Effect of Vitamin C as Antioxidant on Stressed Quail Induced by Hydrogen Peroxide

Abdullah F. Abdul-Majeed*[1] Saeb Y. Abdul-Rahman[2] Hassan A. Al-krad[3]

[1,2] Department of Animal Production, College of Agriculture and Forestry, University of Mosul / Iraq.
[3] Department of physiology, College of Vet. Medicine, Hama / Syria.

[1]Corresponding author: dr.abdullah@uomosul.edu.iq ; ORCID/ 0000-0001-7331-9969

Abstract

This study aimed to evaluate the effect of vitamin C as an antioxidant and its capability to reduce and/or prevent the H_2O_2-induced oxidative stress and its effects on antioxidant status as well as on some hematological variables of quail. 150 male quail (*Coturnix coturnix*) were randomly distributed into five groups (30 males/group), and treated from 21 to 56 days age as follows:1st group: Control, 2nd group: 0.5% H_2O_2 with drinking water, 3rd group: Vitamin C (300 mg/kg feed), 4th group: 0.5% H_2O_2 with drinking water and Vitamin C (300 mg/kg feed) which added from the beginning of treatment, 5th group: 0.5% H_2O_2 with drinking water and the Vitamin C (300 mg/kg feed) which added after two weeks from the beginning of treatment. Results showed that the hydrogen peroxide induced oxidative stress, as represented by the retraction of the antioxidant status, as well as blood indices (cholesterol, triglycerides, aspartate transaminase and alanine transaminase) of male quail. On the contrary, the addition of substances that have an antioxidant capacity, such as vitamin C, reverse the negative impact of H_2O_2 and improved significantly most of the studied blood indices values, as well as the antioxidant status (increased serum glutathione level and decreased malondialdehyde level compared with the control group) by preventing and/or reducing the bad effects of oxidative stress.

Keywords: Ascorbic Acid, H_2O_2-induced Oxidative Stress, Glutathione, *Coturnix coturnix*.

Introduction

Many previous studies were interested in enhancing the antioxidant role by some supplements that encourage the body's defense systems and reduce the oxidative stress effects by scavenging free radicals in order to maintain the vital functions of cells thus improving the physiological and productive performance [1]. Free radicals attacking the cellular membranes causing polyunsaturated fatty acids oxidation and formation of the Malondialdehyde (MDA) by a process called lipid peroxidation [2, 3], therefore the membrane loses its selectivity [4].

Vitamin C is one of the most powerful water dissolved antioxidants which acts to reduce the risk of oxidative stress, as a scavenger for the Reactive Oxygen Species [5], and it's involved in many cellular redox processes [6].

Kaźmierczak-Barańska *et al.* [7] stated that vitamin C has the ability to protect the cell membrane and its organelles from the oxidative damage by removing the free radicals and making them water soluble compounds, thereby protecting the tissues and reducing the oxidative stress damage that can be associated with many disease cases, oxidative stress can be induced experimentally by using many chemicals, including the

addition of hydrogen peroxide to the drinking water [8].

The current study aimed to evaluate the impact of vitamin C as an antioxidant to prevent or reduce H_2O_2-induced oxidative stress effects in male quail by determining some parameters as serum glutathione (GSH) (one of the most important non- enzymatic antioxidants in the body), malondialdehyde (MDA) (one of the most important products of lipid peroxidation, which is used as an indicator to the cell membranes damage) [2, 9], and some other stress factors as the adrenal cortex hormone (corticosterone), aspartate transaminase (AST), and alanine transaminase (ALT) enzymes.

* Part of PhD. dissertation submitted by the first author.

Materials and Methods

The study was conducted on 150 male quail (*Coturnix coturnix*). They were reared in-ground cages with dimensions 2.0 * 2.5 * 3.0 m in length, width, and height respectively, from the age of 21 to 56 days under standard requirements of temperatures, ventilation, and lighting according to the bird's age. The birds were distributed randomly into five groups (30 males/group, 2 replicates/ group, 15 birds/ replicate), and they were fed on a mixture that was formed according to the American National Research Council [10] which included: growing ration (24.6% crude protein and 2838 kcal/kg metabolizable energy), then replaced at 42 days of age with the finisher ration (22.00% crude protein and 2942 kcal/kg metabolizable energy). The water and ration were offered *ad libitum* along the study. The birds treated as follows:

1st group (Control): birds were given a basal diet and tap water.

2nd group (H_2O_2): birds were given a basal diet and tap water supplemented with 0.5% H_2O_2.

3rd group (Vitamin C): birds were given a basal diet supplemented with 300 mg vitamin C/kg diet and tap water.

4th group (H_2O_2 and vitamin C added from the beginning of the experiment): birds were given a basal diet supplemented with 300 mg vitamin C /kg diet and tap water supplemented with 0.5% H_2O_2.

5th group (H_2O_2 and vitamin C added after two weeks from the beginning of the experiment): birds were given a basal diet supplemented with 300 mg vitamin C /kg diet and tap water supplemented with 0.5% H_2O_2.

At the end of the study (56 days aged) 6 birds were slaughtered from each group, and the blood was collected in tubes without anticoagulant for serum isolation, then kept at -20 °C until the biochemical tests were performed, that included: determination of glucose, cholesterol, triglycerides, Aspartate Transaminase (AST) and Alanine Transaminase (ALT) by using Biosystems Kits.

To estimate the antioxidant status, determined the serum glutathione level (GSH) according to the modified method used by Tipple and Rogers [11], also determined the serum MDA level (as an indicator of lipid peroxidation status) [12] using the modified method of Thiobarbituric Acid Reaction Substance that used by Buege and Aust [13]. Corticosterone hormone was measured by using Corticosterone ELISA Kit (Manufactured by Assay Max).

Statistical analysis:

Statistical analysis was performed by using Statistical Analysis Statics program [14] to analyze the data according to the Complete Randomize Design (C.R.D), one- way analysis of variance, and to test the significance of the differences between the means, the Duncan's Multiple Range Test [15] was used at ($P \leq 0.05$) according to the Steel and Torrie [16].

Results and Discussion

Table 1 showed that the oxidative stress induced by H_2O_2 led to a significant increase in the cholesterol and triglycerides of the hydrogen peroxide group compared with the other group. Our results are in agreement with Al-kattan [17] who confirmed that the addition of 0.5% H_2O_2 in the drinking water of laying hens led to a significant increase in the cholesterol concentration compared with the control group. Gursu *et al.* [18] stated that oxidative stress in birds leads to an increase in serum cholesterol and triglycerides levels. Also, it agreed with Ameen Agha [19] who observed a significant increase in serum cholesterol of chickens that consumed H_2O_2 with the water.

The changes in cholesterol and triglycerides maybe due to disturbances in the metabolism of the lipids as a result of ingestion of hydrogen peroxide [20], or maybe due to hypothyroidism, as a result of a decrease in thyroxine secretion (as a lipolytic hormone) in stressed birds, which leads to negative effects in the excretion of cholesterol with bile, and thus its level in the blood increases [21, 22].

Table 1. Mean (±SE) effect of H_2O_2 and vitamin C on serum glucose, cholesterol and triglyceride of male quail at 56 days aged.

	Parameters		
Treatments	Glucose mg/dl	Cholesterol mg/dl	Triglycerides mg/dl
T_1 (Control)	320.00 ±4.84 AB	208.35 ±8.75 BC	163.62 ±8.31 B
T_2 (0.5% H_2O_2)	338.73 ±4.90 A	247.68 ±7.49 A	220.25 ±20.70 A
T_3 (Vitamin C 300 mg/kg of ration)	290.05 ±12.88 C	177.68 ±7.85 D	114.22 ±12.47 C
T_4 (H_2O_2 + Vitamin C added from the beginning)	303.77 ±7.01 BC	189.88 ±5.81 CD	140.22 ±12.08 BC
T_5 (H_2O_2 + Vitamin C added after 2 weeks from the beginning)	323.77 ±6.30 AB	211.98 ±4.42 B	170.25 ±6.53 B

- Different letters in each column indicate a significant difference at (P≤0.05).

With the addition of vitamin C to the quail ration, it is evident from Table 1 that the treatment with vitamin C led to a significant decrease in the concentration of serum glucose, cholesterol, and triglycerides compared with the control group (P≤0.05). Our results agreed with the findings of Al-Rahawi [23] that stated that the addition of vitamin C to water at 200 mg/liter led to a significant decrease in the level of glucose and triglycerides in the serum of quail (females and males) compared with the control group. While our results were not in agreement with Seyrek *et al.* [24] when he added different concentrations of vitamin C (150, 250 and 500 mg/kg of feed) to the rations of stressed quail, it did not affect the level of glucose, but it led to a significant decrease in the cholesterol and triglycerides levels compared to the control group. The reason for hypoglycemic effect may be due to the effect of vitamin C which inhibits the secretion of the corticosterone hormone from the adrenal cortex (Table 3) and thus inhibits the process of gluconeogenesis and therefore the level of glucose in the blood serum decreases [25, 26]. This opinion is supported by the presence of a significant increase in the level of corticosterone hormone in the blood of the hydrogen peroxide group (Table 3).

Table 1 showed that the addition of vitamin C to the ration of H_2O_2-stressed quail can prevent the H_2O_2 effects serum glucose, cholesterol and triglycerides (when added

from the beginning of treatment), also it can reverse the H_2O_2 effects (when added after 2 weeks from the beginning of treatment), so that it is evident that vitamin C have the ability to prevent and/or reduces and reverse the H_2O_2-induced oxidative stress bad effects. Niki [9] mentioned that vitamin C significantly lowered the level of the corticosterone hormone, improved the cellular antioxidant status, and removed the free radicals, and this is evident to us in Tables (1 and 3), as the level of glutathione increased significantly, while the level of malondialdehyde and corticosterone significantly decreased in the blood of the birds of the vitamin C group compared with the birds of the control group.

From Table 2, it was found that hydrogen peroxide led to a significant increase in the AST and ALT levels compared with the other groups at ($P \leq 0.05$). This result was in agreement with Al-kattan [17], where he found a significant increase in the level of the AST and ALT in the serum of laying chickens when given H_2O_2 for 28 days in drinking water.

Table 2. Mean (±SE) effect of H_2O_2 and vitamin C on the serum enzymes (AST and ALT) of male quail at the age of 56 days.

Treatments	Parameters	
	AST U/L	ALT U/L
T_1 (Control)	301.87 ±9.77 B	9.69 ±0.43 B
T_2 (0.5% H_2O_2)	353.73 ±16.48 A	12.59 ±0.63 A
T_3 (Vitamin C 300 mg/kg of ration)	259.83 ±14.25 B	9.55 ±0.34 B
T_4 (H_2O_2 + Vitamin C added from the beginning)	297.98 ±23.80 B	9.90 ±0.73 B
T_5 (H_2O_2 + Vitamin C added after 2 weeks from the beginning)	303.60 ±10.74 B	10.93 ±0.62 B

- Different letters in each column indicate a significant difference at ($P \leq 0.05$).

This effect may be due to the fact that hydrogen peroxide led to oxidation of polyunsaturated fatty acids in cellular membranes, which disturbs its selective permeability [4], which leads to the leaching of these enzymes from inside to outside the cell, then into the blood, so their elevated level in serum indicates the presence of dysfunction or damage in the cells of these tissues as a result of various oxidative stress factors [8].

Also, Table 2 showed the lowest value of the enzymes was in the vitamin C group alone, and when the vitamin C was given with hydrogen peroxide (T_4 and T_5) it significantly decreased the level of enzymes (AST and ALT) in the blood and prevented or reverse effect of hydrogen peroxide when added with it, and the values of the two enzymes returns to the normal values in the control group. This is because vitamin C is a reductive antioxidant that promotes cellular antioxidant status [5], and it stabilizes the cell membranes by forming complex compounds with fatty acids in their membranes [7].

Results in Table 3 showed that the hydrogen peroxide achieved oxidative stress, as it reduced the level of GSH and elevated the level of MDA in blood significantly compared with the control group, and even with the other groups of the study. The H_2O_2-induced oxidative stress depletes tissues and blood glutathione [27], possibly by increasing the oxidation of GSH and converting it to the oxidative form of glutathione (GSSG) by reducing the activity of pentose phosphate shunt as a result of treatment with H_2O_2 [28, 29], or that the induced oxidative stress may lead to many changes in the antioxidant

enzymes, as Wohaieb and Godin [30] stated that many of changes occurred in the antioxidant enzyme system of the animals that previously exposed to stress.

Table 3. Mean (±SE) effect of H_2O_2 and vitamin C on the antioxidant status (Glutathione and Malondialdehyde) in serum of male quail at 56 days aged.

Treatments	Parameters		
	Glutathione (GSH) µmol/L	Malondialdehyde (MDA) nmol/ml	Corticosterone ng /m
T_1 (Control)	1.576 ±0.06 C	0.739 ±0.04 C	2.537 ±0.12 D
T_2 (0.5% H_2O_2)	1.095 ±0.03 D	1.792 ±0.12 A	15.928 ±0.77 A
T_3 (Vitamin C 300 mg/kg of ration)	2.887 ±0.13 A	0.504 ±0.03 D	1.622 ±0.14 D
T_4 (H_2O_2 + Vitamin C added from the beginning)	1.979 ±0.12 B	0.745 ±0.05 C	4.662 ±0.29 C
T_5 (H_2O_2 + Vitamin C added after 2 weeks from the beginning)	1.553 ±0.08 C	0.944 ±0.04 B	7.977 ±0.33 B

- Different letters in each column indicate a significant difference at (P≤0.05).

The high level of MDA and the low level of GSH is an indicator of the occurrence of lipid peroxidation in the cells, as a result of elevated levels of many of the Reactive Oxygen Species (ROS) that exceed the ability of the antioxidant defenses to removing them, which results in damage to different body tissues [2]. This finding was consistent with Hassan and Al-Ma'atheedi [31] in their research on adult roosters exposed to oxidative stress, as well as agreed with Al-Ma'atheedi [32] when he mentioned that the addition of 0.5% hydrogen peroxide in drinking water for white leghorn cocks led to a significant increase in MDA level and a significant decrease in glutathione level compared with the control group.

Data in Table 3 showed that the addition of vitamin C with ration (T_3) improved and significantly increased glutathione level and significantly decreased the level of malondialdehyde and corticosterone hormone in blood compared with hydrogen peroxide groups and control group at (P≤0.05). And when the vitamin C was given with hydrogen peroxide (T_4 and T_5), it was able to reduce the effect of hydrogen peroxide and improve the state of antioxidants in the body and reduced stress hormone level, as the level of glutathione was significantly increased and the level of malondialdehyde and corticosterone was significantly decreased compared with the stress group (hydrogen peroxide group) (T_2) at (P≤0.05).

These results are in agreement with Al-kattan [17] when he stated that giving vitamin C to stressed laying hens led to elevate the level of GSH and decline the level of MDA compared with the control group. Al-Ma'atheedi and Hassan [33] confirmed that when they adding vitamin C and hydrogen peroxide in the drinking water of White Leghorn cocks.

Vitamin C, is one of the most important non-enzymatic, water-soluble antioxidants, as it works to scavenge the reactive oxygen species by acting as an antioxidant [9], and this is reflected to increase the level of GSH and the reduction of the level of MDA. Abdul-Majeed [8] explained that vitamin C inhibit the secretion of the corticosterone hormone from the adrenal cortex.

Conclusion

In general, the results of the current study revealed that hydrogen peroxide-induced oxidative stress, and had a bad effect on blood

parameters. On the contrary, the addition of a substance that has an antioxidant capacity such as vitamin C enhanced most of the parameter's values, especially the antioxidant status, compared with the control group. On the other hand, the addition of vitamin C with hydrogen peroxide will prevent and/or recover the effects of H_2O_2-induced oxidative stress and returns the parameter values near the level of their values in the control group. The study also revealed that the addition of vitamin C with H_2O_2 from the beginning of the treatment was better than adding it two weeks after beginning the treatment.

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Conflict of Interest

The authors declare that there are no conflicts of interest regarding the publication of this manuscript.

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