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Effect of oral administration of *Myrtus communis* extract on reducing the negative impacts of feed contaminated with mycotoxins on productive performance and some blood characteristics in local male rabbits

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ABSTRACT
Background: Mycotoxins, secondary metabolic compounds released by bacteria, negatively impact the environment, animals, and people. Extraction from *Myrtus communis* may be used instead of antibiotics to treat microbial infections.
Aim: This study investigated the ability of a medicinal plant extract (*M. communis*) to reduce the harmful effect of mycotoxin on the productivity and health status of male rabbits. In addition, it improved the productivity and health status of male rabbits and detected Aflatoxin-B1 residues in kidney and liver tissues using the High-performance liquid chromatography (HPLC) technique.
Methods: Twenty-four local male rabbits, aged 5–6 months with a mean body weight of 1393 ± 20 g, were uncontaminated in three groups based on body weight, each consisting of eight rabbits. The initial control group was provided with a basal diet uncontaminated (C); the second group was given a diet contaminated with state the concentration of mycotoxins in diet mycotoxins (T1); the third group feeds on the same diet as the second group received a diet contaminated with mycotoxins and was treated with *M. communis* at a dosage of 250 mg/head orally (T2).
Results: A significant increase in body weight was observed in the T2 that was treated with *M. communis* after 2, 4, and 6 weeks; the feed intake showed that in the first 2 and 4 weeks, there was a significant increase in T2 compared with C and T1 in all groups of the experiment with no significant change in 6 and 8 weeks. Regarding the blood parameters white blood cells (WBCs), red blood cells (RBCs), Hemoglobin (Hb), and platelets, there was no change among groups; creatinine increased significantly in the T1 and T2 groups, whereas the total protein was unchanged. The liver enzymes AST enzyme increased in the T1 compared with the T2 group, representing improved liver functions. However, alanine aminotransferase was within the average level for the three groups; after detection of AFB1 in the kidney and liver by HPLC, the concentration of AFB1 in T1, T2 was 8.6,1.4 in the kidney tissue, 10.5, 1.6 in also liver T1, T2, respectively, while the control group was under detectable level.
Conclusion: In conclusion, giving *M. communis* extract orally to rabbits can play an important role in improving production performance and reducing the toxic effects of mycotoxins. It was suggested that the activity of this organ to eliminate the mycotoxins by the *M. communis* action. In conclusion, giving *M. communis* orally to rabbits improves their productive performance and minimizes the toxic effects of mycotoxins.
Keywords: *Myrtus communis*, Mycotoxins, Liver enzyme, Rabbits, HPLC.

Introduction

Mycotoxins are secondary metabolic substances secreted by fungi and have many harmful effects on humans, animals, and the environment (Zhou *et al*., 2019; Herman *et al*., 2021).

The most dangerous is transmission in animal secretions such as milk, urine, and feces (Kemboi *et al*., 2020; Lippolis *et al*., 2020; Cha *et al*., 2021; Al-Rubaye *et al*., 2023) as well as in eggs and meat (Mahdi and Atiyah, 2021) as a residue that is only detected by sensitive devices for their presence in tiny proportions for consumption (De Santis *et al*., 2017; Wang *et al*., 2019), which are the permissible ranges according to USA and EU regulations.

Mycotoxins have apparent effects on body weight, feed consumption, milk production, immunosuppression, and antioxidant activity (Huang *et al*., 2018; Hung *et al*., 2018; Cuciureanu *et al*., 2021). Studies confirm

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that 25%–50% of cereals are exposed to mycotoxins on a large scale worldwide. The problem facing the agricultural sector comes from exposure to these toxins, which are transmitted to animals through food and thus transfer to humans through production, and the cycle continues.

To get rid of these toxins, several methods have been used to reduce the damage of mycotoxins (Horky *et al*., 2018; Hussein and Atiyah, 2020; Mahmood and Atiyah, 2021). Medicinal herbs have become common in recent years to support the health situation, treat many diseases, increase animal production, and benefit from the remnants of these plants (Sheikh-Zeinoddin *et al*., 2018; Li *et al*., 2021).

There is global pressure to use potent herbal plants as a food ingredient because plants are widely relevant for treating disease (Damiano *et al*., 2020) and detoxification of mycotoxins (Al-Owaisi *et al*., 2022). The primary components of myrtle oil (up to 0.8% in the leaves) are myrtenol, myrtenol acetate, limonene (23%), linalool (20%), pinene (14%), cineol (11%), as well as p-cymene, geraniol, nerol, phenylpropanoid, and methyleugenol. The anti-fungal efficacy of *Myrtus communis* against *Malassezia* sp. isolated from the skin of individuals with pityriasis versicolor (Gultepe *et al*., 2020). The findings indicate that *M. communis* extraction might serve as an alternative to antifungal medications for treating fungal infections of the skin and mucous membranes, as well as combating dandruff. Another study measured the antioxidant activity of methanol, ethanol, water, and ethyl acetate extract of the leaves and berries. All of the extracts showed significant antioxidant capacity and higher antioxidant content in leaves (Amensour *et al*., 2010). Flavonoids and anthocyanins in berry extract were examined for their activity against free radicals. The myrtle extract demonstrated interesting free radical scavenging activity (Montoro *et al*., 2006).

The *M. communis* extract was used to reduce the concentration of Aflatoxin B1 residue in rabbits' livers and kidneys after mixing it with feed at a rate of 500–1,000 mg /kg diet (Mahmood and Atiyah, 2021). This study was designed to observe the effect of the medicinal plant *M. communis* on reducing the damage of mycotoxins.

Materials and Methods

Twenty-four healthy local male rabbits were brought at the age of about 4–5 months, with a mean body weight (1,393 $\pm$ 20) were kept in cages at the animal house of the veterinary college, Baghdad University. All animals were fed on the same concentrated diet and water, and feed was offered for 3 weeks as a preliminary period. The animals were divided into three equal groups of eight animals each, as follows:

The first group was fed a concentrated diet without contamination with the mycotoxin AFB1 or the addition of *M. communis* and kept as a control group (C).

The second group was fed a concentrated diet naturally contaminated with mycotoxin (T1)

The third group was fed on the concentrated diet contaminated with AFB1 and received *M. communis* at 250 mg/orally (T2).

Preparation of mycotoxin

Mycotoxin was prepared naturally using contaminated corn, which was taken from where the corn was moldy; the corn was mixed with the concentrated diet according to the standard percentage of the control diet after mixing it well and estimating the concentration of mycotoxin three times at the beginning of the study, middle, and end of the study using the ELISA test; as illustrated in Table 1.

Collection of the plant and preparation of the extract

Fresh *M. communis* leaves harvested from local trees in Baghdad were rinsed with tap water, air-dried, and ground into a fine powder using an electric grinder. The aqueous extraction involved placing 50 g of produced powder into 200 ml of distilled water. The solvent from the extracted substance was eliminated using a Soxhlet extractor at 2°C for 4 hours, followed by a rotary evaporator at 60°C for 2 hours. The extracted residue was obtained and stored in the freezer at −20°C for the duration of the investigation, following Dakheel *et al.* (2021).

The samples and parameters of this research involve the following: Body weight (g) was calculated for each group biweekly at weeks (0, 2, 4, 6, and 8) to assess weight changes.

Feed intake (g)

The feed intake was measured daily by subtracting the offered feed (100 g/rabbit) from the remaining quantity the next day. Body weight was also measured to assess overall gain during the experiment.

Blood samples

The samples were collected on weeks 4 and 8 of the experiment to evaluate the abovementioned parameters. Blood samples were (5 ml) from the heart after sterilization of the site of blood drawn by using a disposable syringe. The samples were divided into two parts. The first part of the blood samples was kept in sterile tubes (capacity 10 ml) containing the anticoagulant ethyl diamine tetra acetic acid. The other part of the blood samples was kept in the sterilized tube containing gel and clot activator, and then separated by centrifuge (3,000 rpm) for 5 minutes.

Estimation of AFB_1 residue in liver and kidney by HPLC

Twelve randomly chosen rabbits from each diet group, four per cage, were slaughtered, and their livers and kidneys were removed at the conclusion of the study. Pooling each group's liver and kidneys yielded four tissue samples for each dietary treatment. Refrigerated or frozen samples were placed on a disposable counter. Tissue aflatoxins were eliminated.

Sample preparation

After 25 g were set up in 100 ml methanol/water (70:30 v/v) for 40 minutes and centrifuged for 5 minutes, 5 ml

of the supernatant was extracted, diluted with 20 ml of water, and run through the immunoaffinity column at a rate of no more than 3 ml per minute (the column had been conditioned with 20 ml of distilled water beforehand). After removing the matrix components with 10 ml D.W., the column was allowed to air dry to eliminate any last traces of water. Following the quantitative elution, 1.4 ml of methanol was added to the column and flushed with air. The eluate was then diluted with 2 ml of water and filtered through a 0.45 mm filter before being injected into the HPLC apparatus.

The samples were analyzed via HPLC (model SYKAMN/Germany) according to the methodology outlined by Dan *et al.* (2012): The mobile phase comprised acetonitrile and distilled water in a ratio of 60:40. A C18-ODS column (25 cm × 4.6 mm) was used, with a flow rate of 0.7 ml/min. The detection was performed using a fluorescent detector with an excitation wavelength of 365 nm and an emission wavelength of 445 nm.

Statistical analysis

The Minitab (2022) software was used to detect the effects of different factors on the study. The least significant difference test (*T*-test) was used to compare means significantly ($p \leq 0.005$).

Results

Figure 1 shows that in the first 2, 4, and 6 weeks, body weight significantly increased in T2 G3 compared with C and T1 in all groups of the experiment, with no significant change in 8 weeks. However, Figure 2 showed that in the first 2 and 4 weeks, feed intake was significantly increased in T2 compared with C and T1 in all groups of the experiment, with no significant change in 6 and 8 weeks.

On the other hand, there were no significant changes in WBCs, RBCs, Hb, and platelets among all groups during the study, as shown in (Table 2).

Creatinine

The results of serum creatinine at 4 and 8 weeks are presented in Table 2, showing that there was no significant change in 4 weeks and a significant increase ($p \leq 0.05$) in T1 compared to c and T2 in 8 weeks.

Total protein

Table 2 also shows the serum total protein levels during the 4 and 8 weeks, and there were no significant changes among the three groups during these periods.

Serum alanine aminotransferase (ALT)

Table 2 displays the serum ALT values at 4 and 8 weeks. The ALT enzyme results indicated a substantial rise ($p \leq 0.05$) and no significant change at 4 weeks. T1 in contrast to c and T2.

Serum aspartate aminotransferase

The ALT enzyme results revealed that there was no significant change in 4 weeks and a significant rise ($p \leq 0.05$) in T1 compared to c and T2, whereas the results of serum AST at 4 and 8 weeks are shown in Table 3.

The concentrations of AFB_1 in the kidney and liver

The results showed that the concentration of AFB1 in the kidney was 8 and 6 ppb compared with the group treated with M.C.E, where it was 1.4 ppb. In this instance, the level of AFB1 in the liver was measured at 10.5 ppb, which dropped to 1.8 ppb following daily oral treatment with M.C.E. This is illustrated in Figures 3 and 4, confirming a highly significant increase in AFB1 concentration in the kidney and liver when

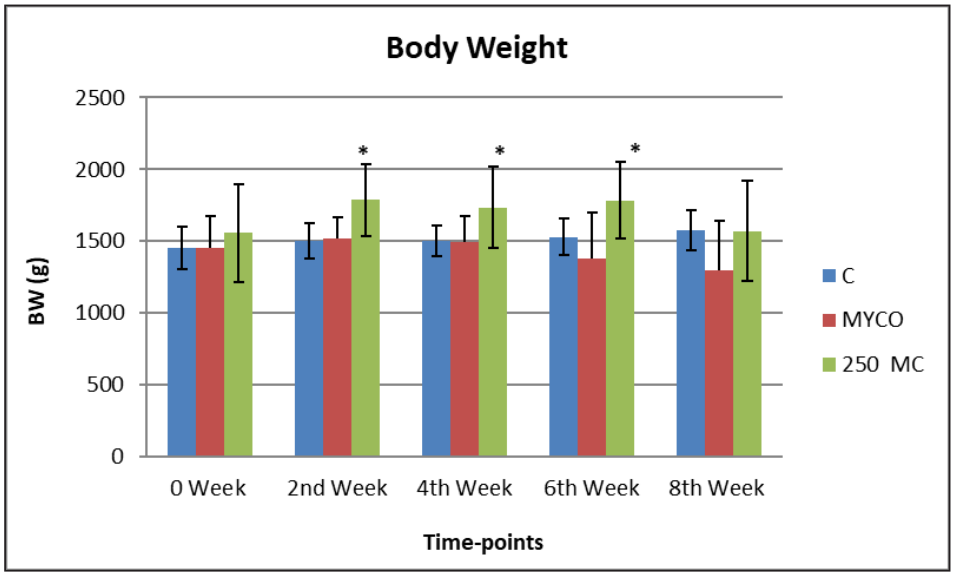

Fig. 1. The body weight of treated groups at 0, 2nd, 4th, 6th, and 8th weeks (mean ± SE), and stars indicate the significant differences at ($p \leq 0.05$).

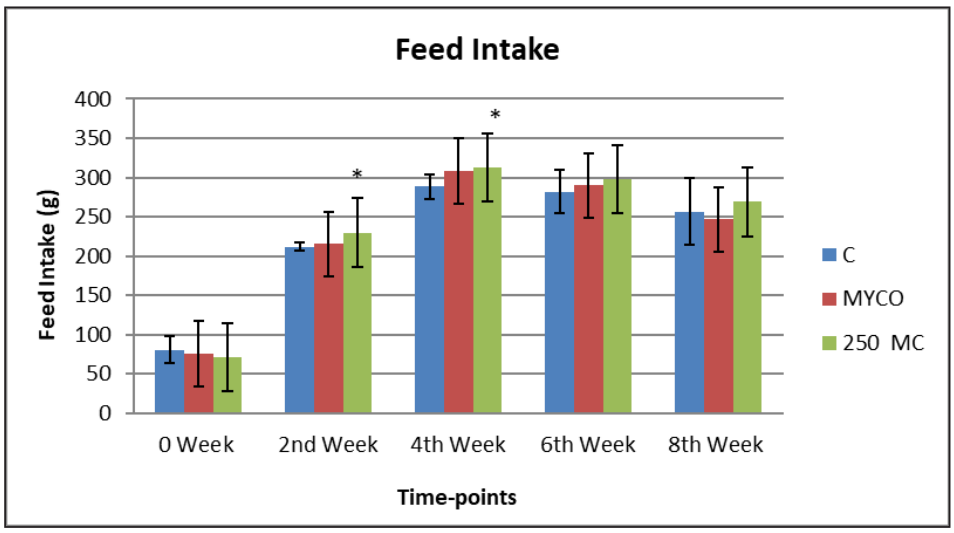

Fig. 2. The feed intake of treated groups at 0, 2nd, 4th, 6th, and 8th weeks (mean ± SE), and stars indicate the significant differences at ($p \leq 0.05$).

Table 1. Concentration of mycotoxin in contaminated dietary feed according to the ELIZA test.

Mycotoxin	Concentration of mycotoxins in feed
Aflatoxin (ppb)	11.8
Ochratoxin (ppb)	1.9
T2 toxin (ppb)	8.7
Fumonisin (ppm)	5.4

ppb = parts per billion; ppm= parts per million.

compared to the group that obtained M.O.E orally after a diet contaminated with AFB1.

Discussion

After 2 weeks in T2, this trial showed a noticeable shift in body weight. Mycotoxin is the cause of the drop in body weight. According to several studies, different breeds of developing or adult rabbits treated with AF showed decreased body weight (Fayed, 1999; Ibrahim, 2000; Shehata, 2002). In general, Hafez *et al.* (1983) found that aflatoxin-B1 combined with aflatoxin-G caused a reduction in body weight in both male and female rabbits. Abd El-Hamid (1990) also produced a closely comparable work. The diet of Baladi rabbits consisted of AFs B1, B2, G1, and G2.

Lower feed conversion caused by AFB1 handling could be attributed to aflatoxicosis or maybe its impact on the regulation of the hypothalamic feed intake core, in addition to the dangerous influence of AFB1 on the digestion and absorption of various nutrients. The changes in hormonal balance among animals cared for with AF may lead to a slight reduction in body weight and other functional requirements. Moreover, reduced feed intake and weight gain among animals fed AF-contaminated diets have been documented in commercial ducks (Han *et al*., 2008) and mice (Kocabas *et al*., 2003). In broiler chickens, feed consumption was reduced to 2.5 ppm/ diet AF (Shareef *et al*., 2019).

The current results also agreed with previous results mentioned that there was a diminishment in body weight in various breeds of growing or adult rabbits handled by AF (Shehta *et al*., 2002; Hussein and Atiyah, 2020; Mahdi and Atiyah, 2021). Further, Hafez *et al*. (1983) reported a decrease in body weight for male and female rabbits through AFB with AFG. The decreased body weight with increasing aflatoxin concentrations in the diet was mainly related to the reduced feed intake. With increasing dietary aflatoxin concentrations, feed intake was linearly decreased. The current study did not agree with the previous research, which showed no effect change in body weight (Hussein and Atiyah, 2020; Mahdi and Atiyah, 2021; Abdulhussein and Dakheel, 2022). Previous research indicates comparable beneficial outcomes, including enhanced feed intake and egg yield in laying hens when myrtle plant extract is incorporated into drinking water at various levels (Goudarzi *et al*., 2016). Mahmoodi-Bardzardi *et al*. (2014) concurred with these findings, noting an increase in feed consumption and live weight in chickens with the addition of myrtle extract to the feed, whereas Biricik *et al*. (2012) reported a decrease in live weight.

Bülbül *et al*. (2014) observed enhanced egg production in chickens administered myrtle plant extract at a dosage of 1000 mg/kg. Saei *et al*. (2013) reported that myrtle plant extract did not produce a significant effect on live weight gain in broilers when compared to the control group. It nonetheless decreased feed consumption and

Table 2. The complete blood count values of treated rabbits at 0, 2nd, 4th, 6th, and 8th weeks.

Groups / Parameters	Control		Mycotoxins		250 MC	
	1st period	2nd period	1st period	2nd period	1st period	2nd period
WBCs ($_X10^6$/µl)	5.24 ± 2.29	6.82 ± 1.71	4.95 ± 3.64	7.12 ± 1.17	4.05 ± 2.55	6.18 ± 1.58
RBCs ($_X10^3$/µl)	5.19 ± 0.93	5.67 ± 0.56	4.80 ± 0.64	5.85 ± 0.51	5.02 ± 0.47	5.62 ± 0.63
Hb (g/dl)	12.62 ± 1.16	12.70 ± 1.30	12.33 ± 1.96	11.08 ± 1.47	12.75 ± 1.08	13.55 ± 0.96
Platelets ($_X10^3$/µl)	467.00 ± 68.94	430.80 ± 102.44	459.00 ± 125.2	525.67 ± 114.7	466.50 ± 54.37	453.50 ± 109.52

The values are displayed as (mean ± SE).

Table 3. The biochemistry values of treated rabbits at 0, 2nd, 4th, 6th, and 8th weeks.

Groups / Parameters	Control		Mycotoxins		250 MC	
	1st period	2nd period	1st period	2nd period	1st period	2nd period
ALT (U/l)	66.25 ± 16.5	49.2 ± 18.3	64.0 ± 14.5	59.7 ± 17.1*	67.3 ± 19.8	51.4 ± 12.9
AST (U/l)	40.5 ± 1.79	50.8 ± 9.18	35.5 ± 2.65	72.1 ± 7.39*	38.5 ± 2.58	64.7 ± 5.60*
Creatinine	0.45 ± 0.10	1.39 ± 0.07	0.46 ± 0.12	1.85 ± 0.19*	0.48 ± 0.14	1.73 ± 0.20*
Total protein	63.6 ± 4.55	61.12 ± 5.94	65.4 ± 4.84	58.8 ± 5.49	68.9 ± 4.08	67.85 ± 5.67

The values are displayed as (mean ± SE), and the stars in the same row indicate the significant differences at ($p \leq 0.05$).

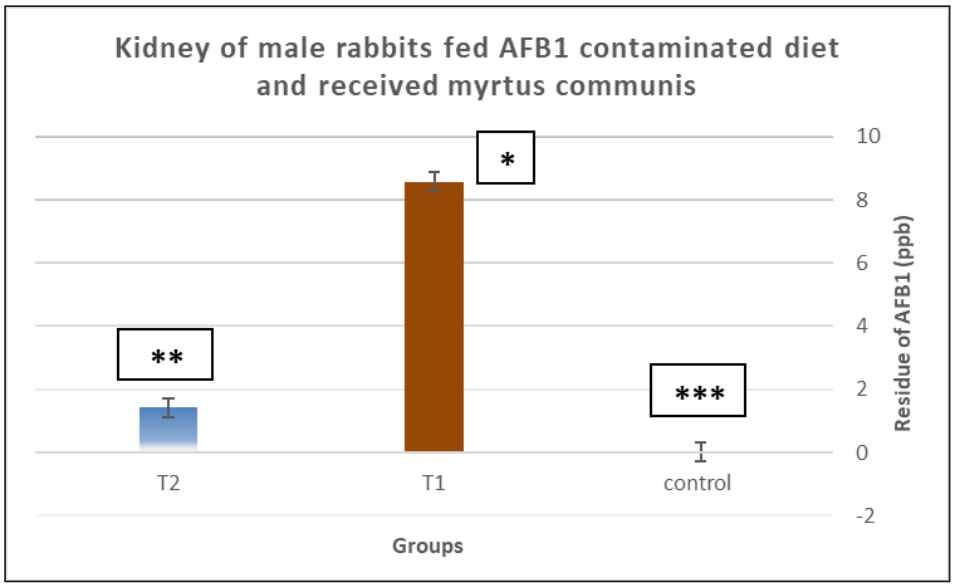

Fig. 3. Concentration of AFB1 residue in the kidney after receiving *Myrtus communis* extract and/or AFB1 in the kidney of male rabbits fed AFB1-contaminated diet. The stars indicate significantly different ($p \leq 0.01$); means ($n = 5$) ± SEM.

enhanced the feed conversion ratio. Taee *et al*. (2017) demonstrated that the inclusion of 1% myrtle powder in the diet of rainbow trout resulted in increased live weight gain and enhanced feed conversion ratios. This finding underscores the significance of incorporating *M. communis* into feed affected by mycotoxins. The beneficial effects observed on growth and feed intake in rabbits exposed to mycotoxins in the current study may be linked to the antioxidant, antibacterial, antifungal, and anti-inflammatory properties of myrtle plant extracts.

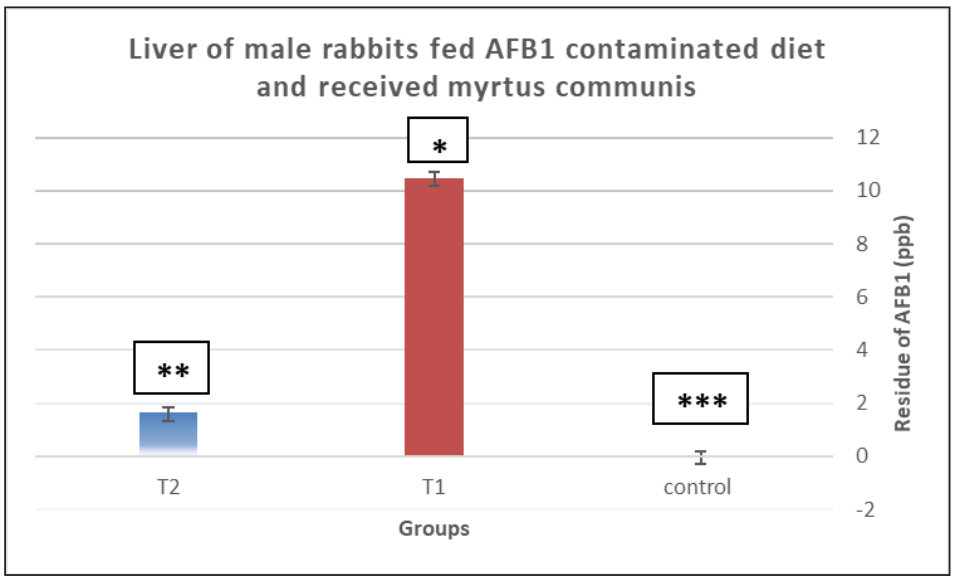

Fig. 4. Concentration of AFB1 residue in the liver after receiving *Myrtus communis* extract and/ or AFB1 in the kidney of male rabbits fed AFB1-contaminated diet. The stars indicate significantly different ($p \leq 0.01$); means ($n = 5$) ± SEM.

Moreover, this result agrees with studies showing decreased total protein after administrating AFB1 (Sun *et al*., 2015). Also, some studies show increased total protein after administration (Zhou *et al*., 2019). The secondary metabolite of herbal plants may cause ameliorative effects on the productive traits and biochemical properties after contaminated feeding a diet with mycotoxins to rabbits (Mahmood and Atiyah, 2021; Abdulhussein and Dakheel, 2022).

Increased creatinine was an important indicator of the action of mycotoxins in the damage of tissue in the kidney; this was observed in our study, where creatinine increased significantly in the group that was fed a diet contaminated with mycotoxins; this agreed with (Vaziriyan *et al*., 2018), the decrease of creatinine concentration in the blood after administration of *M. communis* confirms the positive action of this material, as it contains antioxidants such as flavonoids, which reduce the effect of free radicals that harmful to cell (Montoro *et al*., 2006; Amensour *et al*., 2010).

In the current study, there is an increase in AFB1 compared to the control group, and that result agrees with the authors showing a higher increase in ALT in broiler chick after administrated AFB1 (Hussain *et al*., 2016). T1 shows a slight rise in ALT compared to the control group; this result agrees with a previous study using *M. communis* in water, which shows an increase in ALT (Gultepe *et al*., 2020). Another study shows a decrease in ALT after using *M. communis* (Bagcilar and Gezer, 2020). The result of AST shows no significant change in 4 weeks among the group; in the 8 weeks, there was a significant increase in T2 compared to the other group. The result agrees with another study (Qian *et al*., 2016), which shows an increase in AST after the administration of AFB1. The phenolic compounds in MCE likely protected the liver against toxic agents; thus, the AST decreased in C and T2 compared with T1. Saei *et al.* (2013) concluded that the use of *M. communis* as the treatment reduces the harmful effects of aflatoxin on various aspects, including glucose, creatinine, cholesterol (ALT), (AST), and (ALP) concentration. Flavonoid compounds in the Myrtle leaves, such as rosmarinic acid, caffeic acid, thymol, and carvacrol, are free radical absorbents. Free radicals cause lipid peroxidation, and these compounds are effective against lipid peroxidation (Yazdani *et al*., 2014).

The levels of AFB$_1$ in the kidney and liver

The presence of mycotoxins in the kidney and liver indicates that the body metabolizes mycotoxins. Biochemical analyses indicate a boost in the liver enzyme creatinine, alterations in certain blood parameters, and a decrease in body weight and feed intake in male rabbits. The findings match with those reported by (Hussein and Atiyah, 2020; Mahdi and Atiyah, 2021; Mahmood and Atiyah 2021), indicating that the concentration of AFB1 in the kidney and liver was 10.5 ppb, exceeding the acceptable limit of 8.6 ppb in the USA and EU. The concentration in the group administered MCE orally remained within acceptable limits. Rahmati-Joneidabad (2021), Mahmood and Atiyah (2021), Belmimoun *et al*. (2020), and Muhanna Al-Abdali *et al.* (2019) indicated that M.C.E. may inhibit fungal activity, thereby reducing mycotoxin production, suggesting beneficial treatment effects.

This is consistent with the findings of Gumus *et al.* (2010).

Bioactive compounds derived from medicinal plants, such as phenolic and flavonoid chemicals, are active therapeutic tools for treating many health problems. The active ingredients in *M. communis* are myrtenol, myrtenol acetate, limonene, linalool, pinene, and cineol. Moreover, p-cymene, geraniol, nerol, phenylpropanoid, and methyl eugenol can act against fungi immediately or indirectly as antioxidant materials, supportive immunity, and affirmed reactions (Mohammadi *et al*., 2020; Shaapan *et al*., 2021).

Conclusion

The study concluded that the *M. communis* extract has a positive effect on the health status of male rabbits fed a contaminated diet with mycotoxins, which improved the productive trait and ameliorative effect on liver and kidney function, in addition to decreasing the concentration of aflatoxin-B1 in the kidney and liver.

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Conflict of interest

The authors did not declare any conflicts of interest.

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Authors' contributions

Dr Mohammed Munis Dakheel was the corresponding author for this article. Dr Adil Jabbar Atiyah was responsible for study observations and research management. Shireen Jamal Mahmood was responsible for animal care, clinical parameters, and obtaining blood samples. Article writing was done equally by the authors.

Data availability

All data were provided in the manuscript.

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