ABSTRACT

Gallic acid is a phenolic compound found in many plant sources with strong antioxidant activity. In this study, the bioactivity of Gallic acid was investigated in Japanese quails induced by oxidative stress. The study was performed on four groups of 40-day-old male Japanese quail (Coturnix japonica). Oxidative stress was created for 1 week by adding 0.5% hydrogen peroxide. The study was terminated by administering 100 mg·kg⁻¹ body weight Gallic acid intraperitoneally. Total antioxidant and total oxidant level analyzes from liver tissue homogenates were performed using a ready-made commercial kit. TNF-α levels from blood samples taken for anti-inflammatory activity were investigated by ELISA method. There were no statistically significant results on live weight gain between the experimental groups and control group. However, Gallic acid in liver homogenates together with H₂O₂ increased total antioxidant state (TAS) compared to H₂O₂ application, while it decreased total oxidant state (TOS) in the same groups. Moreover, while the oxidative stress index increased in the H₂O₂ group, it decreased significantly in both the Gallic acid and Gallic acid + H₂O₂ groups. Gallic acid application also caused regression in blood TNF-α expression levels, which were increased by H₂O₂. In quails, Gallic acid showed antioxidant expression by increasing TAS levels and decreasing TOS levels, providing a significant decrease in oxidative stress index. It also provided anti-inflammatory activity by suppressing TNF-α levels. However, advanced molecular analyzes are needed to obtain more detailed information on the subject.

Key words: Gallic acid; growth performance; oxidative stress; inflammation; quails

RESUMEN

El ácido gálico es un compuesto fenólico que se encuentra en muchas fuentes vegetales con fuerte actividad antioxidante. En este estudio, se investigó la bioactividad del ácido gálico en codornices japonesas inducidas por estrés oxidativo. El estudio se realizó en cuatro grupos de codornices japonesas macho (Coturnix japonica) de 40 días de edad. Se creó estrés oxidativo durante 1 semana mediante la adición de peróxido de hidrógeno al 0,5 %. El estudio finalizó con la administración de 100 mg·kg⁻¹ de peso corporal de ácido gálico por vía intraperitoneal. Los análisis del nivel de antioxidante total y oxidante total de homogeneizados de tejido hepático se realizaron utilizando un kit comercial ya preparado. Los niveles de TNF-α de muestras de sangre tomadas para determinar la actividad antiinflamatoria fueron investigados mediante el método ELISA. No hubo resultados estadísticamente significativos sobre la ganancia de peso vivo entre los grupos experimentales y el grupo control. Sin embargo, el ácido gálico en homogeneizados de hígado junto con H₂O₂ aumentó el estado antioxidante total (TAS) en comparación con la aplicación de H₂O₂, mientras que disminuyó el estado oxidante total (TOS) en los mismos grupos. Además, mientras que el índice de estrés oxidativo aumentó en el grupo H₂O₂, disminuyó significativamente, tanto en el grupo de ácido gálico como en el de ácido gálico + H₂O₂. La aplicación de ácido gálico también provocó una regresión en los niveles de expresión de TNF-α en sangre, que aumentaron con H₂O₂. En codornices, el ácido gálico mostró actividad antioxidante al aumentar los niveles de TAS y disminuir los niveles de TOS, proporcionando una disminución significativa en el índice de estrés oxidativo. También proporcionó actividad antiinflamatoria al suprimir los niveles de TNF-α. Sin embargo, se necesitan análisis moleculares avanzados para obtener información más detallada sobre el tema.

Palabras clave: Ácido gálico; desempeño del crecimiento; estrés oxidativo; inflamación; codornices
INTRODUCTION

Recently, especially after the limitation of the use of antibiotics, the use of alternative natural compounds, which are abundant in plants and fungi and have important biological activities, as natural feed additives in poultry farming has been increasing. Polyphenols constitute the most effective groups of phytochemicals added to animal feeds as an alternative to antibiotics [1, 2]. Polyphenols are defined as compounds containing at least one hydroxyl group attached to a phenol ring in their chemical structure. The most widely known of these compounds, which have a wide range of varieties, are Caffeic acid, Ferulic acid, p-Hydroxybenzoic acid, Protocatechic acid, Vanillic acid, Salicylic acid and Gallic acid [3, 4].

Gallic acid (3,4,5-Trihydroxybenzoic acid), an important member of the tannins group of these phenolic compounds, attracts the attention of researchers in scientific studies due to its strong antioxidant bioactivity [5], although Gallic acid, or gallate, is found in many plants [6] and in fungal species [7]. In addition to the antioxidant activity of this compound, which was discovered many years ago, anticancer [8], anti-HIV [9], antileukogenic [10], anti-inflammatory [11], antimicrobial [12] and antifungal [13] have also been reported in studies.

Oxidative stress basically develops when free radicals cannot be adequately removed by antioxidant defense mechanisms. Normally, there are antioxidant defense mechanisms in order to maintain the oxidant-antioxidant balance in living things. When this defense system, which includes enzymatic mechanisms such as superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPx) and nonenzymatic mechanisms such as glutathione, vitamin C, vitamin E and plant polyphenolic compounds, is weakened, the production and accumulation of reactive oxygen species in cells and tissues increases [14]. Oxidative stress is one of the main reasons for the deterioration of animal health and reduced meat quality in poultry farming [15]. Phytochemicals found in plants, especially polyphenols, stand out as the most important supplement compounds in coping with oxidative stress thanks to their strong antioxidant activities [16]. Therefore, these compounds have the potential to be used as feed additives to reduce oxidative stress in poultry and increase productivity in animals [17]. This study was aimed to investigate some parameters of antioxidant and anti-inflammatory activities of Gallic acid in a hydrogen peroxide-induced oxidative stress model in quails (Coturnix japonica).

MATERIALS AND METHODS

Ethical approval

This study was approved by Hatay Mustafa Kemal University Animal Experiments Local Ethics Committee (Decision No: 2021/06-17).

Experimental design

The study was performed on 40–day–old male Japanese quail. The quails were individually weighed on a precision scale (Ohaus NV622, USA), 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 feed starter + Gallic acid intraperitoneally (IP), group II was given commercial feed starter and hydrogen peroxide (HP) added to drinking water; finally group III was applied commercial feed starter + HP + Gallic acid 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 Gallic acid (Sigma Aldrich, EU) dissolved in serum physiologic at a concentration of 100 mg·mL⁻¹ 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).

Determination of biochemical parameters

In the study, liver samples were taken from 5 quails from each group for biochemical parameters. Total antioxidant status (TAS) and total oxidant status (TOS) analyzes from tissue homogenates (Rel assay–TR) were performed using a ready–made commercial kit, following the methods of Kucukgul and Erdogan [18]. The Oxidative Stress Index (OSI) in the experimental groups was calculated by the ratio of these two values. In addition, Tumor necrosis factor alpha (TNF–α) levels from blood samples taken for anti–inflammatory activity were investigated by ELISA method with commercial kits (Kit Origin).

Statistical analysis

Statistical analysis with the help of IBM SPSS 22 package program, the comparison of the groups in terms of the examined features was analyzed with One–way Anova. Differences were determined by Duncan's test.
RESULTS AND DISCUSSIONS

Studies on performance and egg quality of Gallic acid and its derivatives in poultry (Gallus gallus domesticus), especially in layers, are quite limited. However, when reviewing the literature, it is seen that there are studies using grape (Vitis vinifera) seed extract gallic acid abundant in the seeds and skin of the grape. For example, Abdel-Wahab et al. [18] reported that grape seed polyphenols decreased total cholesterol lipid levels, blood sugar and liver enzyme activities, and increased glutathione peroxidase enzyme activity in Japanese quails, especially in 10 to 38-day periods. Again, Silici et al. [20] reported that the addition of 0.5, 1 and 1.5 % ground grape seed to quail compound feeds did not have a negative effect on yield, hatchability and egg quality of quails, and also increased feed efficiency. Moreover, Abu Hafsa and Ibrahim [21] reported that with the addition of 20 g·kg⁻¹ grape seed (GS) to the basal diet, the final live weight and live weight gain of quails increased, their feed conversion ratio increased, but feed intake was not affected. However, they stated that meat significantly increased carcass physical and chemical composition properties, carcass yield, and gizzard percentage. They reported that the addition of 40 g·kg⁻¹ GS significantly reduced the percentage of abdominal fat in poultry. In addition, in a study by Ao and Kim [22], it was reported that the addition of grape seed extract increased feed efficiency, increased antioxidant enzyme activities and immunity, and improved meat quality and body weight gain in Peking ducks (Anatidae).

In the present study, the live weight change rates of quails in the experimental groups (group I, II and III) were found to be lower (4.45 %, 4.25 % and 2.69 % respectively) than the control group (10.52 %). Also, according to the results obtained, feed conversion ratios between the experimental groups and the control group were not found statistically significant. In addition, when the experimental groups were compared among themselves, it was determined that the live weight gain rates changed in parallel with the feed intake amount.

Oxidative stress, known as the disruption of the oxidant–antioxidant balance between reactive oxygen species and the body’s antioxidant defense systems, constitutes one of the most fundamental problems of modern poultry farming [23]. Increasing superoxide and hydroxyl radicals as a result of the disruption of this balance cause serious damage to lipid, deoxyribonucleic acid (DNA), protein and other cellular components, leading to disruption of cell integrity and tissue damage [24]. It is known that oxidative stress affects commercial poultry production, growth performance, productivity and reproductive performance of layer hens in poultry farming. Although there are various strategies for the control of oxidative stress in poultry, natural antioxidant compounds (polyphenols), which are non–toxic and do not leave residues, have recently come into the focus of scientific studies [25]. The health benefits of plant phenolics are mainly due to their antioxidant and anti–inflammatory abilities [26]. Among such plant phenolics, Gallic acid (a trihydroxybenzoic acid) found in various foods and plants can be given as an example [27]. The curative effects of Gallic acid on many metabolic diseases including obesity have been reported [28, 29]. Moreover, several reviews have been published focusing on the therapeutic potential of Gallic acid. In 2013 Locattelli et al. [30] studied alkyl esters of Gallic acid as anticancer agents. Again, Choubey et al. [31] demonstrated the anticarcinogenic, antimicrobial, anti–mutagenic and antiangiogenic properties of Gallic acid and its esters.

When the liver total antioxidant capacity values were compared, TAS value in group I (0.9748 ± 0.15944) was found to be 5 % lower than the control group (1.0306 ± 0.12777), whereas in group II (0.8401 ± 0.09142) decreased by 37 % (P<0.5). However, this value increased 55 % in group III (0.9871 ± 0.13702) compared to group II (P<0.5). According to the results that was obtained in the present study, the TAS levels of Japanese quails decreased with the application of hydrogen peroxide, while the application of hydrogen peroxide together with Gallic acid prevented this decrease and increased the TAS value to the levels of the control group. In a study, Samuel et al. [32] showed that while Gallic acid decreased MDA, it increased plasma SOD and TAS significantly. Moreover, studies have reported that Gallic acid increases the activities of enzymes such as SOD, GPx and CAT, and increases GSH and vitamin C levels [33, 34]. Accordingly with the present study, it is thought that Gallic acid caused an increase in TAS levels by regulating nonenzymatic and/or enzymatic antioxidant mechanisms.

As another finding, when liver TOS values were compared, it decreased by 32 % in group I (13.3718 ± 1.3462) compared to the control group (19.6446 ± 2.41352) (P<0.5), whereas in group II (15.0483 ± 0.81577) a 23 % decrease was observed (P<0.5). However, it was determined that this value decreased by 14 % in group III (12.8825 ± 2.12539) compared to group II (P<0.5) (FIG. 1). In addition, liver OSI values decreased by 28 % in group I (13.7175) compared to the control group (19.0613), while it increased by 23 % in group II (23.5079). However, this value showed a 45 % increase in group III (15.0491 ± 0.81577) compared to group II (P<0.5). According to the results that were obtained in the present study, the OSI levels of Japanese quails decreased with the application of hydrogen peroxide, while the application of hydrogen peroxide together with Gallic acid prevented this decrease and increased the OSI value to the levels of the control group. In a study, Samuel et al. [32] showed that while Gallic acid decreased MDA, it increased plasma SOD and OSI significantly. Moreover, studies have reported that Gallic acid increases the activities of enzymes such as SOD, GPx and CAT, and increases GSH and vitamin C levels [33, 34]. Accordingly with the present study, it is thought that Gallic acid caused an increase in OSI levels by regulating nonenzymatic and/or enzymatic antioxidant mechanisms.

<table>
<thead>
<tr>
<th>TABLE II</th>
<th>Live weight values of experimental groups</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>Initial live weight (g)</td>
</tr>
<tr>
<td>Control</td>
<td>210.52 ± 8.10</td>
</tr>
<tr>
<td>Group I</td>
<td>203.21 ± 5.88</td>
</tr>
<tr>
<td>(Gallic acid)</td>
<td></td>
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<tr>
<td>Group II</td>
<td>210.82 ± 7.39</td>
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<tr>
<td>(H₂O₂)</td>
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<tr>
<td>Group III</td>
<td>210.16 ± 9.08</td>
</tr>
<tr>
<td>(Gallic + H₂O₂)</td>
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<tr>
<td>P</td>
<td>0.878</td>
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</tbody>
</table>

FIGURE 1. Total antioxidant status in liver homogenates of experimental groups (C: Control group, group I: only Gallic applied group, group II: only H₂O₂ applied group, group III: gallic acid+H₂O₂ applied group)
It was found that while H$_2$O$_2$ induced blood TNF–α gene expression levels of Japanese quails, Gallic acid significantly suppressed the expression levels of this gene. It was seen that there are studies investigating the anti-inflammatory potential of Gallic acid. In a study investigating the effects of polyphenols in broilers, it was determined that tea polyphenols (0.03–0.09 g·kg$^{-1}$ body weight) in the long term were found to show anti-inflammatory activity by reducing gene expression levels of pro-inflammatory cytokines such as IL–1β, IL–4, IL–6, IL–10, TNF–α and IFN–γ [39].

**CONCLUSIONS**

In the present study, Gallic acid application did not show a statistically significant change on live weight change rates of antioxidant capacity of muscle tissues and significantly decreased the thiobarbituric acid reactive substances (TBARS) value. Jung et al. [38] showed that in chickens fed a mixture of Gallic acid and Linoleic acid, it reduced the cholesterol level of eggs, and also increased the antioxidant potential by reducing the TBARS level.

Inflammation is a natural, protective response of the organism to pathogens, chemical harmful stimuli or stress. As a physiological process, the main purpose of inflammatory responses is to induce the process of eliminating pathogens and toxins and repairing damaged tissue [39]. In the present study, blood TNF–α values decreased by 5 % in group I (126.8182 ± 21.06224) compared to the control group (134.1212 ± 21.14828) ($P$<0.5), while group II (137.0303 ± 23.78826) showed an increase of 2 % ($P$<0.5). However, this value showed a 7 % regression in group III (127.4545 ± 12.26777) compared to group II ($P$<0.5) (FIG. 4). Oxidative stress is closely associated with inflammation due to the fact that the NF–κB pathway, which is important in the regulation of inflammation, is activated due to the increase of reactive oxygen species [40]. It has been reported that increased ROS may cause nuclear factor kappa B (NF–κB) activation and expression of inflammatory cytokines [41]. Therefore, according to the results obtained, it is thought that Gallic acid may have shown this effect by reducing ROS with its strong antioxidant property.

peroxide. Moreover, when the OSI values are considered, hydrogen peroxide increased the oxidative stress index, while the use of Gallic acid alone or in combination with H$_2$O$_2$ significantly decreased the OSI levels. In a recent study, Ignea et al. [35] reported that Gallic acid GA increases the body’s antioxidant capacity by directly neutralizing reactive oxygen species ROS and free radicals, or both, and reducing plasma malondialdehyde MDA levels. Jung et al. [36] reported that chicken breast meat was significantly larger in the group fed a mixture of 0.5 % and 1.0 % Gallic acid and Linoleic acid compared to the control group. They also reported that a mixture of Gallic acid and Linoleic acid were electron donors capable of neutralizing free radicals in broiler breast meat samples. Samuel et al. [32] reported that 100 mg/kg administration of Gallic acid significantly increased the broiler chest muscle mass compared to the control group, however, there was a decrease in the jejunal crypt depth and an increase in the villus width. Lee et al. [37] reported that the pH value of the broiler leg meat improved and the water holding capacity of the thigh increased in chickens fed with basal feed containing 1 % Gallic acid for 2 weeks. In the same study, it was shown that Gallic acid increased the
Japanese quails. However, it increased liver TAS levels suppressed by hydrogen peroxide and decreased liver TOS levels in quails. Considering the total OSI values, it can be said that it makes a significant contribution to suppressing oxidative stress. In addition, it showed anti-inflammatory activity by significantly suppressing TNF-α, one of the proinflammatory cytokines. As a result, in terms of its antioxidant and anti-inflammatory activities, Gallic acid is a compound that may have antioxidant and anti-inflammatory potentials in Japanese quails. However, further and detailed studies are needed to fully elucidate this argument.

Conflict of interests
The authors of this study declare that there is no conflict of interest with the publication of this manuscript.

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