Open Access **Baghdad Science Journal** **P-ISSN: 2078-8665**
Published Online First: January 2022 **2022, 19(4): 716-724** **E-ISSN: 2411-7986**

DOI: http://dx.doi.org/10.21123/bsj.2022.19.4.0716

Gastroprotective and Immuno-supportive Role of *Alcea kurdica* against Stress Induced Lesion in Japanese Quails

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Received 11/1/2021, Accepted 30/3/2021, Published Online First 20/1/2022, Published 1/8/2022

Abstract:

The conducted research was done in Grda rasha field (Salahaddin University) for one month to compare the impacts of *Alcea kurdica* powder, Rifaxmine, and Ranitidine as anti-lesion and immune-strengthening agents on stress-induced quails which are affecting their growth rate and in severe cases causing gizzard erosion and deep intestinal lesions. To do that, 75 quails (12 weeks old) were grouped into six treatments with different additives. (T0-) = Negative control (Stress-induced Without treatment), (T0+) = Positive control (No stress inducing or treatment). T1= (treated with Rifaximine 200mg/L water mixed), T2= (treated with Ranitidine 200mg/L), T3= (treated with *A.kurdica* extract 100mg/L). The tested groups, except for positive control, were immersed in tap cold water (17°C) as a stress-induced technique. The Macroscopic analysis showed that quails pre-treated with *A.kurdica* extract (T3) had significantly ($p<0.05$) lower relative gizzard koilin layer disruption than those of T1, T2, and T0- groups, respectively. Moreover, the Elisa results indicated higher antibody titers against ND, IBD, and IB viruses for the T3 group with significantly increased HDL and lowered LDL, VLDL, and TCHO for T3 than that of T1, T2, T0+ T0- groups, respectively. Water mixed *A.kurdica* extract showed positive influences on the body weight, lipid profile, immune status and minimized gizzard erosions of breeding quails, which can be considered as a medicinal plant as well as a growth-enhancing agent in the poultry industry.

Key words: *Alcea kurdica*, Gastric lesions, Lipid profile, Cold Stress, Quails' immunity.

Introduction:

Japanese quails are hardy birds that thrive very well in cages and are relatively inexpensive to maintain without stress but unfortunately in comparison with other poultries gained less agricultural interest. Despite their economic importance in animal food resources as efficient egg laying and meat providence bird, Quails can be used in studies of developmental biology as they can be a convenient animal model [1]. Quails immune system is degraded by numerous factors including mycotoxins called Aflatoxins [2]. Another mycotoxin is called Type A-trichothecenes particularly T-2 toxin causing gizzard lesions or erosion. The gizzard erosion occurs despite being a strong organ, which in severe cases causes lesions in the underlying tissues, consequently, affecting quail's growth rate, egg-laying performance, and susceptibility toward immune diseases [3]. Besides, the presence of biogenic amines and tannins in the breeder's diet or breeder age is thought to be the causative factors [4]. Other causes such as pathogenic diseases like Newcastle disease (ND), Infectious Bursal Disease (IBDV/ Gumboro), and Infectious Bronchitis viral diseases (IB) are also reported by [5]. Avian Stomach has been used as an indicator for a bird performance by many researchers [6]. The gizzard is considered as the true stomach in the avian with bigger muscularity than proventriculus, which plays a major role in the grinding food to be further digested by the small intestine [7]. Medicinal plants become more popular in recent years to improve quail's productivity and lowering mortality rates [8].

A.kurdica, is a perennial plant mainly found on limestone in mountain slopes [9]. *A. kurdica* exhibited potent antioxidant activity and its aqueous extract was shown to scavenge free radicals with great antimicrobial properties against the growth of

selected microorganisms [10]. Traditionally, *A.kurdica* was used by people for the treatment of gastric ulcer, duodenal ulcer, pneumonia, and urinary tract infection [11]. Phytochemicals are well known to be used in curing gastric disorders, weight problems and cardiac diseases, as a substitute for drugs with fewer side effects, less expense and lower toxicity [12]. Phenolic (Gallic acids) and flavonoid (Quercetin) ingredients in herbal products possess great biological effects as antiulcer, increased free radical scavenging activity (69.4% ± 1.19%) and anti-inflammatory agents [13]. Pharmacologically, ranitidine and rifaximin have shown anti-lesion effectivity and healing capability against drug-induced enteropathy [14,15]. The current study mainly aims to compare *A. kurdica* powder efficiency with rifaximin, and ranitidine as anti- ulcerative, immune-supportive, and lipid lowering agents in tap water (17°C) stress-induced quails.

Materials and Methods:

Sample Collection and Preparation

A. kurdica was found in a wide geographic range near the Shaqlawa district area. The herb's identification was done in biology Dep./ College of Education/Salahaddin University-Erbil. The washing of plant parts (flowers) was done by running water and air-drying. The dried flowers (100 g) were ground using a grinder for 30 sec. Then, for the maceration of the powder, 1000 mL of boiling distilled water was used and let down for 2 hours to infuse at room temperature. The filtered extract was concentrated in the water bath and then, vacuum dried to be refrigerated at 4 °C for further analysis [16]. The extract yield was 8.6% (w/w).

Experimental Treatments

Adult quails (12 weeks old) were distributed into 5 treatments, each treatment with 3 replicates: 15 chicks per treatment. T0- = Negative control (Immersed in tap cold water (17°C) with no pretreatment), T0+= Positive control (Without pretreatment nor immersion). T1= (Immersed in tap cold water (17°C) with Rifaximine 200mg/L water mixed pretreatment), T2= (Immersed in tap cold water (17°C) with Ranitidine 200mg/L water mixed pretreatment), T3= (Immersed in cold tap water (17°C) with *A.kurdica* extract 100mg/L water mixed pretreatment) [17]. All tested groups fed on standard ad libitum for one month.

(**Vitamine premix**/ kg diet: vit.K3 3 mg; vit.B1 3 mg; vit.B2 7 mg; vit.A12,000 IU; vit.D3 60 µg; vit.E 32.96 IU; vit.B6 4 mg; vit.B12 0.02 mg; nicotinic acid 40 mg; pantothenate 8 mg; folic acid 1 mg; biotin 0.045 mg; vit.C 50 mg; 0.1 mg; Se 0.15 mg. choline chloride 125 mg. **Minerals premix:/** kg diet: Mn 80 mg; Fe 40 mg; Zn 60 mg; Cu 5 mg; Co,0.1mg [18].

- On the 15th day and 30th day of the experiment Body weight were measured, and Serum separated from collected blood to estimate blood glucose with a blood glucose analyzer (Accu-Chek Compact, Roche Diagnostics, Mannheim, Germany) [19] and then, the serum was taken to the immunity laboratory to evaluate the determined antibodies titers against Newcastle (ND), Gamboro (IBD) and Infectious bronchitis viral diseases (IB) by using ELISA [5]. To find the quail's lipid profile, we purchased kits known as DiaSys (Diagnostic System, Germany) [20].

At the end of the experiment, 5 quails were randomly chosen from each group, kept at room temperature and starved for 24hours, then Slaughtered, and necropsied after two hours following stress inducing by immersion in tap cold water (17°C) for 5 seconds [21]. Intestinal tracts and the gizzards were dissected and washed with distilled water for observation. Macroscopic screening for intestinal lesion and gizzard erosion was made following the same method done by Džaja *et al.* [21], The degree of gizzard erosion and ulceration (GEU) considered as follows**:**

(Score 0) No intestinal lesion with no disruption of Gizzard koilin layer fed on ad libitum

(Score 1) A mild lesion with few disruptions of Gizzard koilin layer

(Score 2) Intestinal lesions with severe disruption of Gizzard koilin layer, discoloration of a periventricular-gizzard junction

(Score3) multiple oval or cleft-like erosions with hemorrhages. severe disruption of Gizzard koilin layer.

(Score 4) Deep ulcers, single or multiple, with perforation of epithelial surface, Gizzard erosion.

(Score 5) The gizzard Lumina were severely bleed and small intestine, as a consequence of the gizzard ulcers.

Statistical analysis: The data analysis was done by CRD (Completely Randomized Design) by the SAS institute program [22]. One-Way ANOVA tests were used to compare differences among the treatments

Results and Discussion:

Treating quails with mixed water additives has caused some changes in their Body weight and blood sugar as in (Table 1). On the 15th-day, the bodyweight of all treatments showed insignificant differences, but after the 30th day of treatments, significant reductions in body weight were seen in T0-, T0+, T2, and T1 groups, respectively, but the T3 group showed no changes in the body weight. A previous study showed that adding plant additives did not significantly cause differences in the bodyweight of quails [23]. Based on the results, adding *A. kurdica* powder did not interfere with quail's body weight, unlike anti-ulcer drugs such as Ranitidine and Rifaximine which reduced the quail's body weight and eventually decreased their growth rate and immune system capacity. Our data is in accordance with [24], who proved undesired effects of these drugs as H2 receptor blocking agents causing reduction in stomach acid secretions and appetite loss leading to decrease in the body weight. Our data are also following the conclusion that declared adding organic feed containing mackerel, cassava leaves and turmeric powder to quails' diet have no significant effect on their body-weight [25]. Following the 15th and 30th days of treating groups with additives, blood sugar was non-significantly lower in *A.kurdica* (T3) group than that of (T1), (T1), (T0+), and (T0-), respectively. The anti-diabetic activity of Alcea genus was previously shown in alloxan-induced diabetic rates [26]. The hypoglycemic effect of *A.kurdica* can be linked with their antioxidant activity and phenolic contents that have been reported by Dar *et al.* [27] (Table 1).

Table 1. Body Weight and the Blood Glucose Levels in Serum of Quail Groups at 15th and 30th day of the Experimented.

Quail Groups	Body weight (grams)		Blood Sugar (mg/ dl)	
	At 15th day	At 30th day	At 15th day	At 30th day
Neg.Control(T0-)	285.375±7.17	266.66±8.1	244.5±7.4	290.2±28.93
P.Control (T0+)	287.77±19.50	250.5±34.017	257±27.1	288.88±34.04
Rifaximine (T1)	282.33±38.94	278.375±15.72	270.8±33.83	270.8±33.83
Ranitidine (T2)	296.33±24.78	266.36±28.87	247.66±28.13	283.18±26.14
***A.Kurdica* (T3)**	295.11±26.03	286±32.24	274.6±23.53	243.16±27.18
P-value	.735302	.069666	.27775	.115243
S.L	Not Sig.	Not Sig.	Not Sig.	Not Sig.

[a, b, c, d] Means within column with Different Superscripts Differ Significantly at (P≤ 0.05)* . S.L= Significant level.

Table 2. represents the effect of adding *A.kurdica* extract, Rifaximine and Ranitidine mixed water on quails' lipid profile, in which the T3 group showed significantly lower (P≤0.05) TCHO and VLDL values than that of T2, T1, T0+, T0- groups, respectively. The LDL and TG values were lower significantly in T3 than that of T1, T2, T0+, T0-, respectively. The HDL values were significantly higher in the T3 group than that of the T2, T1, T0+, T0- groups respectively. The lipid profile of the T1 and T2 groups were not significantly changed by water mixed additives. On the whole, Our results showed the effectiveness of *A.kurdica* as a hypocholesterolemic agent that may be a good choice to treat obese or hyperlipidemia patients which were not reported before.

Table 2. Serum level of TCHO, HDL, LDL, VLDL and VLDL in Experimented groups.

Quail groups	Serum lipid Profiles (mg/ dl)				
	TCHO	HDL	LDL	VLDL	TG
T0 -	221±18.3 d	27.4±3.9 c	146.5±6.1 d	47.1±4.0 d	429±35.6 d
T0+	193±14.1 c	32.9±2.8 c	123.3±4.8 c	36.8±2.1 c	390±28.5 c
T1	165±15.7 b	48.1±1.5 b	89.4±3.3 b	27.5±1.6 b	341±26.7 b
T2	172±14.9 b	49.5±1.7 b	96.6±3.5 b	25.9±1.7 b	344±27.1 b
T3	146±11.3 a	55.9±1.2 a	80.7±2.4 a	9.4±0.4 a	309±22.3 a
MSE	17.33	3.63	3.8	2.11	22.50
S.L	*	*	*	*	*

TCHO: total cholesterol, HDL = high density lipoprotein, LDL = Low density lipoprotein, VLDL = Very low density lipoprotein, TG = Triglycerides, MSE= mean standard error, S. L= Significant level, a, b, c, d Means within columns with different superscripts differ significantly at (P≤ 0.05) *.

Herbal medicine gained popularity in recent years, motivating researchers to investigate different plant parts in searching for new efficient phytochemicals [28]. The positive impact of *A.kurdica* extract on the quails' lipid profile can be explained by its natural contents with different phytochemicals like Phenolic (Gallic acids) and flavonoid (Quercetin), illustrated to possess different bioactivities including free radical scavenging as well as an immunomodulatory role on peripheral blood mononuclear cells (PBMCs), in addition, flavonoids known to induce innate and adaptive B- and T-cell responses, including TH1, TH2 and regulatory T cells [27,10]. The flavonoid's capability to improve birds' lipid profile is due to the presence of Functional hydroxyl groups that mediate their antioxidant effects by scavenging free radicals and/or by chelating metal ions [29]. In addition, previous research also concluded that adding plant extracts to quails' diet showed significant improvement in the lipid profile, oxidative stress status, body weight, and egg-yolk cholesterol of quails under stress conditions [30, 31].

A.kurdica as an antiulcer agent to cure irritation and mucosa membrane lesions were purchased for many years [11]. Figure 1. shows that gizzard ulcer score distribution in which (T0+ group) had very mild lesions, then increased progressively in other groups with significantly lower lesion scores for *A. Kurdica* (T3) group. Figure 6. shows that quails drinking *A.kurdica* mixed water (T3) had lower disruption of gizzard koilin layer in comparison with other treated groups shown in Figs. 2,3,4, and 5. The levels of damage are considered as discoloration and gross lesions in the surface of the intestinal tract and severe disruption of the eroded gizzard koilin layer (arrow), penetrating submucosa tissues. Our results regarding stressed-induced gizzard erosion are approved by GRABAREVIĆ *et al.* [17]. The present scores show significant protection of gizzard and intestine lining by *A.kurdica* mixed water, which was disrupted with multiple lesion areas in other tested groups (T1, T2, and T0-).

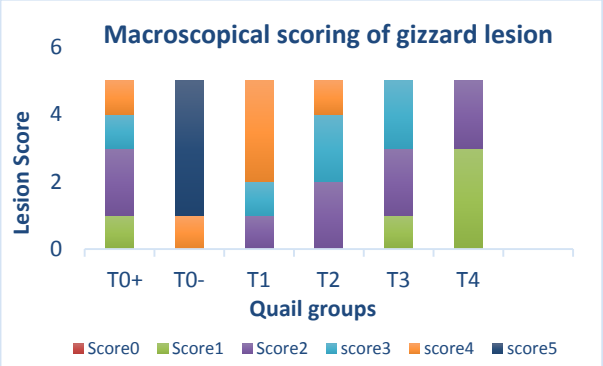

Figure 1. Macroscopic Distribution of Score Lesion in the Experiment treatments

The negative control group of quails (T0-) showed surface lesions on the intestinal tract with disruption and erosion in the gizzard koilin layer, penetrating submucosa tissues as shown in Fig. 2. The positive control (T0+) with no stress inducer had few intestinal damages with a smooth inner surface of gizzard mucosa as shown in Fig. 3. Quails pretreated with Rifaximine (T1) and Ranitidine (T2) had a moderate disruption in the gizzard koilin layer with intestinal lesions as shown in Fig. 4 and Fig. 5.

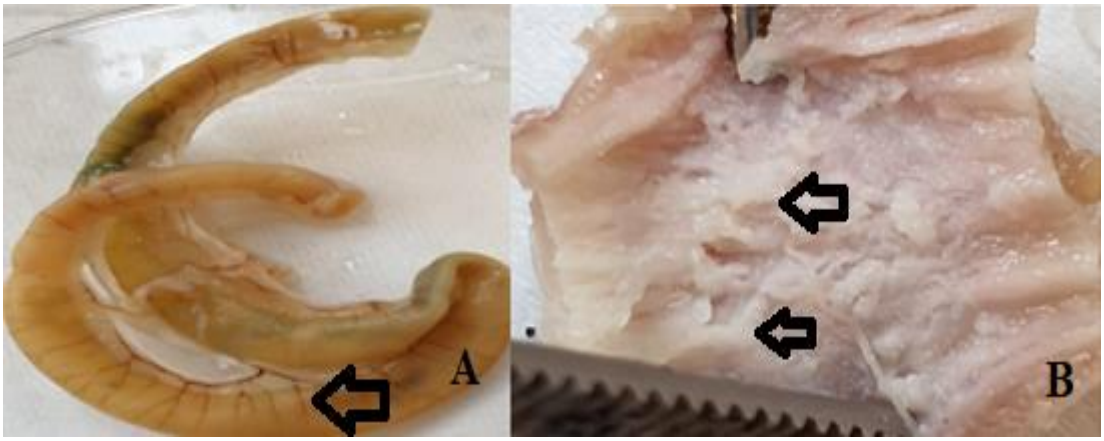

Figure 2. Open gizzards. shows the intestine and gizzard of (T0- group) with the lesions appear as extensive spongy material and diluted isthmus.

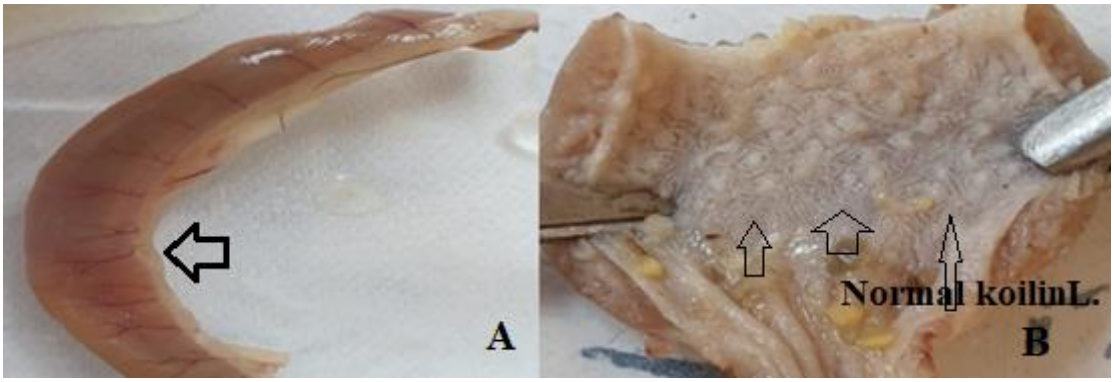

Figure 3. Open gizzards. shows the intestine and gizzard (T0+ group) with few intestinal lesions with normal gizzard groove line and no isthmus damage.

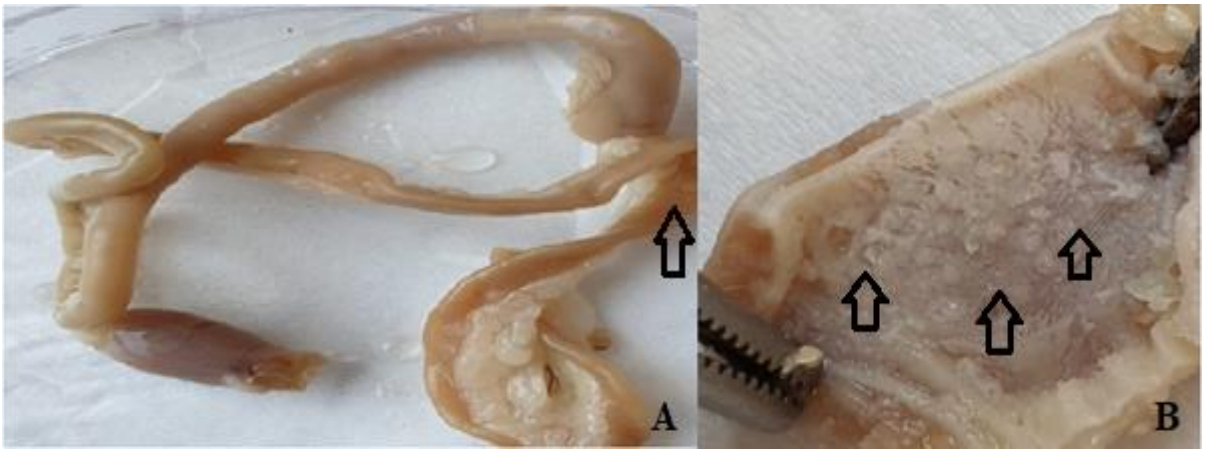

Figure 4. Open gizzards. Shows the intestine and gizzard of (T1 group) with surface intestinal lesions with eroded gizzard groove line and diluted isthmus.

Figure 5: Open gizzards. shows the intestine and gizzard of (T2 group) with clear surface intestinal lesions with eroded gizzard groove line and isthmus damage.

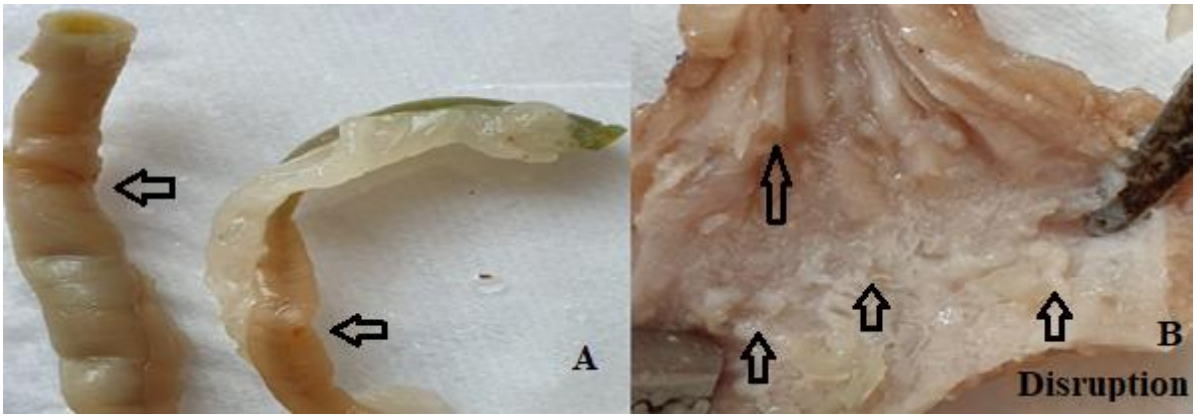

Figure 6. Open gizzards. shows the intestine and gizzard of (T3 group) with no surface intestinal lesions with few gizzard groove line erosion and normal isthmus.

There is no doubt that feeding birds immersed in tap water (17°C) as stress inducer transfers birds into a state with the depression of an optimal immune response: disrupting intestinal mucosa and the koilin layers of gizzard [21]. Lower score lesions in quails under *A.kurdica* mixed water in Fig.1, maybe due to flavonoids and phenolic content of this plant as shown to have antimicrobial, antioxidant activates by scavenging free radicals and immunomodulatory efficacy [27].

Our data results are supported by a previous research article which mentioned that the flowers of *Alcea kurdica* are served as antiulcer and anti-inflammatory agents [12]. Researchers previously mentioned that phytochemicals such as flavonoids, tannins, triterpenoids, saponins, and coumarins possessed significant antiulcer activities [32]. In contrast, some anti-ulcer drugs like Ranitidine may possess several side effects as shown in our study causing a decrease in the quail's body weight in comparison to other tested groups. Making birds more susceptible to various avian diseases affects their growth rate and egg-laying performance. These antiulcer drug side effects have been linked with decreasing gastric acid secretion and appetite loss [7].

The data in Fig. 7 summarizes the Geometric Mean Titers (GMT) for the treated quails against ND, IBD and IB viruses. The GMTs obtained reached the protection level and varied significantly among treated groups and ranged from 845 to 2056. Quails drunk *A.kurdica* mixed water had significantly higher antibody titers against ND, IBD, and IB viruses than that of Ranitidine and Rifaximine respectively. A previous study explained that the development of a bird's specific immunity is reduced by the water that quails drink due to an imbalance between acid and base status [33].

The highest immune titers against ND, IBD, and IB were recorded in the T3 group followed by T2 and T1 respectively. The lowest Immune titers against ND, IBD, and IB were recorded for T0- group. The ELISA results for the immune titers of T1 and T2 against the mentioned diseases did not significantly differ. The data analysis demonstrated that immune titers of T3 quail groups against ND, IBD, and IB were significantly higher more than 2 times than that of the T0+ group under a diet with no additives. Only one group (T3 against ND) had protective antibody titers (GMT > 2000) and the rest showed immune titers (GMT<2000) As shown in Fig. 7.

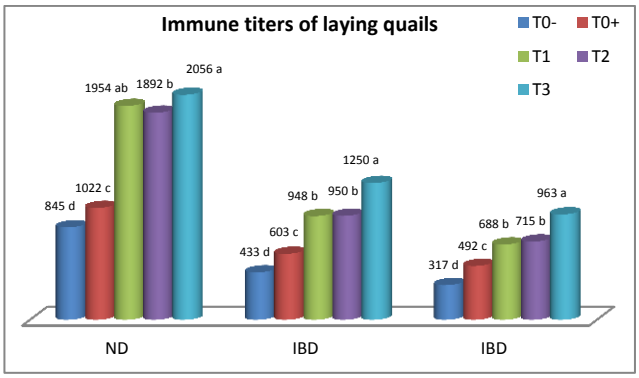

Figure 7. The impact of adding *A.kurdica* T3, Rifaximine T1, and Ranitidine T2 in quails' drinking water on serum antibodies titration against Newcastle (ND), Gamboro (IBD), and Infectious bronchitis viral diseases (IB) by ELISA.

Monitoring serologic antibodies against diseases in the poultry industry is considered an efficient way to evaluate the response of the immunity system toward additives and stress-induced immune diseases [33]. Possessing high immune titers by quails drinking *A.kurdica* mixed water may be due to its phytochemical contents like flavonoids and phenolic compounds. These bioactive compounds possess great antimicrobial

and antioxidant properties by their scavenging and redox properties, through neutralizing and quenching free radical and pathogenic microbes attacking animals' digestive and immune systems [27]. In addition to its terpenoid contents, which are known for their capability in disrupting membranes of microbes with efficient antioxidant and immunomodulatory properties by induction of immune cells [34].

Conclusion:

A.kurdica is a natural efficient plant with valuable phytochemicals. Their flavonoid and phenolic contents possess various biological impacts, acting as anti-cholesterolemia, anti-ulcer, and immune-supportive agents by scavenging free radicals and immune cell activation. Treating quails with the *A.kurdica* extract improves their immune system under stress conditions against various avian diseases, which are considered as risk factors for intestinal lesions and gizzard erosion, causing a decrease in the growth rate and egg-laying performance. Moreover, *A.kurdica* extract can be considered as a substitute for anti-ulcer drugs with fewer side effects as shown to not interfere with the quails' body weight. Further investigations are required to study the long-term physiological impact of *A.kurdica* as an antiulcer and immune booster agent that may be of bird breeder's interest to maximize productivity and decrease mortality rates of birds.

Author's declaration:

- Conflicts of Interest: None.
- I hereby confirm that all the Figures and Tables in the manuscript are mine.
- The author has signed an animal welfare statement.
- Ethical Clearance: The project was approved by the local ethical committee in Erbil Polytechnic University.

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Open Access **Baghdad Science Journal** **P-ISSN: 2078-8665**
Published Online First: January 2022 **2022, 19(4): 716-724** **E-ISSN: 2411-7986**

دور *Alcea kurdica* المعدي والمدعوم للمناعة ضد الآفات الناجمة عن الإجهاد في السمان الياباني

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الخلاصة :
تم اجراء الدراسة في حقل كردة رشة (جامعة صلاح الدين) ولمدة شهر ليقارن تأثير كل من مساحيق A.kurdica و Rifaxmine وRanitidine كمواد المعدي والمدعوم للمناعة للمسان المعرض للإجهاد والتي يأثر علي معدل النمو نتيجة تأكل الحويصلات والآفات المعوية . تم اجراء الدراسة على 75 طير سمان (عمرها 12 أسبوعًا) في ستة معاملات أذ قسمت إلى: (T0-) =تحكم سلبي بدون علاج، = (T0 +) تحكم إيجابي (لا يوجد إجهاد أو علاج (T1) معالج بـ Rifaximine 200mg / L ماء مختلط، (T2) = معالج بـ Ranitidine 200mg/l (T3), =تمت معالجته بمستخلص A.kurdica 100mg / L تم غمر المجموعات المختبرة باستثناء مجموعة التحكم الإيجابية في ماء الصنبور باردة (17 درجة مئوية) كمحفز للتوتر. أظهر التحليل المجهري أن السمان المعالج مسبقًا بمستخلص A.kurdica (T3) كان له تأثير معنوي ($p<0.05$) في طبقة قوانص كويلين أقل اضطرابا من تلك الخاصة بمجموعات T1 و T2 و T0 على التوالي. علاوة على ذلك، أشارت نتائج Eliza إلى زيادة عيار الأجسام المضادة ضد فيروسات ND و IBDو IB لمجموعات T3 مع زيادة HDL بشكل ملحوظ وانخفاض LDL و VLDL و TCHO لـ T3 مقارنة بمجموعات T1 و T2 و T0 + T0 على التوالي. أظهر مستخلص نبات A.kurdica الممزوج بالماء تأثيرات إيجابية على وزن الجسم ، وخصائص الدهون ، والحالة المناعية ، وتقليل تآكل قوانص في السمان، والذي يمكن اعتباره نباتًا طبيًا وكذلك عاملًا يعزز النمو في صناعة الدواجن.

الكلمات المفتاحية: آفات المعدة ، الإجهاد برودة، الدهون ، خطمية كردية ،مناعة السمان