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RESEARCH ARTICLE

Investigating the effectiveness of doum and marjoram powders as dietary supplements in mitigating the negative effects of ochratoxin on broiler chickens health

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ABSTRACT

Mycotoxins pose a significant risk to poultry production as they have detrimental effects on chicken health, immunity, and productivity. Additionally, they present a hazard to human health due to their teratogenic and carcinogenic properties. This work investigated the influence of using doum palm fruit and marjoram leaves' powders separately or in combination against experimentally induced ochratoxicosis in broiler chicks. A total of 100 day-old Cobb chicks were divided into 5 equal groups (20 chick/group with duplicate) as follows: G1: control negative birds, kept on feeding with starter broiler ration free from ochratoxin; G2: control positive birds, fed starter broiler ration with ochratoxin @ 5.4ppb; G3: fed starter broiler ration with ochratoxin @ 5.4ppb and treated with doum fruit powder @ 100g/kg ration; G4: fed starter broiler ration with ochratoxin @ 5.4ppb and treated with marjoram powder @ 15g/kg ration, and G5: fed starter broiler ration with ochratoxin @ 5.4ppb and treated with doum fruit powder @ 100g/kg ration and marjoram powder @ 15g/kg. The dual use of doum and marjoram powder at 15g/kg reduced the adverse effects by improving the renal and hepatic functions and reducing the histopathological as well as immunohistochemical alterations within liver, kidney, spleen, and heart tissues. Also, a significant improvement in the humoral immune response had been detected as elucidated by expression of different vital genes such as TBP, OCLN, MUC-1, JAM-2, CD4, CD8, IL-1b, IL6, TLR-4, BAX, Casp-3, and Gapdh; along with improvement in the antioxidant status. In conclusion, doum fruit powder (100g/kg) and marjoram powder (15 g/kg) can be used as feed additives to reduce the adverse effects induced by ochratoxicosis in broiler chickens.

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INTRODUCTION

Chicken farming is widely recognized for its cost-effectiveness and efficient feed conversion compared to other types of livestock production (Alsulami and El-Saadony, 2023; Salem *et al.*, 2023; Reda *et al.*, 2024a). However, illnesses are considered the primary threat to poultry production (Setta *et al.*, 2024; Mousa *et al.*, 2024;

Ibrahim *et al.,* 2024). Thus, researchers investigate novel feed additives and incorporate them into poultry ration to advance their health, immunity, and birds' productivity (Abd El-Hack *et al.,* 2022a; Arif *et al.,* 2022; Beyari *et al.,* 2024).

Mycotoxicosis is a serious hazard affecting the poultry sector, inducing a detrimental impact on birds' health and productivity, including immune suppression due to the atrophy of thymus and bursal, depletion in bone marrow, glomerular damage and hepatocellular necrosis lesions that reveal retarded growth, decreased production, vaccination failure, increased susceptibility to infections (Khatoon and Abidin, 2021; Salama *et al.,* 2024; Jasim *et al.,* 2025). Ochratoxins, particularly ochratoxin A (OTA), are the second most prevalent mycotoxins contaminating poultry feed after aflatoxins (Khatoon *et al.,* 2023). OTA is known to induce nephrotoxicity, hepatotoxicity, and immunosuppression in broilers (Mehtab *et al.,* 2021; Chen *et al.,* 2022).

On the other hand, herbs and their extracts are considered promising additions to poultry ration as they can be used instead of antibiotics to avoid antibiotic drug resistance and their residual effects in avian products (El-Saadony *et al.,* 2022; Hegazy *et al.,* 2023; El-Sayed *et al.,* 2024). They can also improve birds' immunity and productivity (El-Saadony *et al.,* 2022; Abdel-Moneim *et al.,* 2022). In the search for antibiotic alternatives, the poultry industry is increasingly turning to natural compounds such as phytogenics, probiotics, and prebiotics to enhance chicken immunity (Alagawany *et al.,* 2021a, b; Almuhayawi *et al.,* 2023; Reda *et al.,* 2024b). The secondary metabolites from plants and microbes modulate immune cell activity, provide antioxidant protection, improve gut microbiota balance, and exert antimicrobial effects. By incorporating them into feed, producers strengthen innate and adaptive immune responses, improving disease resistance, vaccine efficacy, and overall flock health (Abd El-Hack *et al.,* 2022b; Yehia *et al.,* 2023, 2024). Since then, many scientists worldwide have become interested in these herbal additives (Abd El-Hack *et al.,* 2022c).

Doum fruit powder (*Hyphaene thebaica*) is a rich source of bioactive compounds, including phenolic acids (e.g., gallic and chlorogenic acids), flavonoids (quercetin, rutin), and dietary fiber, which contribute to its antioxidant, anti-inflammatory, and prebiotic properties in poultry nutrition (Adenowo *et al*., 2024; Elshynrawy *et al*., 2024). These compounds enhance gut health by promoting beneficial microbiota (e.g., *Lactobacillus*) while suppressing pathogens like *E. coli* and *Salmonella* (Saied and El Zubeir, 2024). Studies demonstrate that doum powder inclusion improves growth performance, reduces feed conversion ratio (FCR), and lowers oxidative stress in broilers (Tijjani *et al*., 2024). Similarly, marjoram leaves (*Origanum majorana*) contain potent terpenoids (terpinene-4-ol, sabinene), phenolics (rosmarinic acid, carvacrol), and essential oils (eugenol, linalool), which exhibit antimicrobial, antioxidant, and digestive-enhancing effects (Ghazal *et al*., 2022). Marjoram supplementation has been shown to boost immune response (e.g., higher Newcastle disease antibody titers), improve nitrogen utilization (reducing ammonia emissions), and provide natural anticoccidial activity against *Eimeria* infections (Yousefi *et al*., 2021; Mohamed *et al*., 2021). These phytogenic ingredients offer a synergistic approach to replacing synthetic additives, thus promoting sustainable poultry production.

Chronic exposure to ochratoxin reduces feed efficiency by 12–17% and exacerbates mortality during the infections (Qing *et al.,* 2022) and in-practice mitigation strategies (*e.g.,* adsorbents, improved storage) remain costly or inefficient for small-scale farms (Ben Miri *et al.,* 2024). Thus, this work was planned to explore the possible impact of doum palm fruit powder and marjoram palm powder together in broiler chicken against ochratoxin induced alterations in broiler chickens.

MATERIALS AND METHODS

Experimental design: As seen in Table 1, one hundred-day-old Cobb chicks were reared under a deep litter system being divided into five equal groups (20 chicks/group with a duplicate). All groups were fed starter broiler ration (23 % protein) free from mycotoxin and without any treatment.

After that, from 8 to 14 days, following treatments were followed: G1 (control negative) was fed on starter broiler ration free from mycotoxin. G2 (control positive) was fed on starter broiler ration contaminated with ochratoxin (5.4ppb), G3 was fed starter broiler ration contaminated with ochratoxin (5.4ppb) and treated with doum fruit powder (100g/kg) according to the method of Ibrahim *et al.* (2018), G4 fed starter broiler ration contaminated with ochratoxins (5.4ppb) and treated with marjoram plant powder (15 g/kg) following the method of Ali (2014 a&b) and G5 was fed starter broiler ration contaminated with ochratoxin (5.4ppb) and treated with doum fruit powder (100g /kg) and marjoram plant powder (15g/kg).

In the duration from 15 to 21 days, G1 kept on feeding with a grower broiler ration free from mycotoxin, G2 fed grower ration but contaminated with ochratoxin 10.1ppb, G3 fed grower ration affected with ochratoxin 10.1 ppb and treated with doum fruit powder 100g/kg, G4 fed grower ration with ochratoxin 10.1 ppb and treated with marjoram plant powder 15g/kg ration and G5 fed grower ration with ochratoxin 10.1ppb and treated with doum fruit powder 100g/kg and marjoram plant powder15g/kg. After 22 to 30 days, all groups were fed with grower rations free from mycotoxin and without any treatment. After 30 to 34 days, all groups were fed finisher rations free from mycotoxin and without any treatment.

Vaccines: The birds in all the experimental groups were vaccinated with Gumboro intermediate (IM strain VMG 91) at 14 days through drinking water and LaSot at 18 days by spraying.

Samples collections and parameters determination: The samples were collected at two intervals i.e., day 21 (14 days post-contamination with ochratoxins and herbal powder treatment) and day 34 (12 days after stopping contamination with mycotoxin and herbal powder treatment).

Blood samples were ethically obtained from the wing veins of birds in all groups at the two-time points. Then, serum samples were separated and kept for further detection of hepatic and renal functions, humoral immunity, and antioxidant parameters. Also, two birds/replicates were ethically slaughtered to detect postmortem lesions and other parameter analysis.

Table 1: Experimental design

Groups 20 birds/group (2 replicates with 10 birds)	Different treatments				
	1-7days	8-14 days	15-21days	22-30 days	30-34 days
G1 (Control negative)	All groups were fed with starter broiler ration (23 % protein) free from mycotoxin and without any treatment	Starter broiler ration free from mycotoxin	Grower broiler ration free from mycotoxin	All groups were fed with grower rations free from mycotoxin and without any treatment	All groups were fed finisher rations free from mycotoxin and without any treatment
G2 (Control positive)		Starter broiler ration but contaminated with ochratoxins 5.4ppb	Grower ration contaminated with Ochratoxins 10.1ppb		
G3		a starter broiler ration contaminated with ochratoxins 5.4 ppb and treated with doum fruit powder 100g/1k ration	Grower ration affected with ochratoxins 10.1 ppb & treated with doum fruit powder 100g/1k ration		
G4		starter broiler ration contaminated with ochratoxins 5.4ppb and treated with marjoram plant powder 15g /1k ration	Grower ration with ochratoxins 10.1 ppb & treated with marjoram plant powder 15g /1k ration		
G5		starter broiler ration contaminated with ochratoxins 5.4 ppb and treated with doum fruit powder 100g /1k ration and marjoram plant powder 15g /1k ration	Grower ration with ochratoxins 10.1ppb & treated with doum fruit powder 100g/1k ration & marjoram plant powder 15g/1k ration.		

Serum biochemical analysis: Serum samples were analyzed to detect the levels of alanine aminotransferase (ALT), aspartate-aminotransferase (AST), alkaline phosphates, total protein (TP), globulins, albumin, uric acid, and creatinine (Kind & King, 1954; Reitman & Frankel, 1957; Barham & Trinder, 1972; Doumas *et al.*, 1981).

Antioxidant parameters: estimation of malondialdehyde (MDA), superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (GPx) were determined via utilizing commercial kits (Bio Diagnostic in Dokki, Giza, Egypt) (Nishikimi *et al.*, 1972; Ohkawa *et al.*, 1979; Aebi, 1984).

Gene expression: For real-time quantitative RT-PCR (qRT-PCR) analysis, RNA was isolated from the tissue with Trizol (Invitrogen; Thermo Fisher Scientific, Inc.) as mentioned by Khamis *et al.* (2021). For evaluating the RNA quality, the A260/A280 ratio was analyzed by applying the NanoDrop® ND–1000 Spectrophotometer (NanoDrop Technologies; Wilmington, Delaware, United States) for 1.5µL of the RNA. A High-Capacity cDNA Reverse Transcription Kit cDNA Kit (Applied Biosystems™, USA) was used for cDNA synthesis. The RT reaction mixture was kept for 60min at 45°C, subsequently by 10min at 85°C to inhibit the enzyme in a Biometra 96-well thermal cycler (Applied Biosystems).

A total of 50ng of the total RNA was reverse transcript in a terminal volume of 20µL (50 ng dissolved in 5µl nuclease-free water, 4µL of 5X miRCURY RT reaction buffer, 2.5µL of 10xmiRCURY RT Enzyme Mix, 1.2µL of a predesigned stem-loop primer and 10µL of RNase-free water) with a cycling condition of 42°C for 60 min for the reverse transcription stage and 95°C for the inactivation of the enzyme according to the producer instruction (Qiagen, Germany) then cDNA were aliquot then kept at -20°C until used. The primer design used is summarized in Table 2.

The real-time RT-PCR was adopted in a Rotor-Gene Q 2plex Real-Time PCR System (Qiagen, Germany) using TOPreal™ qPCR 2X PreMIX (SYBR Green with low ROX) (Cat. # P725 or P750) (Enzynomics, Korea) following the manufacturer's instructions. The PCR cycling conditions started with an initial denaturation at 95°C for 12 minutes followed by 40 cycles of denaturation at 95°C for 20 seconds, annealing at 60°C for 30 seconds, then extension at 72°C for 30 seconds. The oligonucleotide-specific primers were synthesized by Sangon Biotech (Beijing, China).

The expression level of the target genes was normalized by applying the mRNA expression of a known housekeeping gene, B-actin. Data are presented as fold-changes compared to the control group following the $2^{-\Delta\Delta CT}$ method (Livak and Schmittgen, 2001).

Histopathological studies: The formalin-preserved chicken's hepatic, renal, cardiac, and splenic tissue specimens were handled in an automated tissue processor and prepared for fixation and dehydration. Samples were then soaked with molten paraffin wax, then embedded then (4–5um) sections were stained with hematoxylin and eosin. These sections were inspected for inflammation, circulatory disturbances, degenerations, necrosis, apoptosis, and other tissue lesions (Suvarna *et al.*, 2013).

Immunohistochemical technique: Paraffin sections from different groups of liver and kidney were stained by immunohistochemistry (IHC) following Hsu *et al.* (1981); Jackson & Blythe (2008) using mouse monoclonal anti-Caspase-3 Ab [ABM1C12] at 5µg/ml dilution, Cambridge, UK., Abcam. The tissue sections from all experimental groups were dewaxed & hydrated. Staining was then performed via the DAB chromogenic agent (expose mouse and rabbit specific HRP/DAB detection kit, Abcam; Ready–to–use; Cat. #: ab80436).

Table 2: Primers, amplicon size, accession numbers

Gene	Forward primer	Reverse primer	Size	Accession No.
TBP	CGTGCACGCTCTGTTTAGTG	AAGCATTCCAGCAAAGCAGC	106	NM_001396193.1
OCLN	AGGTCTGCAACAGCATCACA	ATGCCTTCCCAAAAAGCCCT	157	NM_205128.1
MUC-1	GGGAATCTGTGGCCTGTTGA	TTCTCAGCATCTCTCCCCCA	83	XM_040680153.2
JAM-2	TCCTGCAACGCTGACTTCAT	CGGCCTCAATTACAAGCAGC	138	NM_001397141.1
CD4	ACCGACATCTGTTGAGCAGC	TCCAAGGGAACGCTCTTCAC	197	NM_204649.2
CD8	AACAGTGACAGTGGTGCCTC	CCTGAGTAGGTCGTACGGGA	98	NM_001048080.1
IL-1b	CTGCCTGCAGAAGAAGCCT	TGTCAGCAAAGTCCCTGCTC	164	NM_204524.2
IL6	AACAACCTCAACCTGCCCAA	AGGTCTGAAAGGCGAACAGG	112	NM_204628.2
TLR-4	GTTTGGTGCTTGGAAGCTGG	CGAGCTGTTGCCACTCCTTA	146	NM_001030693.2
BAX	TGACCCTCTGACCCTAGCTC	ATCCCAGCACTTTGAGAGGC	134	NM_001291430.2
Casp-3	AGGTGGAGGAGCTCTCCTATG	CCTGAGCGTGGTCCATCTTT	199	NM_204725.2
Gapdh	CCACATGGCATCCAAGGAGT	GAACTGAGCGGTGGTGAAGA	101	NM_204305.2

Counterstaining by hematoxylin was done. All photos of these sections stained by IHC were captured using a Swift microscope associated with a Swift digital camera. For quantitative analysis, we chose five representative regions with positive cell areas and areas without expression.

Statistical analysis: All statistics were conducted using SAS software version 9. Treatments were arranged in a randomized complete block design. The triplicate data were analyzed using ANOVA, the means were compared using the least significant difference (LSD) test at P<0.05.

RESULTS

General health parameters

Clinical symptoms: Chickens of all groups except G2 were healthy and showed no clinical symptoms throughout the experimental period. G2 showed no clinical symptoms until day 10, and after that, from day 11 until day 26 displayed clinical symptoms of depression as ruffled feathers, dropping off the wings with large chalky white drops in the feces, and soiled around the vent. These symptoms completely disappeared after that.

Mortality rate: Mortality rates were zero in all groups.

Effect on body weight: All groups showed non-significant differences in body weight or feed consumption.

Postmortem (PM) changes: PM examination of sacrificed chicks of all groups was normal and revealed no lesions in different organs except G2. G2 at 22 and 34 days old showed a moderately enlarged liver with distended gall bladders and an inflamed intestine were observed. Congested Kidneys are distended with urates. In addition, congestion in the heart is also observed. On day 34, all the previous lesions were present, and the liver showed some necrotic changes.

Liver functions, kidney functions, and humoral immunity: As seen in Tables 3, 4, and Fig. 1, 2, the effect of marjoram and doum feed supplementation on liver functions, kidney functions, and humeral immunity at day 22 (the first day after stopping contamination with mycotoxin and stopping herbal powder treatment) was as follows: the renal function showed the highest level of creatinine and uric acid in G2 and the lowest parameters were detected in G1, the treated birds in both G3, G4 and G5 showed improvement in renal function when compared with G2. The hepatic functions include ALT and AST. ALP, TP, albumin, and A/G ratio levels showed the ideal parameters in G1 in contrast to G2, the hepatic function indicators showed the highest ALT, AST, and ALP levels with the lowest TP, Albumin, and A/G ratio levels, while birds in treated groups showed improvement in hepatic function parameters in contrast with G2.

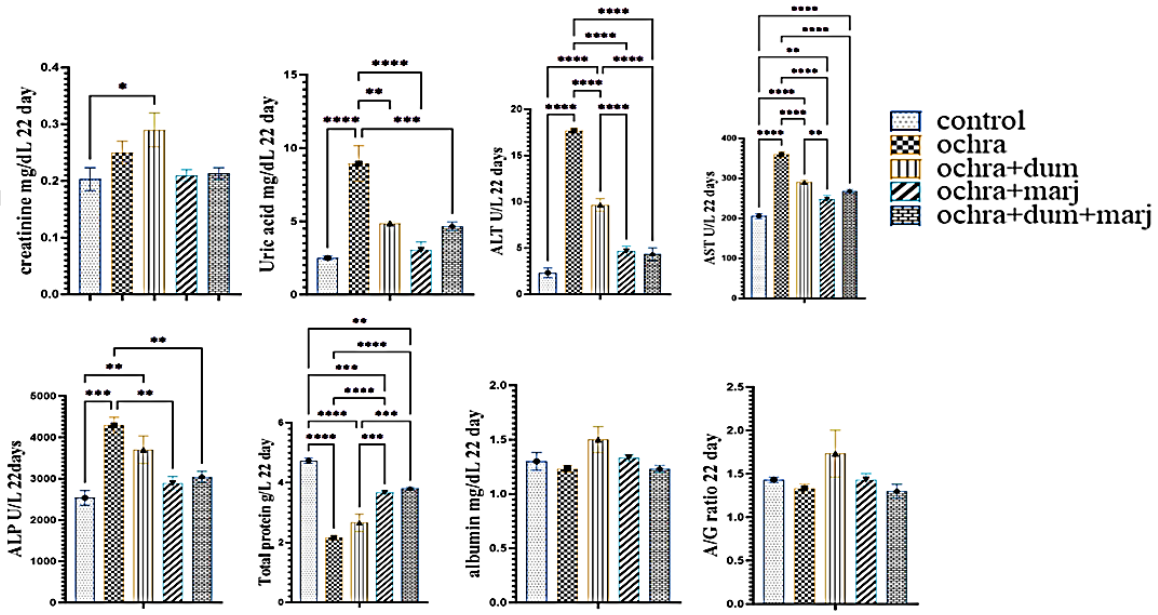

Fig. 1: Effect of marjoram and doum feed supplementation on liver functions, kidney functions, and humeral immunity at day 22 (first day after stopping contamination with mycotoxin and stopping herbal powder treatment). The asterisks above the bars indicate the level of statistical significance: * P<0.05, ** P<0.01, *** P<0.001, **** P<0.0001. This indicates that most of the observed differences are statistically significant, particularly the effects of ochra compared to the control and the protective effects of dum and marjoram against ochra-induced liver and kidney dysfunction.

Table 3: Effect of marjoram and doum feed supplementation on liver functions, kidney functions, and humeral immunity at day 22 (first day after stopping contamination with mycotoxin and stopping herbal powder treatment)

Parameters	G1	G2	G3	G4	G5
Creatinine	0.203±0.02	0.25±0.02	0.29±0.03	0.21±0.01	0.213±0.01
Uric acid	2.5±0.12	8.933±1.23	4.867±0.09	3.033±0.56	4.667±0.28
ALT	2.333±0.52	17.667±0.26	9.667±0.68	4.667±0.52	4.333±0.68
AST	205.7±6.27	359.33±7.65	290±4.4	247.33±9.64	268±4.02
ALP	2534.67±179.30	4291.67±198.65	3700 ±334.57	2890.33±161.88	3047.33±133.34
Total protein	4.733±0.09	2.167±0.07	2.661±0.29	3.667±0.05	3.8±0.04
Albumin	1.3±0.08	1.233±0.03	1.5±0.12	1.333±0.03	1.233±0.03
A/G ratio	1.433±0.03	1.333±0.05	1.733±0.27	1.433±0.07	1.3±0.08

Data are presented mean±SD.

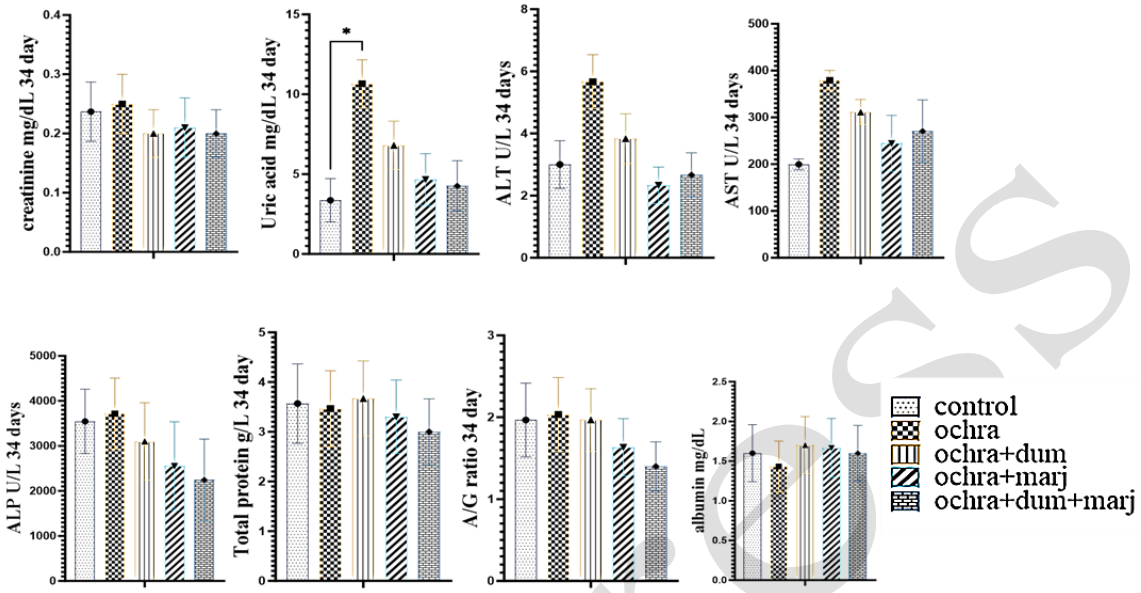

Fig. 2: Effect of marjoram and doum feed supplementation on liver functions, kidney functions, and humeral immunity at day 34 (12 days after stopping contamination with mycotoxin and stopping herbal powder treatment). The statistically significant difference observed is the reduction in ALT and uric acid levels in the ochra + dum + marj group compared to the ochra group.

Table 4: Effect of marjoram and doum feed supplementation on liver functions, kidney functions, and humeral immunity at day 34 (12 days after stopping contamination with mycotoxin and stopping herbal powder treatment.

Renal & hepatic functions	G1	G2	G3	G4	G 5
Creatinine	0.237±0.05	0.25±0.05	0.2±0.04	0.21±0.05	0.2±0.04
Uric acid	3.367±1.35	10.667±1.5	6.8±1.52	4.667±1.62	4.267±1.57
ALT	3±0.77	5.667±0.87	3.833±0.8	2.333±0.59	2.667±0.71
AST	199.51±11.42	379.33±21.03	311±27.5	244.67±59.81	270.67±66.66
AP	3546.33±713.31	3717.33±789.89	3100±858.44	2553.67±982.21	2246±906.64
Total protein	3.567±0.8	3.467±0.76	3.667±0.76	3.3±0.74	3±0.66
Albumin	1.6±0.36	1.433±0.32	1.7±0.36	1.667±0.37	1.6±0.35
A/G ratio	1.967±0.45	2.033±0.45	1.967±0.38	1.633±0.35	1.4±0.30

Data are presented mean±SD.

Gene expression: The impact of marjoram and doum feed supply on the relative expression of the intestinal health markers (OCCU, JAM, and MUC-1) in ochratoxin-challenged broilers was summarized in Table 5 and Fig. 3. The gene expression of OCCU, JAM, and MUC-1 genes appeared normal at G1. At the same time, they reveal a significant lowering in the gene expression of these genes, reflecting intestinal health in G2 that is challenged with ochratoxin. At the same time, G3 and G4 slightly improved their expression. In contrast, the dual treatment of birds by both doum and marjoram post-ochratoxins challenge revealed a significant improvement in gene expression of OCCU, JAM, and MUC-1 compared to G2.

The impact of marjoram and doum feed supplementation on the relative expression of the renal inflammatory (CD-4, CD8, IL-6, IL-1β, and TLR-4) and proapoptotic (Bax and caspase-3) markers in ochre toxin challenged broilers were expressed in Table 6 and Fig. 4. The expression of renal inflammatory markers, including (CD-4, CD8, IL-6, IL-1β, & TLR-4) and proapoptotic (Bax and caspase-3) markers appeared to be normal in G1; on the other hand, they revealed a significant rise in G2. The birds in both G3 and G4 revealed a significant advancement in the expression of the renal inflammatory (CD-4, CD8, IL-6, IL-1β, & TLR-4) and proapoptotic (Bax and caspase-3) markers compared to G2. While the combination of marjoram and doum feed supplementation in G5 revealed a significant improvement in the renal inflammatory (CD-4, CD8, IL-6, IL-1β, and TLR-4) and proapoptotic (Bax and caspase-3) markers when compared to G2.

The impact of marjoram and doum feed supplementation on the relative expression of the hepatic inflammatory (CD-4, CD8, IL-6, IL-1β, and TLR-4) and proapoptotic (Bax and caspase-3) markers in ochratoxin challenged broilers were presented in Table 6 and Fig. 5. The birds in G2 exhibited a significant elevation in the hepatic

gene expression inflammatory (CD-4, CD8, IL-6, IL-1β, and TLR-4) and proapoptotic (Bax and caspase-3) markers in contrast with G1 while both G3 and G4 showed a significant improvement their expression when compared with G2. The hepatic inflammatory (CD-4, CD8, IL-6, IL-1β, and TLR-4) and proapoptotic (Bax and caspase-3) markers revealed a significant improvement in contrast with G2.

Histological examination: No pathological abnormalities could be observed in G1 (Fig. 6). The histopathological findings in G1 at 22 and 34 days were the same as the heart sections showed normal coronary and intermuscular blood vessels, normal cardiomyocytes with preserved sarcoplasm, and sarcomere science. The epicardium and the endocardium are normal, with preserved mesothelial and endothelial cells, respectively. The interstitial tissue appeared normally and free from inflammatory cells or inflammatory oxidase. No pathological changes could be observed. The splenic tissue of G1 showed red pulp of reticuloendothelial cells with round cell infiltration and ill-distance white pulp lymphoid follicles comprising central articulos with ill-distance germinal central's mantle and marginal zone. A thin fibrous splenic capsule and a few strands of fibrous trabocco are within the splenic tissue. The examined section from the liver of G1 showed hepatic lobes with ill distance bounders and preferred located portal triads enclosing some bile ducts and blood vessels (portal vein and hepatic arteries). The hepatocytes appeared as masses of polygonal cells separated by hepatic sinusoidal lined by endothelial cells and Van-Kupffer cells. No degenerative necrotic or inflammatory changes could be observed. The renal section of G1 showed normal avian-tied glomeruli and basophilic epithelial cells enclosed within the bowman capsule with a small empty urinary space. Capillary tuft endothelial cells are ill distance. A group of proximal, distal, and collecting tubules with preserved epithelial lining were seen. A main uni-nephric duct with transitional epithelial lining was also seen. No pathological abnormalities could be observed.

The histopathological sections of different tissues of birds in G2 showed that the heart of this group at 22 days showed mild dilatation of coronary and intermuscular blood vessels with mild edematous changes. Focal marked intermuscular edema associated with atrophy and degenerative changes of the cardiomyocytes with occasional myomalissia could be seen. Focal subepicardial aggregation of lymphocytes and macrophages could be detected. The epicardium and the endocardium showed mild edematous changes. As seen in Fig. 7, at 34 days, the examined section of the heart showed epicardial edema, hemorrhage, and congestion of the epicardial blood vessels. The changes mentioned previously were also detected with characteristic intermuscular vascular dilatation and hemorrhage. Focal vascular wall degeneration represented by vacuolated tonica media and intimal destruction with thrombotic changes could also be observed with marked congestion of the coronary blood vessels. In G2, the section from the spleen at 22 days showed reactive lymphoid follicles with prominent general centers showing follicle diabetic changes. The red pulp is markedly infiltrated by lymphatic cells with multifocal degenerative and apoptotic changes.

At 34 days, the section from the spleen showed focal interstitial necrosis and hemorrhage. Focal histiocytic proliferation of the red pulp could be detected. Some white pulp follicles were normal regarding the cellular population and structure, while adults showed immunosuppressive changes (decreased cellular populations).

The section from the liver from G2 at 22 days showed moderately dilated portal blood vessels with occasionally perivascular edema and lymphocytic aggregates. Focal interstitial aggregates of lymphocytic could be detected. Some bile ducts showed proliferative changes with partial ductal fibrosis and leukocyte infiltration. The periportal hepatocytes showed intercellular edema, pressure atony, and disorganization.

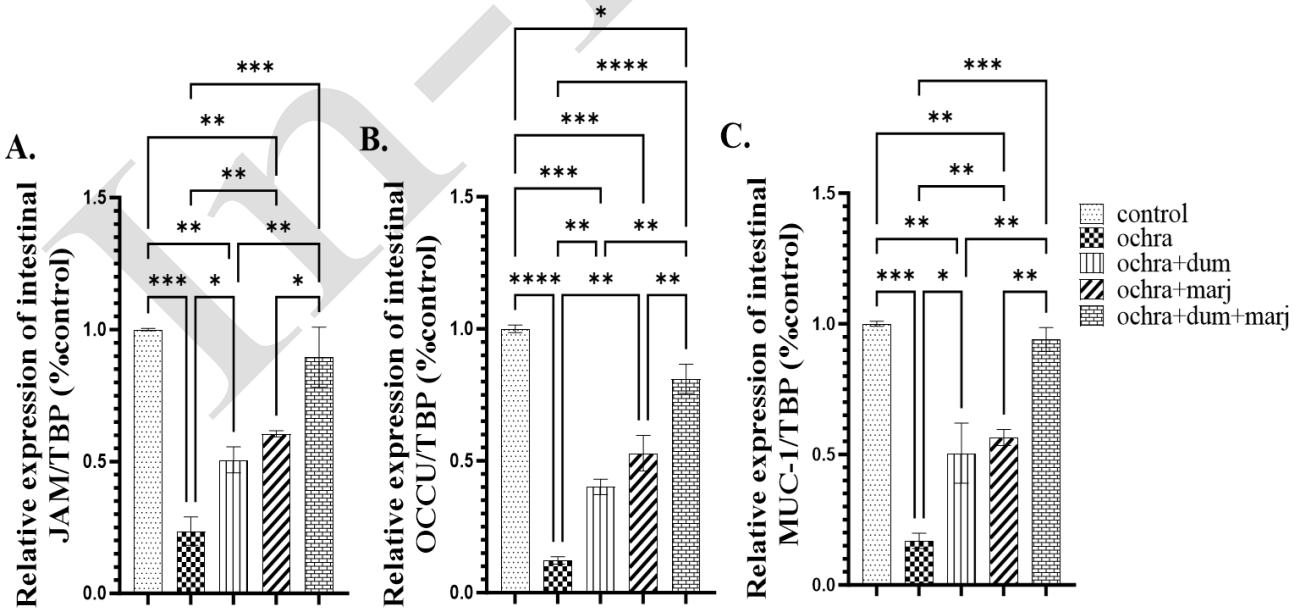

Fig. 3: The effect of marjoram and doum feed supplementation on the relative expression of the intestinal health markers [JAM (A), OCCU (B), and MUC-1 (C)] in ochratoxin-challenged broilers. The asterisks above the bars indicate the level of statistical significance**:** * P<0.05, ** P<0.01, *** P<0.001, **** P<0.0001. This indicates that many of the observed differences are statistically significant, particularly the effects of ochra compared to the control and the protective effects of dum and marjoram extract against ochra-induced changes in intestinal health markers.

Table 5: The effect of marjoram and doum feed supplementation on the relative expression of the intestinal health markers (OCCU, JAM, and MUC-1) in ochratoxin-challenged broilers.

	Genes	G1	G2	G3	G4	G5
Intestine	JAM	1.0000±0.0034	0.2363±0.0381	0.5064±0.0350	0.6050±0.0083	0.8955±0.0804
	OCCU	1.0000±0.0104	0.1219±0.0097	0.4010±0.0208	0.5288±0.0475	0.8104±0.0392
	MUC-1	1.0000±0.0069	0.1690±0.0204	0.5049±0.0815	0.5648±0.0215	0.9400±0.0325

Data are presented mean±SD.

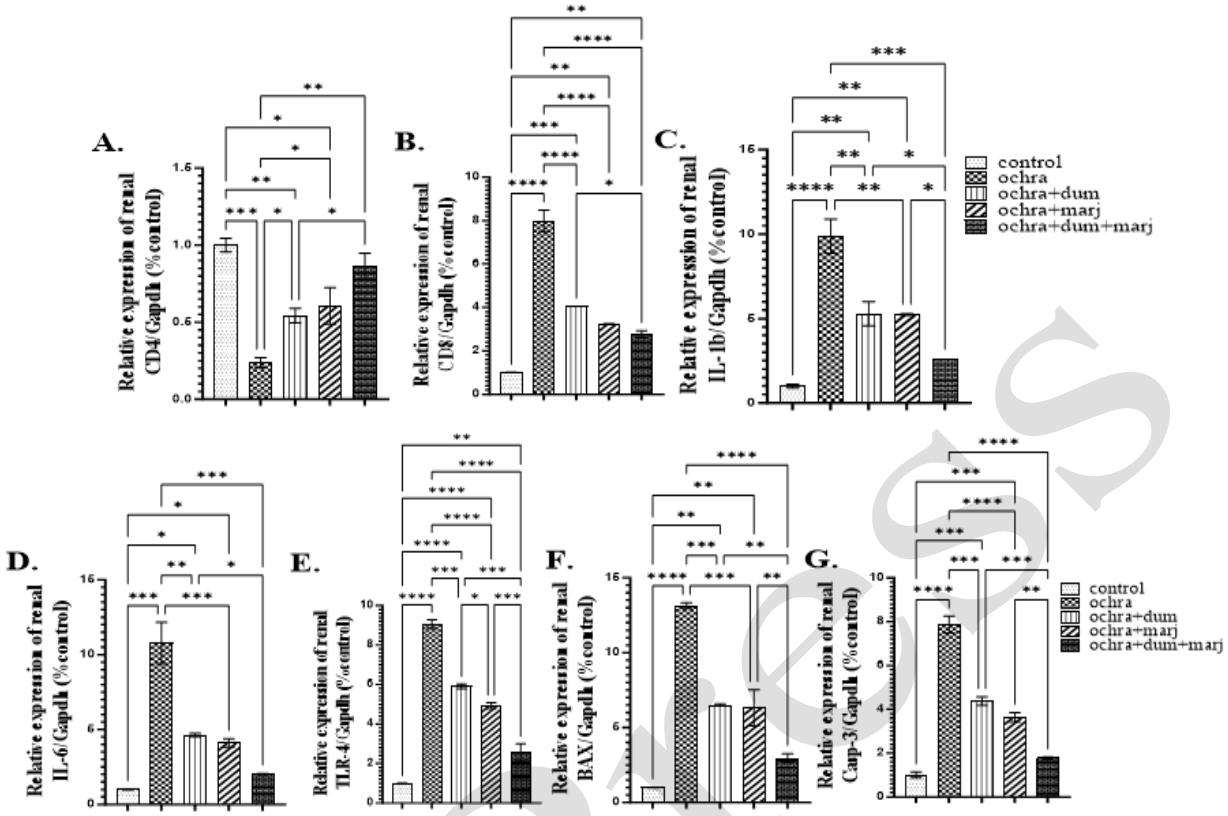

Fig. 4: The effect of marjoram and doum feed supplementation on the relative expression of the renal inflammatory [(A) CD-4, (B) CD8, (C) IL-1β (D) IL-6, and (E) TLR-4) and proapoptotic [(F) Bax and (G) caspase-3) markers in ochre toxin challenged broilers. The asterisks above the bars indicate the level of statistical significance: * P<0.05, ** P<0.01, *** P<0.001, **** P<0.0001. This indicates that most of the observed differences are statistically significant, particularly those comparing the ochra group to the control and the other-treated groups to the ochra group alone.

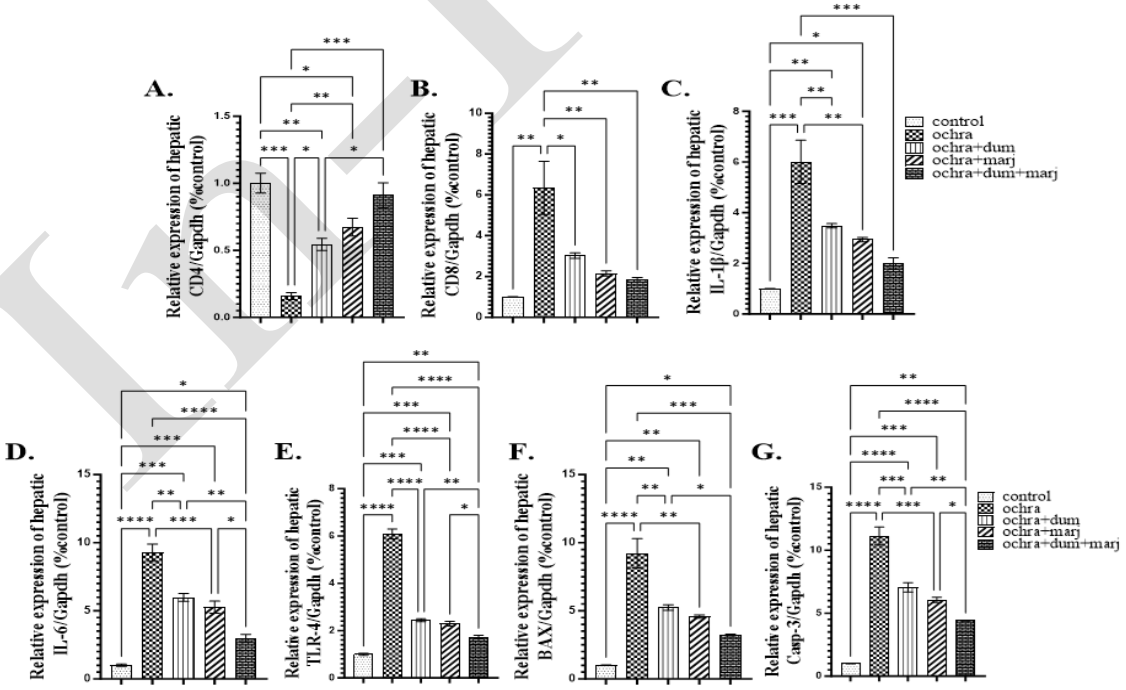

Fig. 5: The effect of marjoram and doum feed supplementation on the relative expression of the hepatic inflammatory [(A) CD-4, (B) CD8, (C) IL-1β, (D) IL-6, and (E) TLR-4) and proapoptotic [(F) Bax and (G) caspase-3) markers in ochre toxin challenged broilers. The asterisks above the bars indicate the level of statistical significance: * P<0.05, ** P<0.01, *** P<0.001, **** P<0.0001. This indicates that most of the observed differences are statistically significant, particularly those comparing the ochra group to the control and the other-treated groups to the ochra group alone.

Table 6: The effect of marjoram and doum feed supplementation on the relative expression of the liver and renal inflammatory (CD-4, CD8, IL-6, IL-1β, and TLR-4) and proapoptotic (Bax and caspase-3) markers in ochre toxin challenged broilers.

	Genes	G1	G2	G3	G4	G5
Kidney	CD-4	1.0004±0.0312	0.2375±0.0221	0.5425±0.0338	0.6066±0.0835	0.8636±0.0597
	CD-8	1.0002±0.0069	7.9804±0.3593	4.3071±0.2512	3.2377±0.0112	2.7726±0.1152
	IL-1β	1.0026±0.0728	9.8752±0.7174	5.2845±0.5112	5.2235±0.0362	2.6117±3.1086
	IL-6	1.0001±0.0173	10.7842±0.9691	4.6274±0.0801	4.1030±0.1989	2.0211±0.0280
	TLR-4	1.0000±0.0104	9.0644±0.1570	5.9181±0.0820	4.9427±0.1027	2.5601±0.3177
	BAX	1.0000±0.0069	13.1326±0.1365	6.4981±0.0450	6.3121±0.8480	2.9461±0.2140
	Casp-3	1.0043±0.0937	7.8672±0.2725	4.3793±0.1365	3.6458±0.1515	1.7779±0.0308
Liver	CD-4	1.0013±0.0520	0.1581±0.0180	0.5443±0.0320	0.6729±0.0465	0.9099±0.0661
	CD-8	1.0000±0.0069	6.3432±0.9168	3.0224±0.0942	2.1454±0.0891	1.8358±0.0826
	IL-1β	1.0000±0.0034	6.0096±0.6019	3.4827±0.0603	2.9389±0.0611	1.9925±0.1584
	IL-6	1.0015±0.0554	9.2644±0.4491	5.9829±0.2072	5.2689±0.3100	2.9858±0.1963
	TLR-4	1.0003±0.0277	6.0857±0.1476	2.4371±0.0422	2.2905±0.0714	1.7239±0.0537
	BAX	1.0001±0.0138	9.2213±0.7652	5.2435±0.1453	4.5795±0.0793	3.2268±0.0447
	Casp-3	1.0000±0.0069	11.1306±0.5011	7.0423±0.2683	6.0436±0.1465	4.4077±0.0305

Data are presented mean±SD.

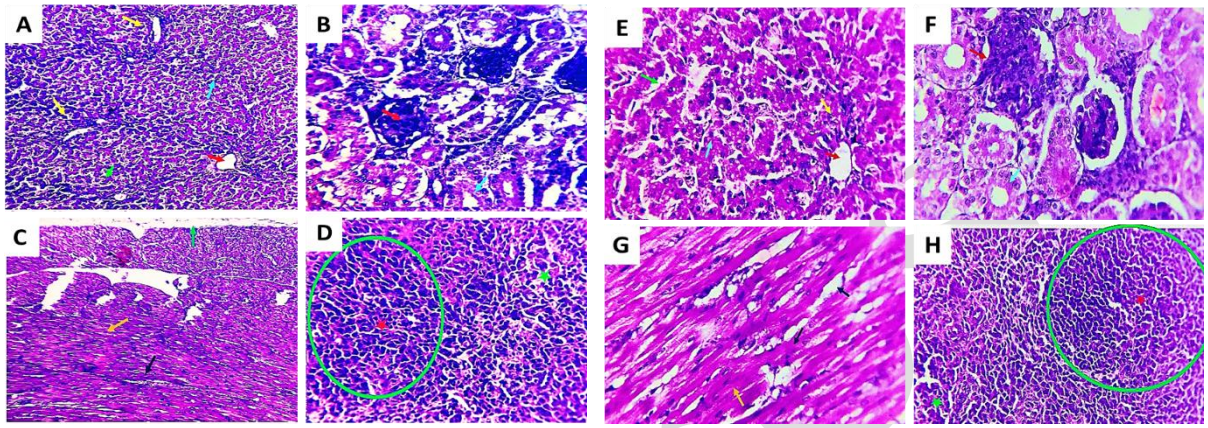

Fig. 6: Photomicrographs from liver, kidney, heart, and spleen of control-free chicks 34 days (from the start of the experiment showing normal micro-morphology of the corresponding organs. A (X 100) & E (X 400): The liver tissue showing A: preserved hepatic portal structures (yellow arrows), central veins (red arrow), sinusoids (green arrow), and hepatocytes (light blue arrow). B (X 100) & F (X 400): The kidneys show normal avian glomeruli (red arrow) and renal tubules (light blue arrows). C (X 100) & G (X 400): The heart represents the normal epicardium (dark green arrow), myocardium (orange arrow), and interstitium (black arrow). D (X 100) & H (X 400): The spleen shows normal white pulp lymphoid tissue (green circle, red asterisk) and red pulp sinusoids and splenic cords (green asterisks). H &E X100 and 400.

At 34 days, a section from the liver showed that focal centrilobular and periportal hepatocellular degenerative and necrotic changes were detected besides the previously mentioned changes. The section from the kidney of birds in G2 at 22 days showed characteristic glomeruli epithelial and endothelial proliferative changes with complete obstruction of urinary species. The tubular epithelial cells showed multifocal epithelial proliferation with large hyperchromatic nuclei. The collecting tubules are widely dilated and partially impacted by bluish-tinged materials and epithelial lining degeneration. Some proximal and distal collected tubules showed degenerative and apoptotic changes with occasionally inter-tubular hyaline cast formation.

The kidney blood vessels are markedly dilated as well as it shows perivascular edema with occasional interstitial hemorrhage. Focal tubular degenerative and apoptotic changes are seen. Thirty-four days showed focal interstitial hemorrhage, focal interstitial lymphocytic aggregation, and renal tubular dilation with epithelium regeneration. Marked congestion of the renal blood vessels is seen. Focal tubular epithelium regeneration and collecting tubule dilatation with inter-tubular homogeneous bluish materials (supposed to be early gouty deposition). The glomeruli epithelial and endothelial cells are hyperplastic and hypertrophic.

In G3, an examined section from the heart of this group at 22 days showed moderate focal interstitial and myocardium degeneration. Cardiomyocyte degenerative and myomalissia changes are also seen. The coronary blood vessels showed perivascular edema and medial wall degeneration with mild epicardial edema. At 34 days, as seen in Fig. 8, an examined section of the heart showed mild intermuscular and perivascular edema. Moderate congestion of the coronary blood vessels with congestion of the intermuscular capillary is seen. Mild interstitial edema and cardiomyocyte degeneration are also present. The section from the spleen at 22 days showed a moderate decrease in the white pulp lymphoid follicles' cellular population and an increase in the red pulp histiocytic cellular population with focal interstitial depletion of the lymphoid tissue represented by necrosis and empty spaces. As seen in Fig. 9, at 34 days, a section from the spleen showed well-formed, organized white and red pulp.

Some lymph follicles are normal, and a few others showed lymphoid depletion, also seen in some areas of the red pulp with characteristic pericapillary histiocytic proliferation with endothelial swelling. Focal depletion of the lymphocytes of the white pulp with pyknotic changes of the nuclei. The sections from the liver at 22 days showed portal pelary proliferation, and few lymphocytic infiltrations were seen. The hepatocytes and other hepatic structures are normal with occasional mild sinusoidal dilatation, Van-Kupffer cells hypertrophy, and hepatocellular hydropic degeneration. Some of the hepatic blood vessels are moderately dilated and engorged with

erythrocytes. No other pathological changes could be detected. At 34 days, sections from the liver showed focal interstitial lymphocytic aggregates and mild hepatocellular degenerative with Focal hepatocellular necrotic.

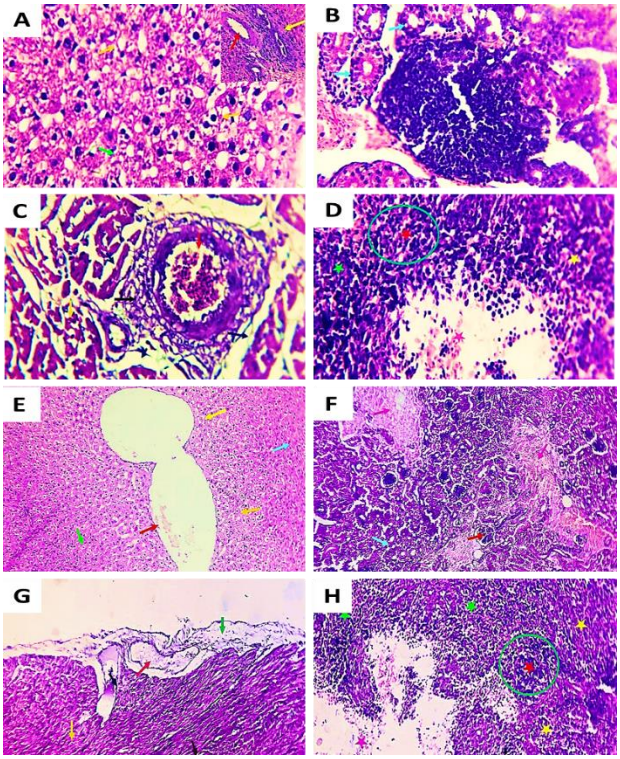

Fig. 7: Photomicrographs from liver, kidney, heart, and spleen of G2 at 34 days, A (X 100) & E (X 400): Liver tissue showing focal centrilobular and periportal hepatocellular degenerative and necrotic changes (orange arrow) beside portal vascular dilatation (red arrow) and biliary proliferation with associated round cells infiltration (yellow arrow). B (X 100) & F (X 400): Kidney lesions are represented by interstitial hemorrhage (purple arrows), focal interstitial lymphocytic aggregation (white arrows), and renal tubular dilation with associated epithelium regeneration (light blue arrow). F: The glomeruli epithelial and endothelial cells are hyperplastic and hypertrophic in some parts and atrophied or lobulated in others (red arrow). C (X 100) & G (X 400): The heart demonstrates epicardial edema, hemorrhage, and congestion of the epicardial blood vessels (green and red arrows). G: Mild dilatation of the intermuscular blood vessels with mild edematous changes can be observed (red and black arrows). Also Cardiomyocytic degenerative and necrotic changes (orange arrow) with moderate interstitial edema can be seen (dark blue arrow). D (X 100) & H (X 400): The spleen shows focal interstitial necrosis and hemorrhage (purple asterisk). H: Immunosuppressive changes decrease in the cellular populations (green circle, red asterisk) and focal histiocytic proliferation of the red pulp (yellow asterisk). The splenic cords appear mildly depleted (green asterisk). H&E X 100, 400.

The sections from the kidney at 22 days showed moderate renal tubular degenerative, peptotic, and necrotic changes with mild to moderate dilation of the kidney blood vessels with perivascular edema. Some of the collecting tubules were dilated. Focal interstitial hemorrhage is also seen. Some renal glomeruli showed hypertrophic epithelial and endothelial cells with obliteration of the urinary space. Focal tubular epithelial regeneration is also seen. At 34 days, the sections showed focal renal tubular epithelial degeneration and necrosis. Some glomeruli show shrinkage of the endothelial cells with focal epithelial regeneration and focal vascular and collecting tubular dilatation. Some renal glomeruli showed proliferative changes of the epithelial endothelial cells with obliteration of the urinary space.

In G4, sections from the heart at 22 days showed normal epicardium, endocardium coronary, and intermuscular blood vessels with normal cardio-myosis. As seen in Fig. 10, at 34 days, the examined section showed the same results. The splenic tissue of this group at 22 days showed normal structure configuration of the white pulp and red pulp with preserved germinal central of the white pulp beside the splenic cord and splenic sinusoids of the red pulp. No histopathological changes could be detected in any of the examined sections. At 34 days, the examined section showed the same results.

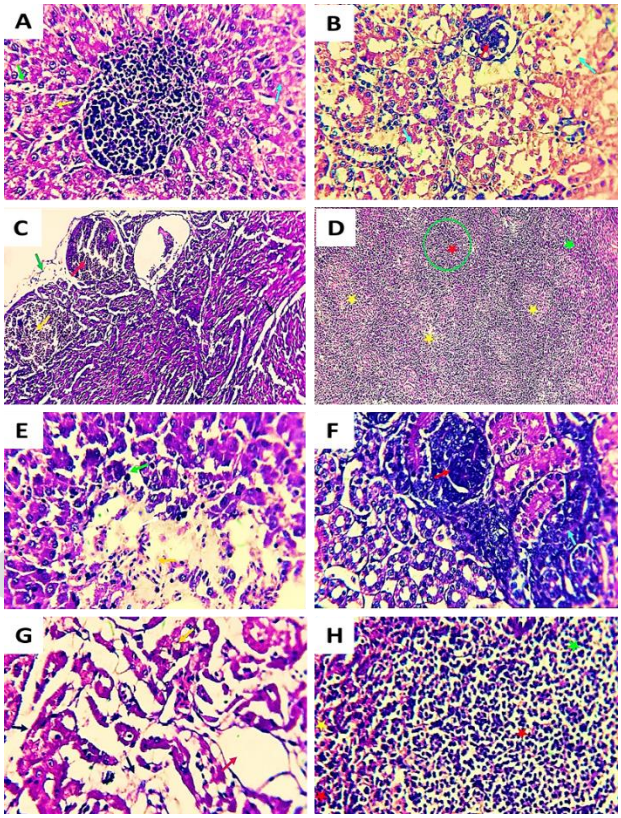

Fig. 8: Photomicrographs from liver, kidney, heart, and spleen of G3 at 34 days, A (X 100) & E (X 400): Liver showing interstitial lymphocytic aggregates (white arrow) and mild hepatocellular degenerative with occasional focal hepatocellular necrotic changes (orange arrow). B (X 100) & F (X 400): Focal renal tubular epithelial degeneration and necrosis with occasional focal epithelial regeneration (black asterisk, light blue arrows) are seen. Some of the glomeruli show shrinkage of the epithelial-endothelial cells. Other renal glomeruli show proliferative changes of the epithelial endothelial cells with obliteration of the urinary space (red arrows). C (X 100) & G (X 400): Mild epicardial (green arrow) intermuscular edema (dark blue arrows) is seen. Congestion of the coronary and intramuscular blood vessels and cardiomyocyte degeneration are also seen (red and orange arrows). D (X 100) & H (X 400): Some of the splenic lymph follicles are normal, and few others showed lymphoid depletion with nuclear pyknosis (green circle, red asterisk), which was also seen in some areas of the red pulp with characteristic pericapillary histiocytic proliferation (yellow asterisks). H&E X 100, 200, 400.

The section from the liver at 22 days showed completely normal and healthy hepatic tissue with preserved portal area structure. Hepatic sinusoids and hepatocytes are normal. No other pathological lesions could be detected. At 34 days, the examined section showed the same results. The section from the kidney at 22 days showed glomeruli epithelial and endothelial proliferative with mild vascular dilatation. Focal tubular epithelium regeneration with mild focal interstitial

hemorrhage (maybe post-vaccinal reactions). At 34 days, the examined section showed mild to moderate epithelial and endothelial proliferative of the glomeruli with mild vascular dilatation.

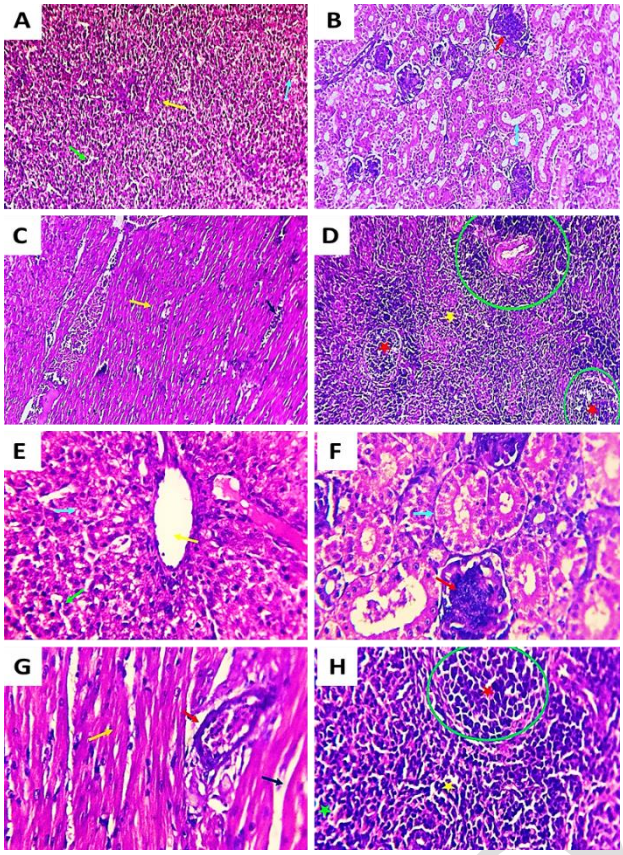

Fig. 9: Photomicrographs from the liver, kidney, heart, and spleen of G4 at 34 days, showing normal and healthy hepatic tissue with preserved portal area structure (red and yellow arrows). **A (X 100) & E (X 400):** Hepatic sinusoids and hepatocytes are normal (green and light blue arrows). **B (X 100) & F (X 400):** There is mild to moderate renal epithelial and endothelial proliferative of the glomeruli. **C (X 100) & G (X 400):** The epicardium (red arrows) and interstitium (dark blue arrows) are normal with normal cardiomyocytes (orange arrows). **D (X 100) & H (X 400):** The spleen shows normal structure configuration of the white pulp and red pulp with preserved germinal central of the white pulp (green circles and red asterisks) beside splenic cord and splenic sinusoids of the red pulp (light green and yellow asterisks). H&E X 100, 400.

In G5, sections from the heart at 22 days showed normal features of epicardium, endocardium, and myocardium. The coronary and intermuscular blood vessels appeared normal in structure and perivascular tissue. The interstitial and cardiomyositic tissue are also normal and free from pathological changes. In a few sections, some of the cardiomyogenic factors appeared vacuolated. Mostly fatty degeneration was present. Some of the intermuscular blood vessels showed vacuolated tonica media with swelling of their endothelial cells. At 34 days, an examined section of the heart showed vocal mild interstitial edema and cardiomyocyte atrophy. The epicardium, endocardium, and coronary blood vessels appeared normally. The remaining cardiomyocytes were also free from any pathological changes. The section from the spleen at 22 days showed normal structure configuration of the white pulp and red pulp with preserved moderate activated germinal central and normal red pulp cords and sinusoids. Focal histiocytic reaction (histocytosis) could be detected at some parts of the red pulp. At 34 days, a section from the spleen showed congestion of the red pulp sinusoids in some spleen areas. Other changes are comparable to those of the previous group.

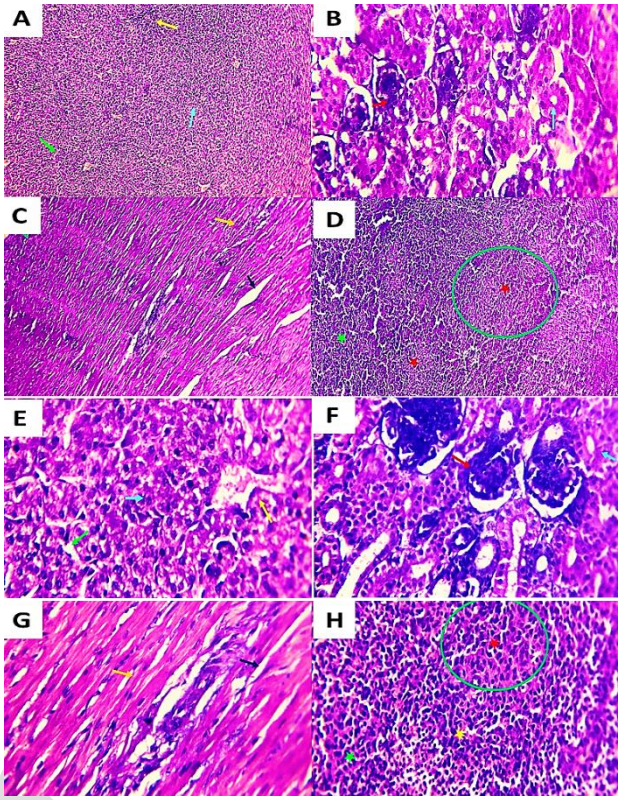

Fig. 10: Photomicrographs from liver, kidney, heart, and spleen of G5 at 34 days **A (X 100) & E (X 400):** showing hepatic tissue with a normal preserved portal area (yellow arrow) hepatocytes and hepatic sinusoids (light blue and green arrows). No pathological changes can be seen. B (X 100) & F (X 400)**:** The kidney reveals a normal renal structure free from any pathological changes (red and light blue arrows**). C (X 100) & G (X 400):** The epicardium (green arrow) appears normal, but mild interstitial edema and cardiomyocyte atrophy (dark blue and orange arrows) are seen. **D (X 100) & H (X 400):** Normal structure configuration of the splenic white pulp and red pulp with preserved moderately activated germinal central (green circle and red asterisks) and apparently normal red pulp cords and sinusoids are seen (yellow and green asterisks). Congestion of the red pulp sinusoids in some spleen areas can be observed (black asterisk). H&E X 100, 400.

The section from the liver at 22 days showed that most hepatic tissue appeared normally with a preserved portal area. Hepatic mass (hepatocytes in poultry normally arranged in an aggregated mass of hepatocytes) and hepatic sinusoids. Hepatic blood vessels and sinusoids are mildly dilated, and a few hepatocytes are mildly degenerated. Very few numbers of round cells were seen in the portal area. At 34 days, liver sections showed normal hepatic tissue comparable to the previous group. No pathological lesions could be detected in the examined sections (Fig. 11). The sections of the kidney at 22 days showed mild focal interstitial hemorrhage and tubular epithelium degeneration. Some of the glomeruli showed epithelial and endothelial reactions. Focal epithelial regeneration with large hyperchromatic nuclei could be seen. Mild focal interstitial lymphocytic aggregations are seen. At 34 days, it showed a normal renal structure free from pathological changes (Fig. 11).

Antioxidant parameters: As seen in Table 7 and Fig. 12, the impact of marjoram and doum feed supply on the antioxidant parameters on day 22 was observed as follows: the MDA levels showed the lowest level in G1 and the highest level in G2. At the same time, G3, G4, and G5 revealed a significant improvement in MDA levels contrasted to G2. At 34 days old, birds in G2 showed a significant increase in MDA level and a significant decrease in SOD, CAT, and GPx levels in comparison with G1. Birds in G3, G4, and G5 revealed a significant improvement in antioxidant parameters compared to G2 (Table 7 and Fig. 12).

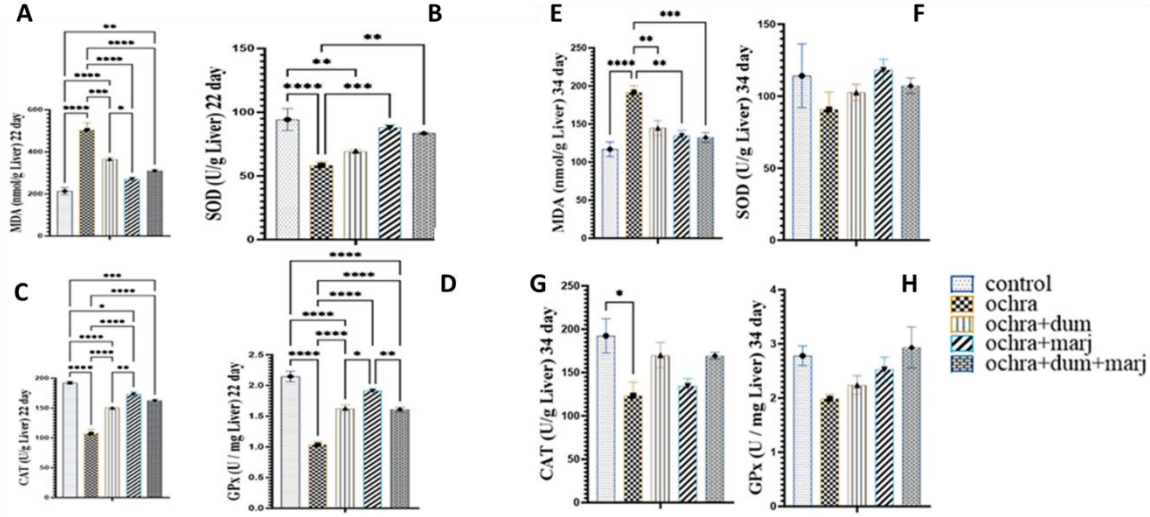

Fig. 11: The effect of marjoram and doum feed supplementation on antioxidant parameters (A and E) refers to the impact of marjoram and doum on lowering MDA at 22 and 34 days old compared to infected control. (B and F) showed the effects of marjoram and doum on enhancing SOD at 22 and 34 days old compared to control. (C and G) showed enhancing CAT at 22 and 34 days old. (D and H) enhancing GPx activity in marjoram and doum-treated broilers compared to the control. The asterisks above the bars indicate the level of statistical significance**:** * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, **** $p < 0.0001$. This shows that many of the observed differences are statistically significant, particularly the effects of ochra compared to the control and the protective effects of dum and marj extract against ochre-induced oxidative stress.

Table 7: The effect of marjoram and doum feed supplementation on antioxidants defense system in Ochra-challenged broiler at day 22 and day 34.

Antioxidant	G1		G2		G3		G4		G5	
parameters	D22	D34	D22	D34	D22	D34	D22	D34	D22	D34
MDA (nmol/g Liver)	117±9.56	213.43±16.4	191.8±8.57	504.77±33.3	144.7±9.80	365.43±7.46	134.66±6.9	270.93±7.05	132.53±6.41	310.3±6.67
SOD (U/g Liver)	114.16±22.1	94.26±8.64	90.77±12.15	58.333±3.06	102.6±5.61	69.53±1.23	118.2±7.52	87.8±2.13	107.21±5.32	83.487±0.95
CAT (U/g Liver)	192.3±19.7	192.33±2.33	123.33±15.5	107.33±6.64	170±14.74	150.33±1.45	134.33±8.68	173±4.61	169.33±3.84	162.66±2.02
GPx (U / mg Liver)	2.78±0.18	2.14±0.08	1.98±0.08	1.0333±0.052	2.24±0.17	1.62±0.059	2.52±0.22	1.913±0.01	82.933±0.37	1.61±0.036

Data are presented mean±SD.

Immunohistochemical finding: As noticed in Table 8 and Fig. 13, the area percentage of liver and kidney caspase 3 appeared to have a significant elevation in G2 in contrast with G1. At the same time, birds in G3, G4, and G5 revealed a significant improvement in the percentage of caspase 3 in the hepatic and renal area, in contrast to G2.

Table 8: The effect of marjoram and doum feed supplementation on the percentage of caspase-3 in liver and kidney

Treatments	Liver	Kidney
G1	1±0.2887	1.1667±0.4409
G2	44.6667±4.0551	40.3333±3.7565
G3	31.3333±1.4529	29±2.08167
G4	20±2.08167	16.3333±2.3333
G5	8.3333±1.4529	6.6667±1.4529

Data are presented mean±SD.

The immunostaining sections of the liver against Caspase-3 (Fig. 13A-E) showed negative expression in the control group feed on normal ration "G1" (Fig. 13A). A marked cytoplasmic expression in abundant hepatocytes was noticed in the group fed on a ration with mycotoxin "G2" (Figure 13B). A moderate count of positive expressed cells was observed in both groups fed on a ration with mycotoxin then treated with doum "G3" (Fig. 13C) and the group fed on a ration with mycotoxin then treated with marjoram "G4" (Fig. 12D). On the other hand, a few immuno-expressed cells were demonstrated in a group fed on a ration with mycotoxin and treated with a combination of doum and marjoram "G5" (Fig. 13E). Area % of expression for Caspase-3 staining within different groups was tabulated in Table 8.

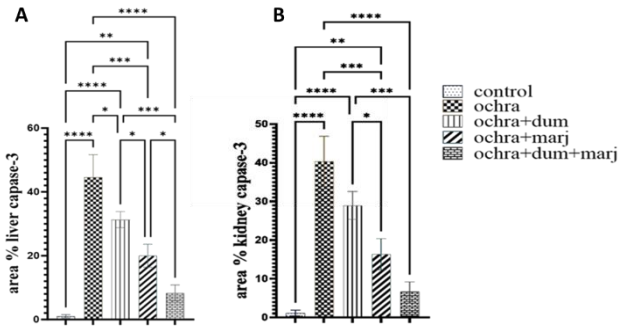

Fig. 12: (A) Area % of liver caspase-3 showed a reduction in apoptosis marker in G5 compared to control. (B) % kidney caspase-3 showed a decrease in apoptosis marker in G5 compared to control. The asterisks above the bars indicate the level of statistical significance**:** * P<0.05, ** P<0.01, *** P<0.001, **** P<0.0001. This suggests that the differences observed between the ochra group and the control group and between the ochra + marj and ochra + dum + marj groups compared to the ochra group are highly statistically significant in both the liver and kidney.

The immunostaining sections of the kidney against Caspase-3 (Fig. 13F-9J) showed non-detectable expression in the control group fed on normal ratio "G1" (Fig. 13F). Strong cytoplasmic expression within numerous renal tubular epithelia was noticed in groups fed on ration with mycotoxin "G2" (Fig. 13G). However, a moderate count of positive cells was observed in both groups fed on a ration with mycotoxin then treated with doum "G3" (Fig. 13H) and the group fed on a ration with mycotoxin then treated with marjoram "G4" (Fig. 13I). Conversely, the labeled cells containing caspase-3 expression were markedly attenuated in a group fed on ration with mycotoxin and treated with the combination of doum and marjoram "G5" (Fig. 13J). The area % of expression for Caspase-3 staining within different groups was tabulated in Table 8.

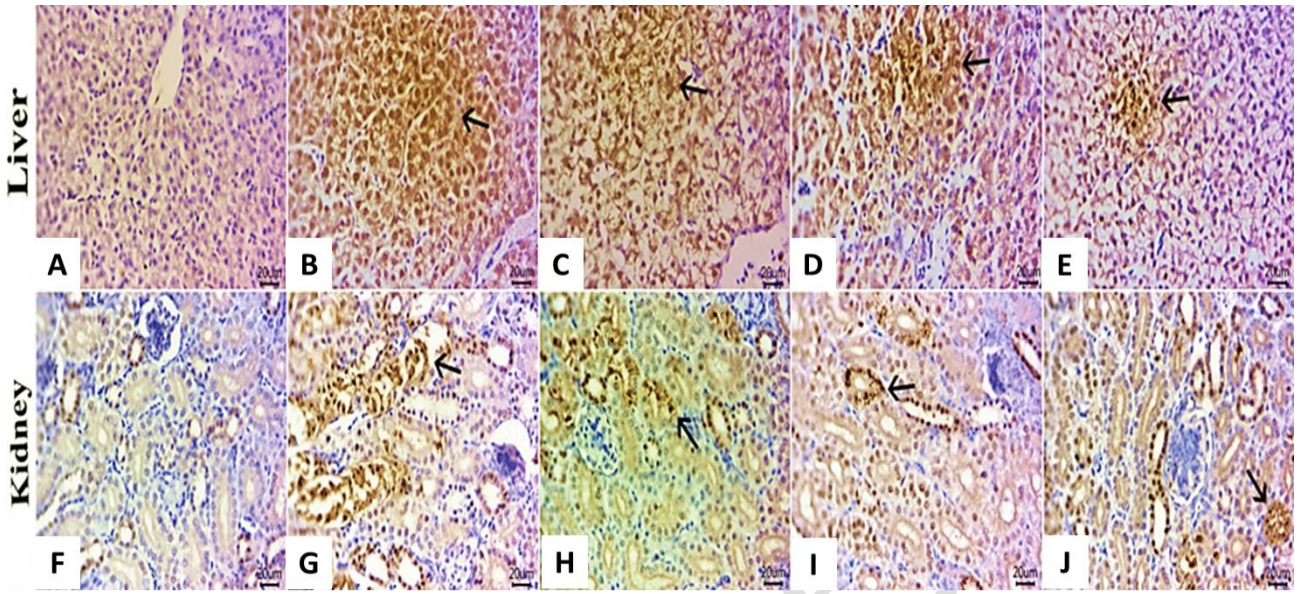

Fig. 13 A-E: Photomicrographs of liver tissue (A-E) stained for caspase-3 reveal negative expression in G1 (A), marked cytoplasmic expression in hepatocytes in G2 (B), moderate positive expression in G3 (C) and G4 (D), and few immuno-expressed cells in G5 (E); similarly, kidney tissue (F-J) shows non-detectable expression in G1 (F), strong cytoplasmic expression within numerous renal tubular epithelia in G2 (G), moderate numbers of positive cells in both G3 (H) and G4 (I), and markedly attenuated immunolabeled cells containing caspase-3 expression in G5 (J), with all tissues counterstained with Mayer's hematoxylin and arrows indicating positively stained cells. Varying levels of caspase-3 expression in both liver and kidney tissues across the different groups. Group G2 consistently shows the highest expression of caspase-3 in both tissues, Group G1 shows no detectable expression, and Group G5 shows the lowest expression among the treated groups. Groups G3 and G4 exhibit moderate expression levels. This visual representation of caspase-3 protein levels provides insight into the apoptotic activity within these tissues under different experimental conditions, as caspase-3 is a key enzyme in the apoptotic pathway.

DISCUSSION

Mycotoxicosis is a serious issue causing severe immunosuppressive effects due to atrophy in the thymus, bursa, spleen, and bone marrow depletion; it also causes renal and hepatic tissue damage, reduced productivity and final body weight of broiler chickens (Okasha *et al.*, 2024; Jasim *et al.*, 2025). Therefore, to improve the performance, immunity, and health of birds and livestock, it was essential for researchers to investigate new feed additives in the avian nutrition system (Salem et al., 2022; Abd El-Hack *et al.*, 2023; Al-Nabati *et al.*, 2024; Hegazy *et al*., 2024). Several findings have reported that using raw plant extracts, derived phytogenic compounds, probiotics and biological nanoparticles as feed additives in avian diet might positively affect birds' health and productivity (Yaqoob *et al.*, 2021; El-Kassas *et al.*, 2022; Alsulami and El-Saadony, 2024). They could reduce the negative impact of different stressors on birds, including environmental stress, especially heat stress, pathogen stress including (viral, bacterial, mycotic, and parasitic), and reduce the adverse effect of most feed contaminants, especially mycotoxins (El-Shall *et al.*, 2022; El-Saadony *et al.*, 2023a; Soliman *et al.*, 2024).

Fruit processing byproducts, such as peels and pomace, are increasingly recognized as valuable reservoirs of bioactive compounds with significant biological functions and potential health benefits for humans and animals (Saad *et al.*, 2021a,b; Mueed *et al.*, 2023, 2024; Alharbi *et al.*, 2024). These materials contain diverse natural substances, including tannins, flavonoids, alkaloids, phlorotannins, terpenoids, and glycosides. Sources rich in these compounds include banana peels, doum fruit and peels, and licorice (El-Saadony *et al.*, 2023b,c; El-Saadony *et al.*, 2024a,b). These naturally occurring compounds contribute substantially to the potential medicinal applications of these often-discarded materials. For example, specific extracts from peels are high in the flavonoid rutin have shown notable promise due to their demonstrated antitumor, antioxidant, and antimicrobial properties (Yu, 2012; Reda *et al.*, 2021). This underscores the potential to transform fruit waste into valuable resources for developing natural health products or functional ingredients.

The current study revealed that all groups showed non-significant differences in BW or feed consumption. In contrast, the PM lesions of sacrificed chicks of all groups were normal and revealed no lesions in different organs except G2 at 22 and 34 days old. The birds showed moderately enlarged livers with distended gallbladders, inflamed intestines, congested kidneys distended with urates, and congestion in the heart. At the same time, on

day 34, all the previous lesions were present, and the liver showed some necrotic changes. Our results concur with several studies that found adding *Origanum majorana* in powder or oil extract did not impact broilers' internal organ weights (Shawky *et al.,* 2020; Vlaicu *et al.,* 2020).

On the other hand, Ocak *et al.* (2008) found that the supplementation of marjoram plant extract significantly ($P<0.01$) enhanced the broilers' body weight, weight gain, and feed intake of broiler chickens during the last stage at day 29-36), the body weight of the group supplemented with marjoram plant extract was significantly higher ($P<0.05$). It could be inferred that the addition of marjoram extract as a growth promoter into chickens' diet was followed by a significant enhancement of broilers' development measures. This result could be due to the role of the herbal extract applied herein in improving nutrient digestion, balancing the intestinal microbial environment, and evoking the secretion of endogenous digestive enzymes, which in turn can result in the enhancement of broiler performance parameters (Ocak *et al.,* 2008).

Also, the broiler response to feed supply with prebiotics, probiotics, or herbal mix (*Origanum majorana*, *Carum carvi*, and *Foeniculum vulgare*) as alternatives to antibiotics showed that the herbal mixture group noted the highest productive measures (Enas *et al.,* 2019). From our observations, the effect of marjoram and doum feed supplementation on liver functions, kidney functions, and humeral immunity at day 22 indicated that the renal function showed the highest level of creatinine and uric acid in G2 and the lowest levels in G1, the treated birds in both G3, G4 and G5 showed improvement in renal function when compared with G2. The hepatic functions include ALT and AST. ALP, TP, albumin, and A/G ratio levels showed the ideal parameters in G1 in contrast to G2, the hepatic function indicators showed the highest ALT, AST, and ALP levels with the lowest TP, Albumin, and A/G ratio levels, while birds in treated groups showed improvement in hepatic function parameters in contrast with G2.

This improvement may be attributed to the fact that doum fruit has a high-quality protein varied between 2.86 and 5.01%, the high proportion of lysine and cysteine of crude protein varied between 4.09–4.16% and 0.2–1.62%, respectively, the limited amino acid threonine, crude fat varied between 1.2 and 8.4%, crude fiber varied between 52.26 and 66.5%, the most important carbohydrates component was mannose varied between 13 and 75.9%, also the presence of calcium, magnesium, potassium, iron sodium and negligible amount of nickel, cobalt and molybdenum (Auwal *et al.,* 2013). Phytochemical compounds of doum fruit, such as tannins, saponin, steroids, glycosides, flavonoids, terpenes, and terpinoids were found at low and moderate levels, reducing sugars, glycosides, and some minerals, like cobalt, iron, and copper, may contribute to these impacts by motivating the production and maturation of blood cells (Auwal *et al.,* 2013).

Also, the improvement of humoral immune response may be owed to the fact that doum palm fruit could promote the synthesis of RBCs, WBCs, and other blood elements in the hematopoietic organs, resulting in the immune-modulatory impact and augmented disease resistance, as WBCs are known to be the vital players of the innate immune system (Whyte, 2007).

In addition, Coombe & Parish (2015) noticed that dietary doum palm fruit was found to enhance the non-specific immunological defenses (phagocytic activity, lysozyme, NO, and serum bound α 2,3-ST and α 2,6-ST also, doum palm fruit increased sialoglycans and a higher degree of glycosylation states by entrapping terminal sialic acid moieties, included in the glycoproteins. Different documents mentioned the impact of dietary date palm fruit extracts, phoenix dactylifera, a plant in the same family of doum palm, in enhancing growth, immunity, and antioxidant enzyme activity of common carp, *Cyprinus carpio* (Hoseinifar *et al.,* 2015, 2017). Likewise, marjoram at the level of 2% resulted in a significant elevation ($P<0.05$) in gamma globulin; it also appears to have an impact effect on immune responses and is considered as an immunity enhancer for broiler chicks (Toghyani *et al.,* 2010).

TP, albumin, creatinine, and urea were not changed by adding *Origanum majorana* powder (Shawky *et al.,* 2020). Globulin increased, while the ratio of albumin/globulin was decreased with *Origanum majorana* powder (Abdel-Wahab, 2019). Also, they suggested that the significant elevation in globulin indicates the Origanum's ability to enhance the immunity of broiler chicks; also, *Origanum majorana* has been reported to induce hypocholesterolemia (Abdel-Wahab, 2019; Saleh *et al.,* 2021).

In the current research, the effect of marjoram and doum feed supplementation on antioxidant parameters at day 22 was observed as follows: the MDA levels showed the lowest and highest levels in G2. At the same time, G3, G4, and G5 revealed a significant improvement in MDA levels compared to G2. At 34 days old, birds in G2 showed a significant increase in MDA level and a significant decrease in SOD, CAT, and GPx levels in comparison with G1. Birds in G3, G4, and G5 revealed a significant improvement in antioxidant parameters compared to G2. The antioxidant activities were improved when the ingested quantity of doum flour was increased, indicating that the effectiveness of its antioxidant and antibacterial features would have a positive impact on antioxidant status (Mohamed *et al.,* 2010a, b).

Also, Kolla *et al.* (2021) observed that the efficacy of the phenolic agents in the doum fruit extracts to donate hydrogen advances the scavenging features. Flavonoids & polyphenols are known to scavenge oxygen-free radicals and stop the fatty acids' lipo-peroxidation in the cell membrane and cellular oxidation (Ayoub *et al.,* 2011). High concentrations of these phytochemicals in it may, therefore, contribute to the antioxidant features of doum palm fruit (Mohamed *et al.,* 2010a; Ayoub *et al.,* 2011). In addition, *Origanum majorana* is characterized by its antimicrobial, antioxidant, immune stimulator, and metabolism-inducing features (Kordali *et al.,* 2022a, b).

The findings showed that OCCU, JAM, and MUC-1 gene expression appeared normal at G1. At the same time, they reveal a significant lowering in the gene expression of these genes, reflecting intestinal health in G2 that is challenged with ochratoxin. In contrast, G3 and G4 showed a slight improvement in their expression. In contrast, the dual treatment of birds by both doum and marjoram post-Ochratoxins challenge revealed a significant improvement in OCCU, JAM, and MUC-1 gene expression compared to G2. Also, the expression of renal inflammatory markers,

including (CD-4, CD8, IL-6, IL-1β, & TLR-4) and proapoptotic (Bax and caspase-3) markers appeared to be normal in G1 conversely, they revealed a significant increase in G2. The birds in both G3 and G4 revealed a significant improvement in the expression of the renal inflammatory (CD-4, CD8, IL-6, IL-1β, & TLR-4) and proapoptotic (Bax and caspase-3) markers compared to G2. At the same time, the combination of marjoram and doum feed supplementation in G5 revealed a significant improvement in the renal inflammatory (CD-4, CD8, IL-6, IL-1β, & TLR-4) and proapoptotic (Bax and caspase-3) markers when compared to G2.

In addition, the birds in G2 showed a significant elevation in the hepatic gene expression inflammatory (CD-4, CD8, IL-6, IL-1β, & TLR-4) and proapoptotic (Bax and caspase-3) markers in contrast with G1 while both G3 and G4 showed a significant improvement their expression when compared with G2. The hepatic inflammatory (CD-4, CD8, IL-6, IL-1β, & TLR-4) and proapoptotic (Bax and caspase-3) markers revealed a significant improvement in contrast with G2. These results may be owed to the fact that *Origanum majorana* has antioxidant, antimicrobial, immune stimulator, and metabolism-inducing features, and the active compounds in marjoram could protect against hepatic damage, thus lowering the level of hepatic enzymes (Yen & Park, 2021; Kordali *et al.,* 2022b). Also, doum palm fruit contains phytochemical compounds like tannins, steroids, saponin, flavonoids, glycosides, terpenes, and terpinoids that stimulate the synthesis and maturation of blood cells and improve hepatic and renal functions (Auwal *et al.,* 2013). Likewise, Demir *et al.* (2005) reported that the marjoram powder (1g/kg) in boiler feed increases total serum protein.

In this study, the histopathological alterations and immunohistochemistry of liver, kidney, spleen, and heart tissues were improved in the groups treated with doum palm fruit powder and marjoram palm powder separately or in combination. In a parallel study, Abdelatty *et al.* (2021) found that the intestinal villi length slight increase and intestinal absorption increased with *Origanum majorana* powder which led to enhancements in productivity accompanied by a numerical rise in broiler BW also, in the muscular layer, the cecal wall was thickened with *Origanum majorana* powder supply, scarce findings take morphometrical parameters for the cecum, despite its role in immunity, water absorption, digestion, and fermentation (Hunt *et al.,* 2019; Wang *et al.,* 2019). Caspase-3, a crucial executioner caspase, plays a pivotal role in apoptosis, a highly regulated process of programmed cell death essential for maintaining tissue homeostasis and normal development (Eskandari and Eaves, 2022).

Changes in caspase-3 expression directly reflect the level of apoptotic activity within tissues; increased expression typically signifies elevated apoptosis triggered by cellular stresses such as DNA damage, oxidative stress, or inflammatory signals, while decreased expression suggests a reduction in apoptosis, potentially due to protective mechanisms or pathway dysregulation (Eid and El-Shitany, 2021; Huang *et al.,* 2022).

Upon activation by initiator caspases like caspase-8 or -9 in response to apoptotic stimuli, caspase-3 initiates a cascade of events by cleaving numerous cellular substrates, leading to the hallmark morphological features of apoptosis, including DNA fragmentation, chromatin condensation, cell shrinkage, membrane blebbing, and the formation of apoptotic bodies (Halder *et al.,* 2024). Consequently, elevated caspase-3 activity and the resulting apoptosis can directly contribute to observed histopathological changes, such as tissue damage manifesting as lesions or necrosis, and potentially triggering secondary inflammation or altering cell proliferation patterns as tissues attempt to compensate for cell loss (Shoshan-Barmatz *et al.,* 2023).

In the context of the provided figure, the heightened caspase-3 expression observed in the ochra (ochratoxin A) group strongly indicates that ochratoxin A induces apoptosis in both liver and kidney tissues, while the subsequent reduction in caspase-3 expression in groups treated with doum and/or marjoram suggests a potential protective effect of these substances in mitigating ochratoxin A-induced apoptosis, which would likely correlate with less severe histopathological alterations in these treatment groups compared to those exposed to ochratoxin A alone.

Moreover, adding a mixture of *Origanum majorana* and another medicinal herbal plant to laying hen diets improved their performance, involving the FCR and egg quality and quantity (Saleh *et al.,* 2021). Similarly, Al-Khalaifah *et al.* (2020) improved the morphometric and histopathology of different tissues of the African catfish, *Clarias gariepinus*, supplied with doum palm fruit powder.

Conclusions: The dual use of doum fruit powder 100g/kg ration and marjoram powder 15g/kg ration reduced the negative impact of experimental ochratoxins associated adverse effects in broiler chickens ration as they improve the humoral immune response, decreased the histopathological and immunohistochemistry alterations of liver, kidney, spleen, and heart tissues also, they improve the antioxidant status and TBP, OCLN, MUC-1, JAM-2, CD4, CD8, IL-1b, IL6, TLR-4, BAX, Casp-3, and Gapdh genes expression. Doum fruit powder 100g/kg ration and marjoram plant powder 15 g/kg are recommended to efficiently ameliorate the adverse effects of ochratoxicosis in broiler chickens.

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