Protective Effect of Peppermint on the Toxicity Induced by Blue Green Algae in Poultry

Gehan B. A.Youssef[1], Elzoghby R.R[2], Reda EL Kammar[3], Walaa S ElDin[4], Rania M Waheed[1]

[1]Forensic Medicine dept,Faculty of Veterinary Medicine Benha University, New Valley Faculty of Veterinary Medicine, [2]New Valley University, [3]Veterinary Teaching Hospital. Pharmacology Dept.,[4] Histology Dept, Physiology dept, Faculty of Veterinary Medicine Benha University, New Valley Faculty of Veterinary Medicine

Abstract

This study was done on 5 groups of poultry chicks from one day up to 45 days of age. We used blue green algae as source of feed additives added to the water and ration in addition to peppermint to evaluate the protective effect of peppermint against the toxicity induced by the blue green algae. The administration of blue green algae in chicken food and water caused hepato- and cardio toxicity indicated by significant increase in liver (ALT and AST) and heart enzymes (CK and LDH) and decrease in MDA and CAT. The algae showed histopathological alterations in the liver in form of congestion in the central vein, degeneration in the hepatic cords and circumscribed areas of lymphocytic infiltrations between the hepatic cords. While it was mild in heart tissues as it showed also lymphocytic infiltration and congestion of myocardial blood vessels. The biochemical parameters were corrected except the CK and LDH. The histopathological alterations were corrected by the addition of peppermint.

Key words. *Blue green algae- peppermint -protective effect- liver, heart*

Introduction

The cyanobacterium is wide distributed in most of water lakes in Egypt and lead to environmental contamination by the excreted cyanotoxin of the blue green algae (1 ,2).

The water toxins produced via the metabolism of the algae and cyanobacterium. The degree of toxicity depends mainly up on the signs which was occurred in the tissues. when it caused liver toxicity (dangerous) and mild when it caused dermatitis (3).

The toxic effect of blue green algae was discovered for the first time in Australia in sheep as it starts by nervous manifestations (4).

Corresponding author.
Gehan B. A.Youssef1
drgehan222@yahoo.com

The cyanotoxin are produced by the dead cyanobacterium cells when it consumed by another microorganism. the sun light changes the toxin to nanotoxic form which is the dangerous form and may lead to liver cancer (5). These toxins were toxic to all types of domestic and wild life including birds (6)

The blue green algae toxin includes microcystins (targeted for liver and heart), cylindrospermopsins, anatoxins and saxitoxins (targeted for nervous tissues and system. (7)

The microcystins toxicity may lead to death in severe hepatic intoxication in animals, birds and fishes (8)

Peppermint is a member of the Labiate family and one of the world's oldest medicinal herbs. (9). The Labiate family, rich in essential oil, has commercial and medicinal values. These herbs are widespread

throughout the world and are widely use in food, flavor, cosmetic, and pharmaceutical industries. [10,11]. The chemical components of peppermint are menthol, menthone, 1,8-cineole, methylacetate, methofuran isomenthone, limonene, b-pinene, a-pinene, germacrene-d, trans-sabinene hydrate, and pulegone. Menthol is the main phenolic component in oil of peppermint, which has antibacterial activities. [12].

The peppermint has numerous properties which includes, antiseptic [13,14], disinfectant and antispasmodic action [15]. When it added to the ration, it made improvement in broiler meat production. [16]

The chemical composition of the peppermint varied according to the types of the cultivated land and the climatic condition in which the leaves grow up [17, 18,19].

Material and Methods

5 groups of chicken were chosen for this experiment (cobb broiler chicks)

The blue-green alga dose was calculated according to [20]. The dose of peppermint 15g/kg was calculated according to [13].

Group 1 control group fed on normal growing ration without any additives

Group 2 fed on growing ration in addition to peppermint 15g/kg

Group 3 fed on blue green algae alone in food 13mg/kg daily

Group 4 fed on blue green algae alone in water 13mg /kg daily

Group 5 fed on blue green algae 13mg /kg in addition to peppermint 15g/kg in food daily

The chickens were raised for 45 days then slaughtered at the end of the experiments. The serum collected for biochemical analysis. The liver and heart tissues were collected for histopathological findings and antioxidants enzymes.

Preparation of algae extract:

Each sample of the marine algae was washed again with distilled water several times, followed by 5% ethanol to remove any epiphytes or any salt precipitates [21]. Samples were spread on white sheets and left for air drying. They were cut into small pieces and powdered in a mixer grinder.

Extraction of grounded algal samples was done using Ethanol: Dichloromethane (1:1) and stored in adark place. Remain extracts were filtered and concentrated in a rotatory evaporator at 35 °C. The weighted crude extracts were suspended in the dimethyl sulfoxide (DMSO) to a final concentration of 20 mg/ml and stored in a refrigerator at 4ºC [22,23].

Biochemical analysis:

Determination of liver functions:

Biochemical parameters were measured in the collected serum samples; Alanine aminotransferase (ALT), aspartate aminotransferase (AST) are measured in serum according to the methods described by [24]

Antioxidant parameters were measured in liver tissues CAT activity was measured as described by [25], and Malondialdehyde (MDA) was measured by method described by [26].

Determination of heart creatinine kinase (CK) and lactate dehydrogenase (LDH) parameters:

These parameter was measured according to [27] for CK and [28] for LDH. The biochemical analysis was done at the Central Lab of Faculty of Veterinary Medicine, Benha University.

Histopathology

The liver and heart tissues were fixed in neutral formalin, processed for preparing the slides. It was stained according to [29].

Statistical analysis: Statistical analysis of data was performed and expressed by means (± SE) using one way analysis of variance (ANOVA) to determine if there were significant differences between the control and experimental groups. $P \leq 0.05$

Results

Biochemical parameters:

The results of biochemical parameters are summarized in Table (1)

Effects on serum biochemical parameters:

The administration of peppermint alone (G2) did not cause any significant alterations in serum liver and heart enzymes (ALT, AST and LDH) except CK level was increased significantly compared to the control group (G1) while, the administration of blue green algae in chicken food and water caused hepato- and cardio toxicity indicated by significant increase in liver (ALT and AST) and heart enzymes (CK and LDH) in 3rd and 4th group (blue green algae treated group) compared to the control group (G1).While, treatment with peppermint and (G5) caused the decreased levels of ALT, AST, CK and LDH in 5th group compared to the (G3 and G4).

In regard to the route of administration of blue green algae (in food or water), there was no significant change in serum ALT, AST, CK and LDH levels between the two experimental routes (G3 and G4).

Effects on malonaldhyde (MDA):

The administration of peppermint alone (G2) caused non-significant decrease in MDA concentration while the administration of blue green algae alone in chicken food and water (G3 and G4) caused significant decrease in MDA concentration compared to the control group (G1). While, the administration of peppermint with blue green algae (G5) caused the lowest significant MDA level compared to the control and other treated groups.

In regard to the route of administration of blue green algae, the administration of blue green algae in water (G4) caused significant decrease in MDA concentration compared to the group administered blue green algae in food (G3).

Effects on catalase enzyme:

The administration of peppermint alone (G2) and blue green algae (blue green algae) alone in chicken food and water (G3 and G4) caused significant decrease in catalase (CAT) level compared to the control group (G1) while, the administration of blue green algae alone in chicken food and water (G3 and G4) caused significant decrease in CAT level compared to (G2). Moreover, the administration of peppermint with blue green algae (G5) caused the lowest significant CAT level compared to the control and other treated groups.

In regard to the route of administration of blue green algae, the administration of blue green algae in water (G4) caused significant decrease in CAT level compared to the group administered blue green algae in food (G3).

The heart CK and LDH were increased in all groups in compare to the (G1). It showed significant changes in (G5).

Cont... **Table (1).**

Table (1).

parameters	G1 (control)	G2 (peppermint)	G3 (algae in food)	G4(algae in water)	G5 (alga +peppermint
ALT serum liver	4.89c±0.17	4.74c±0.63	8.15b±0.46	6.40bc±0.30	6.16a±1.09
AST serum liver	186.30c±7.98	209.53c±9.23	249.33b±3.72	260.90ab±11.25	246.40a±10.22
MDA tissue	58.76a±2.34	47.13ab±2.07	41.036b±0.14	27.25c±3.50	21.13c±8.32
Catalase tissue	516.32a±23.27	346.13b±12.82	275.75c±33.12	210.30d±3.60	155.85d±10.89
CK-heart serum	1319.7c±131.59	2849.3b±118.97	3063.3b±78.17	3482.7b±202.09	4679.3a±412.34
LDH-heart serum	608.63c±44.40	728.53c±30.64	898.57b±46.26	929.83b±0.67	1140.7a±44.89

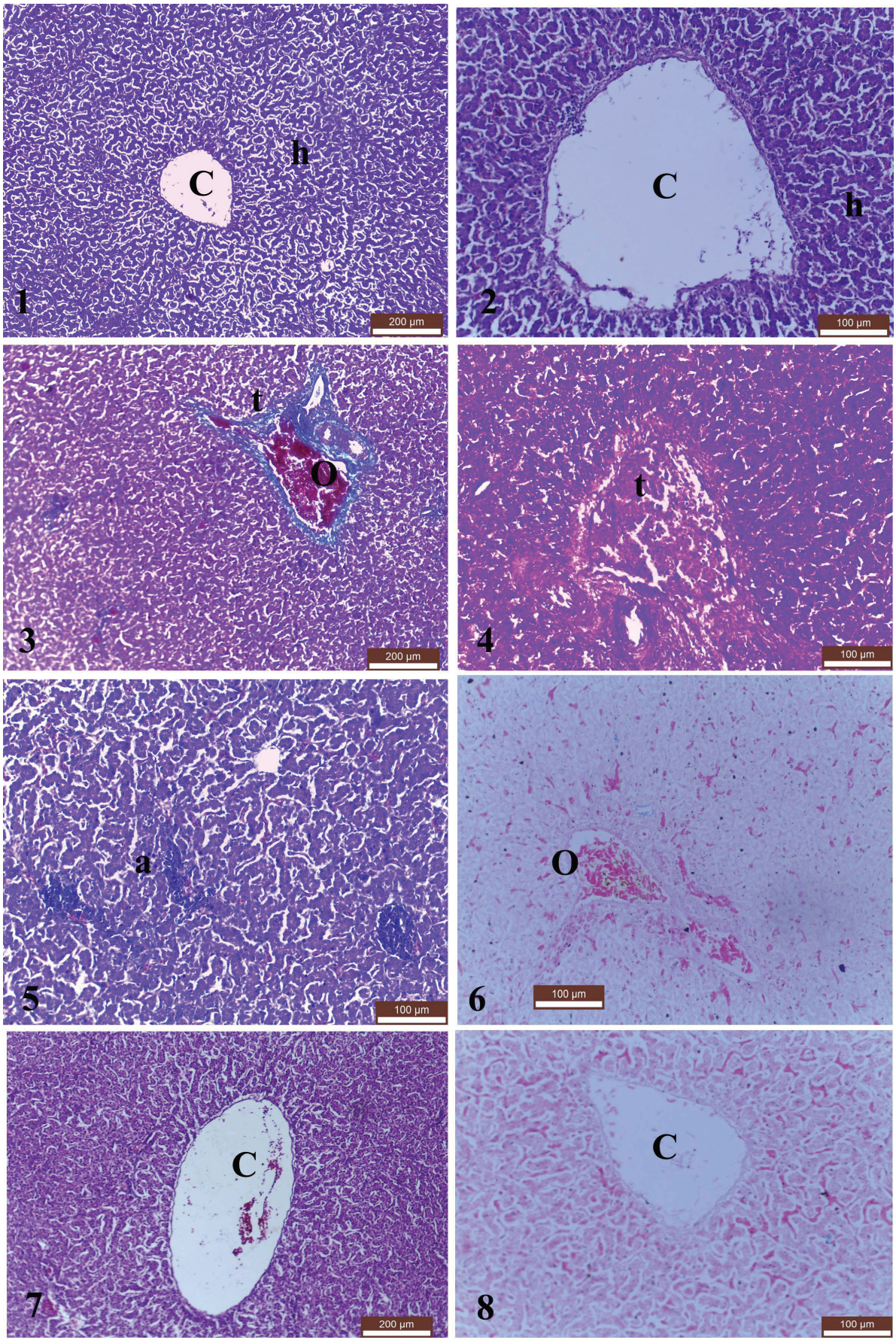
The mean values with different superscript letter within the same row differed significantly at ($P < 0.05$)

Histopathological results

The liver of the control group showed normal arrangement of the hepatocytes around the central vein (Fig.1). The hepatocytes arranged in the form of distinct cords (Fig.2). The blue green alga when added to the food and water made alterations in the architecture of the portal structure, in the form of congestion and edema of the central vein and hepatic vein (Fig.3). Loss of the architecture of the hepatocytes especially around the triad areas with proliferation of the fibrous tissues (Fig.4). Circumscribed areas of lymphocytic infiltrations between the hepatic cords were noticed in the portal lobules (Fig.5). PAS reaction was negative in the hepatocytes and faint positive in the CT fibers around the central vein (Fig.6). The addition of peppermint had protective effect on the alterations induced by the blue green alga as it improved the hepatic alterations (Fig.7). It also improved the histochemical reactions of the hepatocytes as it appeared faint PAS positive (Fig.8).

The heart of the chicken showed myocardial muscles which was branched and anastomosing in nature (Fi.9). Fine collagen fibers were spread between the cardiac muscles (Fig.10). the addition of blue green algae made mild histopathological alterations in the cardiac muscles, in form of patches of degeneration in the cardiac striations (Fig.11) and congestion of the cardiac blood vessels in addition to lymphocytic infiltrations between the myofibrils of the cardiac muscles (Fig.12).

The uses of peppermint protect the myocardial muscles from the hazard effect of blue green alga in the form of well organized cardiac muscle (Fig.13), although some lymphocytes still persist betwen the myocardial fibrils (Fig.14).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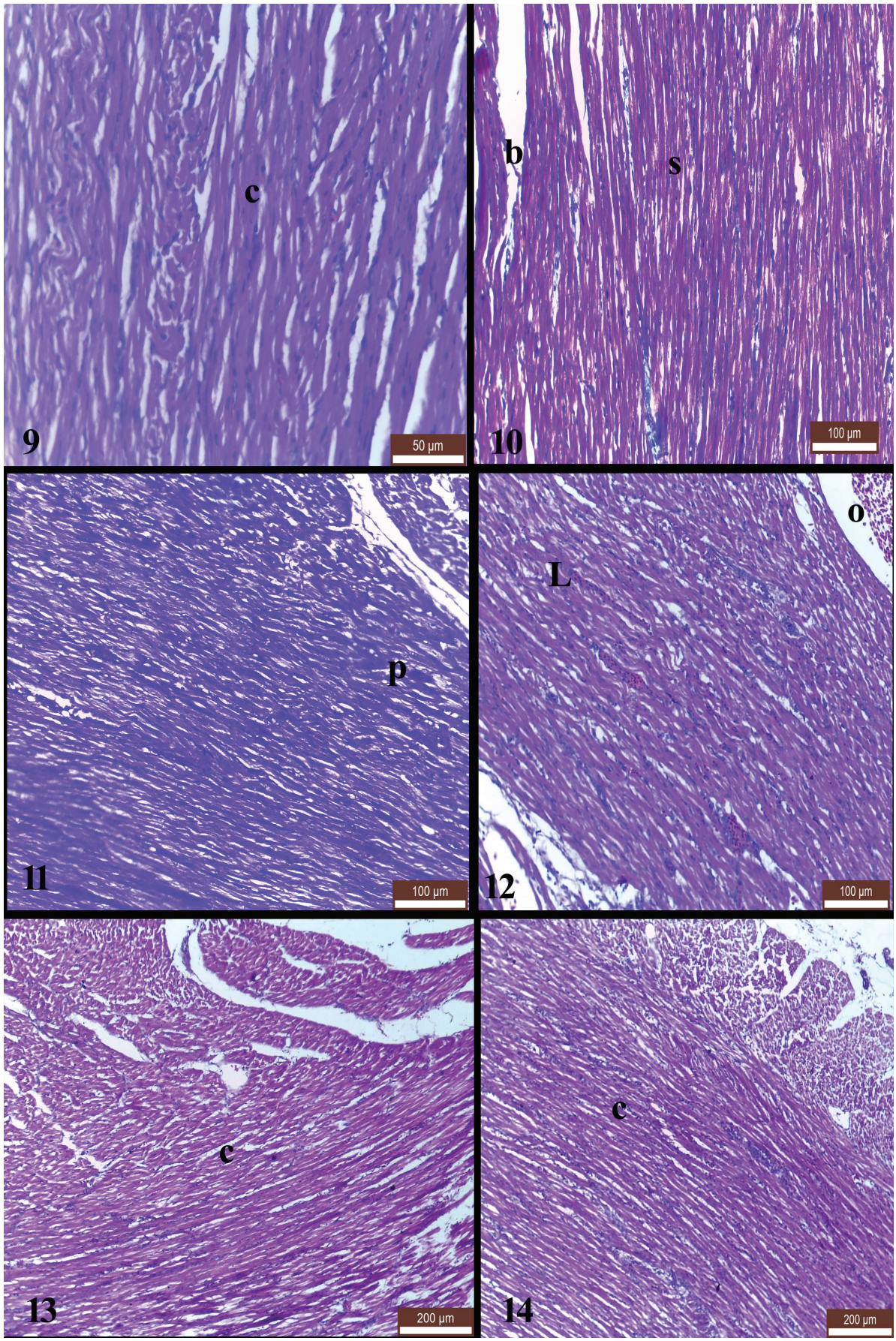
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The liver of the control group showed normal arrangement of the hepatocytes (h) around the central vein (Fig.1). H&E scale bar 200. The hepatocytes arranged in the form of distinct cords around central vein (c) (Fig.2) H&E scale bar 100. Alterations in the architecture of the portal structure in (G3), in the form of congestion and odema (o) of the central vein and hepatic vein (Fig.3) Masson trichrome scale bar 200. proliferation (t) of the fibrous tissues (Fig.4) H&E scale bar 100. Circumscribed areas of lymphocytic infiltrations (a) (Fig.5) H&E scale bar 100. PAS reaction was negative in the hepatocytes and faint positive in the CT fibers (Fig.6) PAS scale bar 100. improve the hepatic alterations in group (G5) (Fig.7) H&E scale bar 200. It also improved the histochemical reactions of the hepatocytes as it appeared faint PAS positive (Fig.8) PAS scale bar 100.

The heart of the chicken showed myocardial(c) muscles (Fi.9) H&E scale bar 50. Fine collagen fibers (s) were spread between the cardiac muscles (Fig.10) Masson trichrome scale bar 100. Mild histopathological alterations in the(s) cardiac muscles, in form of patches (p) of degeneration in the cardiac striations in group (G3) (Fig.11) H&E scale bar 100. Congestion of the cardiac blood vessels in addition to lymphocytic infiltrations (L) between the myofibrils of the cardiac muscles (Fig.12) H&E scale bar 100.

well organized cardiac (e) muscle in group (G5) (Fig.13) H&E scale bar 200, Some lymphocytes still persist betwen the myocardial fibrils (Fig.14) H&E scale bar 200.

Discussion

The blue green algae (cyanobacterium) was widely distributed in water. the cyanotoxin were the main cause of numerous veterinary problems in animal kingdom [30] and also in human [31]

Most cases of blue green algae toxicity occurred in summer where the water is worm. the algae grow and made a layer of pea green slime over the water surfaces. These slime was collected and introduced to the chicken as feed additive. These slime contain the cyanotoxin which cause liver toxicity and sever nervous manifestation. [32]

in this study, administration of blue green algae in chicken food and water caused hepato- and cardio toxicity indicated by significant increase in liver (ALT and AST) and heart enzymes (CK and LDH) compared to the control group. Also the administration of blue green algae alone in chicken food and water caused significant decrease in MDA concentration compared to the control group. These results suggested that blue green algae could exert hepato- and cardio toxic effect and/or radical scavenging activities with toxic dose used in this experiment. This hypothesis is supported by the findings of [7,33]

The blue green algae when added to the food and water made alterations in the architecture of the portal structure, in the form of congestion and edema of the central vein and hepatic vein. Loss of the architecture of the hepatocytes especially around the triad areas with proliferation of the fibrous tissues. The algae toxin caused extensive haemorrhage in the liver with initial perilobular distribution. This was accompanied by necrosis of hepatocytes .[34]. These findings were support our results in which congestion and odema were most prominent histopathological alterations in algae added groups

Circumscribed areas of lymphocytic infiltrations between the hepatic cords were noticed in the portal lobules. These results were supported by the findings of [20] who stated that the toxin was principally hepatotoxic causing massive hepatocellular necrosis and biliary hyperplasia in lethally affected chickens.

Peppermint (Mentha piperita L.) was used in this experiment to protect against the hepato and cardio toxic effect of blue green algae [35,36].

The administration of peppermint alone did not result in significant alterations in biochemical parameters compared to control chickens due to low dose which used in this experiment according to [37]

and [38] who reported that Peppermint leaves can be used as an effective nutritional bioagent up to 40 mg/kg in rats and 15 g/kg in chickens respectively.

Furthermore, the administration of peppermint with blue green algae improve the hepato- and cardio toxic effect for some extent due to low dose of peppermint which used in this experiment and avoid using excess of peppermint to avoid any hazard effects [38].

peppermint also improving feed efficiency which build up the immune system that face off the toxic effect of the cyanobacterium toxins.[39]. Moreover, the antiseptic property of peppermint prevents harmful bacterial growth in the digestive system that led to better digestion and absorption .[40].

. The antiseptic property of peppermint results from the presence of menthol. [41]. peppermint strengthened the stomach and intestinal slow motion because of alpha humlone. [42]

The addition of peppermint had protective effect on the alterations induced by the blue green algae as it improves the hepatic alterations. It also improved the histochemical reactions of the hepatocytes as it appeared faint PAS positive as it returned back to store glycogen in their cytoplasmic granules .[43].

Microscystin toxins had toxic effect on heart and cardiovascular system in human [44]. The blue green algae caused congestion in cardiac blood vessels and pathological alterations in myocytes. Similar results were support our findings. Also the CK and LDH showed significant increase [45]. The addition of peppermint has non significant effect on the correction of the heart enzymes although it made some histopathological corrections in compare to the normal histological structure

Conclusion

The blue green algae were toxic to the chicken when added as feed additive. It had hepato and cardiac toxicity through histopathological alterations and elevation of (ALT,AST, CK, LDH) and decrease MDA. The peppermint when used as co treatment with the blue green algae in food or water made correction in the biochemical and histopathological alterations.

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