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The Effect of Adding Different Levels of Aqueous Extract of Cloves to the Drinking Water of Broiler Chickens (Ross 308) on Blood Biochemical Characteristics and Some Indicators of Oxidative Stress

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ABSTRACT: This study was conducted in the poultry field of the Department of Animal Production College of Agriculture - University of Karbala for a period of 35 days, from 6/10/2022 to 9/11/2022, to demonstrate the effect of the aqueous extract of cloves added to the drinking water of broiler chickens on blood traits. Biochemistry and some indicators of oxidation, where 240 Ross308 broiler chickswere used, one day old, unsexed, and the average weight of the chicks was 40 gm, distributed randomly into 4 treatments and each treatment 3 replicates of 20chicks "for each replicate, and the aqueous extract was added to the drinking water Since the first day, my agencies: T1: control treatment without addition, T2: adding 10 ml / liter of water, T3: adding 20 ml / liter of water, T4: adding 30ml / liter of water, and the most important results that we obtained were summarized:

- A highly significant increase ($p \leq 0.01$) in the blood serum glucose concentrationof the T4 treatment compared to the control treatment. As for the T3 treatment, itexcelled at the level of ($p \leq 0.01$) in the concentration of total protein, albumin and globulin, as well as cholesterol, triglycerides and LDL, compared to the control treatment.
- The results showed a highly significant increase ($p \leq 0.01$) in the ALT enzymefor the T3 treatment. As for the AST enzyme, its concentration increased in the T2 treatment, which was similar to the control treatment in the significance of this enzyme. In the same results, a highly significant ($p \leq 0.01$) superiority was observed in the concentration of GPX enzyme for T4, T3 treatment over T2, T1 treatments. As for the CAT enzyme, its concentration increased at a significant level ($p \leq 0.01$) in T3 treatment birds, but it was significantly similar to the controltreatment. As for the T4 treatment, it increased significantly in the MDA rate compared to the control treatment.

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KEYWORDS: oxidative stress, broiler, plant extract, medical plant.

1. INTRODUCTION

Aromatic medicinal plants have occupied a distinguished position over time in the field of animal production and the nutritional program for domestic birds in particular because of their effective role as an alternative to medicines and antibiotics [1], where these medicinal plants or herbs have been widely usedbecause of their distinctive components and compounds effective biological activity and their impact on growth, production, immune system support, and animal health [2; 3]. The different forms and additions of these plants worked tomaintain the digestive balance, as they play a "functional" distinctive role by increasing the secretion of digestive enzymes and affecting the types of microorganisms in the intestine [4].Many plant extracts have been used to treat many conditions that are impossible to treat with antibiotics. Vital, including garlic, onions, yaas, cloves and others [1].

Cloves are unopened flower buds that are brown in color and have a pungent odor. They are often used as a topical antiseptic. As for clove oil, which we get by distillation, it helps digestion and is used as a broad-spectrum antibacterial [5].Cloves contain volatile oils and tannins, in addition to containing a group of essential and effective compounds such as (Vanillin, Acetyl, Galllotannic acid, Flavinoias, carbohydrates and vitamins B, C [6]. Clove oil and its aqueous extractshow antiseptic, anti-inflammatory and antioxidant activity, and act as an appetitestimulant and digestion [6]. 7; 8] Clove is considered a very important plant because of its enormous potential as a food preservative and as a rich source of antioxidant compounds. And the biological activity of its

components worked toconfirm the development of many medical products used for animals for centuries[9]. Clove has many benefits as it is an active and effective antioxidant, in addition to containing phenolic compounds that have an antibacterial and antibacterial effect, and its availability in the local markets and its cheap price isan alternative solution to industrial antioxidants that are not permanently available, so the aqueous extract of cloves was added to the drinking water of broiler chickens in our current study Also, the effects of different levels of the extract on blood characteristics and oxidative stress indicators were evaluated andcompared with the control treatment.

2. MATERIALS AND METHODS

This study was conducted in the poultry field of the Department of Animal Production / College of Agriculture - University of Karbala for a period of 35 days, from 6/10/2022 to 9/11/2022, to demonstrate the effect of the aqueous extract of cloves added to the drinking water of broiler chickens on blood traits. Biochemistry and some indicators of oxidation, where 240 Ross308 broiler chickswere used, one day old, unsexed, and the average weight of the chicks was 40 gm, distributed randomly into 4 treatments and each treatment 3 replicates of 20chicks "for each replicate, and the aqueous extract was added to the drinking water Since the first day of the experiment, the transactions were as follows

T1: control treatment without addition T2: add 10 ml of clove extract/ L of waterT3: Add 20ml of clove extract/L of water T4: Add 30 ml of clove extract/L water

Prepare the aqueous extract of clove powder

Clove sticks were purchased from one of the markets of the holy city of Karbala, where the clove sticks were ground using a small electric mill to be in the form of powder, and then the extract was prepared according to the [10] method, by mixing a gm of dry powder with 2 ml of distilled water using an electric mixer Then the solution is placed in a water bath at a temperature of 60 °C for one hour, and the solution is left for 24 hours at room temperature, after which the solution is filtered by a number of layers of sterile medical gauze to beready for use in the experiment.

Food treatment...

The chicks were fed on a starter diet (23.04% protein and 3021.45 kilocalories/kg of feed) from the age of one day until the third week of the birds'life, after that they were replaced with growth diet (20.06% protein and 3194.92kilocalories/kg of energy). Fodder) until the end of the fifth week, fodder and water were provided freely, ad libitum, and the diet used, as shown in Table (1).

Table (1) shows the percentages of the components of the diet used in

Feed material	Feed startup	Feed growth
Yellow corn	30	40
Wheat	28.25	24
(48% protein)soybean	31.75	24.8
Protein concentrate	5	5
Sunflower oil	2.9	4.4
Limestone	0.9	0.6
Dicalcium phosphate (DCP)	0.7	0.9
A mixture of vitamins and minerals	0.2	0.2
Nacl	0.3	0.1
Total	100	100

Crude Protein (%)	**23.04**	**20.06**
Calculated representative energy (kcal / kg feed)	**3021.45**	**3194.92**
Lysine %	**1.27**	**1.07**
Methionine%	**0.41**	**0.38**
cysteine %	**0.35**	**0.30**
Methionine+cysteine %	**0.82**	**0.78**
Phosphorous %	**0.41**	**0.43**
c/p Energy Ratio: Protein%	**131.14**	**159.77**

Chemical analysis of the suspension was calculated according to] 11[

Characteristics studied

1. Biochemical characteristics of blood

At the age of 35 days, blood samples were taken from the birds immediatelyafter the slaughter process and collected in tubes that did not contain anticoagulant, and then the serum was separated from the blood using a centrifugeat a speed of 3000 rpm for 15 minutes in the laboratory of the College of Agriculture. / University of Karbala, blood plasma standards were measured in the laboratory of the College of Veterinary Medicine of the same university, andthe level of total protein and albumin in the blood serum was estimated using theready-made analysis kit (kit) from the French company Orphee, which was basedon the Biuret method, based on [12], As for globulin, its level was estimated in the serum according to what was indicated by [13] and according to the following law:

Globulin concentration (gm / 100 ml of blood serum) = total protein level - albumin level .

Glucose concentration (mg / 100 ml) in blood serum was also measured using a measuring kit (kit) from the French company Orphee and according to the methodof [14]. French Orphee and based on the method indicated by [15] and the examination was conducted based on the steps indicated by the supplying company in the attached guide. As for measuring triglycerides and lipoproteins, a ready-made estimation kit was used, which is based on the sedimentation of LDL, VLDL, and Chylomicron by Phosphotungesticacid and magnesium ions Mg + 2, and the survival of HDL in the upper filtrate, which can be estimated using the cholesterol estimation kit, and the reading was done at a wavelength of546

The high-density HDL proteins were calculated in the blood serum by using a kit from the French BIOLO company, and the examination was carried out based on the steps indicated by the equipped company in the attached manual. The samples were read using a spectrophotometer [16]. And LDL proteins werecalculated according to the Friedewald formula [17], as:

(LDL = cholesterol - (HDL + VLD

2. Measurement of the concentrations of aminotransferase (ALT) and(AST) enzymes

To estimate the activity of the enzyme ALT () Alanine Amino Transferase, using a kit prepared from the French company Orphee and accordingto the method of [18], and the test was conducted based on measuring the activityof the enzyme by colorimetric methods by measuring pyruvic acid from alanine,as pyruvic acid is reacted with a compound DNPH for the formation of a complexwith a red color was measured at a wavelength of 546 nm and was estimated according to the international unit / liter As for the determination of the activity of the enzyme Aspartate Amino Transferase (AST), a kit was used for this measurement prepared by the French company 0rphee according to the method of [18], and it is based on This method indicates the enzyme's ability to convert aspartic acid into oxaloacetic acid, which spontaneously converts into pyruvic acid, which in turn reacts with 2,4- dinitrophenyl hydrazine (DNPH) to form a complex with a red color, measured at a wavelength of 546 nm.

3. ESTIMATION OF THE STATUS OF OXIDATIVE ENZYMES

These measurements include Glutathon Peroxidase (GSH-PX), Catalase (CAT)and Monaldehyde (MDA).

3-1. Determination of Glutathon Peroxidase (GSH-PX) Activity

Glutathione was measured using a method [19] that is based on using a precipitation solution containing metaphosphoric acid (Na2EDTA) and adding sodium chloride (NaCl) and placing the solution in a centrifuge at 4500 rpm for 10 minutes. The value of glutathione was estimated as the difference in the absorbance values for samples in the presence or absence of DTNB and at a wavelength of 340 nm

3-2. Determination of catalase (CAT) enzyme activity.

A kit (kit) equipped from the French company Orphee was used and based on [20] and the method that supports spectrophotometry to estimate the activity of catalase, and that method depends on measuring the amount of hydrogen peroxide broken by the action of the catalase enzyme, using Redox dye, and obtaining a change in color intensity at a wavelength of 570 nm or Fluorescenceat a wavelength of 530/545 nm, which indicates the activity of the catalase enzyme in the sample.

3-3. Estimation of malondialdehyde (MDA) level

Its concentration was measured using a measuring kit (kit) from the Frenchcompany Orphee based on [21]. This method determines the amount of lipid peroxides by measuring aldehyde, which is one of the products of lipid peroxidedecomposition. It is done by the reaction of one molecule of Malondialdehyde and two molecules of thiobarbituric acid to form an MDA compound. TBA is redin color and can be measured at a wavelength of 535.

4 RESULTS AND DISCUSSION

4.1 Effect of adding different levels of aqueous extract of cloves added to broiler drinking water Ross 308 on blood biochemical characteristics.

4-1-1. Glucose, total protein, albumin, and globulin.

Table (2) shows the effect of adding different levels of aqueous extract of cloves to the drinking water of broiler chickens on the concentration of glucose, total protein, albumin and globulin, where a high significant increase ($p \leq 0.01$) was observed in the concentration of serum glucose in T4 treatment on all treatments, either treatment T2, T3 did not show any significant difference with the control treatment in the same concentration. Also, a highly significant increase ($p \leq 0.01$) was observed in the concentration of total protein in treatmentT3 on all studied treatments, followed by treatment T2, which was superior to treatment T4, T1, and treatment T4 did not show any significant difference with the control treatment in total protein concentration . In the same table, the resultsof the statistical analysis showed a highly significant ($p \leq 0.01$) superiority of theT3 treatment in serum albumin concentration compared to the experimental treatments, followed by the treatment T2 and T4, where we did not find any significant difference between them, but they were superior to the control treatment in albumin concentration. As for serum globulin, a highly significant increase ($p \leq 0.01$) was observed for treatment T3 over all treatments, followed by treatment T2, which was superior to treatment T4 and T1. As for treatment T4, the concentration of globulin decreased compared to the control treatment.

Cloves contain manganese, a rare mineral that is essential for the metabolism of protein and carbohydrates, and thus the synthesis of protein and amino acids [22;23]. This may explain the reason for the increase in the concentration of protein and albumin in the T3 treatment of our study, which is shown in Table (3). It thusstimulates protein synthesis and increases the activity of thyroid hormones [25].

Table 2: The impact of adding different levels of the host of the centiffil to water drinkingroster 306 in the concentration of the kolksuit, the total protein, the bull and the sylolin in the serum a 35-day blood serum.

Transactions	standard error ± Averages			
	Glucose (mg / 100 ml)	protein (g /100 ml)	Albumin (g / 100 ml)	Globulin (g / 100 ml)
T1	1.20± 271.67 b	0.04± 3.07 C	0.02± 1.21 c	0.05± 1.89 C
T2	1.45± 272.33 b	0.03± 3.44 b	0.01± 1.32 b	0.03± 2.12 B
T3	317± 270.33 b	0.02± 5.42 A	0.01± 1.94 a	0.02± 3.48 A
T4	1.15± 298.00 a	0.01± 3.01 C	0.03± 1.33 b	0.02± 1.68 D
	**	**	**	**

**** Different letters within one column indicate a significant difference at ($p \leq 0.01$) level**

4-1-2. Concentration of cholesterol, triglycerides, high-density lipoprotein(HDL), and low-density lipoprotein (LDL)

Table (3) shows the effect of adding different levels of aqueous extract of cloves to the drinking water of broilers on the concentration of cholesterol, triglycerides, and high-density lipoproteins (HDL) and low-density lipoproteins (LDL) in the blood serum of birds at 35 days of age, where a highly significant increase was observed. ($P \leq 0.01$) in the level of blood serum cholesterol for the birds in the T3 treatment over all treatments. We also notice a decrease in its valuefor the T4 and T2 treatments compared to the control treatment. The T4 treatmentrecorded the lowest levels of cholesterol concentration during the duration of theexperiment. As for triglycerides, it was observed A significant increase ($P \leq 0.01$)in its rate of treatment T3 compared to all experimental parameters, followed bytreatment T2, which was superior to treatment T4, T1. As for the control treatment, it suffered a significant decrease ($P \leq 0.01$) in the rate of triglyceridescompared to the addition treatments. The statistical analysis table also showed a significant increase ($P \leq 0.01$) in the concentration of high-density lipoproteins (HDL) for the T4 treatment. However, it did not show any significant differencein the concentration of these proteins with the control treatment. At the same time,treatment T2 was similar to treatment T3 in the significance of this characteristic.The results also showed that the T3 treatment was significantly superior ($P \leq 0.01$)in the concentration of low-density lipoproteins (LDL) compared to all the treatments studied. This was followed by the T2 treatment, which did not show any significant difference with the control treatment, but it was superior to the T4treatment. In the concentration of low-density proteins. When reviewing the results of our study, we notice a significant increase in the level of triglycerides and LDL for the T3 treatment birds. This increase may be explained by the fact that the phenolic compounds found in cloves are very important for the oxidativestability of polyunsaturated fatty acids and that they also contain trace minerals necessary to increase the synthesis of acids. Fatty and cholesterol [23], but manystudies have proven that the active and effective component in cloves is the compound eugenol, which is a powerful polyphenolic compound [26], as this compound works as an antioxidant and anti-inflammatory, and it reduces cholesterol, low-density lipoproteins, and triglycerides, so it is Anti-hyper lipidemia [27] This may explain the low level of these fats in some treatments ofadding aqueous extract of cloves.

Table 3: The effect of adding different levels of aqueous extract of cloves to the drinkingwater of Ross 308 broilers on the concentration of cholesterol, triglycerides, high-densitylipoprotein (HDL), and low-density lipoprotein (LDL) in blood serum at the age of 35 days.

Transactions	standard error ± Averages			
	Cholesterol (mg / 100 ml)	iglycerides(mg/ dl)	HDL (mg / dl)	LDL (mg / dl)
T1	1.80± 87.18 B	1.57± 96.45 C	1.58± 77.11 a	1.33± 75.07 b
T2	1.52± 66.01 D	b 0.72± 105.45	2.35± 56.28 b	1.50± 72.91 b
T3	2.48± 95.73 A	a 2.06± 126.06	1.09± 61.49 b	0.87± 101.78 a
T4	1.17± 72.65 C	2.08± 77.06 D	1.41± 72.03 a	1.30± 62.29 c
	**	**	**	**

**** Different letters within one column indicate a significant difference at ($p \leq 0.01$) level**

4-2. The effect of adding different levels of aqueous extract of cloves added to the drinking water of broilers Ross 308 on transaminase enzymes and some indicators of oxidation.

The results of Table (4) indicate the effect of adding different levels of aqueous extract of cloves to the drinking water of broilers on the concentration of the enzyme Alanine Amino Transferase (ALT), the enzyme Aspartate AminoTransferase (AST), the enzyme Glutathione Peroxidase (GSH-PX), the enzyme Catalase (CAT), and the compound Malondialdehyde (MDA) in the blood serumand at the age of 35 days, a significant increase was observed at a level ($p \leq 0.01$)in the concentration of the ALT enzyme in treatment T3 over treatments T4 and T1. As for treatment T2, it was identical significantly with treatment T3 on the one hand and with treatment T4 on the other hand. On the other hand, the control treatment recorded the lowest concentrations of this enzyme. As for the

concentration of the AST enzyme, the results showed a significant increase ($p \leq 0.01$) in the level of this enzyme in treatment T2 over treatments T4 and T3, but it did not show any significant difference with the control treatment. As for treatment T4, it was superior to treatment T3 in the concentration of AST. It wasalso noted in the statistical analysis table that a highly significant ($p \leq 0.01$) superiority of treatment T4 and T3 in the concentration of the enzyme glutathioneperoxidase (GPX) over treatment T2 and T1 did not appear, and no significant difference appeared between treatment T3 and T4 as well as between treatment T2 and the control treatment in Same focus. As for the CAT enzyme, catalase, itsconcentration increased significantly ($p \leq 0.01$) in treatment T3 compared to treatments T4 and T2, and did not show any significant difference with the controltreatment. Also, treatment T2 was similar to treatment T4 in the significance of this characteristic. The results also showed Treatment T4 was significantlysuperior ($p \leq 0.01$) to all studied treatments in malondialdehyde (MDA) concentration, followed by treatment T3, which was superior to treatment T2, butdid not show any significant difference with the control treatment. The significantincrease in the concentration of oxidative enzymes in the treatments of adding aqueous clove extract may be due to the fact that clove compounds prevent or delay the oxidation of fats and other biomolecules by suppressing the initiation and spread of oxygen reactions [28] and since cloves contain a high percentage of polyphenol compounds with antioxidant activity. For oxidation [29], the radical scavenging effect of clove extract led to an increase in the levels of GPXand CAT. The decrease in the level of MDA in treatment T2 may be attributed tothe antioxidant activity of eugenol, the active component of cloves, which formsa complex compound with iron and oxygen, thereby preserving iron and copper in their reduced forms. Which reduces fat oxidation processes [30].

Table 4: The effect of adding different levels of aqueous extract of cloves to the drinkingwater of broiler chickens on the concentration of the enzyme Alanine Amino Transferase(ALT), the enzyme Aspartate Amino Transferase (AST), the enzyme Glotathon Peroxidase (GSH-PX), the enzyme Catalase (CAT), and the compound Malondialdehyde(MDA) in Blood serum and at 35 days old.

Transactions	standard error ± Averages				
	ALTU/L)	AST (U/L)	GPX(U/L)	CAT(U/L)	MDA(U/L)
T1	068± 2.92 c	24.58± 479.00 A	1.34± 26.82 b	0.74± 90.58 a	1.20± 16.21 b
T2	1.59± 35.44 ab	1.20± 492.33 A	1.08± 24.79 b	1.56± 80.28 b	0.74± 6.03 c
T3	0.28± 36.50 a	2.96± 204.33 C	1.53± 31.83 a	3.57± 96.16 a	1.02± 20.21 b
T4	0.96± 32.88 b	1.46± 250.33 B	1.26± 32.17 a	2.18± 82.48 b	2.11± 28.91 a
	**	**	**	**	**

**** Different letters within one column indicate a significant difference at ($p \leq 0.01$) level**

CONCLUSION

Adding the aqueous extract of cloves to the drinking water of broilers at a concentration of 20 ml/liter of water in treatment T3 resulted in the protection of vital molecules from oxidation and damage, which led to an improvement in the biochemical characteristics of the blood and the activation of antioxidant enzymes. Treatment with the aqueous extract at a concentration of 20 ml/liter ofT3 water gave the best results in general in terms of biochemical blood characteristics and oxidative stress indicators.

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