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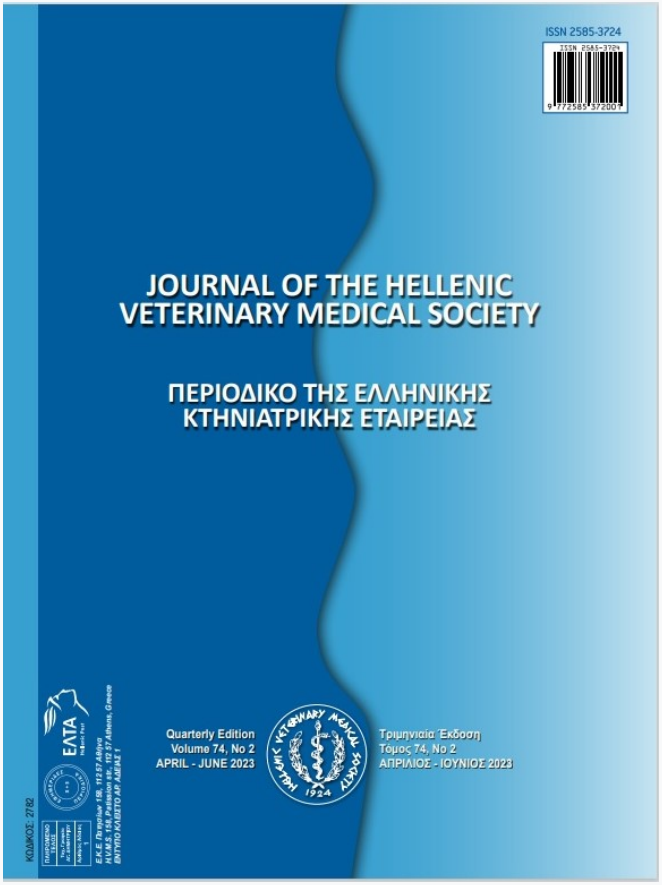

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Research article
Ερευνητικό άρθρο

Investigation of the Bioactivity of Escin in Hydrogen Peroxide-Induced Oxidative Stress

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ABSTRACT: Escin is a triterpene saponin obtained from the horse chestnut tree *Aesculus hippocastanum* L. (*Hippocastanaceae*). The aim of this study is to investigate the bioactivity of escin against hydrogen peroxide-induced oxidative stress in quails. The study was performed in male Japanese quails (n=10) with similar pre-experimental weights (*Coturnix japonica*) on average. In order to create oxidative stress in quails, hydrogen peroxide was given to drinking water *ad libitum* for 1 week. At the end of the period, escin was injected intraperitoneally into the quails twice, every other day. The weights of the experimental animals were measured at the beginning and the end of the experiment and compared. In addition, the 10-day live weight change rate and feed intake rates of the experimental animals were also compared. Total antioxidant (TAC), total oxidant (TOC), and oxidative stress index (OSI) were measured colorimetrically from serum and liver homogenates of animals in the experimental groups. In addition, IL-1β translation levels from serum samples were investigated by the ELISA method. According to the data obtained from the study, 10-day body weight change rates increased by 33% in the hydrogen peroxide added group compared to the control group, while an increase of 133% was observed in the escin-treated group. Besides, in serum and liver samples, it was found that escin showed strong antioxidant properties by decreasing the amount of TOC and increasing TAC levels in the group escin administered with hydrogen peroxide, compared to the group administered only with hydrogen peroxide. It was also observed that escin significantly suppressed the levels of IL-1β induced by hydrogen peroxide. As a result, escin was thought to be an alternative molecule in improving growth performance by showing significant antioxidant activity in the prevention of oxidative stress and inflammation in quails. However, multiple trials and detailed molecular analyzes are needed to obtain more detailed information on the subject.

Keywords: Escin; oxidative stress; quail; inflammation; growth

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INTRODUCTION

Reactive oxygen species (ROS) and certain levels of reactive nitrogen species (RNS) are signaling molecules involved in homeostasis. Excessive increase in ROS and RNS levels or the inability of the antioxidant defense system to sufficiently eliminate these molecules causes oxidative stress (Kurutaş, 2016). It is known that hydrogen peroxide (H_2O_2) stimulates oxidative stress by causing an increase in reactive oxygen species. H_2O_2 can cause various degrees of oxidative stress, lipid peroxidation, and deterioration of intestinal morphology and barrier function in piglets (Celik and Ozkaya, 2002; Yin et al., 2015).

Horse chestnut (*Aesculus hippocastanum*) has been used for centuries as a traditional medicine for the treatment of certain conditions, including varicose veins, hematoma, and venous congestion, and hemorrhoids (Bombardelli et al., 1996; Chauhan et al.2012). One of the most important bioactive components obtained from the horse chestnut tree (*Hippocastanaceae*) is the escin compound, which is a triterpene saponin. For many years, the leaves, bark, and seeds of this plant have been used in the treatment of various diseases such as arthritis, brain trauma, stroke, venous congestion, and thrombophlebitis. In addition, it is known that the extracts of this plant are used for therapeutic purposes in forms such as oral tablets, injections, and topical gels. It has been determined that escin mainly has biological activities such as antiapoptotic, anti-edema, anti-inflammatory, and antioxidant, and is widely used in clinical treatment (Chen et al. 2021; Celep et al., 2012; Xiao and Wei, 2005; Xin et al., 2011; Puvača et al., 2022). Besides, some studies have shown that escin has anti-inflammatory effects against liver injury induced by endotoxin (Jiang et al., 2011), methyl parathion (Du et al., 2012), and carbon tetrachloride (Singh et al., 2017) in animals. Recently, studies in the literature have also revealed the medicinal properties of escin such as antitumor, antiviral, antifungal, and antioxidant effects (Sirtori, 2001; Pittler and Ernst 2012). Wang et al (2014) reported that escin showed significant bioactivity in increasing antioxidant capacity by increasing the activities of various antioxidant enzymes. Horse chestnut and its bioactive component, escin, are used in the treatment of chronic venous insufficiency, edema, and also in the treatment of diabetic retinopathy. Escin has been reported to be a 20 times more potent superoxide and reactive oxygen species scavenger than ascorbic acid (Masaki et al., 1995; Sirtori, 2001).

In summary, the present study, it was aimed to investigate some parameters of antioxidant and anti-inflammatory activities of escin, the most basic bioactive component of the horse chestnut plant, in a hydrogen peroxide-induced oxidative stress model in quails.

MATERIAL AND METHODS

Ethical Approval

This study was approved by Hatay Mustafa Kemal University Animal Experiments Local Ethics Committee (Decision No: 2021/06-18).

Bioactive chemicals and experimental setup

The study was performed on 40-day-old male Japanese quail (*Coturnix japonica*). The quails were individually weighed and their average body weight was recorded and distributed to the experimental groups (n=10). In the study groups, the control group (C) was fed only commercial starter feed group I was administered commercial starter + Escin intraperitoneally (IP), group II was given commercial starter and hydrogen peroxide (HP) added to drinking water, finally group III was applied commercial starter + HP + Escin IP. Oxidative stress was created for 1 week by adding 0.5% hydrogen peroxide (Tekkim, Turkey) to the drinking water of quails in groups II and III. Then, the study was terminated by administering escin (Sigma Aldrich, EU,) dissolved in serum physiologic at a concentration of 100 mg/ml/body weight intraperitoneally for 2 days to the experimental groups I and III every other day. In the study, quails were fed with commercial growth feed (Table 1). In the study, first body weight and final body weight, 10-day Body Weight change rate (%) and Average Daily Feed Intake Amount (g) were measured (n=10). Afterwards, tissue and blood samples were taken and oxidative stress parameters and biochemical-molecular analyzes were performed (n=5).

Preparation of lysate from liver tissue

At the end of the experiment, approximately 0.4 g of liver tissue of each experimental animal was weighed and the samples were crushed with a homogenizer in ice-cold homogenization buffer (pH 7.4) containing 10 mM Tris, 1 mM EDTA, 25 mM $MgCl_2$, 0.1 mM dithiothreitol, 0.25 M sucrose.

Oxidative stress analyzes

Total antioxidant and total oxidant capacities in blood and liver samples taken in the study were per-

Table 1. Nutrient composition of commercial grower feed

Nutrient values	Amount (%)
Crude protein	20.0
Crude Fat	3.00
Crude fiber	3.00
Crude ash	4.80
Lysine	1.12
Methionine	0.51
Calcium	0.880
Total phosphorus	0.440
Sodium	0.140
Vitamin-Mineral premix*	0.250
Metabolizable energy kcal/kg	2859.3

*1 kg of the premix provided: 15.000.000 IU of Vitamin A, 5.000.000 IU of Vitamin D3, 100.000 mg of Vitamin E, 3000 mg of Vitamin K3, 5000 mg of Vitamin B1, 8.000 mg of Vitamin B2, 60.000 mg of niacin, 15.000 mg of D-calcium pantothenate, 5000 mg of Vitamin B6, 20 mg of Vitamin B12, 200 mg of D-biotin, 2000 mg of Folic acid,100.000 mg of Vitamin C, 0.02 mg of Cyanocobalamin, 74 mg of Mn (from MnO), 45 mg of Zn (from ZnO), 4 mg of Cu (from CuO), 12.5 mg of Fe (from FeSO4), 0.3 mg of I (from KI), 0.15 mg of Se (from NaSe).

formed with ready-made commercial kit (Rel Assay, Turkey) according to the method developed by Erel (2007). The oxidative stress index was determined by the ratio of these two values. TAC values are given as mmol Trolox Equiv. /L and TOC values as μmol H_2O_2 Equiv. /L.

Detection of Protein Translation Levels

IL-1β translation levels in serum samples taken from the experimental groups were determined by following the protocol of the ready-made ELISA kit (BT-lab, Korea). The data obtained are given as ng/L.

Statistical analysis

The one-way analysis of variance (ANOVA) and post hoc Duncan tests were performed on the data to examine the differences among groups using the SPSS statistical software package (SPSS22). The results were presented as average mean ±SD. A value of $p< 0.5$ was considered significant.

RESULTS

Live weight and feed intake results

In the study, the values of the groups in terms of initial body weight, final body weight, and average daily feed intake are given in Table 2. When the table is examined, it is seen that the differences between the groups in terms of the examined characteristics are not statistically significant ($P>0.05$). While the initial body weight values were lower in the other groups compared to the control group, it was determined that the final body weight value in group I was numerically higher than the control. The average daily feed intake value was lower in the control group than in experimental groups.

Oxidative stress and Antioxidant levels results

Effects of Escin and Hydrogen peroxide on TAC level in liver homogenates

According to the data obtained, the liver TAC level decreased by 36% in group II (0.44±0.20) compared

Table 2. Live Weight values of the experimental groups (average mean±SD)

	Initial Body Weight (g)	Final Body Weight (g)	Average Daily Feed Intake Amount (g)
	200.96±6.61	213.18±8.28	25.40±1.00
Group I	191.74±6.11	219.12±9.60	28.00±3.60
Group II	192.74±4.00	208.15±4.74	28.80±1.60
Group III	183.69±6.40	198.61±4.74	28.50±4.50
P	0.246	0.243	0.850

to the control group (0.69±0.13), while in group III (0.82±0.08), this level was found to increase by 86% compared to the group II (Figure 1).

Effects of Escin and Hydrogen peroxide on TOC level in liver homogenates

According to the data obtained, liver TOC level increased by 4% in group II (18.24±2.92) compared to the control group (17.54±1.48), while in group III (15.29±1.39), this level was found to be reduced by 16% compared to group II (Figure 2).

Effects of Escin and Hydrogen peroxide on oxidative stress index in liver homogenates

According to the data obtained, it was observed that the liver OSI level increased by 64% in group II (41.30) compared to the control group (25.26), while this level decreased by 54% in group III (18.62) compared to group II (Figure 3).

Effects of Escin and Hydrogen peroxide on TAC level in serum samples

According to the data obtained, serum TAC levels were found to decrease by 37% and 17% in group II (0.86±0.20) and in group I, respectively, compared to the control group (1.34±0.09). However, it was found that this level increased by 18% in the group III (1.04±0.07) compared to group II (Figure 4).

Effects of Escin and Hydrogen peroxide on TOC level in serum samples

According to the data obtained, serum TOC level increased by 37% in group II (16.25 ± 3.93) compared to the control group (11.81 ± 0.67), while in group III (11 03 ± 0.43), this level was found to decrease by 32% compared to group II (Figure 5).

Effects of Escin and Hydrogen peroxide on oxidative stress index in serum samples

According to the data obtained, the serum OSI level increased by 116% in group II (19.00) compared to the control group (8.79), while this level was reduced by 44% in the group III (10.56) compared to group II (Figure 6).

IL-1β translation levels

According to the data obtained, serum IL-1β level increased by 6% in group II (14.09±1.15) compared to the control group (13.21±1.28), while this level decreased by 27% in group III (10.27±1.79) compared to group II (Figure 7).

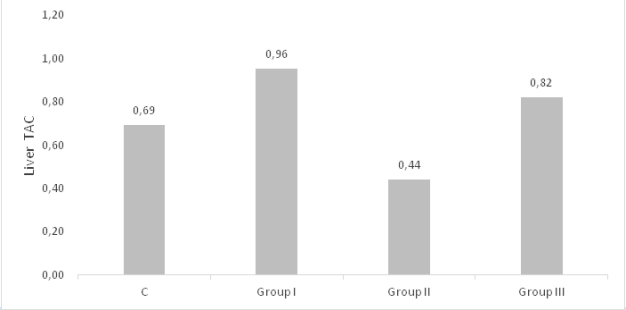

Figure 1. Effects of Escin and Hydrogen peroxide on TAC level in liver homogenates

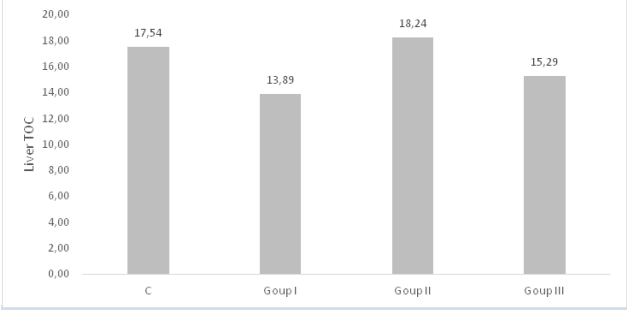

Figure 2. Effects of Escin and Hydrogen peroxide on TOC level in liver homogenates

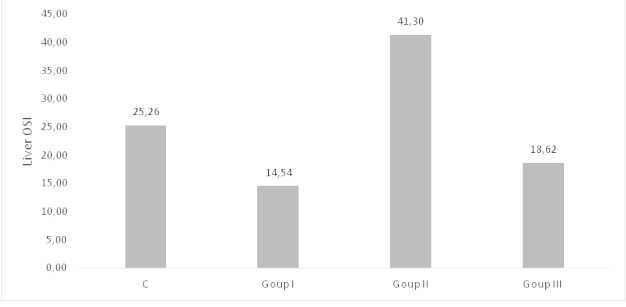

Figure 3. Effects of Escin and Hydrogen peroxide on oxidative stress index in liver homogenates

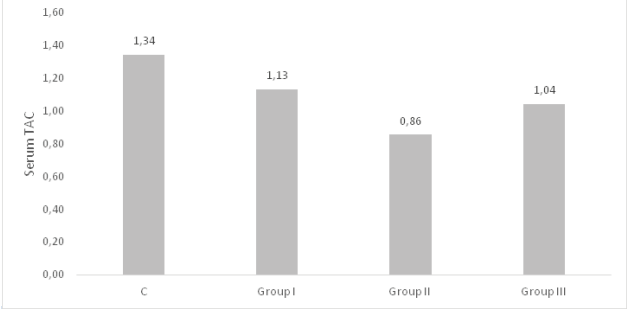

Figure 4. Effects of Escin and Hydrogen peroxide on TAC level in serum samples

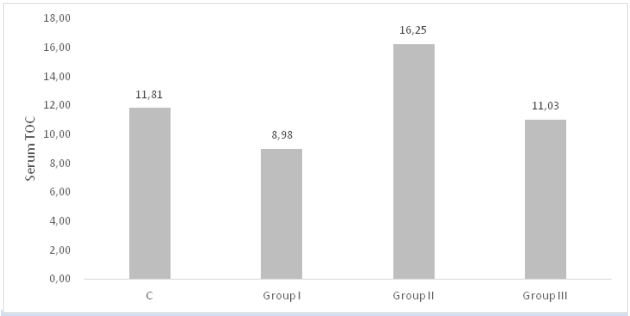

Figure 5. Effects of Escin and Hydrogen peroxide on TOC level in serum samples

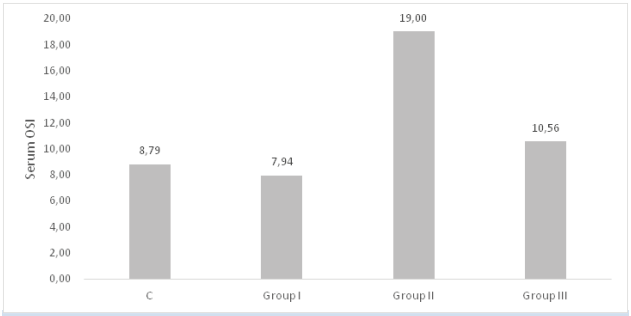

Figure 6. Effects of Escin and Hydrogen peroxide on the oxidative stress index in serum samples

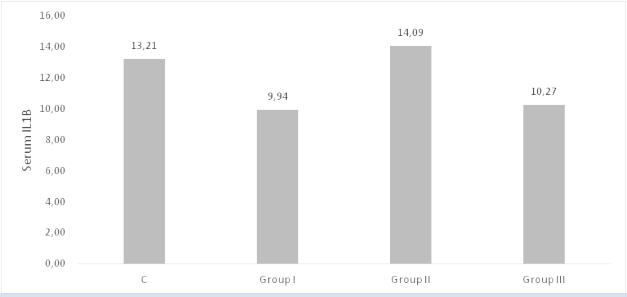

Figure 7. Effects of Escin and Hydrogen peroxide on IL-1β translation levels in serum samples

DISCUSSION

In poultry farming, with the prohibition of the use of antibiotic growth promoters in the European Union due to their harmful effects, the focus has been on alternative products that can be used as additional feed support for poultry to increase growth performance and improve feed efficiency (Canoğulları et al., 2009; Lika et al., 2021). For this reason, research on the use of medicinal plants as a dietary supplement in poultry production has recently gained importance thanks to the bioactive compounds in their content (Alagawany et al., 2015a, 2015b; Puvača 2022). In our study, escin, which is the main bioactive component of the horse chestnut tree (*Aesculus hippocastanum* L.) and is known for its strong antioxidant and anti-inflammatory activity, was used as a feed additive in quails. According to the data obtained, although there was a numerical difference in the experimental groups in terms of final body weight and average daily feed intake compared to the control group, it was not statistically significant ($p>0.05$) (Table 2). Studies conducted with the addition of various feed additives with different antioxidant properties to the rations have reported similar results to the control group (Çiftçi et al., 2013; Sarıkaya et al., 2018; Singh and Kumar, 2018; Youssef et al., 2021; Lakram et al., 2019; Chaudhary et al., 2018). As a result of the study, it was determined that there was a similarity between the control group and the experimental groups. Antioxidants can cause a mild course of adverse health problems by repairing or preventing damage at the cell level caused by reactive oxygen radicals.

Reactive oxygen species mainly consist of singlet oxygen, hydrogen peroxide, superoxide anion, and hydroxyl radicals. These radicals cause major damage to proteins, DNA, and lipids and therefore affect normal cellular functions (Apel and Hirt, 2004; Foyer and Noctor, 2005). Many studies have also reported that hydrogen peroxide often induces oxidative stress in animal models by the generation of potent ROS and nitrogen-oxygen species (Imlay et al. 1988; Ma et al., 2018; Mousa et al., 2022). H_2O_2 exerts its detrimental effects on tissues by lipid peroxidation, which is formed by the conversion of H_2O_2 to hydroxyl radical as a result of the Fenton reaction (Ganie et al. 2011). According to the data we obtained in the study, while hydrogen peroxide decreased the total antioxidant level in both blood serum and liver homogenate samples, it increased the total oxidant level significantly. However, escin showed a strong antioxidant effect in oxidative stress caused by hydrogen peroxide, both in blood serum samples and liver homogenates, by decreasing the total oxidant capacity and increasing the total antioxidant capacity. Similar to the findings in our study, Vaskova et al. (2015) reported that *Aesculus hippocastanum* extracts and escin showed strong antioxidant activity by scavenging superoxide anion

and hydroxyl radicals. Again, similar to the findings in our study, Küçükkurt et al (2010) in their study on mice, showed that escin ethanolic mixture (100 mg/kg) obtained from *Aesculus hippocastanum* increased the antioxidant defense system and prevented lipid peroxidation compared to the control and high-fat diet group. They reported that it removes the negative effects of oxidative stress and has a protective effect on the liver. In the light of this information, it can be said that escin shows a protective effect from oxidative stress by increasing the gene expression levels of antioxidant enzymes.

Another finding we obtained in our study was that hydrogen peroxide added to quail water caused inflammation by increasing IL-1β translation level significantly, but escin application significantly eliminated this negative picture. Similar to the results we obtained, Xin et al. (2011) reported in their study that the use of corticosterone together with escin significantly reduced the levels of NO, TNF-α and IL-1β secreted by LPS-stimulated RAW264.7 macrophage cells. These findings, which alone cannot significantly inhibit the release of inflammatory factors, suggest that escin may synergize with glucocorticoids to increase its anti-inflammatory effects. Again, similar to our findings, Liu et al. (2012) found that escin significantly reduced LPS-induced cytotoxicity in human periodontal connective cells (hPDL) in a concentration-dependent manner, and the gene expression of TLR2 was partially suppressed. They also reported that escin also decreased the increase of proinflammatory cytokines (IL-1β, TNF-α, and IL-6) induced by LPS and showed protective activity against LPS-induced inflammation in hPDLs. Moreover, Lee et al. (2019) reported that escin administration reduces Acetaminophen (APAP)-induced liver injury in a dose-dependent manner (0.5-4 mg/kg). Moreover, escin has been found to decrease hepatic myeloperoxidase (MPO) activity and the levels of hepatic proinflammatory cytokines (TNF-α, IL-1β, IL-6, and IL-17). In addition, the administration of escin was found to reduce the expression of ERK hepatic phosphorylation. They reported that escin exerted protective effects on APAP-induced hepatotoxicity in a dose-dependent manner through the anti-inflammatory mechanism and inhibition of the ERK signaling pathway. In addition, Jiang et al. (2011) observed that escin administration prevented the migration of inflammatory cells, alleviated the degree of necrosis, and decreased serum ALT and AST activities in the LPS-stimulated liver inflammation model. They also reported that escin downregulated the levels of inflammatory mediators (TNF-α, IL-1β, and NO) and 11βHSD2 expression in the liver. According to the data we obtained in our study and the literature reviews on the subject, it can be concluded that escin has a significant anti-inflammatory activity by reducing the expression levels of proinflammatory cytokines such as IL-1β.

CONCLUSIONS

In the study, the 10-day live weight change rates of quails increased significantly with escin application. In addition, it was determined that escin application showed strong antioxidant properties by increasing TAC levels and decreasing TOC levels in serum and liver samples. In addition, it was found that escin showed strong anti-inflammatory activity by significantly reducing IL-1β levels. As a result, it was determined that escin significantly prevented hydrogen peroxide-induced oxidative stress and inflammation. According the data obtained from the study, it was revealed that escin could be an alternative molecule in preventing oxidative stress and inflammation and improving growth performance in quails. However, more multiple trials and more detailed molecular analyzes are needed to fully elucidate the issue.

CONFLICT OF INTEREST

The authors declare that there are no conflicts of interest.

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