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Therapeutic Effects of *Silybum marianum* and *Taraxacum officinale* Extracts on Serum Biochemistry, Immune Function, Growth Performance, CYP3A4 Gene Expression, and Organ Integrity in Broilers Exposed to Mycotoxins

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Abstract | Poultry production is negatively influenced by mycotoxins, which can also harm various organs. This study investigated the therapeutic effects of *Silybum marianum* (milk thistle) and *Taraxacum officinale* (dandelion) extracts when administered to broilers previously fed a mycotoxin-contaminated diet. This study represents the second phase of a two-phase experiment. In the first phase, broilers were fed a diet containing 350 ppb aflatoxins and 180 ppb ochratoxins from 10 to 30 days of age. In the second phase (30–40 days), which constitutes the current study, 90 male Ross-308 chicks previously exposed to the mycotoxin-contaminated diet were transitioned to a mycotoxin-free diet and assigned to three groups: a control group (no herbal supplements), a group receiving milk thistle extract (300 mg/kg BW), and a group receiving dandelion root extract (300 mg/kg BW). The study measured growth performance, white blood cell counts, ALT, AST, ALP, creatinine, albumin, triglycerides, CYP3A4 gene expression, and histopathological evaluations of various organs. Results showed that both milk thistle and dandelion extracts significantly enhanced feed intake, body weight, and weight gain in broilers compared with the control group. Milk thistle supplementation achieved the best feed conversion ratio (1.53 ± 0.02), followed by the dandelion extract group (1.54 ± 0.02). Both extracts significantly increased WBC counts, with milk thistle extract (21.4 ± 0.07), and dandelion root extract (20.13 ± 0.05). Both extracts enhanced heterophil percentage relative to the control. They also lowered AST, ALT, and ALP levels, indicating reduced hepatic damage, and downregulated CYP3A4 gene expression (which catalyzes the bioactivation of aflatoxin to its toxic metabolite) compared to the control group. In contrast to the control group, which showed significant organ damage, both extracts improved liver, kidney, and spleen structure compared to the control group. Overall, milk thistle and dandelion extracts effectively mitigated the detrimental effects of mycotoxins when administered following mycotoxin exposure in broilers.

Keywords | Milk thistle, Dandelion, Growth performance, CYP3A4 gene expression

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INTRODUCTION

Mycotoxins, toxic by-products of fungal metabolism, are natural contaminants of agricultural products (Pitt *et al*., 2012). These compounds pose a significant concern for both food and feed safety because of their stability and resistance to common processing methods. Cereals such as maize, wheat, and barley, key components in livestock and poultry rations, are particularly vulnerable to fungal invasion under conditions of high humidity, inadequate drying, or poor storage (Monson *et al*., 2015). The mycotoxins of greatest relevance to animal health and human food safety include aflatoxins (AFs), fumonisins, and ochratoxins. Aflatoxins are chiefly generated by *Aspergillus flavus* and *Aspergillus parasiticus* (Dutta and Das, 2001). Fumonisins, mainly produced by *Fusarium* species, interfere with sphingolipid metabolism and are linked to neurological disorders, liver damage, and reduced growth performance in animals. Ochratoxins, including OT-A, OT-B, and OT-C, are generated by species such as *Aspergillus niger*, *Aspergillus ochraceus*, and *Penicillium verrucosum*. These toxins are nephrotoxic and have been implicated in immunosuppression and carcinogenesis. Mycotoxins undergo metabolic transformation primarily in the gastrointestinal tract, liver, and kidneys, with the pathways depending on their chemical structures and properties (Sklan *et al*., 2001). Among the wide spectrum of mycotoxins, certain types are recognized as highly toxic to both animals and humans. The World Health Organization (WHO) has classified aflatoxin B1 (AFB1) as one of the most hazardous compounds due to its strong carcinogenic potential (FAO/WHO, 2018). This risk is largely attributed to its biotransformation in the liver, where AFB1 undergoes conversion by hepatic microsomal CYP450 enzymes into the reactive intermediate AFB1-8,9-epoxide, a potent pro-carcinogen that binds to DNA and initiates mutagenic and carcinogenic processes (Rawal *et al*., 2010).

In poultry, exposure to mycotoxins, commonly referred to as mycotoxicosis, leads to profound negative effects on production performance. These effects include reduced body weight gain, impaired feed conversion ratio (FCR), and decreased egg weight, alongside systemic organ damage, particularly hepatotoxicity and nephrotoxicity. Such impairments often result in increased flock mortality and substantial economic losses in the poultry industry (Abidin *et al*., 2011; Alhidary *et al*., 2017; Tahir *et al*., 2022). To mitigate the risks associated with contaminated feed, various detoxification approaches have been investigated. These include physical methods such as thermal inactivation and irradiation, biological strategies such as microbial degradation of toxins, and chemical or nutritional interventions, most notably the incorporation of adsorbent materials into animal diets to bind and reduce toxin bioavailability (Pasha *et al*., 2007; Nazarizadeh and Pourreza, 2019).

In recent years, medicinal herbs have attracted considerable attention as natural feed additives to counteract the harmful impacts of mycotoxins. Among these, milk thistle (*Silybum marianum* L.) is widely recognized for its therapeutic use in managing liver disorders, primarily due to its key bioactive constituent, silymarin (SIL) (Nazarizadeh and Pourreza, 2019). Silymarin is a complex mixture of flavonolignans, with silybin accounting for about 50–60%, silicristin around 20%, silidianin approximately 10%, along with several minor compounds (Saller *et al*., 2007). Milk thistle exhibits multiple beneficial properties, including hepatoprotective, anti-inflammatory, cytoprotective, and anti-carcinogenic activities. Additionally, milk thistle is known to enhance immune responses, making it a valuable candidate for supporting animal health under conditions of mycotoxin exposure (Wilasrusmee *et al*., 2002; Nazarizadeh and Pourreza, 2019). Milk thistle also demonstrates strong antioxidant and anti-inflammatory properties, largely attributed to silymarin's (SIL) ability to function both as a free radical scavenger and as an inhibitor of lipid peroxidation (Juráňová *et al*., 2018). Furthermore, milk thistle has been shown to stimulate lymphocyte proliferation, which is linked to enhanced production of key cytokines, including interleukin (IL)-4, and IL-10, thereby supporting immune modulation (Wilasrusmee *et al*., 2002). Silymarin provides additional hepatoprotective effects by reducing the rise of liver enzymes during hepatic injury, enhancing liver regeneration, and inhibiting the transformation of stellate hepatocytes into myofibroblasts (Romanucci et al., 2018; Adetuyi *et al*., 2021). In cases of aflatoxin (AF) intoxication, SIL helps mitigate toxin-induced damage by modulating cytochrome P450 (CYP450) enzyme activity, thereby reducing the formation of the highly reactive AF-epoxide. The detoxification of aflatoxins primarily occurs through conjugation of the epoxide metabolite with hepatic glutathione (GSH), SIL supports conserving hepatic glutathione reserves, and aflatoxin-induced hepatotoxicity (Campos *et al*., 1989; Girolami *et al*., 2022).

Taraxacum officinale (dandelion), a member of the Asteraceae family, is a perennial herb noted for its broad pharmacological potential, encompassing hepatoprotective, anti-inflammatory, antioxidant, antibacterial, and anticancer (Qureshi *et al*., 2017). Traditionally, it has played an important role in Chinese medicine, where it has been prescribed for conditions such as disorders of the spleen and liver, indigestion, gallbladder dysfunction, and digestive complications (Schütz *et al*., 2006; You *et al*., 2010). Dandelion's therapeutic value is linked to its abundance of bioactive constituents such as phenolics, terpenes, carbohydrates, proteins, and fatty acids. Its roots and leaves are nutritionally rich, providing essential vitamins, minerals, along with micronutrients, dietary fiber, lecithin, and choline (Qureshi *et al*., 2017). The high levels of hydroxycinnamic acid derivatives, mainly chicoric, chlorogenic, and caffeic acids, contribute substantially to its pronounced antioxidant and anti-inflammatory effects (Jędrejek *et al*., 2017; Grauso *et al*., 2019). A recent investigation revealed that dandelion supplementation can improve feed efficiency in broiler chickens by strengthening the intestinal barrier, reducing proinflammatory cytokine levels, and positively modulating the gut microbiota composition (Mao *et al*., 2022). Additionally, several studies have reported that dandelion extract is rich in inulin, comprising about 40% of its content. Inulin is a complex carbohydrate that functions as a prebiotic, helping to preserve microbial balance within the gastrointestinal tract. Recognized as one of the most effective agents, it stimulates the proliferation and function of probiotic bacteria, including *Bifidobacterium* and *Lactobacillus*, thereby supporting gut integrity and boosting immune defense in animals (Noor *et al*., 2021). In contrast to most previous investigations that primarily examined the prophylactic administration of herbal extracts concomitant with mycotoxin exposure, the present study emphasizes their therapeutic application following the establishment of mycotoxin-induced pathology. This experimental design addresses a critical knowledge gap and provides a basis for developing effective therapeutic strategies against mycotoxicosis in poultry production.

MATERIALS AND METHODS

Animal Ethics Statement: The broiler care and use protocol was approved by the Animal Research Ethics Committee (AREC), Faculty of Veterinary Medicine, Suez Canal University, Ismailia, Egypt with Ethical number of (2022045).

Materials

Milk thistle extract (HEPATICUM®), containing 1 g of silymarin per 100 mL and standardized to more than 45% silybin, was purchased from Medical Union Pharmaceuticals (MUP), Cairo, Egypt. Dandelion root extract was obtained from Nature's Way, USA. Kits for the determination of albumin, triglycerides, ALP, AST, ALT, and creatinine were procured from Biodiagnostics Co., Giza, Egypt. The expression of the CYP3A4 gene was quantified by real-time PCR (Stratagene MX3005P) using the QuantiTect® Probe RT-qPCR Kit (Cat. No. 204443). Total RNA was isolated with the RNeasy Mini Kit (QIAGEN, Manchester, UK), and expression levels were normalized to the reference gene β-actin (ACTB). The primer sequences employed are listed in Table 1.

Table 1: Oligonucleotide sequences of sense and antisense primers.

Reference	Primer sequence (5'-3')	Gene
Yuan *et al*., 2007	CCACCGCAAATGCTTCTAAAC	*B. actin*
	AAGACTGCTGCTGACACCTTC	
Chen *et al*., 2023	GTGGACTTCCTGCAGCTGAT	*CYP3A4*
	CCTTCTCCCTGGCAGACTTG	

Experimental chicks, housing and management

Ninety male Ross-308 broiler chicks, aged 30 days, were sourced from Al-Wadi Poultry Company (Al-Beheira, Egypt). Birds were housed in a deep-litter floor system, with thorough disinfection of all equipment and housing areas prior to placement. Temperature control began at 33 °C for the first three days and was gradually lowered by 1°C every three days, reaching 26°C by week six. Throughout the trial, humidity was maintained at 60–70%, with unrestricted access to feed and water.

Experimental design

The experiment was carried out in two phases. In Phase I (10–30 days of age), the chickens were fed a diet containing 350 ppb aflatoxins and 180 ppb ochratoxins. The contaminated diets were formulated following the method of Mgbeahuruike *et al*. (2018) by replacing the corn in the control diet with naturally contaminated corn. The corn used was field-contaminated rather than artificially spiked. Mycotoxin levels in the experimental diets were analyzed prior to the feeding trial using ELISA kits (Elabscience Biotechnology Inc., Total Aflatoxin, Catalog No: E-TO-E006, USA; and Ochratoxin A, Catalog No: E-TOE001, USA), as described by Kara *et al*. (2022). Phase II (30–40 days of age) included 90 positive control birds from Phase I. During this phase, which represents the current study, the birds were switched to a mycotoxin-free diet and divided into three groups (30 birds per group), with each group further subdivided into three replicates. The first group served as the control (CON), where birds were fed a commercial broiler diet without any herbal supplementation in the drinking water. The second group received the same commercial diet supplemented with milk thistle extract (MTE) in the drinking water at a dose of 300 mg/kg BW. The third group was fed the commercial diet supplemented with dandelion root extract (DRE) in the drinking water at the same dose (300 mg/kg BW), as shown in Figure 1. The birds were weighed daily, and the dosage was adjusted according to their body weight (300 mg/kg BW). The calculated dose was then dissolved in four liters of water and provided to the birds once daily to ensure that each bird received the intended dose as accurately as possible. A starter feed was offered to the chicks from day 0 to 10, after which they were switched to a grower ration until day 40. Diet formulations were based on the nutritional requirements recommended by Council and Nutrition, 1994 (Table 2).

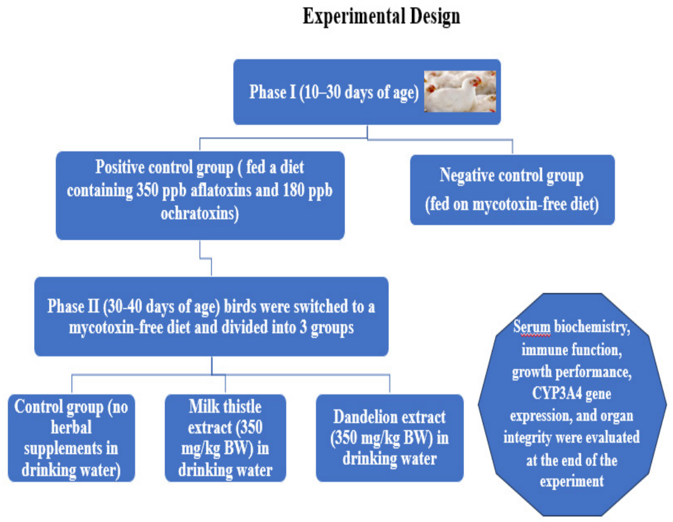

Figure 1: Experimental design.

Table 2: Formulation of the basal diet of broilers.

Ingredients (Kg/100 kg)	Starter (0-10 day)	Grower (10-40 days)
Corn gluten (62%)	6.5	6
Soya bean meal (44%)	32.85	28
Yellow corn (grains)	54	59.2
Soya oil	2.7	2.5
Di-Ca phosphate (22%Caand19%P)	1.46	1.52
Lime stone	1.51	1.8
Premix	0.3	0.3
L-Lysine	0.1	0.1
DL-Methionine	0.28	0.28
Common salt	0.3	0.3
Calculated composition		
Calorie. (ME/kg)	3200	3194
Crude protein (C.P %)	23	21
Available phosphorus%	0.42	0.42
Calcium %	1.1	0.93

** Each 3 kg contains the following vitamins and minerals: Vit. A 12 mIU, vit. D_3 2 mIU, vit. k_3 1000mg, vit. B_1 1000mg, vit. E 1000mg, vit. B_6 1500mg, vit. B_2 5000mg, vit. B_{12} 10mg, pantothinic acid 10g, biotin 50mg, folic acid 1000mg, nicotinic acid 30g, iron 30g, manganese 60g, copper 4g, zinc 50g, iodine 300mg, cobalt 100mg, selenium 100mg, carrier($CaCO_3$) to 3kg.

Growth performance parameters

The parameters evaluated were feed intake, final body weight, body weight gain, and feed conversion ratio (FCR). Calculation of FCR was performed using the following equation:

FCR = Total feed intake (g)/ Total body weight gain (g)

Blood sampling

At 40 days of age, five birds from each group were randomly selected for blood collection. Samples collected in tubes containing EDTA were immediately used to determine the differential leukocyte count (DLC) following the method of Schalm and Jain (1986). A second set of blood samples was collected in plain tubes without anticoagulants, and the sera were separated and stored at –20 °C for subsequent determination of albumin, triglycerides, ALP, AST, ALT, and creatinine.

Organ histopathological assessment

At day 40 of the experiment, liver, kidney, spleen, and bursa samples were collected from each group for histopathological evaluation. The tissue samples were trimmed and preserved in 10% neutral buffered formalin, followed by dehydration through a graded ethanol series (70–100%), clearing in xylene, and embedding in paraffin wax. Serial sections of 3–5 µm were cut using a rotary microtome and subsequently stained with Mayer's hematoxylin and eosin for histological evaluation under a microscope.

CYP 3A4 gene expression

RNA extraction, cDNA synthesis, and qPCR

At 40 days of age, about 30 mg of liver tissue was collected and placed into a 2 ml screw-cap tube containing 600 µl of Buffer RLT. The samples were homogenized using a TissueLyser for 2 minutes at 30 Hz, then centrifuged for 3 minutes at 14,000 rpm. Up to 700 µl of the resulting supernatant was loaded onto an RNeasy spin column in a 2 ml collection tube and centrifuged for 1 minute at 14,000 rpm, after which the flow-through was discarded. The column was washed with 700 µl of Buffer RW1, and RNA was eluted by adding 50 µl of RNase-free water and centrifuging for 1 minute at 10,000 rpm. One microgram of the isolated RNA was then reverse-transcribed into cDNA using the QuantiTech® Reverse Transcription Kit. The synthesized cDNA was used to assess CYP3A4 gene expression, and quantitative PCR was conducted on a LightCycler 480 system under the cycling conditions described in Table 3.

Analysis of RT-qPCR results

Amplification curves and ΔCt values were generated using Stratagene MX3005P software. Relative gene expression was quantified with the comparative Ct (ΔΔCt) approach (Livak and Schmittgen, 2001). For each sample, expression of the target gene was normalized against the reference (housekeeping) gene as follows:

$$\Delta Ct_{sample} = Ct_{target,sample} - Ct_{reference,sample}$$

The difference between treatment and control was then calculated as:

$$\Delta\Delta Ct = \Delta Ct_{treatment} - \Delta Ct_{control}$$

Table 3: SYBR Green RT-qPCR cycling conditions.

Gene	Reverse transcription	Primary denaturation	Amplification (40 cycles)			Dissociation curve (1 cycle)		
			Secondary denaturation	Annealing	Extension	Secondary denaturation	Annealing	Final denaturation
ß. actin	50°C 30 min.	94°C 15 min.	94°C 15 sec.	51°C 30 sec.	72°C 30 sec.	94°C 1 min.	51°C 1 min.	94°C 1 min.
CYP3A4	50°C 30 min.	94°C 15 min.	94°C 15 sec.	55°C 30 sec.	72°C 30 sec.	94°C 1 min.	55°C 1 min.	94°C 1 min.

Table 4: Effects of milk thistle and dandelion extracts on the growth performance of experimental broilers.

Group/ Parameter	CON	MTE	DRE
Total feed intake (g)	2893.17±34[c]	3051.83±31.85[b]	3146.33±35.97[a]
Final body weight (g)	1819.50±29.97[c]	1922.33±27.90[b]	2043.67±33.36[a]
Total weight gain (g)	1848.83±30.97[c]	1952.33±27.90[b]	2002±33.23[a]
Total FCR	1.59±0.03[a]	1.53±0.02[c]	1.54±0.02[b]

Data are presented as mean ± standard deviation (SD), with n = 3. Values within the same row bearing different superscript letters indicate significant differences at P < 0.05.

Relative fold change (treatment versus control) was reported as:

$$\text{Fold change}= 2^{-\Delta\Delta Ct}$$

Statistical analysis

The obtained data were analyzed statistically by analysis of variance (ANOVA) using SPSS software version 14.0.0 (Inc, 2004), with significance considered at P < 0.05. Differences among means were further assessed by Duncan's multiple range test. Results are expressed as arithmetic means ± standard deviation.

RESULTS AND DISCUSSION

Effects of milk thistle and dandelion extracts on the growth performance of experimental broilers

As shown in Table 4, the present findings demonstrated that both milk thistle and dandelion extracts significantly enhanced feed intake, body weight, and weight gain in broilers compared with the control group. Milk thistle supplementation produced the greatest improvement in growth performance, achieving the best feed conversion ratio (1.53 ± 0.02), followed by the dandelion extract group (1.54 ± 0.02). Malekinejad et al. (2015) reported that aflatoxin-contaminated feed reduced growth rate and feed efficiency while increasing mortality in broilers. Several studies have shown that dietary supplementation with silymarin improved feed consumption and body weight (Tedesco *et al*., 2004; Chand *et al*., 2011; Muhammad *et al*., 2012, Fani Makki et al., 2013). Kalorey *et al*. (2005) observed that silymarin enhanced body weight and feed intake in broilers exposed to aflatoxin B1, although it did not influence the feed conversion ratio. Noor *et al*. (2021) reported that supplementing broiler diets with dandelion leaf powder at concentrations of 1.0 or 2.0 g/kg had a positive impact on body weight, weight gain, and FCR compared with the control group. Similarly, Guo *et al*. (2023) demonstrated that dietary supplementation with *Taraxacum officinale* extract significantly increased average daily weight gain and reduced FCR in broilers. In pigs, the inclusion of *T. officinale* extract improved average daily weight gain, feed intake, and nutrient digestibility relative to controls (Yan *et al*., 2011). However, Qureshi et al. (2015) observed no significant differences in cumulative feed consumption between dandelion-treated and control broilers, although FCR was significantly improved, consistent with the findings of Al-Kassie and Witwit (2010). Supplementation with dandelion has been shown to enhance feed efficiency in broiler chickens by reinforcing the intestinal barrier, lowering proinflammatory cytokine levels, and beneficially modulating gut microbiota composition (Mao *et al*., 2022). The extract is also a rich source of inulin, which acts as a prebiotic and helps maintain microbial balance within the gastrointestinal tract (Noor *et al*., 2021).

The effect of milk thistle and dandelion extracts on differential leukocytic count in experimental chickens

Leukocytes (WBCs) serve as key defensive cells that protect organisms against pathogenic microorganisms (Mohammadi *et al*., 2020). The present results indicated that both extracts significantly increased WBC counts compared with the control group, with milk thistle extract (21.4 ± 0.07) showing the strongest effect, followed by dandelion root extract (20.13 ± 0.05). Both extracts enhanced heterophil percentage relative to the control. While milk thistle increased monocyte and basophil percentages compared with the control, eosinophil percentages remained unchanged across all groups (Table 5).

Table 5: The effect of milk thistle and dandelion extracts on differential leukocytic count in experimental chickens.

Parameter/ Group	WBCs (10^3/ mm3)	Lymphocytes	Heterophils	Monocytes	Eosinophils	Basophils
CON	18.2±0.7[c]	9.48±0.6[b]	5.945±0.15[c]	1.71±0.02[b]	0.585+0.015[a]	0.48±0.01[b]
MTE	21.4±0.7[a]	11.36±0.3[ab]	7.06±0.8[a]	1.87±0.02[a]	0.6±0.01[a]	0.52±0.01[a]
DRE	20.13±0.5[b]	10.77±0.4[ab]	6.56±0.4[b]	1.73±0.03[b]	0.59±0.01[a]	0.49±0.01[b]

Data are presented as mean ± standard deviation (SD), with n = 5. Values within the same column bearing different superscript letters indicate significant differences at P < 0.05.

Table 6: The effect of milk thistle and dandelion extracts on blood biochemical parameters in experimental chickens.

Parameter/ Group	AST (U/L)	ALT (U/L)	ALP (U/L)	Albumin (g/ dL)	Creatinine (mg/dL)	Triglycerides (mg/dL)
CON	280.33±24.44[a]	13±1[a]	4854±33.18[a]	2.293±0.04[a]	0.33±0.02[a]	25±1[ab]
MTE	225.33±25.79[c]	10.67±1.53[c]	3724±32.23[b]	2.29±0.04 [a]	0.31±0.03[c]	25.67±2.52[a]
DRE	231.67±23.12[b]	11±1.73[b]	3680.33±26.08[c]	2.29±0.05 [a]	0.32±0.05[b]	25±2[ab]

Data are presented as mean ± standard deviation (SD), with n = 5. Values within the same column bearing different superscript letters indicate significant differences at P < 0.05.

Several studies have reported a significant reduction in WBC and RBC counts in chicks fed mycotoxin-contaminated diets (Celik et al., 2000; Elaroussi *et al*., 2006; Pande *et al*., 2006). The decline in leukocyte numbers has been attributed mainly to a reduction in lymphocytes, and to a lesser extent, monocytes or heterophils (Chang *et al*., 1979; Mohiuddin *et al*., 1993). This lymphocytopenia may serve as a sensitive and reliable indicator of mycotoxicosis, possibly resulting from the direct toxic effects on the germinal centers of lymphoid tissues. In addition, stress hormones such as corticosteroids are known to exert suppressive effects on total leukocyte counts (Naseem *et al*., 2018).

Supplementation of broiler diets with milk thistle extract positively influenced erythropoiesis, hemoglobin synthesis, and leukopoiesis (Bagno *et al*., 2021). Additionally, Sultan *et al*. (2018) reported improvements in hematological parameters in the presence of silymarin. Moreover, milk thistle has been shown to stimulate lymphocyte proliferation, which is associated with increased production of key cytokines such as interferon-gamma, interleukin (IL)-4, and IL-10, thereby contributing to immune modulation (Wilasrusmee *et al*., 2002). In rainbow trout, supplementation with dandelion extract significantly increased WBC counts compared with the control group (Lee *et al*., 2004; Nya and Austin, 2009; Hosseini et al., 2021; Köse and Arıman Karabulut, 2022). Higher lymphocyte and monocyte values were also observed in T. officinale-treated trout (Köse and Arıman Karabulut, 2022). In contrast, Yan *et al*. (2011) reported that dietary administration of dandelion extract powder at 1 g/kg for 10 weeks did not influence blood characteristics in pigs compared with controls. Dandelion's therapeutic effects are linked to its rich mixture of bioactive molecules such as phenolics, terpenes, carbohydrates, proteins, fatty acids, vitamins, minerals, fiber, lecithin, and choline (Qureshi *et al*., 2017).

The effect of milk thistle and dandelion extracts on blood biochemical parameters in experimental chickens

As shown in Table 6, and Figure 2, both extracts significantly lowered AST, ALT, and ALP levels key markers of hepatic damage with milk thistle extract showing the strongest effect across all parameters. Albumin concentrations remained unchanged among the groups, whereas creatinine levels exhibited slight but significant reductions in extract-treated birds, indicating a supportive role in maintaining renal function.

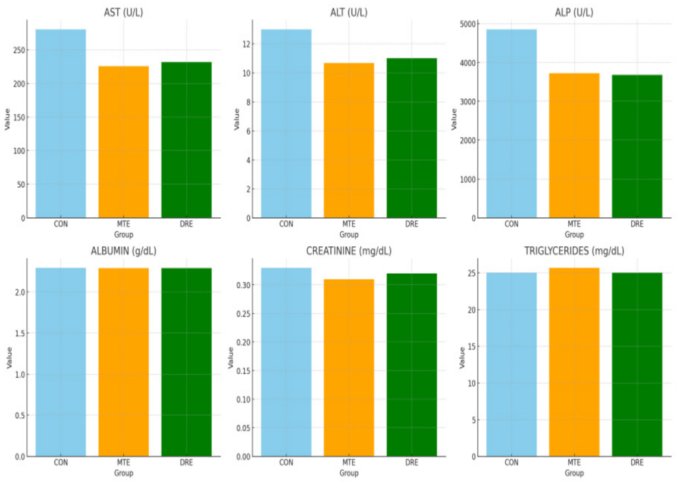

Figure 2: The effect of milk thistle and dandelion extracts on blood biochemical parameters in experimental chickens.

Mycotoxins are metabolized primarily in the gastrointestinal tract, liver, and kidneys (Sklan *et al*., 2001). Abou-Shehema *et al*. (2016) reported that aflatoxin B1-contaminated diets significantly elevated serum ALT, AST, and ALP levels, whereas supplementation with silymarin reversed these effects. Baer-Dubowska *et al.* (1998) demonstrated that silymarin downregulates the cytochrome P450 system, thereby limiting aflatoxin B1 activation. In addition, milk thistle exhibits potent antioxidant and anti-inflammatory activities, largely due to silymarin's role as both a free radical scavenger and an inhibitor of lipid peroxidation (Juráňová *et al.*, 2018). Moreover, silymarin stabilizes cellular and mitochondrial membranes, protecting them from xenobiotic-induced damage (Münter *et al.*, 1986). It also binds to toxins and blocks their uptake into hepatocytes by occupying specific binding sites (Faulstich *et al.*, 1980). According to Amiridumari *et al.* (2013), dietary exposure to aflatoxin B1 (500 ppb) led to significant reductions in high-density lipoprotein, calcium, and glucose levels, alongside an increase in serum creatinine; however, supplementation with milk thistle seeds mitigated these adverse effects. Aflatoxin B1-contaminated diets substantially decreased serum protein concentrations, whereas silymarin supplementation (10 g/kg) restored protein levels Muhammad *et al.* (2012).

Several studies have highlighted the hepatoprotective effects of dandelion leaves (Park *et al.*, 2007; Tabassum et al., 2010; Qureshi et al., 2015). Methanolic extract of *T. officinale* significantly restored liver enzyme levels, along with improvements in bilirubin, lipid profile, and antioxidant enzyme activity (Herrera Vielma *et al.*, 2025). Treatment with dandelion leaf extract also markedly reduced CCl_4-induced elevations in hepatic enzymes (AST, ALT, and LDH) in Sprague–Dawley rats and corrected the impaired release of triglycerides and cholesterol into the serum (Park *et al.*, 2010).

The effect of milk thistle and dandelion extracts on CYP3A4 gene expression in experimental chickens

Cytochrome P450s are key phase I enzymes responsible for the oxidative metabolism of a wide range of drugs (Guengerich, 2001). Among these, CYP1A2, 2D6, 3A4/5, and 2C9 represent the predominant hepatic isoenzymes (Zanger *et al.*, 2013). As shown in Table 7, and Figure 3, the present study demonstrated that supplementation with milk thistle and *T. officinale* extracts significantly downregulated CYP3A4 gene expression in broiler chickens when compared to the control group. Consistent with these findings, several studies have shown that silymarin extracts and their individual constituents inhibit CYP3A4 activity (Beckmann-Knopp *et al.*, 2000; Venkataramanan et al., 2000; Zuber *et al.*, 2002). Silybin, the major active component of milk thistle, inactivated purified recombinant CYP3A4 and CYP2C9 in a mechanism-based manner. The inactivation was time, concentration, and NADPH-dependent, and enzyme activity was not recovered following removal of silybin (Sridar *et al.*, 2004). During aflatoxin (AF) intoxication, silymarin mitigates toxin-induced damage by modulating cytochrome P450 (CYP450) activity, thereby reducing the formation of the highly reactive AF-epoxide (Campos *et al.*, 1989; Girolami *et al.*, 2022). Similarly, dandelion leaf extract significantly downregulated the overexpression of cytochrome P450 2E1 associated with CCl_4-induced hepatic injury in Sprague–Dawley rats (Park *et al.*, 2010).

Table 7: The effect of milk thistle and dandelion extracts on CYP3A4 gene expression in experimental chickens.

Group/ Parameter	CON	MTE	DRE
CYP3A4-fold change	1^a	0.20 ± 0.02^c	0.48 ± 0.06^b

Means within the same row with different superscripts are significantly different (P<0.05).

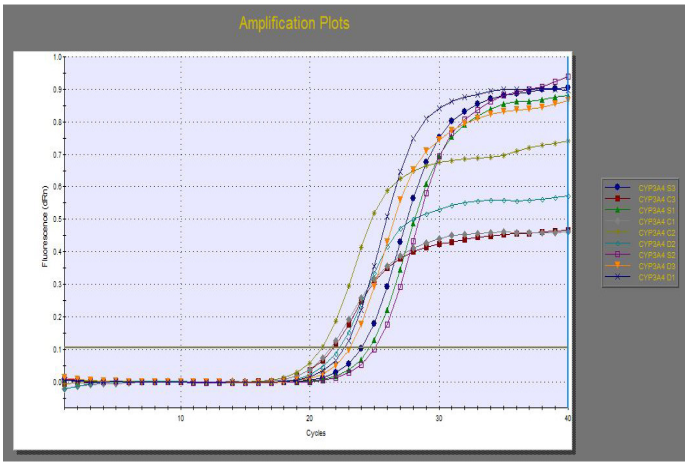

Figure 3: The effect of milk thistle and dandelion extracts on CYP 3A4 gene expression of the experimental chickens.

The effect of milk thistle and dandelion extracts on the histopathological features of the liver, kidney, bursa, and spleen in experimental chickens

The present findings revealed that the control group (without herbal supplements) exhibited pronounced hepatic damage, characterized by vascular congestion, perivascular fibrosis, and coagulative necrosis. In contrast, the MTE group displayed mild sinusoidal dilation and normal tissue architecture, vasculature and cellular details (Figure 4). Examination of kidney tissues showed that the control group developed severe renal lesions, including intertubular hemorrhage, tubular degeneration, and marked vascular congestion. In contrast, DRE group showed normal renal cortex, intact glomeruli and mild perivascular inter tubular edema. MTE showed mild intertubular extravasated erythrocytes normal renal cortex with intact glomeruli and renal tubules (Figure 5). The control group showed moderate pathological alterations in bursa of Fabricius, such as interfollicular and subepithelial edema, whereas the MTE group showed noticeable interlobular edema. By comparison,

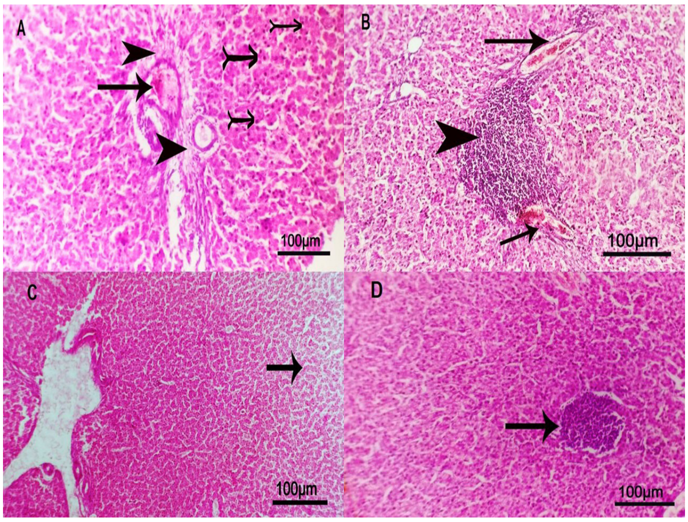

Figure 4: Photomicrograph of H and E stained section of chicken LIVER at 40 days old (scale bar =100μm). A and B represent the control group, C represents MTE, and D represents DRE. (A) CON liver with vascular congestion (arrow), perivascular fibrosis (arrows head) and coagulative necrosis represented by pyknosis (tailed arrows). (B) CON liver with focal massive mononuclear cells infiltration (arrowhead) with diffuse vascular congestion (arrows). (C) MTE liver with mild sinusoidal dilation (arrow) and normal tissue architecture, vasculature and cellular details. (D) DRE liver with focal mononuclear cells infiltration (arrow), normal tissue architecture, vasculature and cellular details.

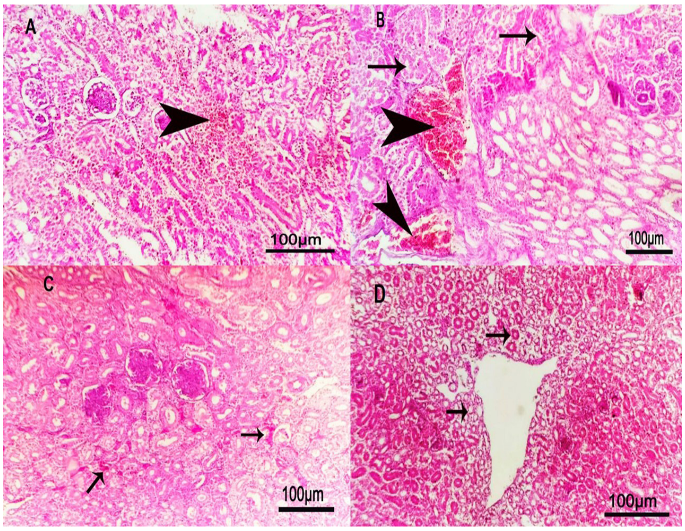

Figure 5: Photomicrograph of H and E stained section of chicken KIDNEY at 40 day old (scale bar = 100μm). A and B represent the control group, C represents MTE, and D represents DRE. (A) CON kidney with focal areas of inter tubular extravasated erythrocytes (hemorrhage) (arrowhead). (B) CON kidney with degeneration of some renal tubules (arrow) and severe vascular congestion (arrows head), (C) MTE kidney with mild intertubular extravasated erythrocytes (arrows) normal renal cortex with intact glomeruli and renal tubules. (D) DRE kidney with normal renal cortex, intact glomeruli and mild perivascular inter tubular edema (arrows).

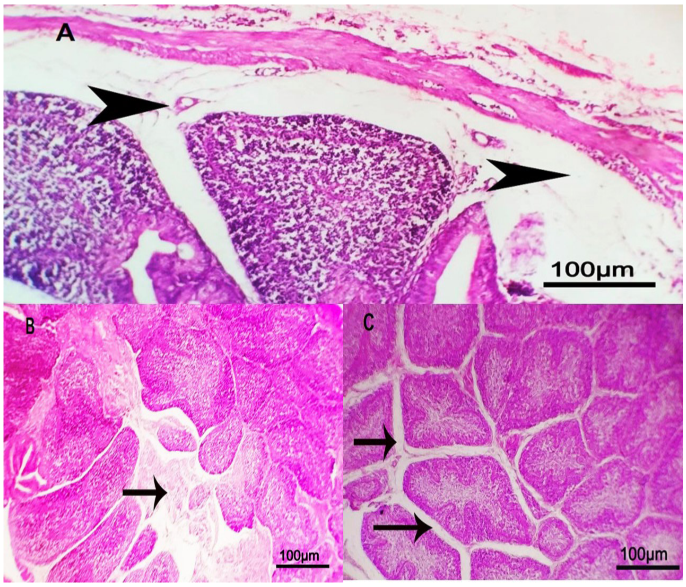

Figure 6: Photomicrograph of H and E stained section of chicken BURSA at 40 days old showing (scale bar = 100μm). (A) represent the control group, B represents MTE, and C represents DRE. (B) MTE bursa of Fabricius with interlobular edema (arrow). (C) DRE bursa of Fabricius with normal lymphoid follicles and interfollicular edema (arrows).

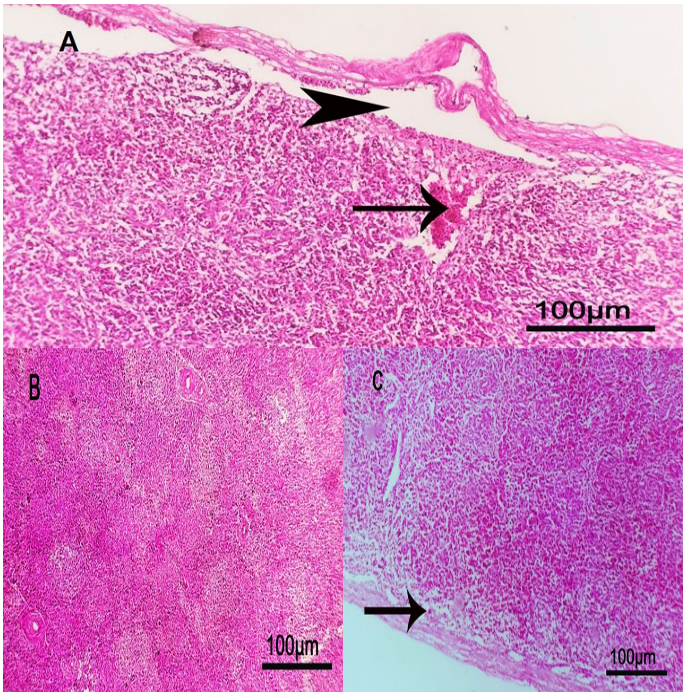

Figure 7: Photomicrograph of H and E stained section of chicken SPLEEN at 40 days old showing (scale bar = 100μm). A represent the control group, B represents MTE, and C represents DRE. (A) CON spleen with subcapsular edema (arrowhead) with capsular splitting and vascular congestion (arrow). (B) MTE spleen with normal vasculature, parenchyma of both red and white pulp. (C) DRE spleen with normal vasculature, parenchyma of both red and white pulp except mild subcapsular depletion of lymphocytes from white pulp (arrow).

the DRE group showed normal lymphoid follicles and interfollicular edema (Figure 6). Splenic examination demonstrated obvious lesions in the control group, including subcapsular edema, capsular splitting, and vascular congestion, while MTE group showed normal vasculature, parenchyma of both red and white pulp. DRE group showed spleen with normal vasculature, parenchyma of both red and white pulp except mild subcapsular depletion of lymphocytes from white pulp (Figure 7).

Stoev *et al*. (2021) reported that in lymphoid organs, including the bursa of Fabricius and thymus, the most pronounced degenerative lesions occurred in chicks exposed to OTA, followed by those treated with OTA plus SIL. In the liver, supplementation with SIL reduced cloudy swelling as well as granular and vacuolar degeneration of hepatocytes. Similarly, Tedesco *et al*. (2004), observed less severe hepatic lesions in AFB1-treated chickens when SIL-phytosome was administered. Egresi *et al*. (2020) also noted focal lymphocytic and histiocytic interstitial infiltrates, which were markedly milder in birds supplemented with milk thistle seed at 0.5%/kg of diet. In pigeons, hepatic lesions such as necrosis with multifocal portal infiltration of mononuclear cells, were observed and were not alleviated by SIL administration at 10–100 mg/kg BW (Grizzle et al., 2009). Nevertheless, in cases of aflatoxin (AF) intoxication, SIL has been shown to mitigate toxin-induced injury by modulating cytochrome P450 enzyme activity, thereby reducing the formation of the highly reactive AF-epoxide (Campos *et al*., 1989; Girolami *et al*., 2022).

Several studies have shown that *T. officinale* can ameliorate hepatic microvesicular steatosis and liver injury (Domitrović *et al*., 2010; Al-Malki *et al*., 2013; Favari et al., 2013; Ahmad et al., 2014). Dandelion extract was also found to reduce epithelial cell necrosis, decrease inflammatory cell infiltration, and alleviate vascular congestion in the kidneys, liver, and heart compared with the ethylene glycol group (Al-Daoudi et al., 2024). Dandelion extract is rich in phenolic compounds, particularly chicoric acid, chlorogenic acid, and caffeic acid, which are regarded as the primary mediators of its antioxidant and anti-inflammatory properties (Jędrejek *et al*., 2017; Grauso *et al*., 2019).

CONCLUSION AND RECOMMENDATIONS

The study concluded that supplementation with milk thistle extract (each 100 mL containing 1 g of silymarin, standardized to more than 45% silybin) and dandelion extract through drinking water effectively mitigated the detrimental effects of mycotoxins when administered as a therapeutic regimen following mycotoxin exposure in broilers. These extracts improved growth performance by increasing body weight gain and reducing the feed conversion ratio, enhanced immune responses, and regulated liver enzyme activities. Furthermore, they mitigated the toxic effects on the liver, kidneys, and spleen. The supplements also downregulated CYP3A4 gene expression, which may contribute to reduced CYP3A4 activity and, consequently, a lower generation of toxic mycotoxin metabolites. Further investigations employing different approaches such as incorporating the herbs into feed, characterizing the bioactive components of the dandelion extract, and testing various extract doses are recommended.

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NOVELTY STATEMENT

In contrast to most previous studies that primarily focused on the prophylactic use of milk thistle and dandelion extracts administered concurrently with mycotoxin exposure, the present study highlights their therapeutic application after the onset of mycotoxin-induced pathology. The findings demonstrate that both milk thistle and dandelion extracts significantly improved growth performance, modulated liver enzyme activities, downregulated hepatic CYP3A4 gene expression, and mitigated the toxic effects on the liver, kidneys, and spleen in broilers previously fed a mycotoxin-contaminated diet.

AUTHOR'S CONTRIBUTION

The research strategy was done by Prof. Dr. Abdelfattah M. Abdelfattah. Yehia El-Sayed M. Badawi composed the article and conducted the experimental work. Statistics and language revision were done by Khalil F. Waleed. The finished manuscript was examined and revised by Sahar Ez-Eldin and Prof. Dr. Hassan M. Fayez.

Generative AI and AI-assisted technology statement

All authors of this work declare that generative AI technologies including large language models (e.g., ChatGPT, Copilot) and text-to-image generators were not utilized in any capacity during the preparation, writing, or editing of this manuscript.

Conflict of interest

The authors have declared no conflict of interest.

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