effect of the hesperidin on lipid peroxidation in L-NAME-induced pre-eclampsia rats.

#### Effects of L-Name and Hes On NSE Activity

The effects of L-NAME on NSE activities were shown in Figure 2. The data were presented as mean  $\pm$  SD.

NSE activity has significantly increased in the L-NAME group compared to the Control group ( $*p=0.001$ ). However, in contrast to the L-NAME group, its activity decreased in HES, L-NAME-HES and L-NAME P-HES groups significantly ( $*p=0.001$ ,  $p=0.001$ ,  $p=0.002$ , respectively). Our findings showed that hesperidin had a potent diminishing NSE activity.

In some studies, experimental pre-eclampsia model was created by administrating L-NAME [2]. Similarly, in our study, we successfully mimicked pre-eclampsia in pregnant rats by introducing of L-NAME and observed maternal hypertension and proteinuria, high stillbirth rate, and fetal growth retardation.

Flavonoids are widely present mainly in fruits and vegetables and have a potent both biological and pharmacological effect. Therefore, the diet rich in flavonoids is found very effective in reducing the oxidative stress and in the regulation of brain function [18,19]. Therefore, this study is designed to investigate the role of HES, a flavonoid found in citrus fruits, in preventing oxidative damage induced by the L-NAME. The NSE can be detected in both blood and cerebrospinal

fluid. As a result of this feature of the NES, it is promising to be a good biomarker for appraising both neuronal damage and injuries [9,20-21]. Because there are very few studies on the relationship between NSE activities and neurologic diseases in gestational conditions. In addition, research on mechanisms to explain the relation is still nonexistent [22]. A number of studies also showed that hypertension was associated with raised oxidative stress [23].

According to our results, in with the L-NAME group, while the GSH levels decreasing, LPO levels significantly increased. This was similar to findings of Yang et al. and Kumar et al. [18,24]. After treating with HES in the L-NAME group, while the GSH levels increasing, the LPO levels decreased significantly. However, this decrease was found to be more in the L-NAME P-HES groups. We think that this is related to the prophylactic effect of HES administration for 7 days before pre-eclampsia occurs. This effect of HES is the first report that HES may be used as a complementary treatment in the prevention of oxidative damage of pre-eclampsia.

A number of studies reported that a raised risk of ASD in descendant have disclosed to pre-eclampsia. This can be exemplified by Buchmayer et al. study: They did a population-based, case-control study which covered 1,216 cases and 6080 controls (7,296 individuals). They found that pre-eclampsia formation in offsprings accompanies to more than 50 % increase in the risk of ASD [25]. A similar finding was also reported by y Burstyn et al. in a study of totally 218,890 live births [26]. Some studies reported that women who have had pre-eclampsia with no other acute disease have a raised risk of lasting cognitive failure for a long time of period after the pregnancy. The women with the earlier high blood pressure problems demonstrated brain atrophy and cognitive impairment even decades after the gestation [27-28].

Additionally, women who had pre-eclampsia have a greater risk of cardiovascular complications [27,29]. These findings might be an indication of the entirety of the blood vessel wall and blood-brain barrier in women who suffered from pre-eclampsia. As Bergman et al. reported, the data obtained from these studies confirmed that NSE levels are increased in women with pre-eclampsia [27]. Similarly to Bergman et al. study, we observed that the NSE activities dramatically increased in the experimental pre-eclampsia model.

## CONCLUSION

The most remarkably, this study is the first report on the effect of the HES on the NSE activities, an important biomarker for neurodevelopmental disorders in the pre-eclampsia model, and a risk factor for ASD. We observed that the NSE activities increased significantly in the L-NAME group when compared to the Control. Conversely, when we compared the L-NAME group with HES groups, we observed that NSE activities decreased significantly. As a result, it can be said that HES has a significant effect on both the LPO levels and the NSE activities in the case of pre-eclampsia with a high probability of ASD formation. Accordingly, it is recommended to consume HES, a potent antioxidant compound, in daily diet.

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## AUTHORSHIP CONTRIBUTIONS

Authors equally contributed to this work.

## DATA AVAILABILITY STATEMENT

The authors confirm that the data that supports the findings of this study are available within the article. Raw data that support the finding of this study are available from the corresponding author, upon reasonable request.

## CONFLICT OF INTEREST

The author declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

## ETHICS

Our study was examined by Uskudar University Animal Experiments Local Ethics Committee and approval of ethics committee was obtained as a result of 2019-10 meeting.

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