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AI HOSNY, MH KHAIRY, AM ASY, EA ABOZEID

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## Ameliorative effect of tocotrienol and selenium yeast against the adverse effect of florfenicol in broilers' liver

A.I. Hosny<sup>1</sup>, M.H. Khairy<sup>1</sup>, A.M. Asy<sup>2</sup>, E.A. Abozeid<sup>2</sup>

<sup>1</sup> Department of Pharmacology, Faculty of Veterinary Medicine, Zagazig University, Zagazig, Egypt

<sup>2</sup> Department of Biochemistry, Animal Health Research Institute, Benha-Branch, ARC, Dokki, Giza, Egypt

**ABSTRACT:** The prolonged use of florfenicol can lead to detrimental side effects in poultry. This work focuses on the role of tocotrienol and selenium yeast to mitigate the adverse effects of florfenicol in broilers' liver. One hundred and fifty, one-day-old Cobb broiler chicks were equally divided in 5 experimental groups according to the following experimental design: Group (1) control group chicks fed to a balanced diet only. Group (2) Chicks treated with florfenicol (20 mg/kg b.w.) per bird for 3 successive days and the florfenicol was administered in other groups by the same dose and for the same period of time. Group (3) chicks treated with florfenicol and tocotrienol (170 mg/kg b.w.) for 7 successive days. Group (4) Chicks treated with florfenicol and selenium yeast (0.15mg /kg b.w., on feed) for 7 successive days. Group (5) Chicks treated with a combination of florfenicol, tocotrienol and selenium yeast. Chickens treated with florfenicol exhibited an increased level in hepatic malondialdehyde (MDA), as well as a decreased level in hepatic superoxide dismutase (SOD) and reduced glutathione (GSH). Tocotrienol and selenium yeast decreased the MDA and increased SOD and GSH in hepatic tissue as well as return ALP, cholesterol, triglyceride and VLDL to their normal levels. Treated chicks with tocotrienol returned serum (ALT) to normal activity but serum total protein and albumin levels were increased. The selenium yeast treated groups showed an increase of serum total globulin. Histopathologically, florfenicol treated group had focal hepatic leukocytic infiltration and focal coagulative necrosis of hepatocytes but chickens with the combination of tocotrienol and selenium yeast had activated Kupffer cells and revealed less evident necrotic changes in liver. In conclusion, tocotrienol and selenium yeast administered alone or in combination highlighted improved antioxidant effects and mitigated the lipid peroxidation in broilers' liver treated with florfenicol. Thus, tocotrienol and selenium yeast can improve the safety of using florfenicol in broiler chickens under experimental conditions.

**Keywords:** Florfenicol, Tocotrienol, Selenium yeast, Liver, Antioxidants, Broilers.

### Corresponding Author:

Eman Abd-El Moneim Abozeid, Department of Biochemistry, Toxicology and Nutritional Deficiency, Animal Health Research Institute, Benha-Branch, ARI, Giza, Dokki, Egypt  
E-mail address: eman.abozeid89@gmail.com

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## INTRODUCTION

Florfenicol, a synthetic broad -spectrum antibiotic, contains a fluorine atom at the 3-carbon position, instead of the hydroxyl group found in thiamphenicol used in veterinary practice to treat most gram-positive and gram-negative bacteria (Chang et al., 2010). Florfenicol action is mainly bacteriostatic by inhibition protein synthesis of bacteria by binding to 50S and 70S subunits ribosome and abolishes the activity of peptidyl transferase (Khalil et al., 2012). Ronette (2012) reported that florfenicol is used for the treatment of many microorganisms which affect poultry such as *Escherichia coli*, *Klebsiella*, *Ornithobacterium rhinotracheale*, *Pasteurella multocida*, *Mannheimia haemolytica*, *Enterobacter cloacae*, *Haemophilus somnus*, *pneumonia*, *Salmonella typhi*, *Shigella dysenteriae* and *Staphylococcus aureus*. Florfenicol usually made biotransformation effect (more concentrations of reactive oxygen species) with biochemical changes after entering into cells because it is a highly lipophilic drug. Florfenicol damage changes differ according to the dosage of the drug, time of administration and animal (Ren et al., 2014). Florfenicol administration can produce oxidative stress in broiler's liver by inhibiting the expression of antioxidant proteins nuclear factor-erythroid 2-related factor 2 (Nrf2), hemeoxygenase-1 (HO-1) and NAD(P)H dehydrogenase quinone-1 (Han et al., 2020). In addition Wang et al. (2020) reported that administration of florfenicol by different doses (0.15, 0.3, 0.6, 1.2 and 1.8 g/L) in drinking water of broilers for five successive days produced oxidative stress effect on chicks through inhibition of the expression of related factors in Nrf2.

Antioxidants are substances that interact with unstable free radicals and prevent there damage effects (Sies, 1997; Olayinka et al., 2012). The antioxidants can be classified to natural and synthetic. Natural antioxidants are vitamin E, organic selenium, vitamin C and beta carotene. Synthetic antioxidants are butylated hydroxyanisole and butylatedhydroxytoluene (Hurrell, 2003).

Tocotrienols are the primary form of vitamin E which has  $\alpha$ ,  $\beta$ ,  $\gamma$ , and  $\delta$  forms. The main sources of tocotrienols are palm oil extract from *Elaeis guineensis* (African oil palm) contain more than 800 mg/kg of tocotrienol and cereal grains such as wheat, barley, rice and differ from tocopherol by the presence of three trans-double bonds in the hydrocarbon tail. Tocotrienols have hypo cholesterolemic, neuropro-

tection and anticancer effects (Sen et al., 2006). Tocotrienol rich fraction from palm oil can protect cellular membranes from damage effect and it can inhibit protein oxidation and lipid peroxidation in rat liver microsomes (Kamat et al., 1997). Palmitate oil (tocotrienol containing diet) displayed significant decrease in malondialdehyde MDA level with raises superoxide dismutase SOD activity and reduced glutathione GSH level (Khan et al., 2011). Selenium yeast is one of the best organic sources of selenium for poultry and other farmed animals which was approved for chicken consumption in June 2000 by the United States Food and Drug Administration US-FDA, and has a potent antioxidant effect (Wang and Xu, 2008). Elevation selenium level in the diet was led to lowering lipid peroxidation products, free radical elimination and protecting cell membranes (Fan et al., 2009). Diets supplemented with selenium yeast improve activity of hepatic glutathione peroxidase and help for production of oxidized glutathione then stimulate glutathione reductase, which prevent its deactivation by NADPH (Upton et al. 2009). The prolonged use of florfenicol can lead to detrimental side effects in poultry. This work focuses on the role of tocotrienol and selenium yeast alone or their combination to mitigate these side effects in broilers' liver.

## MATERIAL AND METHODS

### Animals

One hundred and fifty, one-day-old, unsexed Cobb broiler chicks were used and purchased from El-Watania Poultry Company - Cairo - Egypt. The birds were allocated in separate units of metal wire-floored battery for five successive weeks. The study was approved by the Ethical Committee for care and use of animals at Animal Health Research Institute Benha Branch, Egypt (25/3/2019).

### Drugs

Three drugs were used in this experimental model. Firstly, florfenicol 10% (Floricol<sup>®</sup>, PharmaSwede Co., Egypt) was administrated for 3 successive days at 20 mg/kg according to the instructions of manufacture. Each ml of the product contained 100 mg of florfenicol base. Secondly, tocotrienol 50% (Tocovid<sup>®</sup>, Hovid Company, Malaysia) was administrated for 7 successive days, at the recommended dose of 170 mg/kg. Finally, selenium yeast 0.2% (Bio-SEL 2000<sup>®</sup>, IBEX International Co. LTD, Egypt) was administrated at 0.15 mg/kg for 7 successive days.

## Experimental design

One hundred and fifty (150), apparently healthy, one-day-old, unsexed, Cobb broiler chicks were used. The chicks were housed in clean and disinfected enclosure, with controlled environmental temperature, and fed with a well-balanced ration throughout the experimental period of five successive weeks. The chicks were equally divided into five groups of 30 chicks each. Group 1, was the negative control group, including chicks which fed with balanced diet only. Chicks in Group 2 were treated with florfenicol (20 mg/kg) for 3 successive days (15<sup>th</sup> - 17<sup>th</sup> day of age) by drinking water. Group 3 was treated with florfenicol and tocotrienol (170 mg/kg, per os) for 7 successive days (15<sup>th</sup> - 21<sup>st</sup> day of age). Group 4 was treated with florfenicol and selenium yeast (0.15 mg/kg, in feed) for 7 successive days (15<sup>th</sup> - 21<sup>st</sup> day of age). Group 5 was treated with the combination from florfenicol, tocotrienol and selenium yeast for 7 successive days (15<sup>th</sup> - 21<sup>st</sup> day of age).

Blood samples were collected from jugular vein of five birds of each group on the 18<sup>th</sup>, 25<sup>th</sup> and 35<sup>th</sup> days of age. The liver of chicks was divided into two parts. One part was obtained immediately for fixed with 10% formalin solution for 48h and the another part was stored in at -20°C for determination of antioxidant /oxidant status in the hepatic tissue on the 18<sup>th</sup>, 25<sup>th</sup> and 35<sup>th</sup> days of age.

## Biochemical analysis (Liver function tests)

Blood samples were collected and sera were separated. The sera were stored -20°C until examination to evaluate alanine aminotransferase (ALT) and aspartate aminotransferase (AST) (Varli, 1974), alkaline Phosphatase ALP (Belfield and Goldberg, 1971), total protein (TP) (Domas, 1975), serum albumin (Dumas, 1971), serum total globulin (Coles, 1974), serum total cholesterol (Flegg, 1973), triglycerides (TG), high-density lipoprotein cholesterol (HDL-C) (Gordon et al., 1977), and low-density lipoprotein cholesterol (LDL-C) (Friedewald et al., 1972) were estimated. Very low-density lipoprotein cholesterol (VLDL-C) was calculated by dividing triglycerides value by 5 (Tietz, 1976). Low-density lipoprotein cholesterol (LDL-C) was calculated by the following equation:  $LDL-C = (Total\ cholesterol) - (HDL-C) - (VLDL-C)$  (Ashayerizadeh et al., 2009).

## Evaluation of antioxidant and oxidant status in hepatic tissue

Liver tissues from each chick were collected im-

mediately and stored in low temperatures for reserve. Prior to dissection, perfuse hepatic tissues with phosphate buffered saline solution (PH 7.4) containing heparin (0.16 mg/ml) to remove any blood cells and clots. Homogenize the tissue in 5 -10 ml cold buffer (I, e, 50mM potassium phosphate, pH 7.5.1 mM EDTA) per gram tissue, using tissue homogenizer. Centrifuge at 4000 rpm for 5 minutes at 4°C. Remove the supernatant for assay and store on ice. If not assaying on the same day, freeze the sample at - 80°C. The sample will be stable for at least one month. The prepared samples were ready to evaluate superoxide dismutase (SOD) activity (Nishikimi et al., 1972), reduced glutathione (GSH) concentration (Beutler et al., 1963) and malondialdehyde (MDA) level (Ohkawa et al., 1979).

## Histopathological examination of liver tissue

Liver samples were fixed in 10% formalin solution for 48 hours for further histopathological examination following standard methodologies to obtain hematoxylin and eosin-stained slides (Bancroft and Stevens, 1977).

## Statistical analysis

Statistical analysis was conducted with the Statistical Package for Social Science (SPSS Inc. Released, 2009) to determine if variables differed between groups, according to Snedecor and Cochran, 1989. The Shapiro-Wilk test was used to test the normal distribution of the data before statistical analysis was performed. Analysis of variance was conducted by one-way ANOVA and compare between means were conducted by Duncan's multiple range test (Duncan, 1955). Probability values of less than 5 % ( $P < 0.05$ ) were considered a significant finding.

## RESULTS

The blood biochemical parameters (liver enzymes) in broilers treated with florfenicol showed a significant ( $P < 0.05$ ) increase in serum ALT on the 25<sup>th</sup> day of age and no significant ( $P > 0.05$ ) differences on the 35<sup>th</sup> day of age while a significant ( $P < 0.05$ ) increase in serum ALP activity when compared with normal group. Other treated group returned serum ALT and ALP activities to normal levels as shown in Table 1.

On the 25<sup>th</sup> day of age, florfenicol treated group showed significant ( $P < 0.05$ ) decreases in serum TP, albumin and serum total globulin when compared with control group. Other groups displayed significant ( $P < 0.05$ ) increases in serum TP and albumin



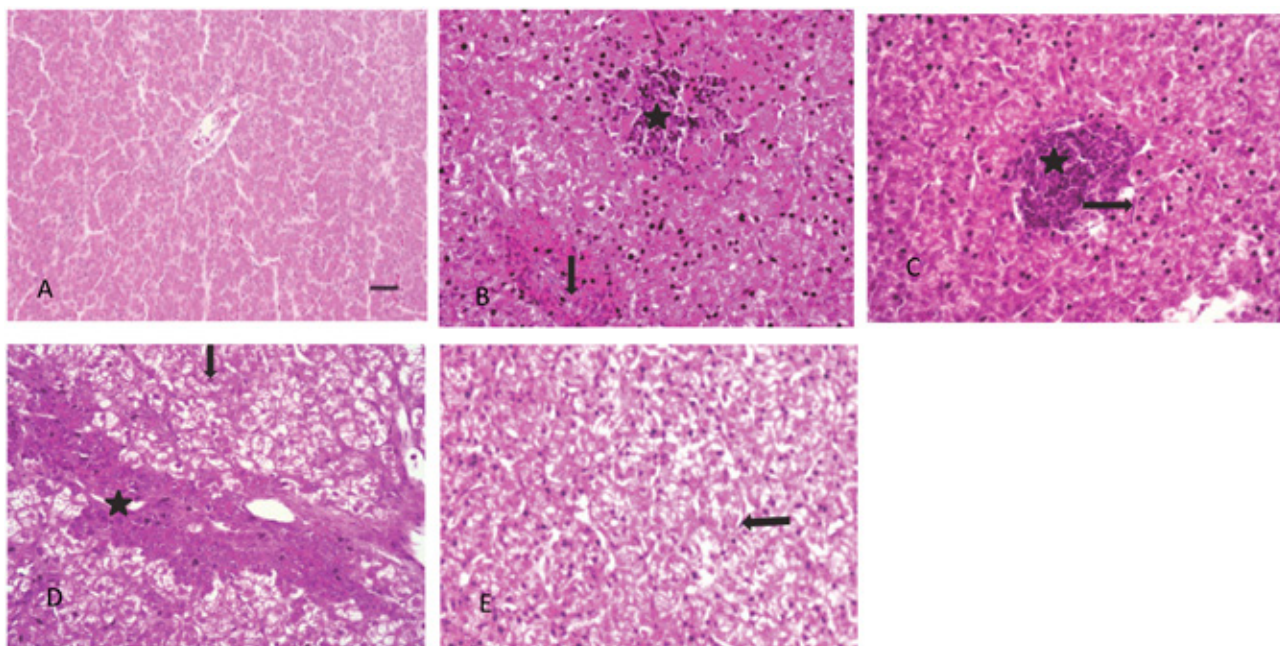
when compared with florfenicol treated group. Selenium yeast and florfenicol treated group alone and the group treated with its combination with tocotrienol displayed a significant ( $P < 0.05$ ) increase in serum total globulin when compared with florfenicol treated group as shown in Table1.

Florfenicol and tocotrienol treated group showed a significant ( $P < 0.05$ ) decrease in serum cholesterol and LDL levels on the 18<sup>th</sup> day of age with no significant ( $P > 0.05$ ) differences on the 25<sup>th</sup> day of age but triglycerides returned to normal on the 18<sup>th</sup> and 25<sup>th</sup> day of age when compared with control group. Selenium yeast and florfenicol treated group alone and the group treated with its combination with tocotrienol returned to normal lipid profile levels as shown in Table1.

Florfenicol increased MDA level in hepatic tissue, but significantly ( $P < 0.05$ ) decrease SOD activity and GSH concentration in hepatic tissue when compared with the control group. Florfenicol, tocotrienol and selenium yeast either alone or in combination showed a significant ( $P < 0.05$ ) decrease in MDA as well as significant ( $P < 0.05$ ) increases in SOD and GSH in hepatic tissue when compared with florfenicol treated

group as shown in Table2.

For histopathological examination five samples from 30 birds (5/30) per group were collected on the 18<sup>th</sup>, 25<sup>th</sup> and 35<sup>th</sup> days of age. As shown in Fig. 1A, the liver section of the negative control group revealed normal hepatic architecture. Liver sections of florfenicol treated group showed focal leukocytic infiltration in the hepatic tissue and focal coagulative necrosis of hepatocytes represented by deeply eosinophilic cytoplasm of the nuclei with karyorrhexis and karyolysis in the liver section of chicks (Fig. 1B). Liver sections of florfenicol with tocotrienol treated group displayed fatty degeneration of some hepatic cells, with eccentric location of the nuclei on the 35<sup>th</sup> day of age and focal mononuclear cell infiltration of the hepatic tissue and activation of the Kupffer cells on the 35<sup>th</sup> day of age (Fig. 1C). Liver sections of florfenicol and selenium yeast treated group revealed focal coagulation hepatocellular necrosis with karyolysis of the nuclei and ballooning degeneration represented by empty cytoplasm and centrally located nuclei in the adjacent hepatocytes (Fig. 1D). Liver sections of florfenicol, tocotrienol and selenium yeast treated group showed activation of Kupffer cells and less evident necrotic changes (Fig. 1E).



**Figure 1.** Histopathological analysis of liver tissue. Note: Liver tissues were fixed and stained with H&E. A) Control group 200X, B) Florfenicol treated group showed focal leukocytic infiltration in the hepatic tissue (star) focal coagulation necrosis of hepatocytes (black arrow) 400X, C) Florfenicol and tocotrienol treated group showed activations of Kupffer cells (black arrow) focal mononuclear cell infiltration of the hepatic tissue (star) 400X, D) Florfenicol and selenium yeast treated group showed focal coagulation necrosis (star) and ballooning degeneration (black arrow) 200X, E) Florfenicol, tocotrienol and selenium yeast treated group showed activation of Kupffer cells (black arrow) and less evident necrotic changes. 200X

**Table 1:** Effect of oral administration of tocotrienol and, selenium yeast on blood biochemical parameters of broilers exposed to adverse effect of florfenicol. (Mean  $\pm$  SEM)

Parameters	Experimental Periods	Groups				
		1	2	3	4	5
ALT (U/L)	18 day	11.00 $\pm$ .58 <sup>a</sup>	6.50 $\pm$ .29 <sup>b</sup>	5.00 $\pm$ .58 <sup>b</sup>	10.50 $\pm$ .29 <sup>a</sup>	10.00 $\pm$ .58 <sup>a</sup>
	25 day	4.50 $\pm$ .29 <sup>c</sup>	10.00 $\pm$ .29 <sup>ab</sup>	3.50 $\pm$ .29 <sup>c</sup>	9.00 $\pm$ 1.15 <sup>b</sup>	11.50 $\pm$ .29 <sup>a</sup>
	35 day	12.50 $\pm$ .29 <sup>b</sup>	15.00 $\pm$ 1.15 <sup>ab</sup>	12.00 $\pm$ 1.73 <sup>b</sup>	16.50 $\pm$ .87 <sup>ab</sup>	17.50 $\pm$ 2.02 <sup>a</sup>
AST (U/L)	18 day	12.50 $\pm$ .87 <sup>a</sup>	12.00 $\pm$ .00 <sup>a</sup>	12.00 $\pm$ .58 <sup>a</sup>	12.00 $\pm$ .58 <sup>a</sup>	12.50 $\pm$ .29 <sup>a</sup>
	25 day	16.00 $\pm$ 1.73 <sup>bc</sup>	11.00 $\pm$ 1.15 <sup>c</sup>	14.00 $\pm$ 2.31 <sup>bc</sup>	21.00 $\pm$ 3.46 <sup>ab</sup>	25.00 $\pm$ 2.89 <sup>a</sup>
	35 day	21.5 $\pm$ .87 <sup>a</sup>	22.00 $\pm$ 1.73 <sup>a</sup>	23.00 $\pm$ .58 <sup>a</sup>	27.50 $\pm$ 3.75 <sup>a</sup>	27.50 $\pm$ 4.33 <sup>a</sup>
ALP (U/L)	18 day	522.50 $\pm$ 17.03 <sup>b</sup>	608.00 $\pm$ 23.67 <sup>a</sup>	600.00 $\pm$ 10.39 <sup>ab</sup>	595.00 $\pm$ 42.15 <sup>ab</sup>	672.00 $\pm$ 20.21 <sup>a</sup>
	25 day	660.00 $\pm$ 7.50 <sup>b</sup>	735.50 $\pm$ 18.76 <sup>a</sup>	733.50 $\pm$ 10.68 <sup>a</sup>	680.00 $\pm$ 1.15 <sup>b</sup>	680.00 $\pm$ 9.81 <sup>b</sup>
	35 day	618.50 $\pm$ 3.50 <sup>ab</sup>	708.50 $\pm$ 20.50 <sup>a</sup>	540.50 $\pm$ 22.50 <sup>b</sup>	594.00 $\pm$ 158.00 <sup>ab</sup>	519.00 $\pm$ 158.00 <sup>b</sup>
Total Protein (g/dl)	18 day	3.55 $\pm$ .043 <sup>a</sup>	3.55 $\pm$ .043 <sup>a</sup>	3.33 $\pm$ .04 <sup>ab</sup>	3.25 $\pm$ .07 <sup>b</sup>	3.49 $\pm$ .14 <sup>ab</sup>
	25 day	4.27 $\pm$ .12 <sup>b</sup>	3.25 $\pm$ .12 <sup>d</sup>	3.62 $\pm$ .05 <sup>c</sup>	3.99 $\pm$ .08 <sup>b</sup>	4.79 $\pm$ .04 <sup>a</sup>
	35 day	3.60 $\pm$ .058 <sup>ab</sup>	3.35 $\pm$ .09 <sup>b</sup>	3.45 $\pm$ .09 <sup>b</sup>	4.00 $\pm$ .23 <sup>a</sup>	4.00 $\pm$ .17 <sup>a</sup>
Albumin (g/dl)	18 day	1.89 $\pm$ .07 <sup>a</sup>	1.78 $\pm$ .012 <sup>ab</sup>	1.70 $\pm$ .017 <sup>bc</sup>	1.63 $\pm$ .003 <sup>c</sup>	1.80 $\pm$ .055 <sup>ab</sup>
	25 day	2.26 $\pm$ .04 <sup>a</sup>	1.63 $\pm$ .04 <sup>c</sup>	2.03 $\pm$ .12 <sup>b</sup>	2.00 $\pm$ .017 <sup>b</sup>	2.13 $\pm$ .032 <sup>ab</sup>
	35 day	2.00 $\pm$ .06 <sup>a</sup>	1.80 $\pm$ .06 <sup>a</sup>	1.95 $\pm$ .03 <sup>a</sup>	2.15 $\pm$ .20 <sup>a</sup>	2.15 $\pm$ .14 <sup>a</sup>
Globulin (g/dl)	18 day	1.65 $\pm$ .02 <sup>a</sup>	1.75 $\pm$ .01 <sup>a</sup>	1.63 $\pm$ .02 <sup>a</sup>	1.62 $\pm$ .07 <sup>a</sup>	1.69 $\pm$ .08 <sup>a</sup>
	25 day	2.01 $\pm$ .075 <sup>b</sup>	1.61 $\pm$ .08 <sup>c</sup>	1.58 $\pm$ .06 <sup>c</sup>	1.99 $\pm$ .06 <sup>b</sup>	2.66 $\pm$ .07 <sup>a</sup>
	35 day	1.60 $\pm$ .00 <sup>b</sup>	1.55 $\pm$ .03 <sup>b</sup>	1.50 $\pm$ .11 <sup>b</sup>	1.85 $\pm$ .03 <sup>a</sup>	1.85 $\pm$ .03 <sup>a</sup>
A/G ratio	18 day	1.15 $\pm$ .05 <sup>a</sup>	1.02 $\pm$ .00 <sup>b</sup>	1.04 $\pm$ .00 <sup>ab</sup>	1.00 $\pm$ .05 <sup>b</sup>	1.07 $\pm$ .02 <sup>ab</sup>
	25 day	1.12 $\pm$ .02 <sup>ab</sup>	1.01 $\pm$ .03 <sup>b</sup>	1.29 $\pm$ .13 <sup>a</sup>	1.00 $\pm$ .03 <sup>b</sup>	0.8 $\pm$ .03 <sup>c</sup>
	35 day	1.25 $\pm$ .03 <sup>a</sup>	1.16 $\pm$ .02 <sup>a</sup>	1.32 $\pm$ .12 <sup>a</sup>	1.16 $\pm$ .09 <sup>a</sup>	1.16 $\pm$ .05 <sup>a</sup>
Total Cholesterol (mg/dl)	18 day	129.00 $\pm$ 3.46 <sup>a</sup>	138.00 $\pm$ 9.23 <sup>a</sup>	102.50 $\pm$ .29 <sup>b</sup>	127.00 $\pm$ 1.15 <sup>a</sup>	123.50 $\pm$ 2.02 <sup>a</sup>
	25 day	91.00 $\pm$ 10.97 <sup>ab</sup>	63.50 $\pm$ .87 <sup>b</sup>	69.50 $\pm$ 3.17 <sup>b</sup>	91.00 $\pm$ 15.58 <sup>ab</sup>	95.00 $\pm$ 2.88 <sup>ab</sup>
	35 day	55.00 $\pm$ 1.73 <sup>c</sup>	61.00 $\pm$ 4.04 <sup>bc</sup>	75.50 $\pm$ 9.53 <sup>ab</sup>	73.00 $\pm$ 4.04 <sup>ab</sup>	69.50 $\pm$ 3.75 <sup>abc</sup>
Triglycerides (mg/dl)	18 day	101.50 $\pm$ 7.79 <sup>b</sup>	133.00 $\pm$ 5.77 <sup>a</sup>	100.50 $\pm$ 3.18 <sup>b</sup>	101.00 $\pm$ 1.15 <sup>b</sup>	94.50 $\pm$ 1.44 <sup>b</sup>
	25 day	77.50 $\pm$ 4.90 <sup>a</sup>	46.00 $\pm$ 1.73 <sup>b</sup>	67.50 $\pm$ 3.18 <sup>a</sup>	64.00 $\pm$ 7.51 <sup>a</sup>	63.50 $\pm$ 4.33 <sup>a</sup>
	35 day	75.50 $\pm$ 2.50 <sup>b</sup>	69.50 $\pm$ 1.44 <sup>b</sup>	71.50 $\pm$ 6.06 <sup>b</sup>	79.00 $\pm$ 2.30 <sup>ab</sup>	78.50 $\pm$ 2.02 <sup>b</sup>
HDL-C(mg/dl)	18 day	41.00 $\pm$ .58 <sup>a</sup>	39.00 $\pm$ 1.15 <sup>ab</sup>	42.00 $\pm$ 1.73 <sup>a</sup>	41.50 $\pm$ 3.18 <sup>a</sup>	34.50 $\pm$ .29 <sup>b</sup>
	25 day	38.50 $\pm$ .87 <sup>a</sup>	33.00 $\pm$ 2.31 <sup>ab</sup>	34.50 $\pm$ 2.02 <sup>ab</sup>	32.00 $\pm$ 2.31 <sup>b</sup>	31.50 $\pm$ .87 <sup>b</sup>
	35 day	38.00 $\pm$ .58 <sup>b</sup>	41.00 $\pm$ .58 <sup>ab</sup>	31.50 $\pm$ 1.44 <sup>c</sup>	42.50 $\pm$ .29 <sup>a</sup>	37.00 $\pm$ .58 <sup>b</sup>
LDL-C (mg/dl)	18 day	67.70 $\pm$ 2.30 <sup>a</sup>	72.40 $\pm$ 16.00 <sup>a</sup>	40.40 $\pm$ 4.600 <sup>b</sup>	65.30 $\pm$ 7.100 <sup>a</sup>	70.10 $\pm$ 3.500 <sup>a</sup>
	25 day	37.00 $\pm$ 15.80 <sup>ab</sup>	21.30 $\pm$ 6.10 <sup>b</sup>	21.50 $\pm$ .90 <sup>b</sup>	46.20 $\pm$ 20.4 <sup>a</sup>	58.80 $\pm$ 8.00 <sup>a</sup>
	35 day	13.90 $\pm$ 1.50 <sup>b</sup>	15.10 $\pm$ 5.50 <sup>b</sup>	29.70 $\pm$ 11.90 <sup>a</sup>	14.50 $\pm$ 5.70 <sup>b</sup>	16.80 $\pm$ 4.80 <sup>b</sup>
VLDL-C (mg/dl)	18 day	20.30 $\pm$ 2.70 <sup>b</sup>	26.60 $\pm$ 2.00 <sup>a</sup>	20.10 $\pm$ 1.10 <sup>b</sup>	20.20 $\pm$ .40 <sup>b</sup>	18.90 $\pm$ .50 <sup>b</sup>
	25 day	15.50 $\pm$ 1.70 <sup>a</sup>	9.20 $\pm$ .60 <sup>b</sup>	13.50 $\pm$ 1.10 <sup>a</sup>	12.80 $\pm$ 2.60 <sup>a</sup>	12.70 $\pm$ 1.50 <sup>a</sup>
	35 day	15.10 $\pm$ .50 <sup>b</sup>	13.90 $\pm$ .50 <sup>b</sup>	14.30 $\pm$ 2.10 <sup>b</sup>	17.00 $\pm$ .80 <sup>ab</sup>	15.70 $\pm$ .70 <sup>b</sup>

<sup>ab</sup> Mean values within the same row with different superscript letter are statistically different at  $P \leq 0.05$ . SEM = Standard Error of Means. 1) Control group 2) Florfenicol treated group 3) Florfenicol and Tocotrienol treated group 4) Florfenicol and Selenium yeast treated group 5) Florfenicol and Tocotrienol with Selenium yeast treated group.

(ALT: Alanine aminotransferase, AST: Aspartate aminotransferase, ALP: Alkaline Phosphatase, A/G: Albumin/Globulin ratio, HDL: High-density lipoprotein, LDL: Low-density lipoprotein, VLDL: Very low-density lipoprotein).

**Table 2:** Effect of oral administration of tocotrienol and selenium yeast on hepatic antioxidant/oxidant Status in broilers exposed to adverse effect of florfenicol. (Mean  $\pm$  SEM) (n=5)

Parameters	Experimental Periods	Groups				
		1	2	3	4	5
Hepatic SOD (mg/dl)	18 day	80.39 $\pm$ 7.33 <sup>a</sup>	46.72 $\pm$ 2.76 <sup>cd</sup>	62.57 $\pm$ 1.76 <sup>bc</sup>	60.71 $\pm$ 2.33 <sup>bc</sup>	68.79 $\pm$ 6.58 <sup>ab</sup>
	25 day	77.29 $\pm$ 8.75 <sup>a</sup>	47.78 $\pm$ 6.17 <sup>b</sup>	80.85 $\pm$ 15.67 <sup>a</sup>	82.55 $\pm$ 2.63 <sup>a</sup>	85.68 $\pm$ 4.24 <sup>a</sup>
	35 day	53.61 $\pm$ .002 <sup>a</sup>	39.28 $\pm$ 5.18 <sup>b</sup>	61.42 $\pm$ 1.91 <sup>a</sup>	53.56 $\pm$ .26 <sup>a</sup>	57.10 $\pm$ 4.41 <sup>a</sup>
Hepatic GSH (mg/dl)	18 day	4.76 $\pm$ .06 <sup>a</sup>	3.88 $\pm$ .069 <sup>c</sup>	4.68 $\pm$ .037 <sup>a</sup>	4.62 $\pm$ .133 <sup>a</sup>	4.18 $\pm$ .032 <sup>b</sup>
	25 day	4.14 $\pm$ .26 <sup>b</sup>	3.36 $\pm$ .020 <sup>c</sup>	3.58 $\pm$ .254 <sup>c</sup>	4.70 $\pm$ .069 <sup>a</sup>	4.66 $\pm$ .092 <sup>ab</sup>
	35 day	4.83 $\pm$ .060 <sup>d</sup>	4.03 $\pm$ .012 <sup>c</sup>	5.56 $\pm$ .057 <sup>bc</sup>	5.66 $\pm$ .10 <sup>a</sup>	5.32 $\pm$ .008 <sup>b</sup>
Hepatic MDA (mg/dl)	18 day	7.82 $\pm$ .10 <sup>b</sup>	9.95 $\pm$ .32 <sup>a</sup>	7.95 $\pm$ .40 <sup>b</sup>	5.95 $\pm$ .46 <sup>c</sup>	7.00 $\pm$ .72 <sup>bc</sup>
	25 day	6.25 $\pm$ .144 <sup>b</sup>	7.50 $\pm$ .69 <sup>a</sup>	7.67 $\pm$ .043 <sup>a</sup>	5.83 $\pm$ .13 <sup>b</sup>	6.85 $\pm$ .086 <sup>ab</sup>
	35 day	5.20 $\pm$ .12 <sup>c</sup>	6.18 $\pm$ .10 <sup>a</sup>	5.56 $\pm$ .057 <sup>bc</sup>	5.48 $\pm$ .21 <sup>bc</sup>	5.97 $\pm$ .30 <sup>ab</sup>

<sup>ab</sup>Mean values within the same row with different superscript letter are statistically different at  $P \leq 0.05$ . SEM = Standard Error of Means. 1) Control group 2) Florfenicol treated group 3) Florfenicol and Tocotrienol treated group 4) Florfenicol and Selenium yeast treated group 5) Florfenicol and Tocotrienol with Selenium yeast treated group.

(SOD: Superoxide dismutase, GSH: Reduced glutathione, MDA: malondialdehyde).

## DISCUSSION

Florfenicol administration has a damage effect in liver of broiler chickens so tocotrienol and selenium yeast used to mitigate these side effects. In serum biochemical examination, ALP activity returned to normal level in all groups except in the florfenicol treated group. This result was previously explained by Hasan et al. (2018) who stated that tocotrienol derived from seeds of *Bixa orellana* enhance bone structure and bone strength with decrease bone resorption and improve bone formation by increased activity of ALP. Moreover, Norazlina et al. (2002) reported that tocotrienol plays an important role in bone calcification. ALP has a critical role in bone calcification. Therefore, tocotrienol may improve the ALP activity. Our results related to selenium yeast were in accordance with those obtained by Yang et al. (2012) and Invernizzi et al. (2013) in maintaining ALP activity within normal. On the other hand, a significant increase in serum ALP activity of rats fed ontocotrienol (120-130 mg/kg b.w.) for 13 weeks was previously reported by Nakamura et al., 2001. This elevation may be caused by cholestasis or bone remodeling.

Albumin is one of main protein sources formed in the liver to maintain plasma osmotic pressure, providing energy and repairing tissue. Albumin is considered a carrier of nutrients to maintain the body tissue protein dynamic balance (Ahmed et al., 2002). In our results on the 25<sup>th</sup> day of age, all treated groups

showed significant increases in serum TP and serum albumin when compared with the florfenicol treated group. These results were agreed by who reported with Shi et al., (2018) stating that diets supplemented with selenium can improve metabolism of major elements due to elevation of total protein and globulin. Our results related totocotrienol disagreed with those previously described by Tasakia et al. (2008) and Shibata et al. (2012), while our results regarding selenium yeast agreed with those previously described by El-Demerdash and Nasr (2014) and Shi et al. (2018) and disagree with those previously reported by Attia et al. (2010), Invernizzi et al. (2013) and Liu et al.(2020) in serum albumin characterization.

On the 25<sup>th</sup> day of age, the florfenicol treated group showed significant decreases in serum total globulin when compared with the control group, which is in agreement with Shaheen and El-Far (2013). The immunosuppressive effects of florfenicol could be attributed to its protein inhibition. However, our results disagree with those previously published by Allam et al. (2014) who reported a significant increase in serum total globulin of Pekin ducklings.

The selenium yeast treated group alone and combined with tocotrienol displayed a significant increase in serum total globulin when compared with the florfenicol treated group. This result agree with that mentioned by Shi et al. (2018) who proved that elevation



of serum globulines enhance immune system of animals. In addition, these results were unconformity with those of Yang et al. (2012) who showed no significant difference in serum total globulin in broilers fed on 3 ppm selenium yeast from 0 to 3 weeks of age.

The tocotrienol treated group showed a significant decrease in serum cholesterol and LDL levels on the 18<sup>th</sup> day of age when compared with the control group. The selenium yeast treated group alone and combined with tocotrienol returned the lipid profile levels to normal. The hypocholesterolemic effect of tocotrienol may be attributed to the decrease activity of  $\beta$ -hydroxy- $\beta$ -methylglutaryl coenzyme A reductase (Yuet al., 2006). These results referred to tocotrienol agreed with those previously described by Qureshi and Peterson (2001), Yu et al. (2006) and Budin et al. (2009). Our results involving selenium yeast are similar with those published by Sevcikova et al. (2008) and Yang et al. (2012). Our results were not in agreement with Hasselwander et al. (2001) who reported that tocotrienol had little effect on serum lipid levels. Attia et al. (2010) showed significant decreases in serum cholesterol level and triglycerides after selenium yeast treatment.

Florfenicol treated group showed a significant increase of MDA level and significant decrease of SOD activity and GSH concentration in hepatic tissues in comparison with the control group. These results attributed to the ability of florfenicol to inhibit the expression of antioxidant proteins Nrf2, HO-1 and NQO-1 resulted in decrease antioxidant factors SOD and GSH (Han et al., 2020). Firozian et al. (2020) reported that elevation of lipid peroxidation is usually joined by decrease GSH and SOD antioxidant factors. GSH depletion can cause oxidative damage but SOD can directly inhibit reactive oxygen species (ROS) formations which they are considered an important antioxidant defense enzymes. These results are in agreement with those described by Farombi et al. (2001) who reported that MDA level was elevated and glutathione was lowered in liver of rats, which received 28.6 mg/kg chloramphenicol. On the other hand, Elia and Pacini, (2016) reported an increase in liver glutathione levels of rainbow trout treated with a dose of 7.5 and 15 mg/kg b.w. florfenicol. Glutathione levels were 1.5 fold higher in elevated dose of florfenicol attributed to protect liver cells from oxidative damage of florfenicol.

Other treated groups such as the tocotrienol and selenium yeast alone and combination of them showed

significant decreases in MDA level while significant increases in SOD activity and GSH concentration in hepatic tissue when compared with the florfenicol treated group. The result of SOD activity in liver tissue with the addition of tocotrienol was attributed to the disturbance of defense mechanism of liver tissue which stimulates production of superoxide anion radicals which prevent lipid peroxide formations (Lee et al., 2009). Selenium yeast may improve the antioxidant status of broilers by increasing the activity of antioxidant enzymes and inhibiting lipid peroxidation (Jiang et al., 2009; Yang et al., 2012). Gladyshev and Hatfield, (1999) reported that selenium inter in the form of amino acid called selenocysteine (one of selenoprotein) which have important enzymatic functions associated with antioxidant activity. Selenium is important in sulphur amino acid metabolism. In this way, the sulphur amino acids methionine and cystine can spare selenium through their antioxidant role.

Our results referred to tocotrienol of liver tissue were in accordance to those explained by Khan et al. (2011). Moreover, Palozza et al. (2006) reported that tocotrienols decrease MDA in rat liver microsomes (obtained from tissue homogenization then *in vivo* added tocotrienols to suspension) by inhibition of 2,2'-azobis 2-amidinopropane (AAPH) which induced MDA production. This result related to in selenium yeast were also observed by several authors (Mahmoud and Edens, (2003); Petrovič et al., 2006; Bao-wei et al., 2011; Li et al., 2016; Hamidet al., 2018) who concluded that selenium yeast restored or increased the liver antioxidant defense. The antioxidant capacity of selenium is an integral component of glutathione peroxidase (GPx) which plays a crucial role to reduce cellular damage by ROS (Kong et al., 2017). Our results disagreed with those previously explained by Lee et al. (2005) who reported SOD activity in liver of rats was lowered with age when these rats were fed with palmvitee (palm oil). This decrease may be due to the compensating effect of palmvitee which replaces antioxidant enzyme activities. Our results in selenium yeast also disagreed with those described by Holovská et al. (2003) and Chen et al. (2013) who showed no significant differences in SOD activity of hepatic tissue in chickens fed on selenium yeast.

Liver sections of the florfenicol treated group displayed focal leukocytic infiltration in the hepatic tissue and focal coagulative necrotic foci represented by deeply eosinophilic cytoplasm of the nuclei with karyorrhexis and karyolysis in the liver section



of chicks. Our findings were in correlation to those described by Yue and Li-hai (2009) who reported fatty degeneration and necrosis in the livers of seventh day old broiler chicks, which were fed diet containing different doses of florfenicol (200, 400, 800 and 2000 mg/kg) for 14 days. In addition, Reda et al. (2013) noted diffused hydropic degeneration in the liver examined in Nile tilapia (*Oreochromis niloticus*) which received 5 mg/kg of florfenicol for 12 weeks, whereas Isa et al. (2020) described coagulative necrosis with karyopyknosis and karyorrhexis in livers of 8-week-broiler chicks which treated with 250 mg/kg chloramphenicol from the 1<sup>st</sup> day.

Liver sections of the tocotrienol treated group showed fatty degeneration of some hepatic cells with eccentric location of the nuclei on the 35<sup>th</sup> day of age and focal mononuclear cell infiltration of the hepatic tissue and activation of the Kupffer cells on 35<sup>th</sup> day of age. These findings are in agreement with those of Qureshi et al. (2011) who stated that mild chronic inflammation was observed in liver of chickens fed with diet containing 50 ppm  $\delta$ -tocotrienol and Wong et al. (2012) who observed mild fatty changes in the liver of rats which fed a high carbohydrate diet, contained 240 mg TRF/mL palm olein, for 8 weeks. On the other hand, no histopathological changes were observed in the liver tissue of mice and rats treated with different doses of tocotrienol (Husain et al., 2009; Shibata et al., 2012).

In our study, treatment with selenium yeast revealed focal hepatocellular coagulative necrosis with karyolysis and ballooning degeneration represented by empty cytoplasm and centrally located nuclei in the adjacent hepatocytes.

Our results are in agreement with those previously published by Attia et al. (2010) who stated

that breeding hens fed on selenium yeast showed mild focal necrosis in hepatic tissue. Moreover, Hamid et al. (2018) concluded that rats treated with selenium-enriched yeast showed ballooning of liver hepatocytes, mild fatty changes, and mild degree of centrilobular necrosis with partial infiltration of inflammatory cells.

On other hand, Mirjana et al. (2004) reported that chickens orally fed on selenized yeast showed varying degree of intracellular edema and fatty changes in the liver of sacrificed chicks..

Broilers treated with the combination of tocotrienol and selenium yeast revealed focal mononuclear cell infiltration in the fibrous connective tissue of the portal area and activation of the Kupffer cells with little evidence of necrotic changes in the liver. This means that the addition of tocotrienol with selenium yeast mitigated the damage caused by florfenicol on liver tissue.

## CONCLUSION

The addition of tocotrienol, selenium yeast alone or their combination can improve the antioxidant effect and mitigate lipid peroxidation in the livers of treated broiler. Therefore, tocotrienol and selenium yeast can improve the safety of using florfenicol in broilers under experimental conditions.

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## CONFLICT OF INTEREST

None declared.

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