



# Evaluation of the Addition of Different Concentrations of Aqueous and Alcoholic Extract of *Annona muricata* Leaves to Drinking Water on the Blood Characteristics of Laying Hens

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**Abstract** | This study was conducted in the fields of Babylon Governorate, and the field experiment lasted for 112 days. The aim is to know the effect of Garviola leaves on some physiological characteristics of blood for old laying hens. (105) White Lohmann laying hens were raised at 65 weeks of age, 66-69 weeks of age, 70-73 weeks of age, 74- 77 weeks of age, and 78- 81 weeks of age. 21 groups were distributed into 7 experimental treatments consisting of 15 birds. The treatment included three replicates containing five birds. T1: control without addition. (T2:15ml, T3:30ml, T4:45ml/ liter of water of aqueous extract of Graviola leaves at a concentration of 1%). (T5:15ml. T6:30 ml and T7:45 ml/liter of water of alcoholic extract of Graviola leaves at a concentration of 1%). The results of the experiment showed a significant improvement ( $p \leq 0.05$ ) in the concentration of total protein in treatments (4, 6, and 7) and albumin concentration in the fourth period. A decrease in uric acid for treatments (3, 4, 5, 6, and 7) and a reduction in the addition coefficients in the enzyme AST and ALT, and a significant improvement for two treatments (4 and 7) compared to the first control. We concluded that adding Garviola extracts improved blood parameters (protein, albumin, uric acid, AST, ALT, and ALP).

**Keywords** | Aqueous extract, Alcoholic extract, Biochemical parameters, Blood characteristics, Graviola, Laying Hens

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## INTRODUCTION

Today, Medicinal plants major role in the production of medicines, as they are the primary source of active compounds used in the treatment of many diseases, to form some of the primary chemical compounds used in the pharmaceutical industry, these compounds have medicinal properties, such as flavonoids, glycosides, and polyphenols. They are also considered raw materials for the formation

of some chemical compounds used in the pharmaceutical industry. These compounds have medicinal properties that are beneficial to health, such as flavonoids, glycosides, polyphenols, and saponins (Ali *et al.*, 2021; Rahmasari *et al.*, 2022). It is known that the advancement in the age of laying hens leads to a decrease in egg production and a deterioration in the qualitative characteristics of eggs as a result of the increased production of free radicals within the bird's body, which leads to its exposure to oxidative

stress and a decrease in the effectiveness of antioxidant enzymes (Al-Kaissy *et al.*, 2023; Oke *et al.*, 2024). Therefore, recent studies have turned to the use of medicinal plants and their extracts as natural and economic sources to raise the nutritional value of eggs and improve the productive performance of older laying hens, especially since they have very important biological effects such as antioxidants (Abbas and Ali, 2023a) which work to scavenge free radicals and reduce the risks of oxidative stress, in addition to stimulating the functions of the digestive system by increasing the production of digestive enzymes and enhancing the effectiveness of the liver (Ali *et al.*, 2023). They also help reduce serum lipid levels and improve the immune status (Esenbuga and Ekinici, 2023; Tchoffo *et al.*, 2024; Al-Jebory *et al.*, 2024). Among these medicinal plants is the Graviola plant, also known as the custard apple tree or the prickly custard apple, which belongs to the Annonaceae family and the Annona genus (Chatrou *et al.*, 2012). Its original habitat is South America and it is widely cultivated in tropical regions around the world (Moghadamtousi *et al.*, 2015). Graviola is an important source of many active compounds such as flavonoids, tannins, and phenolic compounds in addition to acetogenins, which are the main components of Graviola, in addition to its good content of amino acids and minerals (Daud *et al.*, 2015; Usunobun *et al.*, 2015; Yang *et al.*, 2015). Graviola is an important source of antioxidants, antimicrobial, anticancer, and antiparasitic (Orak *et al.*, 2019; Balderrama-Carmona *et al.*, 2020). Recent research indicates the presence of medical therapeutic benefits for many cancerous diseases as a result of the aqueous and alcoholic extraction of all parts of this tree (fruits, stems, roots, leaves) (Jiwuba *et al.*, 2017; Orak *et al.*, 2019; Maesaroh and Dono, 2022), but global studies are still very rare in the use of parts of this tree in feeding poultry, and most of them were limited to feeding laboratory animals (Son *et al.*, 2021; Al-Shemery *et al.*, 2023; Abbas and Ali, 2023b; Elmas *et al.*, 2024). However, further research is needed to improve the use of Graviola leaf extract in laying hens to improve some physiological traits of aged laying hens. The study aimed to demonstrate the effect of Graviola leaves on the physiological indicators of laying hens and their effect on the studied treatments, whether the aqueous or alcoholic extract and to determine the best concentration of the aqueous and alcoholic extract of Graviola leaves added to the drinking water of laying hens.

## MATERIALS AND METHODS

The field experiment of this study, which was conducted in Babylon fields, lasted for 112 days, starting on November 20, 2023, and ending on March 10, 2024. To determine the best concentrations of Graviola leaves from alcoholic and aqueous extracts (*Annona muricata*) that affected the physiological performance of the performance of laying hens.

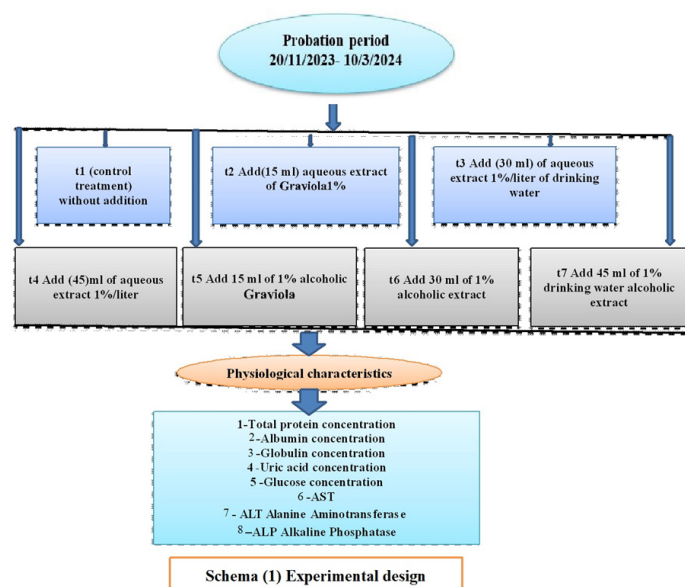
A total of 105 Lohmann laying hens were reared to 65 weeks of age, with a 1-week introductory period, and divided into 4 groups (66-69, 70-73, 74-77, and 78-81) weeks. They were divided into 21 replicates in a randomized order and administered according to the standard guidelines described in the Lohmann-White manual, with 7 experimental treatments per treatment, 15 birds. Each treatment included three replicates, each containing five birds. The experimental treatments were as follows: First treatment: group devoid of any additives. T2: Add 15 ml of aqueous extract of Graviola leaves at a concentration of 1%/l. T3: Add 30 ml of 1% aqueous extract of Graviola leaves to the water. T2: Add 15 ml of aqueous extract of Graviola leaves at a concentration of 1%/l. T3: 30 ml of aqueous extract of Graviola leaves at a concentration of 1%. T4: Increase the volume of Garviola aqueous extract by 45 ml. This treatment is used when the concentration of leaves reaches 1% in 1 liter of water. T5: Add 15 ml of Graviola leaf alcoholic extract 1%/L. T6: 30 ml of 1% Graviola alcohol extract (Plate 1). T7: 45 ml of 1% alcoholic extract of 1% Graviola leaves. The chickens were prepared after being fully vaccinated and fed with the food treatments (Figure 1). After a week of rearing as a preparatory period and adaptation to the hall before starting the experiment, where no data were collected, the chickens were randomly distributed into 21 replicates, and each replicate had 5 chickens at the age of 66 weeks on the treatments. Pyramid pens (Pens) with a pen area of (2×2) m<sup>2</sup> containing (6) batteries, each battery containing (5) cages with dimensions of each cage of (40×40) cm<sup>2</sup>. The cages were equipped with automatic cage nipple outlets for drinking water, and in each cage (2) nipples were fixed on top of the cage. As for feeding, it was done manually in longitudinal feeders. The hall was equipped with all its requirements during the breeding period. The amount of feed consumed by the chicken was calculated according to the bird (100 g/chicken/day) (Table 1). The lighting system was used: 16 hours of light, 8 hours of darkness, and 4 watts/m<sup>2</sup>, according to the recommendations in the bird's guide. The temperature in the hall was recorded throughout the experiment daily (at eight o'clock in the morning and evening) using (4) thermometers placed at the ends of the hall and in the middle of the hall, where electric heaters were used to heat the hall. The following characteristics were measured: total protein, albumin, globulin, glucose, uric acid, and liver enzymes (AST, ALT, ALP) in blood serum (IU/L).

## STATISTICAL ANALYSIS

The completely randomized design (CRD) was used to study the effect of different coefficients in the studied traits. Significant differences between the averages were measured by a Duncan test (1955) multidimensional test under a significant level of 0.05 SAS (2012) was used in statistical analysis.

## PREPARATION OF ALCOHOLIC EXTRACT OF GARVIOLA LEAVES

The alcoholic extract of Garviola leaves was prepared in the laboratory of the College of Science / University of Babylon according to [Ali and Dakhil \(2022\)](#) where 50 grams of it were placed in the incubator E of the Soxhlet apparatus and 250 ml of methanol alcohol with a concentration of 90% was heated in the flask A at a temperature of 80° C and its vapors were condensed in the condenser D, the condensed extract was dripped into the finger containing the raw plant, and it was extracted by contact, when the liquid level in the incubator rises to the top of the siphon tube C, its contents in the incubator descend to A, and the process continues and no drop of methanol solvent remains from the siphon tube, and the soaking process continues for about 6 hours, and compared to other extractions, it is possible to extract a large amount of the plant with a much smaller amount of solvent, thus saving time and energy and thus economic feasibility and applicability, where the raw alcoholic extract was separated by filtration and then the solution using the rotary evaporator until it was a hot water bath at (40 m)the refrigerator ([Figure 3](#)).



**Plate 1:** Experimental design.



**Figure 1:** White Lohmann chicken used in the experiment.

## PREPARATION OF THE EXTRACTION

It was prepared in the College of Science/University of Babylon according to the method of [Yasir and Ali \(2020\)](#) by placing the dried leaves in 1 liter of distilled water and boiling it on low heat (60) degrees Celsius for 10 minutes, then filtering it so that the liquid becomes ready with a concentration of 1% ([Figure 2](#)) and used in doses (15 ml/ liter of and 30 ml/liter of and 45 ml/liter) drinking water.



**Figure 2:** Garviola leaf extract.



**Figure 3:** The alcoholic extract of Garviola leaves used in the experiment.

## RESULTS AND DISCUSSION

[Table 2](#) shows the effects of an alcoholic and aqueous extract of Garviola (*Annona muricata*) for 66–81 weeks on the concentrations of globulin, albumin, and total protein (g/100 ml blood serum) (mean  $\pm$  standard error). There are significant differences in the total protein concentration (g/100 ml) for the first period (66–69) weeks, where the fourth and seventh treatments recorded a significant improvement ( $P \leq 0.05$ ) in the total protein concentration (g/100 ml) compared to the first treatment (control). They recorded 5.81 and 5.37 g/100 ml, respectively, while the first treatment (control) recorded the lowest concentration of total protein, amounting to 4.03 g/100 ml. As for the second, third, fifth, and sixth treatments, they did not differ significantly from the first treatment on the one hand, and from the fourth and seventh treatments on the other hand. On the other hand, the results indicate that there



are no significant differences between all treatments in the second period (73-70) weeks and the third period (74-77) weeks in total protein concentration (g/100 ml), while in the fourth period (78-81) weeks. The third, fifth, sixth, and seventh treatments recorded an improvement in the concentration of total protein, with a significant difference ( $P \leq 0.05$ ) from the first treatment (control), which recorded the lowest concentration of total protein. As for the second and fourth treatments, no significant differences were recorded from the rest of the treatments.

**Table 1:** Production feed used in the experiment.

| Feed material                                   | (%)    |
|-------------------------------------------------|--------|
| corn yellow                                     | 36.5   |
| Wheat                                           | 12     |
| Barley                                          | 12.83  |
| Soybean (44% protein)                           | 25.92  |
| Protein                                         | 2.5    |
| Limestone                                       | 9.25   |
| The Vegetable oil                               | 1.0    |
| The Total                                       | 100    |
| the Chemical analysis **                        |        |
| The Representative energy (kilocalorie/kg feed) | 2700   |
| Crude protein (%)                               | 17     |
| Crude fiber (%)                                 | 3.68   |
| Calcium (%)                                     | 4.13   |
| Available phosphorus(%)                         | 0.42   |
| methionine + cysteine %                         | 0.71   |
| Lysine (%)                                      | 0.92   |
| (DCAB) Dietary Cation-Anion Balance (mg/kg)     | 202.43 |
| Choline(%)                                      | 0.17   |
| Folic acid (mg/kg)                              | 0.54   |
| glycine(%)                                      | 0.73   |
| Glycine+serine (%)                              | 1.58   |
| Histidine(%)                                    | 0.45   |
| Isoleucine(%)                                   | 0.71   |
| Leucine(%)                                      | 1.41   |
| Lysine(%)                                       | 0.92   |
| Methionine(%)                                   | 0.42   |
| Cysteine(%)                                     | 0.29   |
| Phenylalanine(%)                                | 0.82   |
| Tyrosine(%)                                     | 0.70   |
| Phenylalanine + Tyrosine (%)                    | 1.52   |
| Threonine (%)                                   | 0.64   |
| Tryptophan(%)                                   | 0.25   |
| Valine(%)                                       | 0.80   |
| Arginine(%)                                     | 1.07   |

Protein concentrate from the Dutch company Profimi. Each kg contains: 5.9% crude protein, 3600 representative

energy calories/kg, 6.4% calcium, 5.7% phosphorus, 6.5% sodium, 4000 mg/kg iron, 2800 mg/kg zinc, mg /kg 600 copper, 8.35 mg cobalt, 60 mg/kg iodine, 10 mg/kg selenium, 5.9% methionine, 1.5% lysine 5.9% methionine with cysteine, 1200 mg/kg niacin, 400,000 IU vitamin A, 140,000 IU vitamin D3, 2000 mg/kg E, 100 K, 90 mg/kg vitamin B1, 160 ppb vitamin B2, 200 mg/kg vitamin B6 and 1000 mg/kg vitamin B12. \*\*The analysis of the entering feed materials was used to calculate the chemical composition., according to the Lohman Company guide [2020], and according to the American UFFDA program (NRS,1994).

As for albumin concentration, the results (weeks 66-69) were that the seventh treatment was superior in albumin concentration compared to the first treatment, which recorded the lowest concentration, while no significant differences were recorded between the second, third, fourth, fifth, and sixth treatments, and the first and seventh treatments, in (70-73). Weeks and (74-77) weeks, the results showed no differences, but in the fourth period (78-81) weeks, the additional treatments (second, third, fourth, fifth, sixth, and seventh) recorded the highest concentration of albumin compared to the first treatment (control), which recorded the lowest concentration. For albumin 2.06 g/100 ml. As for the globulin concentration (g/100 ml), there were no differences between all treatments.

Total protein works to maintain the balance of fluid volume between blood and tissues and acid-base balance, in addition to its composition in many food compounds, which include carbohydrates, fats, vitamins, and mineral salts, as well as hormones and various tissues (Alkassar and Alaboudy, 2021). The significant improvement in protein concentration in the overall proportion of the Garveola treatments may be due to the direct proportion between the egg production rate and the egg's total protein rate because most of the egg's components are transported through the blood to the ovary and are linked to the protein (Jiwuba *et al.*, 2017). This explains to us the reason for the increase in the production of treatments for adding the alcoholic and aqueous extract of Garviola leaves and the concentration of total protein in blood serum, which reflects a good impression on the health of the birds and in turn is reflected in egg production, and this proves the role of Garviola leaves in working as one of the most important antioxidants (Sawant and Gogle, 2014; Daud *et al.*, 2015), which was reflected in herd immunity and reduced stress resulting from free radical oxidation represented by an increase in total protein concentration. As for the significant improvement in the albumin concentration of Garveola treatments in blood serum, this may be due to an improvement in the total protein concentration, as the protein concentration increased. Total levels in blood serum indicate an increase in the process of protein synthesis and a decrease in the process of protein catabolism (Patterson *et al.*, 1967;

**Table 2:** The effects of adding different amounts of the alcoholic and aqueous extract of Garviola (*Annona muricata*) leaves to drinking water for (66–81) weeks (mean  $\pm$  standard error) were measured in terms of the blood serum's total protein (g/100 ml), albumin (g/100 ml), and globulin (g/100 ml).

| Treatments   | Total protein g/100 ml |                       |                      |                         | Albumin g/100ml      |                       |                      |                         | Globulin g/100 ml    |                       |                      |                         |
|--------------|------------------------|-----------------------|----------------------|-------------------------|----------------------|-----------------------|----------------------|-------------------------|----------------------|-----------------------|----------------------|-------------------------|
|              | First period (66-69)   | Second period (70-73) | Third period (74-77) | Fourth period (78 - 81) | First period (66-69) | Second period (70-73) | Third period (74-77) | Fourth period (78 - 81) | First period (66-69) | Second period (70-73) | Third period (74-77) | Fourth period (78 - 81) |
| T1 (control) | 4.03b $\pm$ 0.29       | 4.38 $\pm$ 0.04       | 4.82 $\pm$ 0.38      | 4.37 b $\pm$ 0.13       | 2.02 b $\pm$ 0.27    | 2.05 $\pm$ 0.34       | 2.50 $\pm$ 0.06      | 2.06b $\pm$ 0.17        | 2.01 $\pm$ 0.01      | 2.33 $\pm$ 0.31       | 2.32 $\pm$ 0.39      | 2.31 $\pm$ 0.31         |
| T2           | 4.46 ab $\pm$ 0.34     | 5.16 $\pm$ 0.37       | 5.12 $\pm$ 0.34      | 4.89 ab $\pm$ 0.22      | 2.27 ab $\pm$ 0.20   | 2.30 $\pm$ 0.16       | 2.66 $\pm$ 0.23      | 2.77 a $\pm$ 0.10       | 2.19 $\pm$ 0.13      | 2.86 $\pm$ 0.54       | 2.46 $\pm$ 0.23      | 2.12 $\pm$ 0.08         |
| T3           | 4.33 ab $\pm$ 0.37     | 5.03 $\pm$ 0.41       | 5.01 $\pm$ 0.32      | 5.11a $\pm$ 0.12        | 2.33 ab $\pm$ 0.24   | 2.41 $\pm$ 0.25       | 2.41 $\pm$ 0.41      | 2.83 a $\pm$ 0.07       | 2.00 $\pm$ 0.14      | 2.62 $\pm$ 0.57       | 2.60 $\pm$ 0.26      | 2.28 $\pm$ 0.37         |
| T4           | 5.41 a $\pm$ 0.25      | 5.47 $\pm$ 0.32       | 5.50 $\pm$ 0.37      | 5.55 a $\pm$ 0.23       | 2.60 ab $\pm$ 0.11   | 2.70 $\pm$ 0.33       | 2.74 $\pm$ 0.16      | 2.82 a $\pm$ 0.05       | 2.81 $\pm$ 0.30      | 2.77 $\pm$ 0.33       | 2.76 $\pm$ 0.41      | 2.73 $\pm$ 0.28         |
| T5           | 4.68 ab $\pm$ 0.37     | 4.78 $\pm$ 0.35       | 4.89 $\pm$ 0.27      | 4.98ab $\pm$ 0.21       | 2.41 ab $\pm$ 0.32   | 2.51 $\pm$ 0.41       | 2.78 $\pm$ 0.07      | 2.66 a $\pm$ 0.13       | 2.27 $\pm$ 0.32      | 2.27 $\pm$ 0.66       | 2.11 $\pm$ 0.25      | 2.32 $\pm$ 0.17         |
| T6           | 5.08 ab $\pm$ 0.29     | 5.16 $\pm$ 0.39       | 5.21 $\pm$ 0.31      | 5.26a $\pm$ 0.15        | 2.64 ab $\pm$ 0.14   | 2.71 $\pm$ 0.11       | 2.74 $\pm$ 0.04      | 2.72 a $\pm$ 0.06       | 2.44 $\pm$ 0.15      | 2.45 $\pm$ 0.44       | 2.47 $\pm$ 0.27      | 2.54 $\pm$ 0.32         |
| T7           | 5.37 a $\pm$ 0.43      | 5.45 $\pm$ 0.48       | 5.47 $\pm$ 0.44      | 5.54 a $\pm$ 0.32       | 2.81 a $\pm$ 0.04    | 2.56 $\pm$ 0.30       | 2.80 $\pm$ 0.09      | 2.83 a $\pm$ 0.05       | 2.56 $\pm$ 0.40      | 2.89 $\pm$ 0.14       | 2.67 $\pm$ 0.33      | 2.71 $\pm$ 0.27         |
| Significant  | *                      | N.S                   | N.S                  | *                       | *                    | N.S                   | N.S                  | *                       | N.S                  | N.S                   | N.S                  | N.S                     |

The symbol N.S. denotes the absence of substantial variations among the coefficients. \* indicates that, at the significance level ( $P < 0.05$ ), there are statistically significant differences between the coefficients. T1 (control). Add the aqueous extract of Garviola leaves T2, T3, and T4 at a dose of 15, 30, and 45 ml/liter/drinking water, respectively. Add the alcoholic extract of Garviola T5, T6, and T7 leaves at a dose of 15, 30, and 45 ml/liter of drinking water, respectively.

**Table 3:** Effects of adding of aqueous and alcoholic extract of Garviola (*Annona muricata*) leaves to drinking water for aperiod of (66–81) weeks were measured in terms of uric acid concentration (mg/100 ml blood serum) and glucose concentration (mg/100 ml blood serum). (mean  $\pm$  standard error).

| Treatments   | Uric acid (mg/100 ml serum) |                        |                      |                           | Glucose (mg/100 ml serum ) |                       |                      |                         |
|--------------|-----------------------------|------------------------|----------------------|---------------------------|----------------------------|-----------------------|----------------------|-------------------------|
|              | First peri- od (66-69)      | Second peri- od(70-73) | Third period (74-77) | Fourth peri- od (78 - 81) | First period (66-69)       | Second period (70-73) | Third period (74-77) | Fourth period (78 - 81) |
| T1 (control) | 5.20 $\pm$ 0.19             | 5.63 $\pm$ 0.13        | 6.34 $\pm$ 0.26      | 6.77 $\pm$ 0.07a          | 380.42 $\pm$ 7.57          | 382.71 $\pm$ 7.60     | 371.82 $\pm$ 8.52    | 381.92 $\pm$ 6.87       |
| T2           | 5.08 $\pm$ 0.19             | 4.78 $\pm$ 0.30        | 5.48 $\pm$ 0.11      | 5.92 $\pm$ 0.44ab         | 356.30 $\pm$ 17.95         | 374.30 $\pm$ 15.31    | 377.63 $\pm$ 11.25   | 360.60 $\pm$ 8.24       |
| T3           | 5.17 $\pm$ 0.32             | 4.93 $\pm$ 0.25        | 5.69 $\pm$ 0.60      | 5.25 $\pm$ 0.32bc         | 386.49 3.591               | 386.44 $\pm$ 16.48    | 375.68 $\pm$ 14.92   | 361.37 $\pm$ 15.16      |
| T4           | 4.99 $\pm$ 0.15             | 4.67 $\pm$ 0.16        | 5.30 $\pm$ 0.40      | 4.92 $\pm$ 0.22bc         | 368.32 14.53               | 371.68 $\pm$ 18.14    | 374.04 $\pm$ 13.97   | 367.07 $\pm$ 14.13      |
| T5           | 4.88 $\pm$ 0.35             | 5.00 $\pm$ 0.31        | 5.77 $\pm$ 0.46      | 5.72 $\pm$ 0.35bc         | 375.24 $\pm$ 14.55         | 375.27 $\pm$ 10.01    | 366.65 $\pm$ 10.76   | 375.20 $\pm$ 9.32       |
| T6           | 5.35 $\pm$ 0.76             | 4.97 $\pm$ 1.62        | 5.67 $\pm$ 0.07      | 5.22 $\pm$ 0.25bc         | 381.63 $\pm$ 14.56         | 391.65 $\pm$ 13.62    | 387.42 $\pm$ 14.59   | 369.71 $\pm$ 15.51      |
| T7           | 5.17 $\pm$ 0.16             | 4.79 $\pm$ 1.27        | 5.21 $\pm$ 0.40      | 4.76 $\pm$ 0.31c          | 385.39 $\pm$ 16.90         | 391.09 $\pm$ 14.22    | 375.49 $\pm$ 11.89   | 365.08 $\pm$ 11.24      |
| Significant  | N.S                         | N.S                    | N.S                  | *                         | N.S                        | N.S                   | N.S                  | N.S                     |

The symbol N.S. denotes the absence of substantial variations among the coefficients. \* indicates that, at the significance level ( $P < 0.05$ ), there are statistically significant differences between the coefficients. T1 (control). Add the aqueous extract of Garviola leaves T2, T3, and T4 at a dose of 15, 30, and 45 ml/liter/drinking water, respectively. Add the alcoholic extract of Garviola T5, T6, and T7 leaves at a dose of 15, 30, and 45 ml/liter of drinking water, respectively.

Orak et al., 2019; Merzah and Ali, 2022), as albumin represents the largest protein portion in the blood, which transports carbohydrates, fatty acids, vitamins, and some mineral elements (Oluwayinka et al., 2017).

(mg/100 ml serum) concentrations on different concentrations of aqueous and alcoholic extracts of Garviola (*Annona muricata*) leaves added to drinking water. (Mean  $\pm$  standard error) Statistical analysis of the first, second, and third periods regarding uric acid concentration showed no statistically significant differences between any of the ex

**Table 3** Effect of uric acid (mg/100 ml serum) and glucose

**Table 4:** Effect of aqueous and alcoholic extract of Garviola (*Annona muricata*) leaves in drinking water on the activity of liver enzymes in blood serum during 66–81 weeks (mean  $\pm$  standard error): AST, ALT, and ALP (IU/L).

| Treatments   | AST (IU/L)             |                       |                       |                         | ALT (IU/L)             |                       |                        |                         | ALP (IU/L)             |                        |                        |                         |
|--------------|------------------------|-----------------------|-----------------------|-------------------------|------------------------|-----------------------|------------------------|-------------------------|------------------------|------------------------|------------------------|-------------------------|
|              | First period (66-69)   | Second period (70-73) | Third period (74-77)  | Fourth period (78 - 81) | First period (66-69)   | Second period (70-73) | Third period (74-77)   | Fourth period (78 - 81) | First period (66-69)   | Second period (70-73)  | Third period (74-77)   | Fourth period (78 - 81) |
| T1 (control) | 28.76a<br>$\pm 0.36$   | 29.12a<br>$\pm 0.32$  | 29.56a<br>$\pm 0.63$  | 31.12a<br>$\pm 1.25$    | 15.48a<br>$\pm 0.55$   | 15.59<br>$\pm 0.93$   | 16.69a<br>$\pm 0.94$   | 19.12a<br>$\pm 0.69$    | 54.54c<br>$\pm 1.30$   | 54.30 c<br>$\pm 2.17$  | 54.34 b<br>$\pm 3.06$  | 58.37b<br>$\pm 1.65$    |
| T2           | 27.93ab<br>$\pm 0.33$  | 28.36ab<br>$\pm 0.04$ | 27.79ab<br>$\pm 0.34$ | 26.97b<br>$\pm 1.20$    | 15.37 a<br>$\pm 0.50$  | 15.25<br>$\pm 0.21$   | 15.35ab<br>$\pm 0.59$  | 14.87b<br>$\pm 1.27$    | 55.72 bc<br>$\pm 0.46$ | 55.42 bc<br>$\pm 0.57$ | 56.88 ab<br>$\pm 0.23$ | 59.00 ab<br>$\pm 0.22$  |
| T3           | 27.49bc<br>$\pm 0.40$  | 27.26bc<br>$\pm 0.18$ | 26.96b<br>$\pm 0.34$  | 25.61 bc<br>$\pm 0.55$  | 15.00 ab<br>$\pm 0.41$ | 15.44<br>$\pm 0.64$   | 15.52 ab<br>$\pm 0.47$ | 14.74b<br>$\pm 1.25$    | 57.06 ab<br>$\pm 0.99$ | 56.62 bc<br>$\pm 1.13$ | 57.39 ab<br>$\pm 1.02$ | 59.09 ab<br>$\pm 0.58$  |
| T4           | 27.1 bc<br>$\pm 0.39$  | 27.10bc<br>$\pm 0.89$ | 26.22b<br>$\pm 0.86$  | 24.11 bc<br>$\pm 0.92$  | 14.46 ab<br>$\pm 0.14$ | 14.22<br>$\pm 0.38$   | 14.99 ab<br>$\pm 0.24$ | 14.19b<br>$\pm 1.22$    | 59.27 a<br>$\pm 0.25$  | 59.97 a<br>$\pm 0.35$  | 59.39 a<br>$\pm 0.38$  | 60.83 a<br>$\pm 0.55$   |
| T5           | 27.72 bc<br>$\pm 0.36$ | 28.16ab<br>$\pm 0.27$ | 27.25ab<br>$\pm 0.67$ | 26.49bc<br>$\pm 0.49$   | 14.92 ab<br>$\pm 0.32$ | 15.70<br>$\pm 0.46$   | 15.73 ab<br>$\pm 0.54$ | 15.30b<br>$\pm 0.32$    | 58.77a<br>$\pm 0.26$   | 58.22 ab<br>$\pm 0.57$ | 58.32ab<br>$\pm 0.41$  | 59.77ab<br>$\pm 0.34$   |
| T6           | 26.77bc<br>0.37        | 27.21bc<br>$\pm 0.38$ | 26.24b<br>$\pm 0.26$  | 24.53bc<br>$\pm 1.27$   | 14.35ab<br>$\pm 0.54$  | 14.78<br>$\pm 0.86$   | 14.88ab<br>$\pm 0.33$  | 14.43b<br>$\pm 0.55$    | 59.36a<br>$\pm 0.40$   | 60.06a<br>$\pm 0.19$   | 59.76a<br>$\pm 0.68$   | 59.99ab<br>$\pm 0.63$   |
| T7           | 26.51c<br>$\pm 0.31$   | 26.21c<br>$\pm 0.60$  | 25.66b<br>$\pm 1.38$  | 23.55c<br>$\pm 0.91$    | 13.93 b<br>$\pm 0.37$  | 14.02<br>$\pm 0.85$   | 14.04 b<br>$\pm 0.39$  | 13.88b<br>$\pm 0.42$    | 59.37a<br>$\pm 0.49$   | 60.12a<br>$\pm 0.17$   | 59.42a<br>$\pm 0.71$   | 61.05a<br>$\pm 0.42$    |
| Significant  | *                      | *                     | *                     | *                       | *                      | N.S                   | *                      | *                       | *                      | *                      | *                      | *                       |

N.S. indicates that there are no significant differences between the coefficients. \* indicates significant changes between treatments at the significance level ( $P \leq 0.05$ ). The first treatment (control) was without any additives. The second, third, and fourth treatments included a 1% aqueous extract of Garviola leaves at concentrations of 15, 30, and 45 ml/liter of drinking water, respectively. The fifth, sixth, and seventh treatments included an alcoholic extract of Garviola leaves at concentrations of 15, 30, and 45 ml/liter of water. Drink 1% concentration consecutively.

perimental treatments. During the fourth period, the first treatment (control group) had significantly higher uric acid concentrations ( $P \leq 0.05$ ) than the lowest uric acid values of the third, fourth, fifth, sixth, and seventh treatments. There were no significant differences between the second treatment and the other experimental treatments. It is noted in the same table that the glucose concentration (mg/100 ml serum) did not differ between all treatments and during all four production periods.

The primary byproduct is uric acid resulting from protein catabolism (Coles and Saunders, 1986). The reason why uric acid concentrations were lower in the treatments containing alcohol and aqueous extracts than in the first treatment (control) can be attributed to the function of Graviola leaves in removing free radicals, which in turn stimulates the synthesis of antioxidants. Enzymatic, which also works to reduce free radicals and protect your RNA for protein synthesis while preventing amino acids from oxidizing. This maintains the balance of amino acids needed for hormone production, enzyme production, muscle protein synthesis, and other processes (Tchoffo *et al.*, 2024). Graviola leaves moderate the hyperactivity of the hypothalamic-pituitary-adrenal cortico-cortical axis by lowering the adrenal gland's secretion of corticosterone, and brain cells. Consequently, they regulate the activity of the hypothalamic-pituitary-adrenal cortico-cortical axis, which

prevents the protein from being catabolized in the blood serum when corticosterone is present (Yang *et al.*, 2015; Nguyen *et al.*, 2020).

**Table 4** The effect of adding different concentrations of aqueous and alcoholic extracts of Garviola (*Annona muricata*) leaves to drinking water on the activity of liver enzymes AST, ALT, ALP (units/L) in blood serum (mean  $\pm$  standard error). In the first period, the concentration of the enzyme AST (aspartate transaminase) increased significantly ( $P < 0.05$ ) in the first treatment (control) and the second treatment, while the enzyme concentration decreased in the third, fourth, sixth, and seventh treatments. During the fifth treatment, no noticeable differences were found between it and the other treatments, or between the first and second treatments. while the second period, the first treatment (control) recorded a significant increase ( $P \leq 0.05$ ) in enzyme concentration. AST compared to the third, fourth, sixth, and seventh treatments, while the second and fifth treatments, there were no significant differences between them and the first treatment on the one hand, and between the rest of the treatments on the other hand, During the third period, the first treatment (control) had the highest enzyme concentration, measuring 29.56 international units/liter. This difference from the other treatments (the third, fourth, sixth, and seventh) was significant ( $P \leq 0.05$ ). The seventh treatment had the lowest

AST enzyme concentration, measuring 25.66 international units/liter, while the second and fifth treatments did not differ significantly from the first treatment, and between the remaining transactions. During the fourth period of the trial, the first treatment (control) maintained its highest AST enzyme content, differing significantly ( $P \leq 0.05$ ) from the second, third, fourth, fifth, sixth, and seventh treatments. ALT enzyme (alanine transaminase) units/liter. It is noteworthy that the ALT enzyme concentration (units/liter) was highest in the first and second treatments, while the lowest concentration was seen in the seventh treatment. It was observed that there were no notable distinctions between the third, fourth, and fifth and the first and second treatment or the seventh treatment. We observe that in the second period, there are no significant differences between any of the experimental treatments; however, in the third period, the first treatment (control) had the highest enzyme concentration, differing significantly ( $P \leq 0.05$ ) from the seventh treatment, which recorded the least amount of ALT enzyme, in comparison to the other experimental treatments, did not significantly differ. During the fourth period of the trial, the first treatment (control) maintained its highest AST enzyme content, differing significantly ( $P \leq 0.05$ ) from the second, third, fourth, fifth, sixth, and seventh treatments. ALT enzyme (alanine transaminase) units/liter. It is noteworthy that the ALT enzyme concentration (units/liter) was highest in the first and second treatments, while the lowest concentration was seen in the seventh treatment. It was observed that there were no notable distinctions between the third, fourth, and fifth and the first and second treatment or the seventh treatment. We observe that in the second period, there are no significant differences between any of the experimental treatments; however, in the third period, the first treatment (control) had the highest enzyme concentration, differing significantly ( $P \leq 0.05$ ) from the seventh treatment, which recorded the least amount of ALT enzyme, in comparison to the other experimental treatments, did not significantly differ.

As for the liver enzymes AST, ALT and ALP, which are the enzymes responsible for transferring amino groups from an amino acid to a keto acid in the process of manufacturing glucose from non-carbohydrate sources in the process of gluconeogenesis (Osho *et al.*, 2023), the ability of treatments to add aqueous and alcoholic extract of Garviola leaves to reduce the enzymatic activity of ALT and AST and increase the concentration of the ALP enzyme may be due to enhancing the antioxidant status and reducing oxidative stress, as the ability of treatments to enhance the activity of glutathione, catalase and superoxide dismutase enzymes and reduce the level of malondialdehyde as well as reduce oxidation catalysts such as metal ions by preventing their release from tissues (bound to iron) in the liver and thus breaking the chains of free radical reactions

and limiting their production and formation, especially free radicals of active oxygen species, thus protecting polyunsaturated fats present in cell membranes from oxidation and liver membranes from oxidative damage and thus preserving the properties of the membrane, the most important of which is its permeability, as its permeability is optional, which prevents the leakage of these enzymes. From inside the cell to the outside (Jiwuba *et al.*, 2017; Oluwayinka *et al.*, 2017; Balderrama-Carmona *et al.*, 2020). The increase in the activity of the ALP enzyme in the blood serum of birds treated with aqueous and alcoholic extracts of Corviola leaves compared to the first treatment (control) is one of the reasons for the increase in the activity of the ALP enzyme in the blood serum or it may be due to the decrease in the concentration of the liver enzymes AST and ALT due to the phenolic compounds, flavonoids and phenolic acids present in the leaves of the Corviola plant, as the phenolic compounds improve the condition of the liver (Abdul Basit *et al.*, 2020).

## CONCLUSIONS AND RECOMMENDATIONS

Adding the aqueous extract of Garviola leaves (concentration: 1% dosage: 30 and 45 ml/liter of drinking water) and the alcoholic extract (concentration: 1% dosage: 15, 30 and 45 ml/liter of drinking water) improves some physiological blood characteristics Concentration of albumin, uric acid, and liver enzymes (AST, ALT, and ALP) compared to the first treatment. We recommend adding the aqueous extract and the alcoholic extract of Garviola leaves to drinking water at a level of 45 ml/liter of drinking water and at a concentration of 1% throughout the breeding period to improve the physiological properties of the yolk of older laying hens. Conduct studies on histological sections of the liver and add Garviola leaves to the diets of laying hens and broiler mothers and study its effect on the resulting chicks since the yolk plays an important role in the hatching process due to the lack of local studies or research on this plant.

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## NOVELTY STATEMENT

The novelty of our study has been its strong evidence that Garviola aqueous and alcohol extract improved physiological blood characteristics of laying Hens, in addition to liver function test for the first time. These results provided new insights that aqueous and alcoholic extract of fresh Garviola leaves can significantly improve the health and per-



formance of laying hens. Extracting the active compounds found in the leaves of Garviola, such as coumarin and as-tiggenin, and using them in the pharmaceutical industry, as they are natural antioxidants and anti-cancer agents.

## AUTHOR'S CONTRIBUTIONS

Diaa Hamza Yasser: conceived the presented idea, re-searched and supervised the results of this work, conducted the experiment, supervised the project, and contributed to the final version of the manuscript.

Nihad Abdul Latif Ali: conceived the presented idea, veri-fied the analytical methods, conducted the experiment, and contributed to the final version of the manuscript.

## CONFLICT OF INTEREST

None.

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