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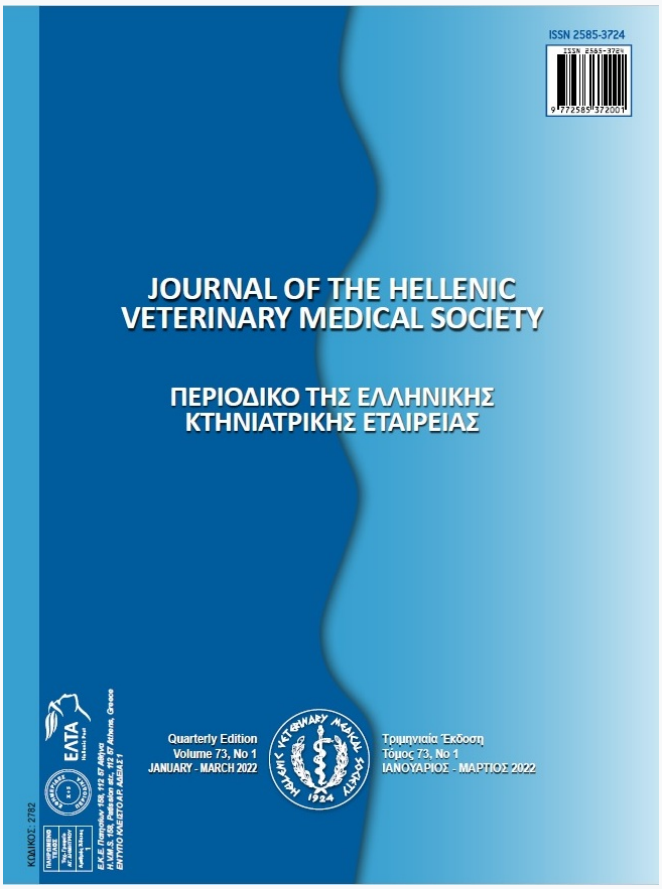

Ameliorative effects of L-carnitine on florfenicol-induced hepatotoxicity in broilers

Nourhan A. Haggag, Mustafa A. Aziz, Abu Elnasr A. Zahra, Soad S. Belih, Hazim Omar Khalifa

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Ameliorative effects of L-carnitine on florfenicol-induced hepatotoxicity in broilers

N.A. Haggag[a], M.A. Aziz[b], A.A. Zahra[b], S.S. Belih[a], H.O. Khalifa[b, c]

[1] *Animal Health Research Institute, Agricultural Research Center, Tanta, Egypt*

[2] *Department of Pharmacology, Faculty of Veterinary Medicine, Kafrelsheikh University, Egypt*

[3] *Department of Infectious Diseases, Graduate School of Medicine, International University of Health and Welfare, Narita, Japan*

ABSTRACT: L-carnitine is a non-essential amino acid derivative naturally occurring and widely distributed in nature. It received a growing interest in its potential uses as a medicinal agent possess protective effects that postulated to be related to its antioxidant action. This study was aimed to evaluate the ameliorative role of L-carnitine on florfenicol induced hepatic toxicity in broilers. A total of 150 broiler chicks were grouped into 6 groups each of 25 chicks. Group one was kept as a control group, while group two and three were treated with florfenicol and L-carnitine, respectively. Group 4 was pre-treated with L-carnitine for three days before florfenicol administration. Groups five and sex were cotreated with L-carnitine and florfenicol and post-treated with L-carnitine for three days after florfenicol administration, respectively. The biochemical analysis, liver indices, antioxidant profile, and histopathological examination were performed to evaluate its ameliorative effects. Results emphasized that florfenicol induced hepatic toxicity in broilers and L-carnitine can ameliorate its action when its usage preceded the florfenicol or when they were used together which reflected by an enhancement in liver indices, antioxidant profile, and histopathological findings. As far as we know this the first study confirming the ameliorative potency of L-carnitine on florfenicol-induced hepatotoxicity.

Keywords: Antioxidant Profile, Florfenicol, Hepatotoxicity, L. carnitine, Liver Indices.

Corresponding Author:
Hazim O. Khalifa, PhD, Department of Pharmacology, Faculty of Veterinary Medicine, Kafrelsheikh University, Egypt. Department of Infectious Diseases, Graduate School of Medicine, International University of Health and Welfare, Narita, Japan
E-mail address: hazimkhalifa@chiba-u.jp, hazem.khalifa1@vet.kfs.edu.eg

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INTRODUCTION

Antibiotics are used in the poultry industry to improve performance by increased feed conversion, growth rate promotion as well as for the treatment and prevention ofa wide range of avian diseases (Mehdi et al., 2018). According to an estimate, more than 60% of worldwide antibiotic production was intended for livestock production, including poultry (Van Boeckel et al., 2015). Owing to its superior spectrum of activity with greater potency besides its fewer adverse effects than other members of amphenicol group of antibiotics; florfenicol has been increasingly used in livestock for treating bacterial diseases replacing chloramphenicol (Wei et al., 2016). Florfenicol is a fluorinated derivative of chloramphenicol where the hydroxyl group at C-3 site issubstituted with a fluorine atom (sams, 1994). Florfenicol is primarily bacteriostatic functions through binding irreversibly to the 50_sribosomal subunit preventing protein synthesis (Dowling, 2013). However, the uncontrolled usage of florfenicol for the treatment and prevention of infectious diseases in animal husbandry has contributed to many impacts as hepatotoxicity (Amacher1998; Hassaninet al., 2014; Jiaoet al., 2009). Consequently, concerns have been put forward to discover hepatoprotective agents that can counteract these impacts.

L-carnitine (LC) is a non-essential amino acid derivative naturally occurring and widely distributed in nature compound which can be obtained either through endogenous biosynthesis or from exogenous sources (Surai, 2015). L-carnitine received growing interest in its potential uses as a medicinal agent possess protective effects which postulated to be related to its antioxidant action (Hassanin et al., 2014). In poultry industry, L-carnitine was purposed for growth promotion, immune system strengthening and antioxidant actions (Adabi et al., 2011). L-carnitine has protective effects against lipid peroxidation by reducing the formation of hydrogen peroxide and buffering of excess acetyl-CoA, which in itself can cause free radicals formation and potentially toxic to the cells (Agarwaland Said, 2004; Bayraktar et al., 2008). L-carnitine acts by reducing the availability of lipids for peroxidation through transportation of fatty acids into the mitochondria for β-oxidation to produce ATP energy (Nouboukpo et al., 2010). L-carnitine acts as an antioxidant in the protection of glutathione peroxidase, catalase and superoxide dismutase enzymes from further per oxidative damage (Kala Iselvi and Panneerselvam, 1998) and by restoration of the endogenous antioxidant enzymes (SOD, CAT, GSH-Px, GR and GST) and non-enzymatic antioxidants (vitamins E and C) in the liver and other tissues of stressed animals, increased intracellular concentration of GSH in liver and other tissues and decreased lipid and protein oxidation (Surai, 2015). Therefore, this trial aimed to evaluate the potential ameliorative role of L-carnitine on florfenicol-induced hepatotoxicity in broilers before, with, and after florfenicol administration through assessment of liver indices including aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP), and total bilirubin (T.Bil). Furthermore, its antioxidant profile was estimated by the evaluation of superoxide dismutase (SOD), glutathione reductase (GSH), glutathione peroxidase (GPx) and malondialdehyde (MDA) levels. To the best of our knowledge, this is the first study elucidate the hepatoprotective effect of L-carnitine against florfenicol-induced hepatotoxicity.

MATERIALS AND METHODS

Drugs

Florfenicol (Floribiotic10%) ® was obtained from ATCOpharma, Co, Egypt. L-carnitine® was obtained from IDPCO for feed additives, Egypt.

Experimental Birds and protocol

A total of 150 one day old broiler chicks (Erbo plus) were used in this trial. They were accommodated in separated pens in experimental construction rooms on deep litter, maintained under natural environmental conditions (25-30 °C), free access to feed, water and continuous lightening program. Chicks were divided into 6 equal groups, each of 25 chicks; group (1) : kept as a control group, group (2) : chicks in this group were given (60 mg/kg b.wt.) florfenicol orally (Marwa et al., 2013) on day 18 of age once daily for 3 successive days, group (3) : chicks in this group were given (50 mg/kg b.wt.) L-carnitine orally (Baumgartner and Blum, 1997) once daily on day18 of age for 3 successive days, group (4) : chicks in this group were given (50 mg/kg b.wt.) L-carnitine orally on day 15 of age old once daily for 3 successive then were given (60 mg/kg b.wt.) florfenicol orally on day 18 of age old once daily for 3 successive days, group (5) : chicks in this group were (60 mg/kg b.wt.) florfenicol together with (50 mg/kg b.wt.) L-carnitine orally once daily on day 18 of age for 3 successive days, group (6) : chicks in this group were given (60 mg/kg b.wt.) florfenicol orally on day 18 of age once daily for 3 successive days then were given (50 mg/kg b.wt.) L-carnitine orally on day 21 of age once dai-

ly for 3 successive days. All experimental protocols were approved by the Committee on Animal Experiments, Faculty of Veterinary Medicine, Kafrelsheikh University.

Biochemical analysis

After the last day of florfenicol administration (day 20), five birds from each group were randomly selected after five days (day 25), seven days (day 27), and nine days (day 29) of the experiment and blood samples were obtained from their wing vein without anticoagulant and placed in a slant position then centrifuged at 3000 r.p.m. for 20 minutes to obtain serum.

Liver indices

Serum ALT and AST activities were assayed by using commercial kits that were obtained from Diamond Diagnostic Company, Egypt, according to the method of (Murray and Kaplan, 1984). ALP activity was assayed according to (Belfield and Goldberg, 1971) by using commercial kits that were obtained from Vitro Scient, Company, Egypt. The concentration of serum total bilirubin was determined according to the method described by Jendrassik and Grof (1938) by using commercial diagnostic kits that were supplied by cell biolabs, USA.

Preparation of tissue homogenate for antioxidant profile assay

From liver samples, 0.5gm was homogenized in 5ml of distilled water using an electrical homogenizer, centrifuged at 3000r.p.m. for 15 minutes. The supernatant was collected and used for the estimation of SOD, MDA, GPx, and GSH. Tissue homogenates were preserved at -20°C until performing the investigations (Sakeran et al., 2014).

Histopathological examination

The livers from all groups were obtained, kept in 10% formalin and processed in paraffine wax. Sections of five-microns thickness were stained with Hematoxylin and Eosin (H&E) and examined microscopically according to Bancroft and Gamble (2008).

Statistical Analysis

The obtained data were statistically analyzed through one way (ANOVA) using the software statistical program (SPASS, ver.16.00, USA). Data are expressed as the mean± SE, and the results were statistically significant at$P \leq 0.05$.

RESULTS

Changes in Liver indices

Our results showed that administration of florfenicol in a dose of 60 mg/kg b.wt. orally for 3 successive days caused hepatotoxicity in broilers as indicated by a significant increase ($P \leq 0.05$) in serum AST, ALT, ALP, and total bilirubin levels compared to the control group. This elevation significantly ($P \leq 0.05$) decreased in previously treated with the L-carnitine group and co-treated with L-carnitine group (G4and G5) compared with florfenicol treated group (G2). AST, ALT, ALP, and total bilirubin levels in co-treated florfenicol with L-carnitine groups (G4 and G5) were significantly decreased ($P \leq 0.05$) when compared with post-treated florfenicol with L-carnitine group (G6), (Table1). Our results confirmed that there are no statistical differences between the results after five days (day 25), seven days (day 27), and nine days (day 29) of florfenicol administration (data not shown).

Table 1. The effect of L-carnitine (50 mg/kg, oral) on florfenicol (60 mg/kg, oral) induced hepatotoxicity in broilers on day nine after florfenicol administration (day 29)

Parameters	Groups					
	G1	G2	G3	G4	G5	G6
AST (IU/L)	16.94±0.15	29.94±0.11*	17.94±0.11#	19.94 ± 0.29#	20.08 ± 0.29#	27.10± 0.31*
ALT (IU/L)	45.54±0.37	54.98 ± 0.45*	44.9 ± 0.25#	49.34 ± 0.22#	48.32 ± 0.20#	51.76 ± 0.08*
ALP (IU/L)	88.54±0.41	140.12±0.41*	95.79±0.27#	121.30±0.49#	121.84±0.14#	138.96± 0.38*
T. Billirubin	0.81 ± 0.03	1.40 ± 0.00*	0.85±0.01#	0.98 ± 0.00#	0.89 ± 0.01#	1.18 ± 0.01*

AST: aspartate aminotransferase, ALT: alanine aminotransferase, ALP: alkaline phosphatase and T.Bil: total bilirubin. G1, control group; G2, florfenicol group; G3, L-carnitine group; G4, previously treated with L-carnitine; G5, Co-treated group with L-carnitine; G6, Post treated group with L-carnitine. Data are presented as (mean ± SE) with *significant at ($P \leq 0.05$) as compared with control group, # significant at ($P \leq 0.05$) as compared with florfenicol treated group.

Changes in hepatic SOD, GSH-Rd, GPx and lipid peroxidation levels

A significant ($P \leq 0.05$) increased in the liver MDA of florfenicol treated group (G2) and post-treated group with L-carnitine (G6) when compared with the control and L-carnitine groups (G1 and G3). At the same time, the data declared significant decreased ($P \leq 0.05$) in the liver SOD, GSH-Rd, and GPx levels of the florfenicol treated group (G2) and post-treated with L-carnitine (G6) as compared with the control and L-carnitine (G1 and G3) groups. As well, the importance of treatment with L-carnitine groups (G4 and G5) has been shown significantly decreased in the MDA and a significant increase in the liver SOD, GSH-Rd, and GPx levels when compared to the florfenicol group (G2) and post-treated groups (G6), (Table 2).

Table 2. The effect of L-carnitine (50 mg/kg. oral) on florfenicol (60 mg/kg, oral) induced hepatotoxicity in broilers on day nine after florfenicol administration (day 29)

Parameters	Groups					
	G1	G2	G3	G4	G5	G6
SOD (u/g)	386.53±0.70	176.64±3.92*	378.05±3.09#	324.35±2.76#	326.33±1.46#	199.38±0.62#
GSH (mg/g)	57.77±0.29	45.55±0.23*	57.86±0.32#	51.18±0.25#	51±0.21#	47.97±0.30*
GPx (u/g)	59.14±0.23	19.15±0.28*	58.35±0.23#	35.58±0.11#	33.82±0.21#	20.52±0.19*
MDA (nmol/g)	9.17±0.28	12.26± 0.22*	9.15±0.11#	9.95±0.08#	11.05±0.24#	12.33±0.36*

SOD: Superoxide dismutase, GSH: Reduced glutathione, GPx: glutathione peroxidase and MDA: Malondialdehyde. G1, control group; G2, florfenicol group; G3, L-carnitine group; G4, previously treated with L-carnitine; G5, Co-treated group with L-carnitine; G6, Post treated group with L-carnitine. Data are presented as (mean ± SE) with *significant at ($P \leq 0.05$) as compared with control group, # significant at ($P \leq 0.05$) as compared with florfenicol treated group.

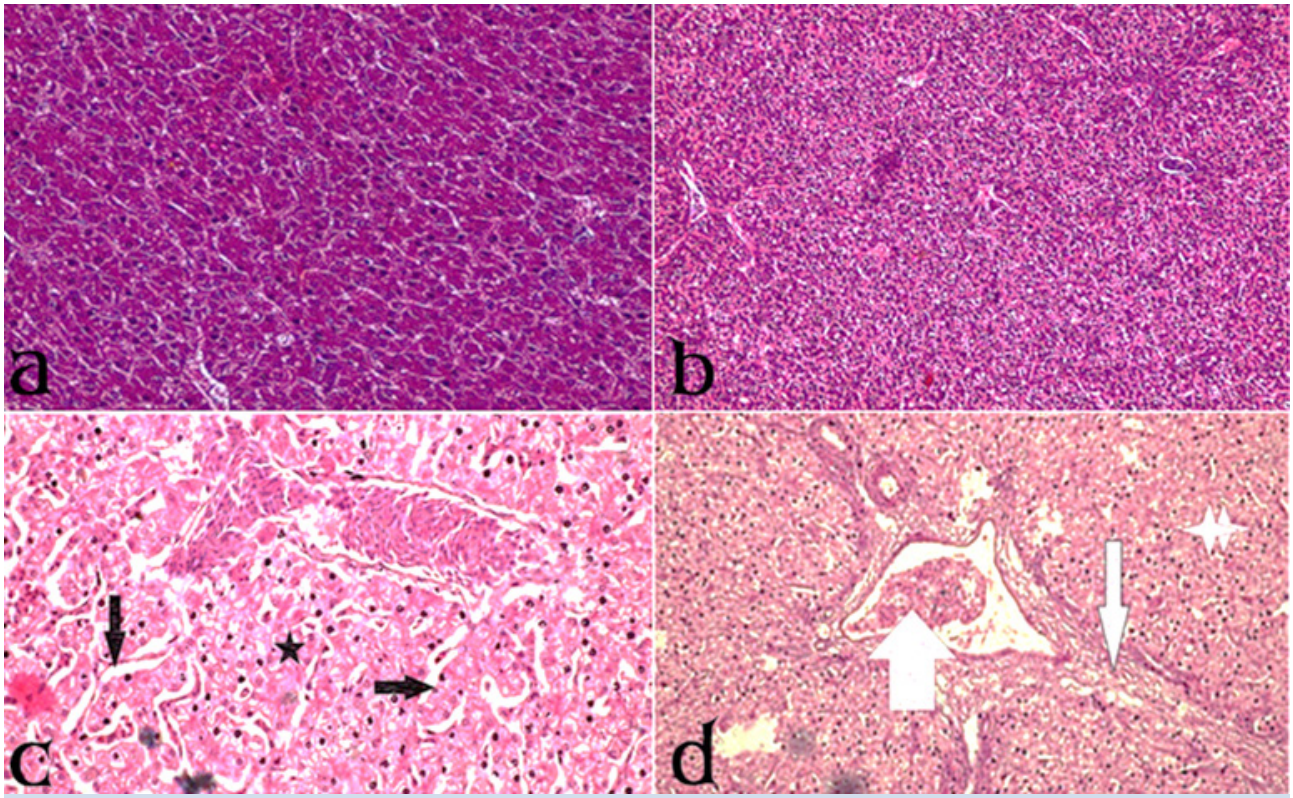

Fig. 1. (a) A liver section of broiler showing normal architecture H&E (X400 *(*. (b) A liver section from a broiler treated with L-carnitine revealing normal hepatocyte H&E (X200 *(*. (c) A liver section from a broiler treated with florfenicol showing severe edema in the hepatic sinusoid (black arrows) and hepatocyte showing necrosis (asterisk) H&E (X400). (d) A liver section from a broiler treated with florfenicol revealing periportal fibrosis (thin arrow), portal blood vessel showing dilatation and engorged with blood (thick arrow), and hepatocytes revealing vacuolar degeneration (asterisk) H&E (X400).The histopathological findings were adapted from the liver of the broilers on day nine after florfenicol administration (day 29).

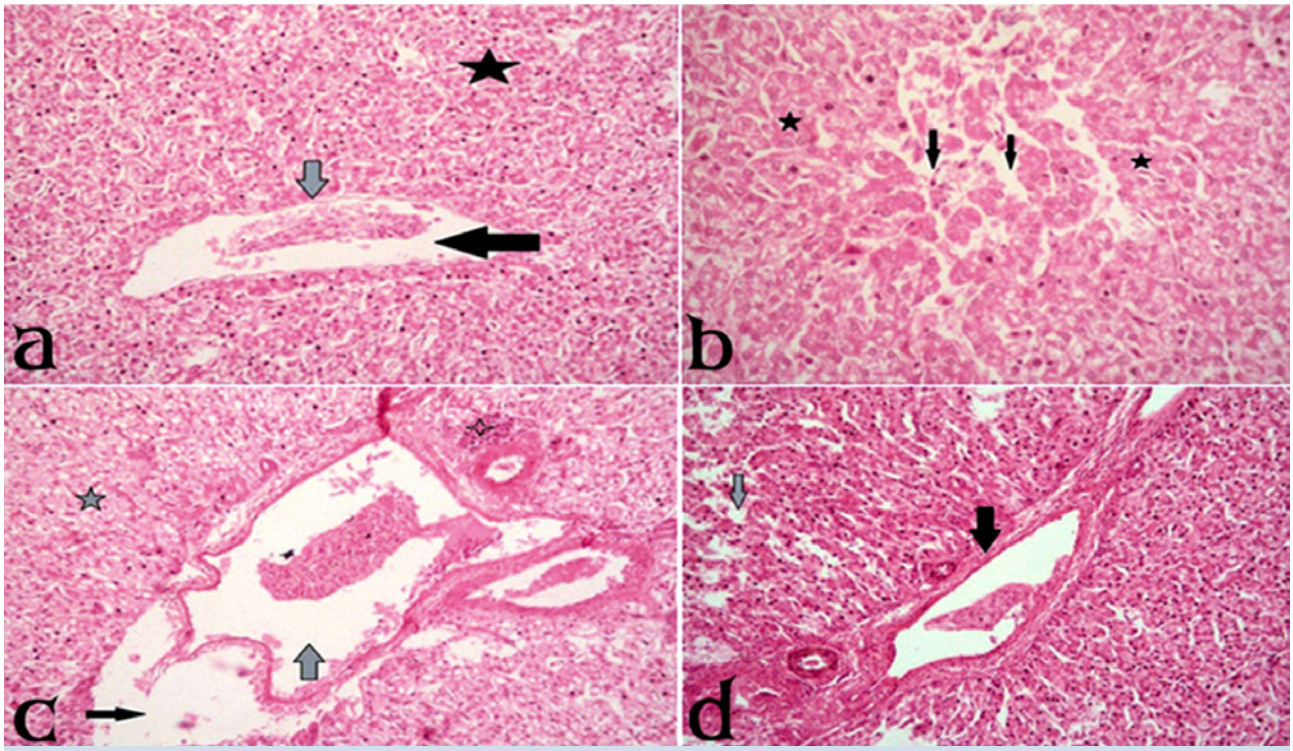

Fig. 2. (a) A liver section from a broiler previously treated with L-carnitine showed mild dilatation of central vein (black arrow), mild pericentral hepatocytic necrosis (grey arrow), and most of hepatocyte revealing vacuolar degeneration (asterisk) H&E (X200). (b) A liver section from a broiler previously treated with L-carnitine showing mild necrosis in the hepatocyte (asterisk) and edema in the hepatic sinusoid (arrows) H&E (X400). (c) A liver section from a broiler co-treated with L-carnitine showed periportal edema accompanied by focal inflammatory cell infiltration (black arrow, black asterisk) with mild necrosis of the hepatocyte (grey asterisk) H&E (X200). (d) A liver section from a broiler post-treated with L-carnitine showed periportal fibrosis (black arrow), dilatation, and edema in the hepatic sinusoid (grey arrow) H&E (X400). The histopathological findings were adapted from the liver of the broilers on day nine after florfenicol administration (day 29).

Histopathological changes

The histopathological changes in the liver of the broilers in the experimental groups are shown in (Fig. 1 and Fig. 2). The broiler liver sections in the control group (G1) and L-carnitine treated group (G3) showed normal hepatocyte and sinusoidal architectures (Fig. 1a, 1b). The liver sections in the florfenicol treated group (G2) showed severe edema in hepatic sinusoid, periportal fibrosis, and hepatocyte showing necrosis and vascular degeneration (Fig. 1c, 1d). The liver sections in previously treated with L-carnitine group (G4) (Fig. 2a, 2b) showed a good degree of improvement in hepatocytes where mild necrosis of hepatocyte and dilatation in hepatic sinusoid. The liver sections in the co-treated group with L-carnitine (G5) (Fig. 2c) showed periportal edema accompanied with focal inflammatory cell infiltration and mild necrosis of the hepatocytes. The liver sections in post treated with L-carnitine group (G6) (Fig. 2d) showed periportal fibrosis, dilatation, and edema in the hepatic sinusoid.

DISCUSSION

Florfenicol is a fluorinated structural analogue of thiamphenicol, is a synthetic broad-spectrum antibacterial drug with a range of activity similar to that of chloramphenicol, commonly used in veterinary medicine (Dowling, 2013). However, high dosages of florfenicol may give rise to hepatotoxicity (Amacher, 1998; Jiao et al., 2009; Hassanin et al., 2014). L-carnitine is a non-essential amino acid derivative widely distributed in nature and had received growing interest in its potential uses as a medicinal agent possess protective effects that postulated to be related to its antioxidant action (Hassanin et al., 2014). This is the first study confirming the hepatoprotective effects of L-carnitine against hepatotoxicity induced by florfenicol.

The previous reports confirmed the complete withdrawal of florfenicol from the serum of broiler after six days in healthy chickens and seven days in infected ones (EL-Banna et al., 2007). To ensure the hepatoprotective effect of L-carnitine after complete withdrawal of florfenicol from the serum of broiler,

we present our results on day nine after florfenicol administration (day 29). In agreement with the previous records, our study revealed that administration of florfenicol induced hepatic damage which was evidenced by increased levels of AST, ALT, ALP, and total bilirubin (Marwa et al., 2013; Er and Dik, 2014). Furthermore, our findings are in agreement with the results of experimental studies of other authors, who reported that elevated levels of these parameters due to their release into the circulation after the cellular damage has occurred as evidence of liver toxicity (Amacher, 1998; Sakeran et al., 2014). The obtained histopathological observations basically supported the results obtained from the serum enzyme assay (Fig. 1c, 1d).

In the current study, supplementation of L-carnitine was found to alleviate the changes in liver indices which induced by florfenicol, where, the group which previously treated with L-carnitine had the more prominent effect (G4) than the co-treated florfenicol with L-carnitine group (G5). The ameliorative effect of L-carnitine was confirmed by the histopathological findings (Fig. 2a, 2b, 2c).Our results were in the same line with some recent studies (Ahmed, 2016; Mousah et al., 2016).The ability of L-carnitine to significantly improve liver function enzymes may be due to its ability to act as a radical scavenger, leading to protection of membrane permeability (prevent the leakage of intracellular enzymes by its membrane stabilizing activity (Augustyniak and Skrzydlewska, 2009).

Oxidative stress is an indicator of the damage that results from a change in the balance between oxidants and antioxidants in favor of oxidants. If the delicate balance between oxidants and antioxidants cannot be maintained in tissues, many pathological changes extending to cellular damage occur (Jahovic et al., 2004). SOD, GPx, and GSH-Rd play a major role in the first line of antioxidant defense (El-Demerdash et al., 2009).SOD prevents the inhibition of glutathione by scavenging superoxide radicals and glutathione in turn prevents the inhibition of SOD by scavenging H_2O_2. MDA is the most important oxidation by-product of lipid breakdown which can show the extent of lipid peroxidation in many organs (Del Rio et al., 2005). In the current study, MDA levels in the florfenicol group were significantly increased, with SOD, GSH-Rd, and GPx levels were significantly decreased when compared with the control group. The current results were similar previously reported by other investigators (Ren et al., 2014; Yuxuan et al., 2019). The ability of florfenicol to produce oxidative stress is by inhibition of antioxidant enzymes or as a result of the generally impaired physiological state of an organism (Wu et al., 2011).Our histopathological result is agreed with Khalil et al., (2012) who stated that such portal fibrosis infiltrated with few lymphocytes, seemed to be due to direct toxic effects of florfenicol.

Our result clearly demonstrated that L-carnitine increased the levels of hepatic SOD, GSH-Rd, and GPx associated with a reduction in MDA levels with no significant difference between previously treated with L-carnitine (G4) and co-treated with L-carnitine (G5). On the other hand, there was a significantly increase in the MDA and significant decreased in the liver SOD and GPx levels in post-treatment with L-carnitine (G6) when compared to co-treatment with L-carnitine groups (G4 and G5).These effects of L-carnitine may result directly from the antioxidant effects against free oxygen radicals (Bayraktar et al., 2008), or from the restoration of the endogenous antioxidant enzymes and non-enzymatic antioxidants in the liver enhanced biosynthesis of enzymatic antioxidants (Surai, 2015).Our results were in the same line with Çekınet al., (2013) who reported that administered of L-carnitine for 4 days prior to ischemia-reperfusion injury in male Westar-Albino rat induced marked protection as detected by a significant elevation in the liver reduced glutathione (GSH) and significant decrease in MDA. Furthermore, the present study agreed with Dokmeci et al., (2006) who recorded that pretreatment with L-carnitine before irradiation, resulted in a marked decrease in MDA values of liver tissue and a significant increase in glutathione (GSH) level in liver tissue. These results indicated that pre-treatment with L-carnitine may potentially protect from florfenicol induced hepatotoxicity, which is also agreed with Abu-El-Zahab et al., (2019) who reported that administration of L-carnitine decreases oxidative stress by reducing lipid peroxidation and increasing GPx and SOD activities in the liver. All these enhancements were confirmed by the histopathological finding where the treatment with L-carnitine showed moderate to good degree of improvement in hepatocytes in (G4 and G5) (Fig. 2a, 2b, 2c). While liver sections in post-treated florfenicol with L-carnitine showed marked disturbance of the hepatocytes, where periportal fibrosis and edema in the hepatic sinusoid were observed (Fig. 2d).

CONCLUSIONS

The current study suggested that oxidative stress

plays a major role in florfenicol induced hepatotoxicity. Administration of L-carnitine preceded the florfenicol usage or when they were used together had a protective effect against florfenicol-induced hepatic damage in broilers more prominent than the post-treatment with L-carnitine. The hepatoprotective effect of L-carnitine is most probably due to its ability to scavenge free radicals and enhance the antioxidant systems. Our study elucidates for the first time the significant importance of admiration of L-carnitine with florfenicol to prevent its undesired hepatotoxic action in broilers.

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CONFLICT OF INTEREST

There is no declared conflict of interest.

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