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# Research Article
# Hepatoprotective and Immunostimulatory Effect of *Ganoderma*, *Andrographolide* and *Turmeric* Against Aflatoxicosis in Broiler Chickens

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## Abstract

**Objective:** The present study aimed to investigate the toxic effects of Aflatoxin (AF) B1 (AFB1) and evaluate the role of *Ganoderma lucidum* (*GL*), *Andrographolide* (*AP*) and *Turmeric curcuma* (*CM*) in reducing these toxic effects in broiler chickens. **Material and methods:** A total of 250 (Ross-308) one-day-old broiler chickens were randomly divided into 5 treatment groups (T1-T5) with 50 chicks per group. All groups except for T5 were fed diets that were contaminated with AFs and the groups were treated as follows: T1: Received 0.2% (2 g $kg^{-1}$) *GL* as a feed additive. T2: Received 0.2% (2 g $kg^{-1}$) *AP* as a feed additive. T3: Received 0.2% (2 g $kg^{-1}$) *CM* as a feed additive. T4: Was a positive control (vaccinated but not treated). T5: Was a negative control (not vaccinated or treated). At ages 7, 15 and 25 days, all groups except T5 were vaccinated against Newcastle Disease (ND) (*La Sota*) and at 12 days, they were vaccinated against Infectious Bursal Disease (IBD) (intermediate D78 strain) (in drinking water). Twenty chicks from each group were challenged with a local virulent ND virus (NDV) isolate ($ELD_{50}$ $10^5$) at 35 days. The AF content was 46.768 ppb in starter diets and 48.661 ppb in the final diet. **Results:** The *GL*-fed chicks produced the highest variable antibody titer (Abs), not significant ($p<0.05$) hepatic and spleen levels of hydrogen peroxide ($H_2O_2$) and malondialdehyde (MDA), significantly different ($p<0.05$) levels of lipid peroxidation (LPO) and highly significant ($p<0.05$) hepatic and spleen levels of antioxidant defense compounds (Glutathione Reductase (GR) and glutathione peroxidase (GSH-Px)), followed by T3 and T2, respectively, in comparison with T5 and T4. In addition, T1, T2 and T3 showed a significant increase ($p<0.05$) in Average Daily Gain (ADG) and a significant decrease ($p<0.05$) in the feed:gain ratio (F:G) compared to T4. However, the Average Daily Feed Intake (ADFI) in the above groups was not significantly different ($p<0.05$) during the trial period. **Conclusion:** The study showed the role of *GL*, *AP* and *CM* in reducing the negative effects of AFs by decreasing oxidative stress and immunosuppression in broiler chickens.

Key words: *GL*, *AP* and *CM*, aflatoxins, immunity, oxidative stress, antioxidant, growth performance

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

## INTRODUCTION

Aflatoxin (AF) is one of the most potent mycotoxins produced by *Aspergillus flavus* and *Aspergillus parasiticus* and is considered a major problem in the poultry industry. The AF toxicity was significantly investigated in broiler chickens by the identification of carcinogenic, teratogenic and mutagenic[1,2], growth[3] haematological and biochemical[4], immunologic[5] and pathologic[6] effects. Diets contaminated with AF caused aflatoxicosis in poultry, which is characterized by depression, listlessness, loss of appetite, low growth rate, poor feed intake, reduced weight gain, decreased weight and egg production, increased susceptibility to environmental and microbial stress and increased mortality. AFB1 is one of the most widespread oxidizing agents of Aflatoxins[7,8], The toxic effect of AFB1 is closely linked to a pro-oxidant, which in turn generates Reactive Oxygen Species (ROS) that inhibit DNA, RNA, lipid, protein and other molecules[9,10]. Thus, several studies have suggested adding antioxidants to poultry diets to increase protection against the toxicity of AFB1 by activating the immune system and providing antioxidants[11,12]. *Ganoderma lucidum* (*GL*) is a traditional medicinal mushroom in China that has been known for more than 2000 years to promote longevity and health[13]. *In vitro GL* polysaccharide plays a major role in the removal of 1,1-diphenyl-2-picrylhydrazyl and oxygen radicals[14]. In mice, the antioxidant activity of GL polysaccharides was demonstrated[15], in addition to the role of these polysaccharides as strong stimulants of spleen mass, lymphocyte proliferation and high antibody production[16,17]. Other *GL* active agents are fatty acids, which have anti-cancer activity and are important immune regulators in mice[18] by stimulating the production of interferon-γ, interleukin-2, interleukin-4 and interleukin-6[19]. *Andrographolide* (*AP*) is a traditional herbal supplement that has anti-inflammatory, hepatoprotective, anti-viral, antioxidant and active immunomodulation properties[20]. The active ingredient of this herb has anti-cancer[21] and anti-HIV properties. Roy *et al.*[22] reported the *AP* extract has antibacterial activities. Curcumin is a polyphenolic compound that is extracted from the rhizomes of *Turmeric curcuma* (*CM*), which has been widely used in spice houses, as a natural food colorant and as a medicinal herb in many Asian countries for thousands of years[23]. It possesses anti-inflammatory, anticancer, radio-protective, chemotherapeutic, antioxidant and detoxification properties in laboratory animals and humans[24]. Several studies have shown that *CM* has the potential to alleviate the adverse effects of AFs in different animal species[25]. In addition, *CM* protects against the adverse effects of AF by improving antioxidant effectiveness[26]. The current study aimed to discover the role of these herbs (*GL*, *AP* and *CM*) in reducing the oxidative stress resulting from an AF-contaminated diet by activating the antioxidant defense system and improving immunity against Newcastle Disease (ND) after vaccination and challenge with virulent field strains.

## MATERIALS AND METHODS

**Dietary AFB1 analysis:** To detect AFB1, 20 g of the diet was mixed with 100 mL of methanol (Fisher, Pittsburgh, PA, USA): Water (30/70 v/v) and shaken for 3 min. Then, the supernatant of the mixture was filtered through a Whatman filter (Whatman Clifton, NJ, USA). The filter was collected and the AFB1 concentration was measured using an ELISA kit (Agra Quantum Aflatoxin B1 Assay, Romer, Singapore).

**Herbs:** Herbs (*GL* and *AP*) were obtained from the Malaysian DXN company, while *CM* was obtained from the commercial market.

**Experimental design:** Two hundred and fifty healthy broiler chicks (Rose 308, of Belgian Origin) were bought from AL-Afrah Hatchery in Baghdad and divided randomly into 5 groups (T1-T5) with 50 chicks in each group:

- **T1:** Received 0.2% (2 g $kg^{-1}$) *GL* as a feed additive
- **T2:** Received 0.2% (2 g $kg^{-1}$) *AP* as a feed additive
- **T3:** Received 0.2% (2 g $kg^{-1}$) *CM* as a feed additive
- **T4:** Was a positive control (vaccinated but not treated)
- **T5:** Was a negative control (not vaccinated or treated)

At age 7, 15 and 25 days, all groups were vaccinated against ND and at 12 days they were vaccinated against Infectious Bursal Disease (IBD) (intermediate D78 strain) (in drinking water). Twenty chicks from each group were challenged with a virulent local ND virus (NDV) isolate ($ELD_{50}$ $10^5$) at 35 days.

**Sample collection:** Five medium-sized birds of different ages were selected from each group. The birds were fasted for 12 h and 2-5 mL of blood from the jugular vein was collected using tubes (without anticoagulation). The blood samples were centrifuged at 1000 rpm for 10 min to separate serum from the blood and then stored at -20°C until analysis. At 35 days, the 5 selected birds were sacrificed and samples of the liver and spleen were collected directly and rapidly frozen at -70°C.

**Oxidative stress of the liver and spleen:** Small and homogeneous pieces (approximately 1 g) of the liver and spleen were placed in ice-cold saline buffer (1:9 wt/v) homogenized by Ultra-Turrax (T8, IKA-Labortechnik, Staufen, Germany). The concentration of the homogenate was 0.1 g $mL^{-1}$ for the analysis. The samples were centrifuged at 1000 rpm for 10 min at 4°C to separate the homogenate, after which the supernatant [containing Glutathione Reductase (GR), glutathione peroxidase (GSH-PX), malondialdehyde (MDA), lipid peroxidation (LPO), hydrogen peroxide ($H_2O_2$)] was collected and stored at -70°C. The concentrations of all compounds were analyzed using commercial assay kits (Nanjing Jiancheng Bioengineering Institute, Nanjing, China) according to the manufacturer's protocol.

**Enzyme linked immunosorbent assay (ELISA) (Synbiotics-USA):** The ELISA was performed according to the ProFLOK® NDV indirect ELISA kit (Synbiotics-USA)[27], which is one of the fastest serological tests used for the detection of ND antibodies in serum-infected and vaccinated (NDV) birds.

**Challenge test:** The challenge was met with the field isolate NDV in titration (100 $ELD_{50}$ $10^5$) according to Reed and Muench[28]. Clinical signs (respiratory and neurological) and mortality of the challenged birds were recorded daily for 10 days.

**Statistical analysis:** The SAS was used to illustrate the effect of factors used in this study[29]. Multiple-level Least Significant Difference (LSD) tests were used to determine the significance level. Differences of $p<0.05$ were considered statistically significant.

## RESULTS

**Newcastle disease immunity:** To determine the maternal immunity against ND, 10 serum samples were randomly selected from 250 one-day-old birds before dividing them into groups. The results of ELISA showed a good maternal immunity with a mean value of 5340.9±170.9. The current study aimed to determine the efficacy of *GL*, *AP* and *CM* with the appropriate ND vaccination program to improve the immune response against ND under the effect of AF in birds of different ages. Table 1 shows that the chicks treated with *GL* produced a highly variable antibody titer (Abs) against ND, followed by T3, T2 and T4, respectively, with significant differences ($p<0.05$) in antibodies compared to T5. In the third, fourth and fifth weeks before the challenge, there was a significant ($p<0.05$) increase in the Abs against ND in all treated groups; in particular, T1 produced the highest Abs, followed by T3 and T2, respectively, compared to T4, which showed differences in Abs of low significance ($p<0.05$). In the sixth week post challenge with the virulent NDV isolate (100 $ELD_{50}$ $10^5$), a significant ($p<0.05$) increase in Abs was observed in all groups but T5 showed a sudden and rapid increase in Abs of immune antibodies.

**Oxidative status and antioxidant defense in liver and spleen:** Table 2 shows the oxidation status of the birds under the influence of AF for 45 days; that the hepatic levels of $H_2O_2$, LPO and MDA were significantly ($p<0.05$) higher in T4 compared with T5. The levels of $H_2O_2$ and MDA were significantly ($p<0.05$) lower in the treated groups, especially T1 compared to T5, followed by T3 and T2, respectively. However, the levels of LPO were not significantly different ($p<0.05$) between the 5 groups. The treated groups also showed a significant ($p<0.05$) increase in the level of the hepatic antioxidant defense compounds GR and GSH-PX, especially in T1 compared to T4 and T5.

The splenic oxidation status results were similar to the liver results (Table 3). T4 showed a significant ($p<0.05$) increase in $H_2O_2$, MDA and LPO levels and a significant ($p<0.05$) decrease in the level of the antioxidant compounds GR and GSH-PX compared to T5. The results of the groups

Table 1: Effects of herbal extracts (GL, AP and CM) on antibody titer (Mean±SE) against Newcastle disease in broiler chickens exposed to aflatoxin for different durations, as determined by ELISA

| | Weeks | | | |
|---|---|---|---|---|
| Groups | Third | Fourth | Fifth* | Sixth |
| **ND antibody titer Mean±SE** | | | | |
| T1 | 2697.1±87.9[a] | 3066.4±119.8[a] | 3756.9±139.3[a] | 5806.9±138.4[b] |
| T2 | 2190.2±60.7[b] | 2561.4±82[b] | 3002.8±72.5[c] | 4262.8±90.7[c] |
| T3 | 2343.1±105.1[b] | 2636.7±114[b] | 3359.2±129.9[b] | 4659.2±137.5[c] |
| T4 | 1695.9±62.3[c] | 1858.4±60.5[c] | 2556.2±124.2[d] | 3436.2±80.1[d] |
| T5 | 315.9±35.2[d] | 0.0±0.0[d] | 0.0±0.0[e] | 8253.1±285.1[a] |
| LSD | 211.56 | 247.34 | 303.89 | 466 |

No. of samples: 10 from each group, *Challenged with a virulent local ND isolate in the fifth week, T1: Administered 2 g $kg^{-1}$ *GL*, T2: Administered 2 g $kg^{-1}$ *AP*, T3: Administered 2 g $kg^{-1}$ *CM*, T4: A positive control, T5: A negative control, The different letters with the means in the same column refer to significant differences among treatment means at $p<0.05$, LSD: Least significant difference

Table 2: Effects of herbal extracts (GL, AP and CM) on the liver oxidative status of broiler chickens fed aflatoxin

| | Index | | | | |
|---|---|---|---|---|---|
| Groups | $H_2O_2$ (mmol $g^{-1}$ pro) | MDA (nmol $g^{-1}$ pro) | LPO (μmol $g^{-1}$ pro) | GR (U $g^{-1}$ pro) | GSH-Px (U) |
| **Mean±SE** | | | | | |
| T1 | 10.94±0.17$^{c}$ | 2.12±0.08$^{c}$ | 0.83±0.01$^{c}$ | 9.104±0.14$^{a}$ | 43.90±0.23$^{a}$ |
| T2 | 12.98±0.21$^{b}$ | 2.48±0.08$^{b}$ | 0.89±0.01$^{b}$ | 6.910±0.17$^{c}$ | 41.11±0.17$^{c}$ |
| T3 | 12.06±0.13$^{bc}$ | 2.36±0.06$^{bc}$ | 0.85±0.01$^{bc}$ | 8.050±0.16$^{b}$ | 42.05±0.17$^{b}$ |
| T4 | 16.14±0.44$^{a}$ | 3.68±0.11$^{a}$ | 1.18±0.01$^{a}$ | 5.820±0.03$^{d}$ | 36.02±0.15$^{e}$ |
| T5 | 9.22±0.26$^{d}$ | 2.46±0.04$^{bc}$ | 0.82±0.01$^{c}$ | 6.370±0.16$^{cd}$ | 40.17±0.12$^{d}$ |
| LSD | 1.12 | 0.345 | 0.055 | 0.6 | 0.73 |

No. of samples: 5 from each group, $H_2O_2$, hydrogen peroxide, MDA: Malondialdehyde, LPO: Lipid peroxidation, GR: Glutathione reductase, GSH-Px: Glutathione peroxidase, T1: Administered 2 g $kg^{-1}$ *GL*, T2: Administered 2 g $kg^{-1}$ *AP*, T3: Administered 2 g $kg^{-1}$ *CM*, T4: A positive control, T5: A negative control, The different letters with the means in the same column refer to significant differences among treatment means at p<0.05, LSD: Least significant difference

Table 3: Effects of herbal extracts (*GL*, *AP* and *CM*) on the spleen oxidative status of broiler chickens fed aflatoxin

| | Index | | | | |
|---|---|---|---|---|---|
| Groups | $H_2O_2$ (mmol $g^{-1}$ pro) | MDA (nmol $g^{-1}$ pro) | LPO (μmol $g^{-1}$ pro) | GR (U $g^{-1}$ pro) | GSH-Px (U) |
| **Means±Standard Error** | | | | | |
| T1 | 13.74±0.18$^{c}$ | 1.85±0.02$^{c}$ | 3.44±0.03$^{e}$ | 9.504±0.08$^{a}$ | 47.58±0.19$^{a}$ |
| T2 | 14.60±0.16$^{b}$ | 1.93±0.01$^{ab}$ | 3.91±0.02$^{c}$ | 7.710±0.18$^{c}$ | 43.61±0.18$^{c}$ |
| T3 | 14.10±0.11$^{bc}$ | 1.87±0.02$^{bc}$ | 3.69±0.02$^{d}$ | 8.850±0.18$^{b}$ | 45.42±0.15$^{b}$ |
| T4 | 17.54±0.11$^{a}$ | 1.97±0.09$^{a}$ | 5.52±0.04$^{a}$ | 5.310±0.13$^{e}$ | 38.44±0.24$^{e}$ |
| T5 | 13.62±0.15$^{c}$ | 1.86±0.01$^{bc}$ | 4.32±0.07$^{b}$ | 6.918±0.04$^{d}$ | 41.65±0.37$^{d}$ |
| LSD | 0.619 | 0.081 | 0.182 | 0.58 | 1.01 |

No. of samples: 5 from each group, $H_2O_2$: Hydrogen peroxide, MDA: Malondialdehyde, LPO: Lipid peroxidation, GR: Glutathione reductase, GSH-Px: Glutathione peroxidase, T1: Administered 2 g $kg^{-1}$ *GL*, T2: Administered 2 g $kg^{-1}$ *AP*, T3: Administered 2 g $kg^{-1}$ *CM*, T4: A positive control, T5: A negative control, The different letters with the means in the same column refer to significant differences among treatment means at p<0.05, LSD: Least significant difference

treated with *GL*, *AP* and *CM* were similar to those of T5. The current study showed that AF-contaminated diets exacerbated the oxidation status of broiler chickens. However, there were negative effects of AF and ameliorative effects of the addition of *GL*, *AP* and *CM* to the diets of broiler chickens by activating the antioxidant defense system in the liver and spleen.

**Effect of dietary supplements on the growth performance of broiler chickens fed aflatoxin:** From 0-21 days, a significant (p<0.05) decrease was observed in the Average Daily Feed Intake (ADFI) of birds fed AF-contaminated diets compared to T5 but the addition of *GL*, *AP* and *CM* to the AF-contaminated diets lead to a significant (p<0.05) increase in the ADFI and Average Daily Gain (ADG), especially in T1 compared to T4 as shown in Table 4. The Feed:Gain ratio (F:G) did not have a significant (p>0.05) effect due to AF or *GL*, *AP* and *CM* supplementation in broiler chickens. From 22-45 days, T4 experienced a significant (p<0.05) decrease in ADFI and ADG compared to the groups treated with *GL*, *AP* and *CM*, which showed a significant (p<0.05) increase in ADG and a decrease in F:G. However, no significant difference (p>0.05) was observed in ADFI, particularly in T1, followed by T3 and T2, respectively and similar growth performance results (ADFI, ADG and F:G) were observed in broiler chickens under the influence of AF during the entire experimental period (0-45 days).

**Clinical signs and mortality of broiler chickens:** Table 5 shows the development of clinical signs and mortality within 10 days after the challenge with a virulent field isolate of ND at 35 days of age. Mortality was reported daily after the challenge, along with the total number of dead birds per group. Birds vaccinated against ND with three consecutive doses (*La sota*) in drinking water produced the lowest rate of morbidity and mortality under the effect of AF compared to T5 birds, which recorded the highest percentage of injury and mortality. The groups treated with *GL*, *AP* and *CM* showed a significant (p<0.05) decrease in the morbidity and mortality of AF compared to T4.

## DISCUSSION

The results of the current study showed that aflatoxine had a significant immunosuppressive effect against ND, as well as increased oxidative stress, with a significant increase in

Table 4: Effects of herbal extracts (*GL*, *AP* and *CM*) on the growth performance of broiler chickens fed aflatoxin

| | Groups | | | | | |
|---|---|---|---|---|---|---|
| Index | T1 | T2 | T3 | T4 | T5 | LSD |
| **0-21 days (Mean±SE)** | | | | | | |
| ADFI (g day$^{-1}$) | 60.48±0.35$^{b}$ | 57.16±0.52$^{c}$ | 58.18±0.4$^{c}$ | 52.74±0.63$^{d}$ | 64.74±0.71$^{a}$ | 2.270 |
| ADG (g day$^{-1}$) | 38.38±0.14$^{b}$ | 35.94±0.16$^{d}$ | 36.80±0.14$^{c}$ | 32.42±0.15$^{e}$ | 41.16±0.25$^{a}$ | 0.747 |
| F:G | 1.57±0.01 | 1.58±0.02 | 1.58±0.01 | 1.62±0.02 | 1.57±0.02 | NS |
| **22-45 days (Mean±SE)** | | | | | | |
| ADFI (g day$^{-1}$) | 139.90±0.85$^{b}$ | 137.16±0.68$^{b}$ | 137.10±0.77$^{b}$ | 138.18±0.48$^{b}$ | 145.08±0.52$^{a}$ | 2.830 |
| ADG (g day$^{-1}$) | 80.40±0.65$^{b}$ | 77.70±0.63$^{c}$ | 78.26±0.47$^{bc}$ | 71.08±0.42$^{d}$ | 85.02±0.48$^{a}$ | 2.261 |
| F:G | 1.73±0.02$^{b}$ | 1.76±0.01$^{b}$ | 1.74±0.01$^{b}$ | 1.93±0.01$^{a}$ | 1.70±0.01$^{b}$ | 0.067 |
| **0-45 days (Mean±SE)** | | | | | | |
| ADFI (g day$^{-1}$) | 100.20±1.83$^{ab}$ | 96.60±1.49$^{b}$ | 98.80±1.36$^{b}$ | 95.40±1.31$^{b}$ | 106.10±1.59$^{a}$ | 6.396 |
| ADG (g day$^{-1}$) | 56.30±0.5$^{b}$ | 53.16±0.4$^{c}$ | 54.68±0.4$^{bc}$ | 48.80±0.36$^{d}$ | 59.44±0.55$^{a}$ | 1.961 |
| F:G | 1.77±0.01$^{b}$ | 1.81±0.02$^{b}$ | 1.80±0.01$^{b}$ | 1.95±0.01$^{a}$ | 1.78±0.03$^{b}$ | 0.1 |

No. of samples: 5 from each group, ADFI: Average daily feed intake, ADG: Average daily gain, F:G: Feed:Gain ratio, equal to ADFI/ADG, T1: Administered 2 g kg$^{-1}$ *GL*, T2: Administered 2 g kg$^{-1}$ *AP*, T3: Administered 2 g kg$^{-1}$ *CM*, T4: A positive control, T5: A negative control, The different letters with the means in the same column refer to significant differences among treatment means at p<0.05, LSD: Least significant difference

Table 5: Development of clinical signs and mortality during 10 days post challenge with a local NDV isolate at 35 days of age

| | Index | |
|---|---|---|
| Groups | Morbidity (%) | Mortality (%) |
| T1 | 60 (12)$^{c}$ | 10 (2)$^{c}$ |
| T2 | 75 (15)$^{bc}$ | 20 (4)$^{c}$ |
| T3 | 60 (12)$^{c}$ | 10 (2)$^{c}$ |
| T4 | 90 (18)$^{b}$ | 45 (9)$^{b}$ |
| T5 | 100 (20)$^{a}$ | 100 (20)$^{a}$ |

No. of chicks for each group: 20, #: No. of chicks showing clinical signs or mortality, T1: Administered 2 g kg$^{-1}$ *GL*, T2: Administered 2 g kg$^{-1}$ *AP*, T3: Administered 2 g kg$^{-1}$ *CM*, T4: A positive control, T5: A negative control, The different letters with the means in the same column refer to significant differences among treatment means at p<0.05, LSD: Least significant difference

MAD, LPO and $H_2O_2$ and a decrease in antioxidants GR and GSH-PX. These results agree with those of many researchers, confirming a significant reduction in Total Protein (TP), albumin, IgA, IgG and IgM in broiler chickens after feeding diets containing 300 µg kg$^{-1}$ AF[30,31]. The results of the current study showed that adding GL to the diets contaminated with AF reduced the negative effects of AF on broiler growth performance. The *GL* is a traditional Chinese herb and has antioxidant properties and hepatoprotective and immunomodulation activities[32,33]. The potent antioxidant activity of *GL* is due to multiple polysaccharides that have the ability to remove DPPH radicals and reduce the increased energy by inhibiting LPO in vitro. In addition, *GL* protects the liver by reducing hepatic AST, ALT and MDA in serum; these compounds reduce Cd(II)-induced hepatotoxicity in mice. The addition of 2-4 g kg$^{-1}$ *GL* to the diets of mice significantly increased the effectiveness of antioxidant enzymes (GSH and GR) and reduced MDA levels in rats (14). Mohan *et al.*[34] showed that feeding *GL* polysaccharides induced significant (p<0.05) increases in muscle TP. Finisher pigs fed *GL* polysaccharides revealed significantly (p<0.05) increased *TP* and IgG levels[35]. Roy *et al.*[22] proved that *AP* protects the intestinal tract against bacterial infections. These results have been verified in many studies[36]. Several researchers have noted the role of *AP* in improving FCR in broiler chickens through increased metabolism by better utilization of nutrients in the diet due to reduced pH, viscosity and thickness of the intestinal lining of the gastrointestinal tract of broiler chickens[37]. The traditional use of this herb represents its role as an anti-inflammatory, anti-viral, hepatoprotective, antioxidant and effective immune regulator[20]. The active ingredient of this herb has anti-cancer and anti-HIV properties and is an active antimicrobial[37]. The protection was highly successful against the hepatic toxic effects of AFs in broiler chickens fed *CM*- and AF-containing diets. Although the broiler chickens were fed AF-contaminated diets, no significant effect was observed on their growth performance. The results of the current study have been consistent with several previous studies that have demonstrated that liver damage caused by AFB1 has disappeared in chickens fed *CM*-containing diets; *CM* plays a protective role in reducing the adverse effects of AFB1[38]. In addition, AFB1 induced highly significant oxidative stress represented by $H_2O_2$, MDA and LPO through reducing the antioxidant susceptibility (GSH, GR, GPX and CAT). On the other hand, *CM* prevented these changes by improving the effectiveness of hepatic GSH-Px[39]. The current study shows that the addition of these herbs plays a significant role in mitigating the negative effects of AFs and improving the immunity and growth performance of broiler chickens.

## CONCLUSION

The results of the current study show the role of AF-contaminated diets in immunosuppression, significantly reduced growth performance and reduced antioxidant susceptibility of broiler chickens. At the same time, they show the role of *GL*, *AP* and *CM* in reducing the negative effects of AFs by decreasing oxidative stress and immunosuppression. The largest role was played by *GL* as the best feed additive used to inhibit aflatoxicosis, which may play a major role in industrial feed applications.

## SIGNIFICANCE STATEMENT

In poultry, AF is considered to be major and important diseases due to significant economic losses from retarded growth rates, high mortality rates, lack of food conversion, increased immunosuppression due to damage to the immune system of infected birds and internal organs damage especially to the liver and spleen. Moreover, the increased human consumption of poultry has placed upon the poultry industry many regulations that act in favor of safety and to prevent contamination of the product with germs and substances that negatively affect human health. AFB1 is one of the most common toxins in poultry diets and the most taxonomic compared to other toxins. When AFs are linked with a pro-oxidant, they generate ROS that impede DNA, RNA, fat, protein and other molecules. In this study, mushroom and the commercial herbs *GL*, *AP* and *CM* decreased oxidative stress resulting from the release of large amounts of LPO, $H_2O_2$ and MDA by increasing antioxidant defenses, repairing hepatic and spleen tissues and enhancing immunity and disease resistance.

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