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Short Communication

Effect of *Silybum marianum* in protecting the layer hens from free radicals induced by hydrogen peroxide

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ABSTRACT:

Silybum marianum (L.) Gaertenr belongs to the Family Asteraceae is wide spread throughout the world. The main medicinal applications of *Silybum marianum* were due to the active constituents such as silymarin and flavonolignan complex. The purpose of the current study was to analyse the effect of *Silybum marianum* and vitamin E on the Layer hens in protecting them from free radical induced by hydrogen peroxide. One hundred twenty Loman brown Layer hens at the age of forty weeks were randomly divided into five treatments with four replicates, (six hens in each replicate). For the first treatment (T_1) hens were fed with experimental diet and normal water (positive control), second treatment (T_2) hens were fed with experimental diet plus 0.5% hydrogen peroxide in drinking water (negative control), T_3, T_4 and T_5 group hens were fed with experimental diet plus 0.5% hydrogen peroxide in drinking water and 10 mg and 15 mg, of *Silybum marianum* per kg diet and 450 mg vit. E/ kg diet respectively. Adding *S. marianum* and Vitamin E resulted in a significant change in peroxide, free fatty acid, ALT, AST, glucose, cholesterol, triglyceride, LDL and had a significant increase in ALP, total protein, albumin, globulin, HDL, glutathione peroxidase, heme iron, catalase.

Keywords:

Silybum marianum, Vitamin E, Layer hen, Antioxidant, Free radical, Hydrogen peroxide.

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INTRODUCTION

The higher ratio of mortality in the hen's flock and the decrease in eggs production especially in the peak period of production without clinical sign for any common disease may be due to the oxidative damage. The continuation of growth with weak self-anti-oxidant system and lack of addition of industrial antioxidant in the feed will lead to damage in vital component of the cells (Mohammed *et al*., 2013a). To prevent the oxidative damage (*Silybum marianum*) can be the industrial or herbal thistle which prevent the oxidative damage and protect the cell membrane from the damage by free radical (Balakumer *et al*., 2010).

The *Silybum marianum* protect the cells by several ways such as antioxidant, glutathione regulator, intracellular, protect the permeability of cell membrane and prevent the permeation of toxic material into hepatocytes, help in regeneration of liver cells by increasing the synthesis of Ribonucleic Acid (RNA), prevent liver stellate cells transformation into myofibroblasts, inhibit the deposition of collagen fibers in liver, and absorption of free radicals leading to the protection of the liver cells (Suchy *et al*., 2008; Fraschini *et al*., 2002).

The protection of body cells from oxidative damage make the body organ work normally especially the liver, which is the organ of yolk synthesis and any disorder in the manufacturing of yolk lead to depression in eggs production. *Silybum marianum* helps in reducing the oxidative damage which was analyzed through the measurement of biochemical traits after adding the *Silybum marianum* and challenged with hydrogen peroxide.

MATERIALS AND METHODS

The experiment was carried out at private sector farm in Bazyan region which belongs to Sulaymaniyah governorate for 12 weeks from 14/2/2015 to 8/5/2015. One hundred twenty loman brown layer hens at the age of forty weeks were distributed randomly in five treatments with four replicate with six hens in each replicate. The hens were reared in floor system chamber.

The chamber was divided into twenty pens (six square meters (2×3 m each)) and the floor were spread with sawdust, lighting was done for 16 h and the temperature was controlled through fuel oil boiler. The hens were fed as per the ration Table 1 while the hydrogen peroxide was added to the drinking water at the level of 0.5%. The drinking water was exchanged twice daily at 8 PM and 8 AM in order to be sure for the continual activity of hydrogen peroxide. The treatments of the study were as follow: First treatment (T_1) normal ration without any addition (control); second treatment (T_2) normal ration with 0.5% H_2O_2 in water twice a day; third treatment (T_3) normal ration with 0.5% H_2O_2 twice a day and 10 mg *Silybum marianum* kg feed; fourth treatment (T_4) normal ration with 0.5% H_2O_2 twice a day and 15 mg *Silybum marianum*/ kg feed; Fifth treatment (T_5) normal ration with 0.5% H_2O_2 twice a day and 450 mg vitamin E/ kg feed. To show the role of *Silybum marianum* and vitamin E in preventing the negative effect of oxidative stress induced by hydrogen peroxide the below parameters were analyzed.

The activity of glutathione peroxides' (GSH-pX) and catalase (CAT) in plasma (Wheeler *et al*., 1990), the concentration of glutathione (GSH) (Moron *et al*., 1979), Malondialdehyde (MDA) (Witt *et al*., 1970), free fatty acid (FFA) and Peroxide Value (PV) (Egan *et al*., 1981), and the levels of hem-iron in liver tissue (Hornsey, 1956 and Basaga *et al*., 1997), the activity of Alanine Amino Transferase (ALT) and Aspartate Amino Transferase (AST), (Reitman and Frankel, 1957) and Alkaline Phosphates (ALP) (King and Armstrong, 1934) were studied. In blood plasma, the concentration of glucose (Asatroo and King, 1954), total cholesterol (Richmond, 1973), triglyceride (Toro and Achermann, 1975), high density lipoprotein (Warnik and Wood, 1995), non high density lipoprotein

(Grundy; 2004), total protein (Varley *et al*; 1980) and albumin (Henry *et al*; 1974) were also analyzed following standard protocols. The concentration of globulin was detected by the difference between total protein and albumin.

For statistical analysis, Complete Randomized Design (CRD) was used in order to show the effect of treatment in the studied characteristics, the significant difference between the means were compared with multiple ranges and Multiple F-test (Duncan, 1955). Statistical Analysis System (SAS, 2010) were used for the statistical analysis of the data.

RESULTS AND DISCUSSION

Table 2 showed that the addition of *Silybum marianum* and vitamin E in the feed of layer hens act like antioxidant and also controlled lipid oxidation and inhibited lipid peroxidation which was clear from the significantly higher ($P \le 0.05$) Peroxide Value (PV) of hydrogen peroxide in T_2 when compared with control and the other treatments and there was no significant difference between T_3, T_4 and T_5. The higher concentration of malondialdehyde in T_2 gave a significant difference ($P \le 0.05$) compared with other treatments and there were no significant differences between the control T_3 and T_4. Also, the higher concentration of free fatty acid was noted in T_2 with a significant difference at ($P \le 0.05$) in comparison with other treatments. The higher peroxide value in T_2 might be due to the inhabitation effect of hydrogen peroxide on the activity of natural antioxidant enzyme which scavenges the free radical and peroxide and increase malondialdehyde value and prevent the liberation of free fatty acid likes glutathione peroxides, glutathione, superoxide dismutase and catalase (Shehate and Yoursif, 2010), while the addition of *Silybum marianum* and vitamin E too work in the shackling of the minerals and decrease it's oxidative effect and stop the first stage of reaction chain and prevent its negative effect like increase in the PV and FFA (Mohammed *et al*., 2013a). The activity of *Silybum marianum* was ten times more than vitamin and this lead to prevent the negative effect of adding H_2O_2 to the drinking water (Adzet, 1987).

Table 1. Experimental diet used in the experiment

S. No	Feed ingredients	Percentage (%)
1	Corn	50
2	Wheat	4
3	Barley	13
4	Soybean cake (44% protein)	20
5	Protein concentrate (40% protein)	4
6	Vegetal fat	1
7	Di phosphate calcium	1.9
8	Lime stone	6
9	NaCl	0.1
	Chemical composition	
1	Crude protein	16.644%
2	Net energy	2718.57 KCl /kg
3	Calcium	3.40%
4	Available phosphorous	0.52%

From Table 3 it can be noticed that the higher concentration of ALT enzyme was noted in T_2 with significant differences ($P \le 0.05$) in comparison with other treatments and there were significant differences ($P \le 0.05$) between T_1 and other treatment. There was no significant difference between T_3, T_4 and T_5. Higher concentration of AST enzyme was also recorded in T_2 with a significant difference between other treatments and there was no significant difference between T_1 and T_3, T_4 and T_5. T_2 gave the lowest value of ALP enzyme with significant differences ($P \le 0.05$) in comparison with other treatments while the highest amount was recorded in T_5 with significant difference ($P \le 0.05$) in comparison with other treatments and there were no significant difference between T_3 and T_4 and the Table 3 explains significant difference ($P \le 0.05$) between control treatment and other treatments. The significantly higher level of AST and ALT in plasma was due to the damage of free radical in liver cells which came from adding

Table 2. The effect of adding *Silybum marianum* and vitamin E in peroxide value, malondialdehyde and free fatty acid in wet liver tissue

S. No	Trait	Treatments					RLSD
		T_1	T_2	T_3	T_4	T_5	
1	PV (ml.equ/kg)	1.78 ± 0.07*c	5.13 ±0.16a	2.06 ±0.12b	2.030.11 ±b	2.070.11±b	0.24
2	MDA (mg/kg)	0.56±0.04b	0.06±1.18a	0.02±0.49c	0.01±0.51b	0.04±0.54b	0.07
3	FFA (%)	0.58±0.03b	0.03±1.71a	0.02±0.54b	0.03±0.58b	0.02±0.56b	0.40

* Mean ± standard error

T_1: normal ration without any addition (control); T_2: normal ration with 0.5% H_2O_2 in water (twice daily); T_3: normal ration with 0.5% H_2O_2 in water (twice daily) and 10 mg *Silybum marianum*/ kg feed, T_4: normal ration with 0.5% H_2O_2 (twice daily) and 15 mg *Silybum marianum*/ kg feed; T_5: normal ration with 0.5% H_2O_2 (twice daily) and 450 mg vitamin E/ kg feed.

H_2O_2 to the drinking water in treatment T_2 but the adding of *Silybum marianum* and vitamin E lead to scavenging of the free radical and prevent it's damaging effect in liver cells and decrease the level of AST and ALT, and this was in agreement with Daniela *et al.* (2014) or may be due to the role of *Silybum marianum* in recovery of the membrane fluidities of liver microsome and mitochondria (Wu, 2003). The significantly low ALP was due to addition of H_2O_2 in drinking water (T_2) which was related to the damage of free radical in body cells. The significantly higher ALP in *Silybum marianum* and vitamin treatments (T_3, T_4 and T_5) relate to the effect of adding *Silybum marianum* and vitamins on bone tissue. The higher ALP was due to the metabolism of calcium after the addition of H_2O_2 which affects the metabolism of calcium and bone building negatively which was in agreement with Mohammed *et al.* (2013a) and Maliheh *et al.* (2014).

Table 4 explains that the higher glucose concentrates in blood plasma was in the T_2 treatment with significant difference ($P \leq 0.05$) in comparison with other treatments as well as there were significant ($P \leq 0.05$) differences between the control and other treatments. T_3, T_4 and T_5 gave the lower glucose concentrate with significant difference ($P \leq 0.05$) in comparison with other treatments but without significant difference between them. From the same table, it was noticed that the lower total protein concentrates was in T_2 with significant difference ($P \leq 0.05$) from other treatments and there was no significant difference between the control, T_3 and T_4 and between control and T_5 and between T_3 and T_4. The higher albumin content was noticed in T_5 with significant difference between other treatment and the lower values was recorded in T_2 with significant difference ($P \leq 0.05$) in comparison with other treatments and there were no significant difference in albumin concentrate between T_1, T_3 and T_4 while the higher globulin content

Table 3. The effect of adding *Silybum marianum* and vitamin E on ALT, AST and ALP in blood plasma

S. No	Treatment	AST IU/L	ALT IU/L	ALP IU/L
1	T_1	28.425±0.49*b	11.000±0.16c	41.950 ±1.61c
2	T_2	42.775±1.05a	18.300±0.24a	28.875 ±0.43d
3	T_3	29.300±0.50b	11.350±0.19b	54.975±0.83b
4	T_4	29.150±0.30b	11.400±0.14b	54.725±0.55b
5	T_5	28.925±0.38b	11.400±0.16b	58.300±0.81a
6	RLSD	13.42	0.35	3.32

* Mean ± standard error

T_1: normal ration without any addition (control); T_2: normal ration with 0.5% H_2O_2 in water (twice daily); T_3: normal ration with 0.5% H_2O_2 in water (twice daily) and 10 mg *Silybum marianum*/ kg feed; T_4: normal ration with 0.5% H_2O_2 (twice daily) and 15 mg *Silybum marianum*/ kg feed; T_5: normal ration with 0.5% H_2O_2 (twice daily) and 450 mg vitamin E/ kg feed.

Table 4. The effect of adding *Silybum marianum* and vitamin E on glucose, total protein, albumin and globulin in blood plasma

S. No	Treatment	Globulin (g/100 mL)	Albumin (g/100 mL)	Total protein (g/100 mL)	Glucose (mg/100 mL)
1	T_1	2.15±0.13[*a]	2.10 ±0.08[b]	4.25±0.13[ab]	238.18±1.93$_b$
2	T_2	1.43±0.16[c]	1.70± 0.10[c]	3.13±0.09[c]	270.78±8.03[a]
3	T_3	1.93±0.13[b]	2.25± 0.13[b]	4.17± 0.12[b]	179.20±0.83[c]
4	T_4	1.87±0.13[b]	2.20± 0.18[b]	4.08± 0.09[b]	181.93±0.41[c]
5	T_5	2.13±0.17[a]	2.33± 0.22[a]	4.35±0.13[a]	180.50±0.58[c]
6	RLSD	0.22	0.40	0.27	32.6

* Mean ± standard error

T_1: normal ration without any addition (control); T_2: normal ration with 0.5% H_2O_2 in water (twice daily); T_3: normal ration with 0.5% H_2O_2 in water (twice daily) and 10 mg *Silybum marianum*/ kg feed, T_4: normal ration with 0.5% H_2O_2 (twice daily) and 15 mg *Silybum marianum*/ kg feed; T_5: normal ration with 0.5% H_2O_2 (twice daily) and 450 mg vitamin E/ kg feed.

was in control but without significant difference in comparison with T_5 and the lower values was noticed in T_2 with significant difference ($P \leq 0.05$) between other treatments and there were no significant difference between T_3 and T_4. The significant decrease in glucose content was due to the addition of *Silybum marianum* which has hypoglycemic effect that decrease the blood glucose without affecting the insulin secretion (Sidra *et al*., 2014), or due to the role of *Silybum marianum* and vitamin E that prevent the negative effect of free radical on beta cells. The significant difference in total protein and albumin between T_1, T_3, T_4, T_5 and T_2 was related to the role of adding *Silybum marianum* and vitamin E that protect the liver cells from the damage of free radical which is produced by the addition of H_2O_2 which causes damage to the liver cells and reduce the synthesis of albumin and globulin and thus significantly lower values (Soto *et al*., 1998; Mohammed *et al*., 2013b). The absence of significant difference in globulin content between T_1 and T_5 indicate the role of vitamin E in the immune response development (Muhammed *et al*., 2012)

From Table 5 it was noticed that the lower concentration of cholesterol, triglyceride, HDL and LDL in T_3, T_4 and T_5 was without significant difference between them but with significant difference ($P \leq 0.05$) between T_1 and T_2. The higher concentration of them in T_2 had significant difference ($P \leq 0.05$) in comparison

Table 5. The effect of adding *Silbum marianum* and vitamin E on cholesterol, triglyceride, HDL and LDL in blood plasma

S. No	Treatments	LDL (mg/100 mL)	HDL (mg/100 mL)	Triglyceride (mg/100 mL)	Cholesterol (mg/100 mL)
1	T^1	141.75±2.06[*b]	49.75±3.86[b]	1095.00±12.91[b]	191.50±2.65[b]
2	T^2	248.50±8.74[a]	24.00±3.37[c]	1222.50±22.17[a]	272.50±9.57[a]
3	T^3	90.25 ±3.59[c]	61.25±1.50[a]	795.00±12.91[c]	151.50±3.11[c]
4	T^4	89.75± 3.59[c]	62.00±1.41[a]	801.25±13.15[c]	151.75±2.36[c]
5	T^5	87.75 ±2.63[c]	63.75±3.50[a]	791.25±13.15[c]	151.50±1.91[c]
6	RLSD	51.5	11.5	127.0	39.75

* Mean ± standard error

T_1: normal ration without any addition (control); T_2: normal ration with 0.5% H_2O_2 in water (twice daily); T_3: normal ration with 0.5% H_2O_2 in water (twice daily) and 10 mg *Silybum marianum*/ kg feed, T_4: normal ration with 0.5% H_2O_2 (twice daily) and 15 mg *Silybum marianum*/ kg feed; T_5: normal ration with 0.5% H_2O_2 (twice daily) and 450 mg vitamin E/ kg feed.

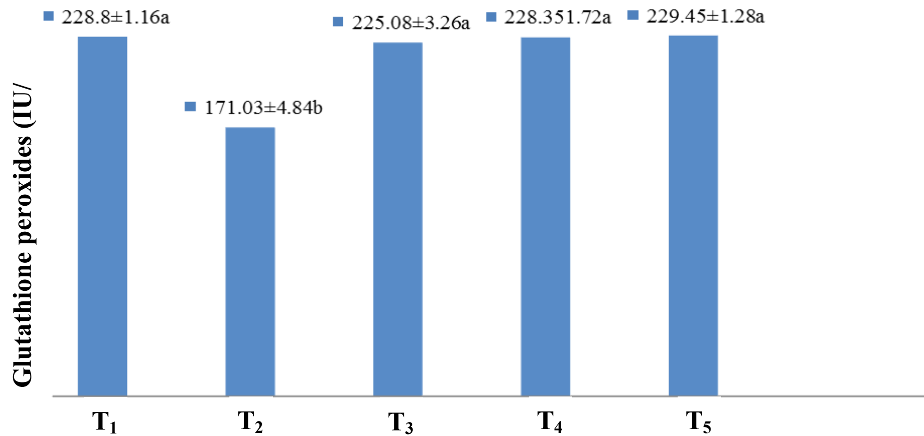

Figure 1. The effect of adding *Silybum marianum* and vitamin E on glutathione peroxides (IU/ML) in blood plasma

with other treatments, whereas the concentration of cholesterol, triglyceride HDL and LDL in control treatment gave significant difference (P≤0.05) when compared with other treatments. The significant lowering of cholesterol in T_3 and T_4 was related to the active component of *Silybum marianum* which is similar to the effect of common drug probucol in lowering cholesterol levels (Panda *et al*; 2008). In general, the lowering of cholesterol, triglyceride, LDL and the increase of HDL in blood plasma when adding *Silybum marianum* and vitamin E was due to the activity of the antioxidants that increase the activity of GSH-P_X and catalyze enzyme in blood plasma which protects blood lipid and low density lipoprotein from oxidation (Spitzer, 2007); stimulate the liver cell receptor for removing the LDL from the blood to the liver (Willimas *et al*., 2004; Krecman *et al*., 1998); increase the activity of lipoprotein lipase which convert low density lipoprotein to fatty acid and use it for the product of energy or send it back to the lipo tissue (Moreno *et al*., 2003) and increase the HDL in blood plasma which encourage the conversely transport operation of cholesterol, lipid and LDL from blood and tissue and then return it to the liver to convert it to gall bladder acid or return the secretion out of the body (Steeg *et al*., 2008).

Figure 1 shows that there was no significant difference between T_1, T_3, T_4 and T_5 in the concentration of glutathione peroxides while the lower values was noticed in T_2 with significant difference (P≤0.05) in comparison with other treatments. Highest glutathione peroxides values in T_3, T_4 and T_5 was due to the role of *Silybum marianum* in stimulating the glutathione reduc-

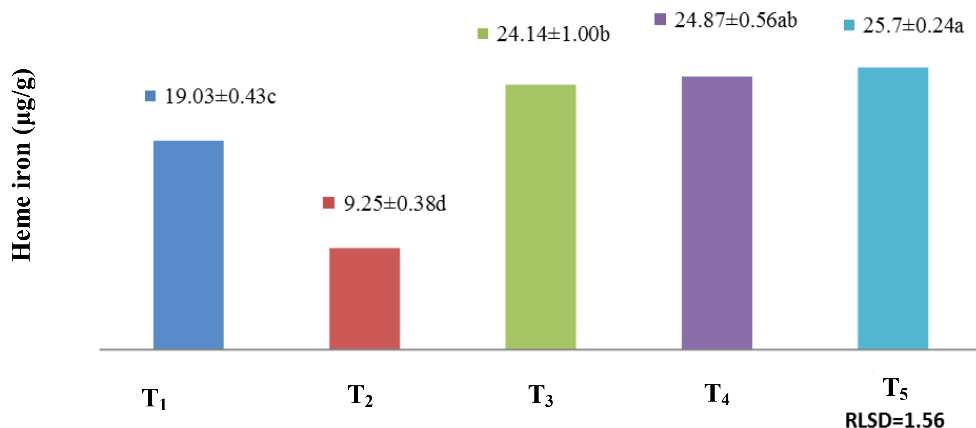

Figure 2. The effect of adding *Silybum marianum* and vitamin E on hem-iron (µg/g) wet liver tissue

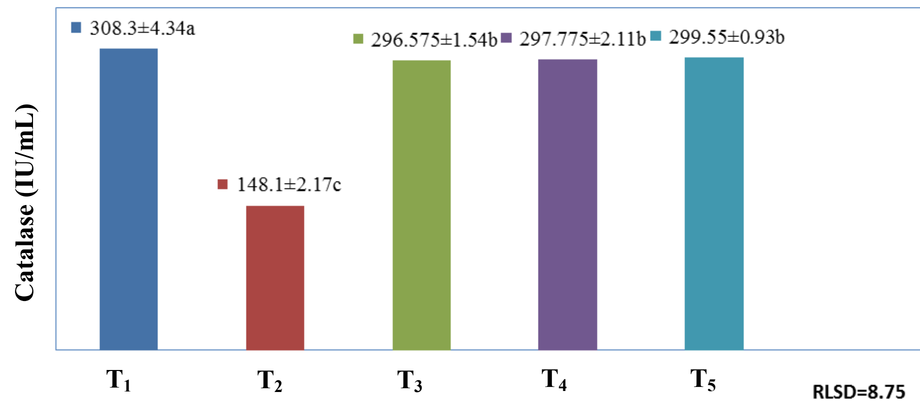

Figure 3. The effect of adding *Silybum marianum* and vitamin E on catalase (IU/ML) enzyme in blood plasma

tase (GSH-RD) enzyme and NADPH that reduces the oxidized form of glutathione (GSSG) to radicalized form of glutathione (GSH) and this was in agreement with Nelson and Cox (2004). The significant decrease in glutathione values in the H_2O_2 treatments added was due to the free radial stress (Suchy *et al*., 2008).

Figure 2 shows the lower heme-iron in wet liver tissue of T_2 with significant difference (P≤0.05) in comparison with other treatment while the higher value was in T_5 with a significant difference in comparison with other treatment except for T_4 and there was no significant difference between T_3 and T_4. The significant decrease in the amount of hem-iron in liver tissue of T_2 indicates that the free radicals destroy the liver cells and the hem-iron emerge from the liver tissue pigment to the blood whereas the addition of *Silybum marianum* and vitamin E act in protecting the liver cells from negative effect of free radical as well as its role's in shackling the hem-iron in liver tissue and limiting its effect in stimulating the oxidation of the cells (Soobrattee *et al*., 2005).

Figure 3 showed that the lower concentration of catalase was in T_2 with significant difference (P≤0.05) with all the treatments while T_1 gave the higher values of catalase with significant difference (P≤0.05) with other treatments. There was no significant difference between T_3, T_4 and T_5. The significant increase in catalase was due to the active ingredient in *Silybum marianum* such as Silibinin, silychristin, silydianin and 2,3-dehydrosilybin and 2,3- dehydrosilychristin (Maureen and Breakspear, 2015). The significant decrease of catalase in was T_2 was due to the stress of free radical which came from H_2O_2 in drinking water.

CONCLUSION

Our results indicated that *Silybum marianum* and vitamin E may be used to protect the liver cells from the free radical damage without any side effect on the health of layer hens.

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