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ORIGINAL ARTICLE

Anti-inflammatory and Antioxidant Effects of *Melissa officinalis* (Lemon Balm) on Paracetamol-induced Hepatotoxicity in Broiler Chickens

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Abstract

BACKGROUND: This study aimed to investigate the hepatoprotective effects of *Melissa officinalis* against paracetamol N-acetyl-p-aminophenol (APAP)-induced hepatotoxicity in broiler chickens.
MATERIALS AND METHODS: A total of 48 one-day-old Cobb healthy chicks were divided into four groups of 12 chicks. The control group received a basal diet for 21 days, while the APAP group received APAP at 2 g/kg body weight daily from the 15th day onward and until the termination of the experiment. The *M. officinalis* + APAP group was offered a 10 g/kg diet for 2 weeks followed by APAP for 1 week, and the APAP + *M. officinalis* group received APAP for 1 week followed by *M. officinalis* for 2 weeks. Hematological and biochemical determination of oxidative stress biomarkers involves gene expression analysis. In addition, histopathological examination of the liver specimens was performed.
RESULTS: Supplementation of lemon balm in the feed of broilers intoxicated with APAP significantly elevated the levels of hematological parameters such as red blood cells count, hemoglobin content, hematocrit%, white blood cells, lymphocyte%, monocyte%, total protein, and albumin, and reduced the activities of alanine aminotransferase, aspartate aminotransferase, and alkaline phosphatase, compared with the APAP group. Furthermore, lemon balm decreased malondialdehyde content and raised glutathione levels and the activities of the antioxidant enzymes superoxide dismutase and catalase. Moreover, a remarkable decrease in the mRNA expression of interleukin-1β) and toll-like receptor-4 with an increase in superoxide dismutase and glutathione peroxidase was recorded in hepatic tissues of *M. officinalis* + APAP and APAP + *M. officinalis*.
CONCLUSION: Administration of *M. officinalis* leaves offers hepatic protection against paracetamol-induced hepatotoxicity in broilers.

Keywords: Broilers, Hepatotoxicity, *Melissa officinalis*, Oxidative stress, Paracetamol

1. Introduction

The large-scale poultry business is expanding worldwide day by day to cope with the increasing demand for food in terms of meat and eggs. Compared with red meat, poultry meat is easily digestible, has a pleasant taste, and is relatively less expensive. It includes high biological value proteins, iron, and zinc, as well as vitamins B6, B12, riboflavin, and thiamin [1]. Poultry meat is not only a product that is being consumed, but its viscera are also consumed for both industrial and food purposes. The most important organ is the liver, followed by the gizzards.

As a significant organ, the liver regulates numerous essential functions, including the

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metabolism, storage, secretion, and detoxification of both endogenous and foreign substances. Thus, it is viewed as a target organ for several toxins [2]. Liver damage in chickens can result from chemical poisoning or metabolic problems [3]. Oxidative stress is believed to be a key contributor to liver damage or is implicated in the cause of liver illnesses because it occurs when the liver is exposed to free radicals produced from specific foreign substances and medications [4].

There are several techniques for treating liver disorders in poultry, one of which is the restricted use of chemical hepatoprotectives to protect consumers of chicken products from the hazards of drug residues. As a result, herbal therapy seems to be the only viable method for treating liver diseases in poultry farms [5]. Worldwide, the use of herbal medications to treat liver problems has grown owing to the idea that these medications are safe and do not cause serious side effects. In addition, nature makes it simple to collect them. A further factor driving the use of alternative medicines, such as herbal remedies, is the restricted therapeutic options and sometimes unpleasant therapeutic failure of traditional medicine [6].

Lemon balm is an important plant in herbal medicine that is being added to poultry feed as an additive to enhance the quality of meat and the health of poultry birds [7]. The medicinal effects of lemon balm are due to the presence of several medically important chemicals such as antioxidant components, including hydroxycinnamic acid derivatives such as coumaric, rosmarinic, and caffeic acids, and flavonoids such as hesperidin and naringin [8]. Published studies have suggested lemon balm as a growth promoter and a better alternative to antibiotics [9]. This study was conducted to investigate the potential protective effects of *Melissa officinalis* (lemon balm) against paracetamol-induced hepatotoxicity in broilers.

2. Materials and methods

2.1. Drugs

Lemon balm plants were purchased from a local market in Mansoura (El Nekety market) at a dose of 10 g *M. officinalis* leaves/kg basal diet [9]. Phytochemical investigations of *M. officinalis* have revealed the presence of various phytochemicals, including terpenes (mono-terpenes, sesqui terpenes, and triterpenes) and phenolic compounds (phenolic acids, flavonoids, and tannins). The main active constituents of *M. officinalis* are volatile compounds (e.g. geranial, neral, citronellal, and geraniol), triterpenes (e.g., ursolic acid and oleanolic acid), and phenolics (e.g. cis-and trans-RA isomers, caffeic acid derivatives, luteolin, naringin), and the major phytochemicals that have been isolated and characterized from *M. officinalis*. *M. officinalis* medicinal activities are mainly attributed to its essential oil and phenolic compounds [10]. Paracetamol (Pfizer Pharmaceutical Company, Dokki, Giza Governorate) was used as a reference drug for hepatic damage. Each capsule contained 500 mg of paracetamol. It was obtained from a local pharmacy and administered orally at a dose of 2 g/kg body weight [11].

2.2. Experimental design

A total of 48 Cobb clinically healthy nonvaccinated chicks (1 day old with an average weight of 45 ± 3 g were purchased from a local poultry farm in Gamasa city and reared for 1 week before starting the experiments for accommodation under environmentally controlled conditions with wood shaving as litter material at 10 cm depth. All chicks were provided with *ad libitum* access to water and feed. The basal diet was formulated according to the Broiler Performance and Nutrition Supplement for Cobb 500 broilers. Chicks were randomly assigned to four groups (12 chicks/group). The first group (control) received a basal diet for 21 days. The second group was administered paracetamol (APAP) at 2 g/kg body weight orally starting on the 15th day onwards and until the end of the experiment [11]. The third group; received 10 g of lemon balm/kg diet for 2 weeks from the first day to the 14th day followed by APAP at 2 g/kg body weight for 1 week till the end of the experiment from the 15th day to the 21st day, while the fourth group received APAP at 2 g/kg body weight for 1 week from the first day to the seventh day followed by lemon balm 10 g/kg diet for 2 weeks from the eighth day to the 21st day [12].

2.3. Collection of samples

On the day following the conclusion of the EDTA experiment, blood samples were collected from the wing veins of six chicks in each group for laboratory analysis. To separate the serum for biochemical research, further blood samples were collected and allowed to coagulate. After that, all of the chosen chickens were anesthetized by an intraperitoneal injection of xylazine and ketamine at 0.6 and 0.7 ml/kg of body weight, respectively [13]. The liver was removed. It was then split into three sections, the first of which was homogenized and centrifuged to extract the supernatant, which was then used to measure oxidative stress indicators. The second portion was

preserved in 10% formalin for histological analysis, whereas the remaining component was kept at −80 °C to determine the expression pattern of certain genes. The Research Ethics Committee, Faculty of Veterinary Medicine, Egypt approved the experimental design and animal handling protocols of this study (approval number: Ph. D/72).

2.4. Hematological analysis

Blood was gently suctioned into a red blood cell (RBC) pipette to a point of 0.5, and the dilution fluid was extracted to the 101 mark. The blood and dilution fluid were mixed by vigorously shaking the pipette with a vigorous 3-min shake. A small amount of the dilution solution was discarded. At a 45° angle, the pipette was inserted into the gap between the coverslip and the counting chamber. To fill the void between the two grooves, a drop of diluted blood was allowed to fall beneath the cover glass. Two minutes were given for the blood cells to settle. High-power counts of erythrocytes were conducted in five sizable secondary squares, a central square, and a square from each corner. The cells contacting the left and the square's low line were counted as well. Erythrocytes were counted using the technique proposed by Haile and Chanie [14].

Colorimetric analysis was used to determine hemoglobin (Hb) levels using the technique outlined by Wintrobe [15]. A test tube containing 5 ml of the reagent solution was pipetted with ~0.2 ml of blood. The reaction mixture was used to rinse the pipette several times. After 3 min, the working solution was combined with sample A (absorbance). White blood cells (WBCs) were counted in the four corner WBC squares of the hemocytometer using a diluent Natt and Herrick solution, as described by Natt and Herrick [16]. Various types of leukocytes have been identified. Anticoagulants were not added when collecting the blood samples. A small drop of blood was placed on a coverslip and then turned upside down on a slide. Leishman's stain was used to stain blood films to determine the various types of leukocytes according to Sastry's instructions [17].

2.5. Biochemical analysis

The levels of aspartate aminotransferase (AST) and alanine aminotransferase (ALT) in the serum were determined using a calorimetric method based on the methodology developed by Young [18]. According to Henry [19], a spectrophotometer (the spintronic 20 D; Milton Roy Company) was used to measure the serum total protein amount calorimetrically using certain kits. A spectrophotometer (Spectrophotometer; Milton Roy Company) was used to perform calorimetric determination of the blood albumin level using certain kits, as per the findings of Doumas *et al*. [20]. Serum creatinine levels were measured calorimetrically using the Dj [21] method following serum deproteinization because creatinine forms a color complex with picrate in alkaline media. A colorimetric technique was used to measure serum uric acid levels according to Koller and Kaplan [22].

2.6. Assessment of oxidative stress and lipid peroxidation in liver tissues

The tissues were then maintained at 80 °C. The tissue (at least 100 g) was minced with a razor blade on a glass plate or dish of ice. A 12−75 cm glass tube was filled halfway with phosphate buffer at a 1 : 3 ratio. A motor-driven Teflon pestle homogenizer was used thrice for 30 s to homogenize the tissue on ice. The sample was placed in a 1.5 ml Eppendorf tube. A Vibra Cell cup horn sonicator was set to 40% power and sonicated for 1 min in 30 s bursts, chilling on ice in between. All operations were performed at 4 °C.

The method of Ohkawa *et al*. [23] was used to assess the concentration of malondialdehyde (MDA) (Bio Diagnostic Company, Catalog Number MD 25 29) in homogenized liver tissue. The activity of the antioxidant enzyme catalase (CAT) was determined as described by Aebi [24]. Reductions in glutathione (GSH) (Bio Diagnostic Company, Catalog Number GR 25 11), a nonenzymatic antioxidant biomarker, were measured by the colorimetric determination method using the Beutler and Mary [25] approach, and superoxide dismutase (SOD) antioxidant enzyme activities were measured as reported by Weydert and Cullen [26].

2.7. RNA extraction and reverse transcription

The manufacturer's recommendations for Direct-ZolTM RNA Miniprep (catalog No. R2050) Trizol reagent was used to extract total RNA from hepatic tissues. By applying a Nano Drop ND-1000 Spectrophotometer, the amount of isolated RNA was measured and verified. Following the manufacturer's instructions, cDNA was generated from each sample using the SensiFAST cDNA synthesis kit from Bioline (product number Bio 65,053). The reaction mixture consisted of 1 μl reverse transcriptase, 4 μl 5 × TransAmp buffer, 1 μl total RNA of up to 1 μg, and 20 μl DNase-free water removed in a total volume of 20 μl. After the final reaction solution was placed in a heat cycler, the following procedure was performed.

2.8. Quantitative real-time PCR analysis

The relative concentrations of the antioxidant enzymes SOD, glutathione peroxidase 4 (GPX4), toll-like receptor 4 (TLR4), and inflammatory interleukin-1β (IL-1β) were measured using SYBR Green PCR Master Combine (2 × Sensi Rapid TM SYBR, Bioline, track number Bio-98,002) and real-time PCR. The primer sequences of the target genes are displayed in Table 1 as a basic reference for standardization. Glyceraldehyde-3-phosphate dehydrogenase was used as a housekeeping gene. Concerning the glyceraldehyde-3-phosphate dehydrogenase gene, the relative expression of each gene in each sample was determined using the 2−ΔΔCt (CT: cycle threshold) method in comparison to a control [31].

2.9. Histopathological examination

Liver tissues were preserved in 10% formalin. Following processing, the samples were fixed in hard paraffin, sliced into 5 μm thick sections, and stained with hematoxylin and eosin according to Bancroft and Gamble [32].

2.10. Statistical analysis

Statistical calculations were performed using SPSS, version 20 (SPSS Inc., Chicago, Illinois, USA) for Windows on the collected data. The data are presented as the mean ± SE. Tukey's test was used to compare the data from each experimental group after a one-way analysis of variance. Statistical significance was defined as P value less than 0.05 [33].

3. Result

3.1. Hematological changes

The RBCs count, Hb content, and hematocrit (HCT%) of chickens treated with paracetamol were significantly lower than those in the control group ($P < 0.0001$) (Table 2). As for the paracetamol group, the administration of *M. officinalis* resulted in a significant increase in RBC count, HCT%, and Hb content ($P < 0.0001$).

When comparing the paracetamol-treated chickens to the control group, Table 3 demonstrates a substantial decrease in the WBC count, lymphocyte percentage, and monocyte percentage. In contrast, the group that received paracetamol had a significantly higher heterophil percentage ($P < 0.0001$) than the control group. Concurrently, the administration of *M. officinalis* resulted in a significant increase in WBC, lymphocyte, and monocyte counts, as well as a decrease in the percentage of heterophils, compared with the paracetamol group ($P < 0.0001$).

3.2. Biochemical determinations

3.2.1. Serum protein profile, hepatic, and renal biomarkers

The activities of serum ALT, AST, and alkaline phosphatase (ALP) were significantly increased

Table 1. Primer sequences of target genes.

Genes	Forward primer (5′-3′)	Reverse primer (5′-3′)	GenBank accession number	Annealing temperature (°C)	References
IL-1β	ATGTCGTGTGTGATGAGCGGC	AGGCGGTAGAAGATGAAGCGG	FJ537852.1	58	[27]
TLR4	GTTCCTGCTGAAATCCCAAA	TATGGATGTGGCACCTTGAA	NM_001030693	58	[28]
SOD	AGG GGGTCATCCACTTCC	CCCATTTGTGTTGTCTCC AA	NM_205064.1	58	[29]
GPX4	GCCACCTCCATCTACGACTTC	TTGGTGATGATGCAGACGAAG	NM_204220	56	[30]
GAPDH	ATGACCACTGTCCATGCCATCCA	AGGGATGACTTTCCCTACAGCGTT	NM_204305.1	56	[28]

GAPDH, glyceraldehyde-3-phosphate dehydrogenase; GPX4, glutathione peroxidase 4; SOD, superoxide dismutase; TLR4, toll-like receptor-4.

Table 2. Effect of Melissa officinalis (10 g/kg basal diet) on total erythrocyte count, hemoglobin content, hematocrit% in paracetamol-intoxicated chicken.

Parameters Groups	RBCs	Hb	HCT
Control	5.25 ± 0.23^{a}	15.77 ± 0.54^{a}	36.75 ± 0.25^{a}
APAP	3.45 ± 0.14^{d}	8.83 ± 0.11^{d}	30.37 ± 0.55^{d}
Melissa officinalis + APAP	4.45 ± 0.15^{bc}	11.00 ± 0.44^{bc}	33.35 ± 0.23^{c}
APAP + *Melissa officinalis*	4.77 ± 0.13^{b}	12.02 ± 0.36^{b}	35.25 ± 0.47^{b}

Data are expressed as mean ± SE ($N = 6$ chickens).
APAP, N-acetyl-p-aminophenol (paracetamol); C, control; Hb, hemoglobin; HCT, hematocrit; RBCs, red blood cell count.
The different small letters in the same row elucidated significant differences ($P < 0.0001$).

Table 3. Effect of Melissa officinalis (10 gm/kg basal diet) on white blood cells, heterophil%, lymphocyte%, and monocyte% in paracetamol-intoxicated chicken.

Parameters groups	WBCs	Heterophil	Lymphocyte	Monocyte	Eosinophil	Basophil
Control	18.70 ± 0.60^{a}	37.09 ± 0.64^{c}	58.01 ± 0.91^{a}	3.15 ± 0.27^{a}	1.16 ± 0.05	0.59 ± 0.007
APAP	11.55 ± 0.47c	45.71 ± 1.12^{a}	51.67 ± 0.74^{e}	1.77 ± 0.08^{c}	0.85 ± 0.03	0
Melissa officinalis + APAP	13.30 ± 0.24^{b}	42.38 ± 0.30^{b}	54.37 ± 0.52^{cd}	2.27 ± 0.21^{b}	0.98 ± 0.04	0
APAP + *Melissa officinalis*	18.35 ± 0.25^{a}	38.73 ± 0.20^{c}	57.12 ± 0.16^{bc}	3.10 ± 0.14^{a}	1.05 ± 0.03	0

Data are expressed as mean ± SE ($N = 6$ chickens).
APAP, N-acetyl-p-aminophenol (paracetamol); C, control; WBCs, white blood cell count.
The different small letters in the same row elucidated significant differences ($P < 0.0001$).

($P < 0.0001$) in chickens administered paracetamol only compared with those in the control group. In contrast, in the paracetamol group, compared with the control group. In contrast, the groups that received *M. officinalis* along with paracetamol exhibited a significant decrease ($P < 0.0001$) in ALT, AST, and ALP activities in comparison with the chickens that received paracetamol (Table 4). The levels of total protein and albumin in the serum were markedly decreased ($P < 0.0001$) in the paracetamol group relative to the control one. In contrast, the groups that received *M. officinalis* along with paracetamol exhibited a significant increase in total protein levels in comparison with the chickens that received paracetamol (Table 5). The results revealed that the serum levels of creatinine and uric acid were significantly increased ($P < 0.0001$) in chickens intoxicated with paracetamol compared with those in the control group. In contrast, a significant reduction ($P < 0.0001$) in the levels of creatinine and uric acid was observed in chickens treated with *M. officinalis* and paracetamol (Table 6).

Table 5. Effect of Melissa officinalis (10 gm/kg basal diet) on liver function biomarkers (serum level of total protein and albumin) in paracetamol-intoxicated chickens.

Parameters groups	Total protein	Albumin
Control	4.33 ± 0.11^{a}	3.91 ± 0.18^{a}
APAP	2.71 ± 0.08^{b}	1.96 ± 0.12^{d}
Melissa officinalis + APAP	2.91 ± 0.01^{b}	2.69 ± 0.06^{c}
APAP + *Melissa officinalis*	4.17 ± 0.07^{a}	3.25 ± 0.11^{b}

Data are expressed as mean ± SE ($n = 6$ chickens).
APAP, N-acetyl-p-aminophenol (paracetamol); C, control.
The different small letters in the same column elucidated significant differences ($P < 0.0001$).

Table 6. Effect of Melissa officinalis (10 g/kg basal diet) on serum level of creatinine and uric acid in paracetamol-intoxicated chickens.

Parameters groups	Creatinine	Uric acid
Control	0.48 ± 0.02^{d}	3.34 ± 0.09^{d}
APAP	1.58 ± 0.06^{a}	7.22 ± 0.10^{a}
Melissa officinalis + APAP	0.88 ± 0.02^{b}	6.11 ± 0.14^{b}
APAP + *Melissa officinalis*	0.65 ± 0.02^{c}	3.72 ± 0.09^{c}

Data are expressed as mean ± SE ($N = 6$ chickens).
APAP, N-acetyl-p-aminophenol (paracetamol); C, control.
The different small letters in the same column elucidated significant differences ($P < 0.0001$).

3.3. Oxidative status and antioxidant biomarkers in hepatic tissues

The use of paracetamol only led to a significant increase in the MDA content and the activities of GSH, SOD, and CAT compared with the control group. The chickens administered *M. officinalis* concurrently with paracetamol showed lower levels and activities of these indices relative to the paracetamol group (Fig. 1). The administration of APAP only led to a significant increase ($P < 0.0001$) in MDA content and a reduction in GSH, SOD, and CAT activities compared with the control group. The chickens administered *M. officinalis* before or after APAP displayed a remarkable decline in MDA content and a significant increase in GSH content

Table 4. Effect of Melissa officinalis (10 g/kg basal diet) on liver function biomarkers (serum level of alanine aminotransferase, aspartate aminotransferase, and alkaline phosphatase) in paracetamol-intoxicated chickens.

Parameters groups	ALT	AST	ALP
Control	151.00 ± 0.91^{d}	143.50 ± 1.19^{d}	203.75 ± 2.39^{d}
APAP	285.00 ± 1.77^{a}	254.25 ± 4.04^{a}	295.25 ± 2.25^{a}
Melissa officinalis + APAP	212.75 ± 1.10^{b}	177.50 ± 4.01^{b}	271.00 ± 2.48^{b}
APAP + *Melissa officinalis*	157.50 ± 1.04^{cd}	152.50 ± 2.02^{c}	226.25 ± 5.54^{c}

Data are expressed as mean ± SE ($N = 6$ chickens).
ALP, alkaline phosphatase; ALT, alanine aminotransferase; APAP, N-acetyl-p-aminophenol (paracetamol); AST, aspartate aminotransferase; C, control.
The different small letters in the same column elucidated significant differences ($P < 0.0001$).

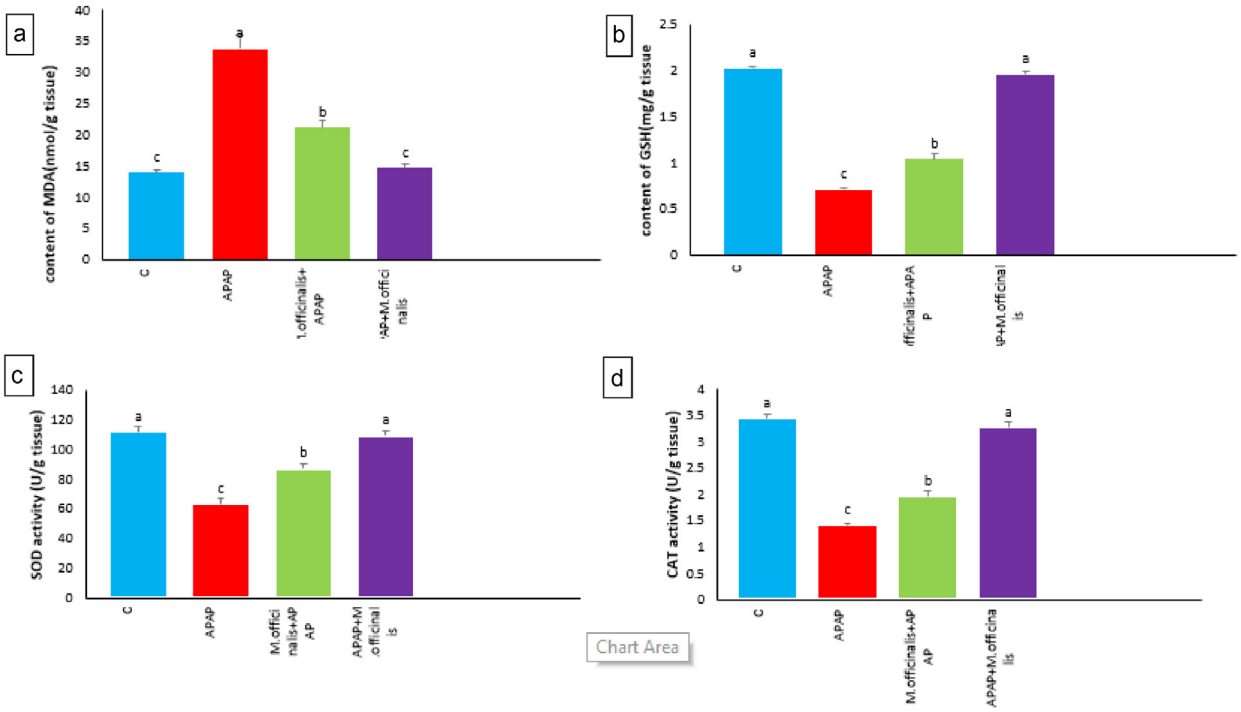

Fig. 1. Effect of Melissa officinalis (10 g/kg basal diet) on oxidative stress biomarkers (MDA, GSH, SOD, and CAT) in paracetamol-intoxicated chickens. (a) MDA level, (b) GSH activity, (c) SOD activity, and (d) CAT activity. Data are expressed as mean ± SE (n = 6 chickens). Each bar carrying different letters is significantly different (P < 0.0001). APAP; N-acetyl-p-aminophenol (paracetamol); C, control; SOD, superoxide dismutase.

and SOD and CAT activities relative to chickens treated with APAP only (Fig. 1).

3.4. Expression of interleukin-1β, toll-like receptor-4, superoxide dismutase, and glutathione peroxidase 4 genes in hepatic tissues

Figure 2 demonstrates a significant upregulation ($P < 0.0001$) in the expression of IL-1β and TLR4 genes in the APAP group compared with the control one. In contrast, a significant downregulation ($P < 0.0001$) in the expression of these two genes was observed in chickens treated with *M. officinalis* and APAP (either before or after APAP). The APAP group showed remarkable downregulation ($P < 0.0001$) in the expression of SOD, GPX4 genes compared with the control group. In contrast, a significant upregulation in the expression of SOD and GPX4 genes was recorded in *M. officinalis* + APAP and APAP + *M. officinalis* groups relative to the APAP group.

3.5. Histopathological examination

Liver histopathological screening revealed hepatocytes with one or two nuclei (thin arrow) and nucleoli (Fig. 3a). The APAP group displayed moderate numbers of plasma cells, lymphocytes, and macrophages (star) admixed with numerous reactive fibroblasts with an increased number of small irregular bile ducts (thick arrows, H&E, × 400) (Fig. 3b). The *M. officinalis* + APAP group showed restoration of up to 90% of the normal hepatic architecture with minimal, focal hepatic necrosis admixed with few leukocytes (thin arrows) (Fig. 3c). Interestingly, the APAP + *M. officinalis* group showed normal histological appearance of the hepatic parenchyma (H&E, X400; Fig. 3d).

4. Discussion

Our results revealed a significant decrease in RBCs number, Hb content, HCT%, WBC number, lymphocyte%, and monocyte% in paracetamol-treated chickens compared with the untreated group. However, compared with the control group with paracetamol treatment, there was a statistically significant increase in heterophil% ($P < 0.0001$), which is in line with the findings of Marmat and Rathore [34], who found that Hb, total RBCs, and mean corpuscular Hb concentrations all demonstrate a clear reduction in the paracetamol group. In addition, Ranganathan *et al.* [35] reported that the levels of Hb and WBC were markedly decreased in

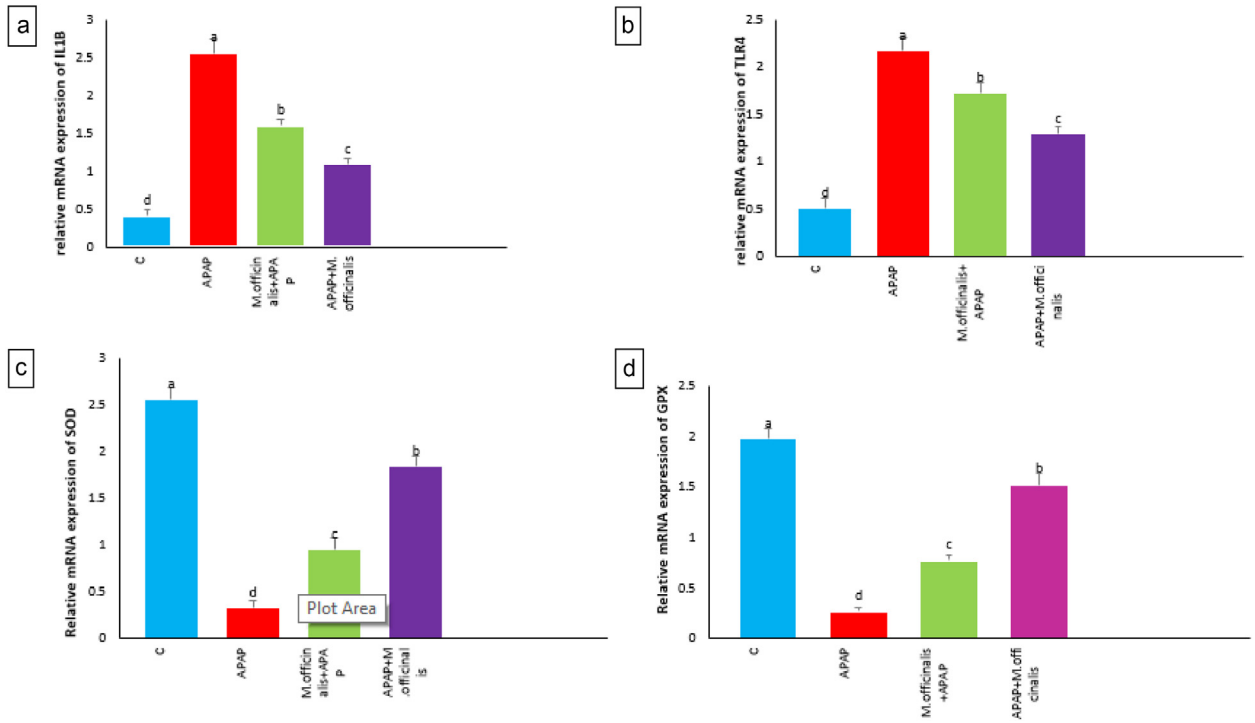

Fig. 2. Effect of Melissa officinalis (10 g/kg basal diet) on target genes (IL-1B, TLR4, SOD, and GPX) in paracetamol-intoxicated chickens. (a) IL-1B, (b)TLR4, (c) SOD, and (d) GPX. Data are expressed as mean ± SE (n = 6 chickens). Each bar carrying different letters is significantly different (P < 0.0001). APAP; N-acetyl-p-aminophenol (paracetamol); C, control; GPX, glutathione peroxidase; IL-Iβ, interleukin-1β; TLR4, toll-like receptor-4; SOD, superoxide dismutase.

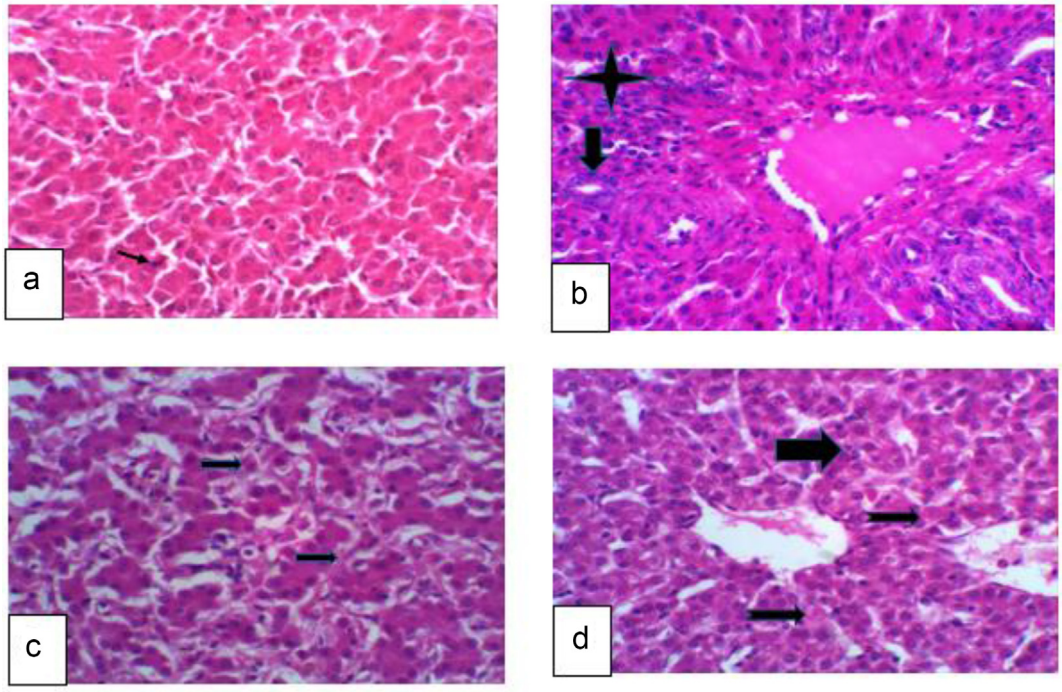

Fig. 3. Representative photomicrograph of poultry liver from different treatments. (a) C group liver showing the normal histological appearance of the hepatic parenchyma, H&E, × 400. (b) APAP group 2 showing biliary hyperplasia characterized by piling up epithelial cells with moderate anisokaryosis (thick arrow) and abundant periductular aggregations of fibroblasts (fibrosis) (arrowhead), numerous lymphocytes and plasma cells (star) admixed with few numbers of newly formed bile ductules (thin arrow), H&E, × 400. (c) Melissa officinalis + APAP group showing restoration of up to 90% of normal hepatic architecture with minimal, focal hepatic necrosis admixed with few leukocytes (thin arrows). (d) APAP + M. officinalis group showing the hepatic architecture of up to 90% of tissue sections appeared normal except for occasional individual cell necrosis (thin arrows) with occasional inflammatory cells (thick arrow).

birds that received paracetamol relative to the control.

Meanwhile, the administration of *M. officinalis* elevated the RBC level, HCT%, lymphocyte count, and monocyte% and decreased the heterophil% relative to the paracetamol group ($P < 0.0001$). The RBC count, HCT, and Hb concentration may be increasing because of the presence of flavonoids, glycosides, and fumaric acid, which are responsible for most of the pharmacological properties of lemon balm. These compounds are potent antioxidants that shield cells and tissues from oxidative damage and lipid peroxidation as well as improve hemostasis and productivity under stress. Farahi *et al*. [36] showed that hematological parameters and immunity improved when *M. officinalis* was added to a fish diet. Furthermore, Bilen *et al*. [37] and Mohammadi *et al*. [38] reported that the application of *M. officinalis* extract to fish diets increased in HCT value, Hb%, and RBC and WBC counts.

The levels of ALT, AST, and ALP were greatly increased in chickens administered paracetamol, in contrast to those in the control group. Compared with the untreated group, the serum levels of total protein and albumin in the paracetamol group were considerably lower. Similarly, Rasooli *et al*. [5] reported a significant elevation in ALT, AST, and ALP levels after the administration of paracetamol in the treated group. Moreover, Ranganathan and colleagues [35]observed that blood concentrations of AST and ALT were significantly increased in birds when paracetamol was administered. Conversely, in comparison to chickens treated with acetaminophen alone, the groups administered *M. officinalis* together with paracetamol showed a significant decrease in ALT and AST levels and an improvement in the levels of total protein. In contrast, the groups that received *M. officinalis* along with paracetamol exhibited significant decreases in ALT and AST activities and an increase in total protein levels in comparison with the chickens that received only paracetamol. Our data are in agreement with Namjoo *et al*. [39], who reported that when compared with the untreated group, mice receiving 1350 mg/kg of lemon balm had a significant decrease in ALT enzyme activity. The obtained data are supported by Zarei *et al*. [40], who reported that *M. officinalis* extract decreased AST, ALP, ALT, and cholesterol in the treated group. Tao *et al*. [41] showed that whole flavonoids improve hepatic histopathology and decrease serum AST and ALT levels. Similar findings were previously mentioned by Poorghasemi *et al*. [42], who found enhancements in total proteins and albumin plasma levels in the group that took 0.5 g of *M. officinalis* supplements. Our findings were in accordance with Elsadek and Habib [43] who stated that the fatty liver group (untreated) had significantly higher levels of liver marker enzymes (ALT and AST) than both the normal and lemon balm-treated groups ($P \leq 0.0001$). In addition, there was a significant ($P \leq 0.0001$) increase in serum total protein in the lemon balm-treated groups compared with the untreated fatty liver group. The groups that received two doses of lemon balm leaves at 10 and 20 g/kg had significantly ($P \leq 0.0001$) higher serum albumin levels than the group that received no treatment for fatty liver disease. Decreased protein synthesis and hepatic dysfunction may be the cause of this decrease.

The findings showed that compared with the hens in the control group, paracetamol-intoxicated birds had considerably higher blood levels of uric acid and creatinine. In contrast, hens treated with *M. officinalis* coupled with paracetamol showed considerable suppression in the levels of creatinine and uric acid. These results are in accordance with those reported by Namjoo *et al*. [39], who showed that following treatment with an extract of lemon balm (*M. officinalis*), the serum activity of urea and creatinine levels significantly decreased in BALB/c mice.

In addition, Elsadek and Habib [43] reported that compared with the normal control group, the untreated fatty liver group (intoxicated by oxytetracycline) showed a highly significant ($P \leq 0.0001$) increase in serum urea and creatinine. However, the effects of oxytetracycline on serum urea and creatinine levels were somewhat mitigated by the administration of two doses of lemon balm toward the conclusion of the experiment. Similar findings were previously reported by Purena *et al*. [44] who reported that by lowering blood urea nitrogen and serum creatinine, boosting the activities of GPX, GR, SOD, and CAT, and lowering the level of MDA in the kidneys, treatment with *Emblica officinalis* leaf extract significantly reduced renal damage.

Eivani *et al*. [45] stated that in comparison to the group that was intoxicated with lead, kidney function markers were significantly lower in the *Melissa* extract group.

The results showed that exposure to paracetamol led to a significant elevation in MDA content and GSH, SOD, and CAT functions in comparison to the untreated group. The chickens administered *M. officinalis* concurrently with paracetamol showed a reduction in the levels and activities of the mentioned indices in comparison with the paracetamol group. The effect of paracetamol is in accordance with Aziz *et al*. [46], who found that MDA levels were significantly higher in the paracetamol

group than in the control group. In addition, Marmat and Rathore [34] reported that paracetamol decreases GSH levels and increases lipid peroxidation. These results are in agreement with the findings of Adelifar *et al*. [47], who reported that athletes who received lemon balm had higher levels of MDA and overall antioxidant capacity. In addition, Tao *et al*. [41] reported that SOD and GSH activities increased and MDA levels decreased in groups treated with total flavonoids. The presence of flavonoids in *M. officinalis* extract has the potential to reduce the production of free radicals and possesses antioxidant and anti-inflammatory properties, thereby protecting liver cells [40]. Schulz *et al*. [48] reported that in treatment groups, *M. officinalis* reduced the activity of hepatic enzymes. Polyphenolic compounds can neutralize free radicals and reduce their harmful effects because of their antioxidant properties. Yoshikawa *et al*. [49] revealed that flavonoids, in particular, are antioxidant substances that protect the liver against the harm caused by chemicals and free radicals. Bolkent *et al*. [50] demonstrated that the administration of an extract of *M. officinalis* L. enhanced the levels of GSH in their tissues. Similar to Dastmalchi *et al*. [51], the ingestion of *M. officinalis* increased plasma levels of CAT, SOD, and GPX enzymes. Zarei *et al*. [40] showed that applying an infusion of *M. officinalis* led to a considerable increase in serum levels of total antioxidant capacity and the potent antioxidant qualities of *M. officinalis* extract are believed to be responsible for its capacity to lower liver enzymes. Phenolic compounds in this plant act as antioxidants. Flavonoids inhibit the cytochrome system and protect the liver from free radical-induced damage. Because flavonoids increase the activity of antioxidant enzymes, such as CAT, oxidase, and reductase, they can also shield cells from GSH depletion. Tao *et al*. [41] reported that total flavonoids enhance the levels of glutathione S-transferase protein to increase oxidative stress and greatly decrease MDA. Similar findings were previously reported by Tao *et al*. [41], who reported that the enzymes glutathione peroxidase (GSH-Px) and SOD were markedly elevated by total flavonoids, showing that total flavonoids suppress oxidative stress. The data obtained were supported by Vasques *et al*. [52], who reported that total flavonoids increased liver tissue and glutathione S-transferase levels. Another study confirmed that polyphenolic compounds are the most powerful antioxidants [53].

The obtained data were supported by Saberi *et al*. [54], who stated that *M. officinalis* methanolic extract greatly lowered MDA levels, a biochemical marker of oxidative damage and lipid peroxidation, and increased SOD and GPX levels, indicators of the antioxidative qualities of cells. The effect of *M. officinalis* methanolic extract may be mediated by its effective natural antioxidant components, such as tocopherol, rosmarinic acid, and flavonoids, or by an increase in the levels of the antioxidant enzymes SOD and GPX, or a decrease in MDA, a biochemical indicator of cell injury and lipid peroxidation.

Elsadek and Habib [43] reported that, in comparison to the fatty liver group, the administration of *M. officinalis* (lemon balm) significantly decreased the level of MDA and increased the level of SOD. El Gamel [55] explained that *M. officinalis* supplementation reduced serum MDA levels and increased GSH content in liver tissue homogenates compared with positive intoxicated rats (control group).

A significant upregulation in the expression of IL-1β and TLR4 was observed in the paracetamol-administered group compared with the control group. In contrast, an important downregulation was observed when these two genes were expressed in chickens treated with either *M. officinalis* or paracetamol. Moreover, *M. officinalis* lowers hippocampal antioxidant capacity and decreases MDA synthesis. Following ischemia, there was a noticeable increase in mRNA levels of proinflammatory cytokines such as TNFX and IL-1B, in addition to hypoxia-inducible factor-1α. Hypoxia-inducible factor-1α expression was inhibited by the consumption of *M. officinalis* extract. In addition, research has demonstrated that the *M. officinalis* extract can be used as a protective agent in various neurological conditions linked to cerebral ischemia [56]. In the liver, total flavonoids dramatically reduce mRNA levels of TNF-α, IL-6, and IL-1β. These findings demonstrate that total flavonoids may protect against liver damage by inhibiting oxidative stress and inflammatory responses [41]. In control chickens, blood cells from chickens fed *M. officinalis* extract showed a lower inflammatory response to LPS. There was a reduction in blood cell IL-1β expression at D30 [57].

Choi *et al*. [58] reported that when three different doses of MLD (a mixture of lemon balm and dandelion extracts) were administered, CCl4 significantly increased the mRNA and protein levels of IL-1β. Conversely, CCl4 significantly inhibited the increase in mRNA and protein levels of proinflammatory cytokines. These changes were identified by real-time PCR. The acquired information showed that the bleomycin group's IL-1β levels were significantly higher than those in the control group. It is interesting to observe that bleomycin rats administered *M. officinalis* extract had significantly

less amount of IL-1β than bleomycin animals, bringing all inflammatory cytokines and cardiac enzymes within normal ranges [59]. The paracetamol group showed remarkable downregulation in SOD and GPX gene expression levels compared with the control group; however, a noticeable upregulation in the expression of SOD and GPX genes was recorded in *M. officinalis* + paracetamol relative to the paracetamol group.

5. Conclusion

M. officinalis leaves offer hepatic protection against paracetamol-induced hepatotoxicity in broilers by reducing oxidative stress and inflammation. However, further research is needed to establish the characteristics of lemon balm elements.

Authors' contributions

Conceptualization: Magdy S. Amer, Sara T. Elazab. Data curation: Ghada A. Abou Zead. Formal analysis: Ghada A. Abou Zead, Ahmed I. Ateya. Funding acquisition: Ghada A. Abou Zead. Investigation: Ghada A. Abou Zead, Azza E. Hassan.

Conflicts of interest

There are no conflicts of interest.

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