Original Article

Assesment of legalon on kidney functions and Lipids profile in broiler chickens exposed to Hydrogen Peroxide

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ABSTRACT

Milk thistle (Silymarin) is a worldwide valuable medical plant which has been used for many years in the modern and alternative medicine to treat liver diseases. The current study aimed to evaluate the probable effect of legalon (standard hepatic drug) on improvement the kidney functions, lipids profile and body weight in broiler chickens. Twenty-four Ross commercial broiler of thirty days old male were randomly distributed into four treatments of six birds each. The treatment was continued for 3 weeks as follows: 1st group (control) drinking tap water, 2nd group given 0.5% H_2O_2, 3rd group 0.5% H_2O_2 and 6mg/kg legalon, and 4th group 6mg/kg legalon single oral dose once daily. The birds' weight was measured at the end of each week. At the end of the experiment, the broilers were killed and blood parameters were taken for measuring of total cholesterol, triglycerides, HDL-C, LDL-C, VLDL-C, risk index creatinine, and urea. The results indicated that hydrogen peroxide-treatment significantly increased serum total cholesterol, triglycerides, LDL-C, VLDL-C and decreased level of HDL-C, risk index as compared with control at $P< 0.05$. Concentration of 6mg legalon had significantly good impact on lipids profile and kidney functions, but did not affect significantly the body weight and better effect was obtained in the last two weeks when legalon was administrated alone.

Keywords: legalon, hydrogen peroxide, broiler, kidney function, lipid profile

Introduction

Chemicals of plants, especially flavonoids, are highly studied in human as well as animal trials dealing with health status, defense against diseases and food protection mechanisms [1]. In poultry farms, stress can reduce productive performance. Aqil *et al.* [2] and Ahmed *et al.* [3] associated the production of free radicals and reactive oxygen species at cellular level with fear in poultry. Scientists have reported medicinal plants positive effect on performance of broiler challenged with stress [4]. Medicinally, both growth boost and feed conversion ratio improvement in poultry farming require the herbals medicine to be involved [5]. Milk thistle (Silybum marianum) is among them, which is a very natural popular worldwide plants used for many centuries to cure some disorders such as spleen, renal, hepatic, bile ducts, and liver cirrhosis [6]. The mode of action is preventive through the protection of hepatocytes from the toxic effect of alcohol, drugs, medicine, heavy metals, and pesticides. In addition, it detoxifies the liver from all harmful substances [7, 8] through having a high percentage 70-80% of flavanolignans consisting of four subunits including Silybin (50-60%), Isosilybin (5%), Silydianin (10%), and Silychristin (20%). Silybin is the major component and shows better biological activities, all together known as silymarin that have been reported to have a very active biological preventive action on liver [9]. Many studies posted the anticancer effect of silymarin through inhibiting the proliferation of neoplastic cells of prostate, breast, ovaries, colon, lung, and urinary bladder [10, 11]. Many scientists mentioned that silymarin has the ability to cure aflatoxin toxicity in poultries [12]. Moreover, silymarin has a preventive effect on fatty liver in dairy cows on last period of pregnancy and a positive effect on offspring health [13]. In the past centuries, milk thistle extracts have been used for healing

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diseases [14], silymarin in especial, remains highly regarded as safe and has multiple health benefits. Currently, milk thistle, under the trade name Legalon (MADAUS, Koln, Germany), is used to improve high level of glucose for type 2 diabetes [15]. Crocenz and Roma [16] mentioned its strong anticholestatic activity in their review of clinical studies regarding the Hepatoprotective effect of silymarin. [17] Suggest the probability of silymarin direct impact on the cholesterol metabolism by means of silymarin biosynthesis inhibition. Silybin stimulates renal cells by the same way in hepatocytes. Silychristin and Silybin increase proliferation rate as well as protein and DNA biosynthesis in renal cells that have been damaged in laboratory by all vincristine, cisplatin and paracetamol. Silybin treatment before or after the chemical-induced injury works on preventing or reducing nephrotoxic effects [18]. So, Silymarin seemed to have the potential as a renoprotective agent against nephrotoxic medications due to its antioxidant, anti-apoptotic actions and anti-inflammatory features. When silymarin is used on cats and dogs after administering large doses of oxytetracycline which has a hepatotoxic effect, silymarin has been shown to be effective [19]. Silymarin is already reported to prevent damages induced by other environmental toxins like benzoyl peroxide [20]. However, the effect of oral administration with legalon 70 on kidney functions, lipids profile and body weight needs to be assessed. Therefore, the objective of the current study was to evaluate the effect of oral administration of herbal drug alone and in combination with hydrogen peroxide on growth performance, renal functions and lipids profile of chickens.

Material and Methods

Experimental design:

The experiment was conducted in Veterinary Medicine college, University of Mosul. Twenty-four Ross broiler of thirty-day old males provided from local hatchery, were randomly assigned into four groups of six birds each. The birds of each group were housed in individual suspended wire cage, under controlled conditions of temperature, humidity and artificial light with 12 hours photoperiod. The birds kept in animals' house for 30 days before the experimental work, feed and water were given *ad libitum*. After adaptation, the broilers groups were treated for 3 weeks as follows: 1st group (control) were given drinking tap water, 2nd Group 0.5% H_2O_2, 3rd group 0.5% H_2O_2 plus 6mg/kg legalon, and 4th Group 6mg/kg legalon alone.

Treatment solutions:

Hydrogen peroxide 30% was obtained from Spain (E.L Gato perez Schar lab.) which was diluted with tap water to 0.5% [21]. Legalon (standard drug of milk thistle seeds) was obtained from Madaus, GmbH (Germany) on tablets which were powdered by an electrical mill and instantly dissolved with distil water then given to animals by oral gavege needle [22].

Biochemical Assays:

The chickens were slaughtered from the neck after an overnight fasting for 12 hours at the end of the trial period (3weeks). Then, the blood samples were collected in disposable plain tubes, and centrifuged at 3000 (rpm) for 10 minutes. The collected serum was stored at -20 c^0 until biochemical analysis. Commercial kits provided by (Biolabo, Miazy, France) was used to measure the cholesterol serum total, triglycerides, HDL-cholesterol, LDL-cholesterol, VLDL-cholesterol [23], urea as well as creatinine. The risk index was estimated from the equation: Risk index = LDL- C / HDL-C [24]

Statistical Analysis:

Data were presented as the mean ± S.R. The results were analyzed using one way analysis of variance (ANOVA), and all groups were tested with Sigma statistical version 3 [25]. Differences of $p < 0.05$ were considered to be significant by using Duncan [26].

Results:

The blood parameters of lipids profile in Table (1) show that the cholesterol, triglycerides, LDL-C, and VLDL-C concentration were significantly higher in hydrogen peroxide given at 0.5% (2nd group) compared with the control ($p < 0.05$). Legalon administration significantly lowered the level of cholesterol, triglycerides, LDL-C, and VLDL-C in the 3rd group and 4th group compared with the 2nd group. Also, HDL-C concentration was lower significantly in the 2nd group compared with the 1st group ($p < 0.05$). The treatment with legalon pretends significantly higher of HDL-C level in the 4th group compared with the 1st group as well as 2nd group. The risk index was higher significantly in the 2nd group compared with the control ($p < 0.05$). The treatment with legalon shows significant reduction in the level of risk index in the 3rd group and 4th group compared with the 2nd group.

Table (2) shows that the concentration of urea and creatinine significantly higher in 2nd group compared with the control ($p < 0.05$). Administration of legalon significantly lowers the level of urea and creatinine in the 3rd group and 4th group compared with the 2nd group and 1st group.

Table 1. Legalon drug effect on lipids profile

1st Group control	2nd Group H_2O_2 0.5%	3rd Group Legalon 6mg/kg + H_2O_2 0.5%	4th Group Legalon 6mg/kg

Cholesterol	84.88±3.53 a	177.35±7.14 B	72.33±4.24 A	73.01±2.28 A
Triglyceride	80.49±5.39 a	111.78±1.48 B	27.80±2.59 C	75.51±4.16 A
HDL-C	34.45±0.96 a	25.00±0.95 b,c	23.72±1.85 C	50.05±2.79 D
LDL-C	64.24±5.36 a	174.71±7.87 B	54.21±3.01 c,a	38.05±2.49 C
VLDL-C	13.81±2.42 a	22.36±0.29 B	5.55±0.52 C	15.10±0.83 A
Risk index	1.87±0.16 a	7.05±0.24 B	2.33±0.24 A	0.77±0.19 C

The values represent mean ± SR of four experiments with six chicks in each group ($p < 0.05$) as compared with the control.

Table 2. Legalon drug effect on kidney functions

	1st Group control	**2nd Group H_2O_2 0.5%**	**3rd Group Legalon 6mg/kg + H_2O_2 0.5%**	**4th Group Legalon 6mg/kg**
Creatinine mg/dl	٢,٧±٠,٣٩٠ A	4.16±0.306 B	2.58±0.306 A	2.79±0.237 A
Urea mg/dl	18.73±1.29 a,b	24.89±1.06 a	19.73±2.389 a,b	17.638±1.213 B

The values represent mean ± SR of four experiments with six chicks in each group compared with the control.

Table 3. Legalon drug effect on Body weight

	Weeks			
	1	2	3	4
1st Group Control	1040±60.548 A a	1414±89.964 B b	1784±102.222 C c	1917±78.167 C d
2nd Group H2O2 0.5%	890±52.563 A a	946.667±39.715 A b	941±33.379 A b	1090±117.535 A a
3rd Group Legalon 6mg/kg + H2O2 0.5%	905.833±57.616 A a	1011.667±28.156 A b	886.667±34.916 A b	900±88.009 A a
4th Group Legalon 6mg/kg	1169.167±114.682 A a	1322.143±102.509 A b	1690.714±123.034 B b	2046.667±175.180 B c

The values represent mean ± SE of four experiments with six chicks in each group. Means in a column followed by different small letters, or in a row by different capital letters are significantly different compared with the control.

In Table (3), no significant difference ($p < 0.05$) was found in the chicks' weight at the first week of all treatments. In the next three weeks, there were significant ($p<0.05$) increase in the control weight compared to the rest groups, but the treatment with the 2nd group and 3rd group seemed to be insignificantly ($p < 0.05$ different the all duration of experiment; while, the fourth group chicks' weight was significantly increased ($p < 0.05$) in the last two weeks.

Discussion:

Liver and kidney are exposed to a lot of oxidant substances that are both from exogenous and endogenous sources. Kidney is the most target organ of toxicity [27]. Several chemicals, heavy metals and drugs show change in their function and structure [28]. Silymarin, a very strong antioxidant compound, has been recorded for attenuation of oxidant mediated renal damage induced by various xenobiotics [29]. Therefore, the study was devoted to determine the beneficial effects of silymarin on H_2O_2-induced kidney injury and lipid profile besides growth

performance in broiler. According to the findings, hydrogen peroxide is an oxidant agent led to reduction in body weight which is in agreement with Aziz [30] who used oral administration of H_2O_2 (1%) in mice, silymarin did not show improvement growth performance in birds which were treated with H_2O_2. The same effect in Japanese quails for 42 days was observed by Behboodi *et al.* [31] which also contrasts with Ebrahimi results *et al.* [32]

Ebrahimi used powdered silymarin in various concentrations (0, 100, and 200 mg/kg) in broilers. The species differences and experimental doses might have led to that difference. Also, it was mentioned that toxins produced through the destruction of intestinal epithelial cells or released during changes in the intestinal ecosystem cause an adverse impact on performance [33]. A significant increase ($p < 0.05$) was observed in the body weight of the fourth group compared with the other groups in the last two weeks after addition of silymarin drug. This might be because of milk thistle (silymarin) increasing the elimination of toxins directly from the intestines without absorption and perhaps because they prevent fat accumulation in the liver. Therefore, milk thistle may be considered multipurpose feed growth promoter and may be promising in improving broiler performance, particularly feed efficiency, weight gain by the action of silymarin. This results in close agreement with those of Bouhalit *et al.* [34] who reported increased body weight by silymarin in rat and so as with Khaleghipour *et al.* [35] who observed a significant increase ($p < 0.05$) in the body weight of Japanese quail which were fed with diets containing 1000mg/kg silymarin. Creatinine and urea measurements are considered a mean for clinical diagnosis of renal dysfunction following acute and chronic. H_2O_2 exposure possibly causes cellular damage due to the excess free radical production [36]. In our study, the treatment with H_2O_2 led to a significant increase ($p < 0.05$) of urea and creatinine, these markers are the end products of various metabolic pathways that are excreted in the urine via glomerular filtration [37]. The treatment of silymarin alone and in combination with H_2O_2 led to decrease of urea and creatinine. These results were in accordance with those reported by Hashmi *et al.* [38] who mentioned a significant decrease ($p<0.05$) in urea and creatinine after oral administration of silymarin (2.5mg/kg) in albino rabbits for 28 days. Similar observation was reported by Khan and Siddique [39]. It was reported that Milk thistle has hepatoprotective and hepatorestorative functions and protects liver and kidney from both exo- and endo toxins all this were reported by Chakarverty and Parsad [40]. Recent evidence suggests that silymarin may be important for kidney health, silymarin concentrates in kidney cells, where it aids in repair and regeneration by increasing protein and nucleic acid synthesis [41]. There are many studies referring to hydrogen peroxide and oxidants effect on liver toxicity and impairing of liver function [42]. The current results indicate that treatment with hydrogen peroxide significantly increases ($p<0.05$) serum triglycerides, cholesterol, LDL-C, VLDL-C and risk index and significantly decreases HDL-C. We found that oral administration of silymarin alone and with H_2O_2 decreased the lipids profile and increased the HDL-C. Our results are in agreement with Behboodi *et al.* [31]. who showed that oral administration of silymarin (1ml /kg B.W.) to Japanese quails significantly reduced triglycerides and total cholesterol. Hasani-Ranjbar *et al.* [43] observed a significant decrease in levels of total cholesterol and LDL-C after treatment with silymarin. LDL-C, or so called bad cholesterol is caring cholesterol in the blood. If too much LDL-C cholesterol is found in the blood, it can slowly accumulate in the arteries walls feeding the brain and heart. In addition with other substances it can cause plaque, a thick, hard deposit that can clog those arteries. The risk of heart disease increases with high level of LDL[44]. It has been observed in vitro that silybin and silymarin have anti oxidant LDL activity. However, Silybin effects are limited in vivo because of its low bioavailability which can be improved by complex of silybin with phosphotidylcholine[45]. Banaee *et al.* [46] reported that oral administration of silymarin (100 and 400mg/kg) to fish significantly decreased total cholesterol levels. Tumova *et al*.[47] showed that administration of silymarin led to decrease in serum cholesterol levels and mild increase in HDL-C. It might be due to fat mediated improved bioavailability or/and inhibition of reabsorption of dietary cholesterol. Indeed, silymarin can effectmembrane lipids quantities and qualities such as phospholipids and cholesterol [48]. Oral administration of silymarin decreases liver content of cholesterol and level of serum cholesterol in rats [49]. Sobolova′ *et al.* [50] reported that when silymarin discourages the cholesterol absorption, it may play an important role in regulation of rats' cholesterol profile serum. On the other hand, cholesterol acyltransferase activities inhibition might be a basic way to decrease lipoprotein biosynthesis and cholesterol absorption.

Generally, administration of legalon alone affects growth performance especially in the last two weeks, but legalon in combination with H_2O_2 did not. Blood serum concentration has showed a reduction in birds treated with legalon urea, creatinine, triglycerides, total cholesterol, LDL-C, VLDL-C, risk index and greater concentration of HDL-C. H_2O_2 increases the kidney functions, and lipids profile except HDL-C.

In conclusion, administration of legalon significantly improved the Hydrogen peroxide induced disturbances and has good effect on lipids profile and kidney functions as well as on body weight when given alone in chickens.

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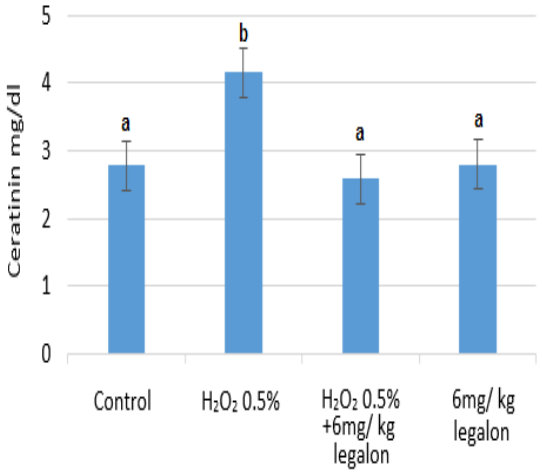

Figure 1: Effect of Legalon 70 on serum Creatinine

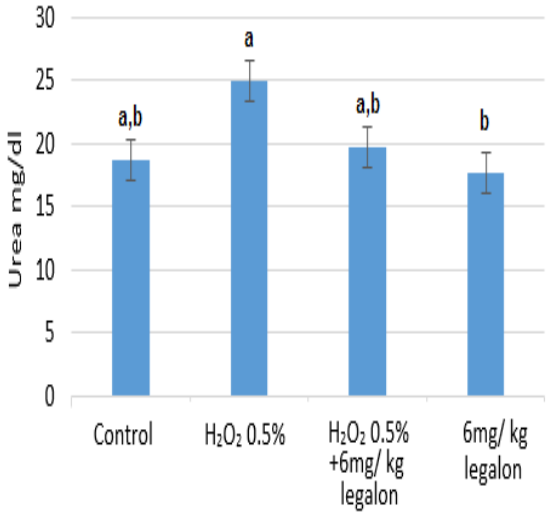

Figure 2: Effect of Legalon 70 on serum Urea

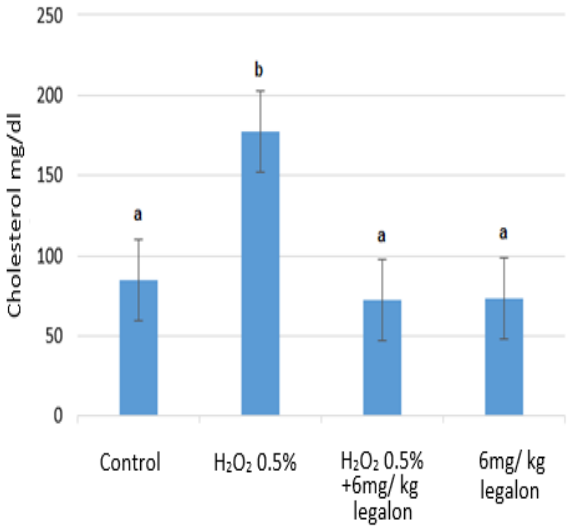

Figure 3: Effect of Legalon 70 on serum Cholesterol

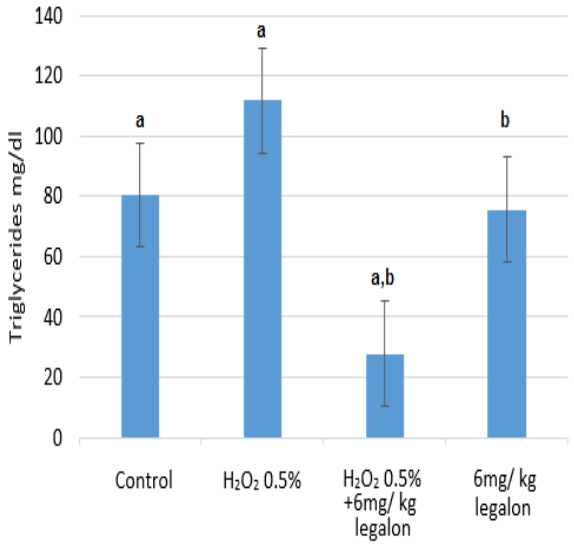

Figure 4: Effect of Legalon 70 on serum Triglycerides

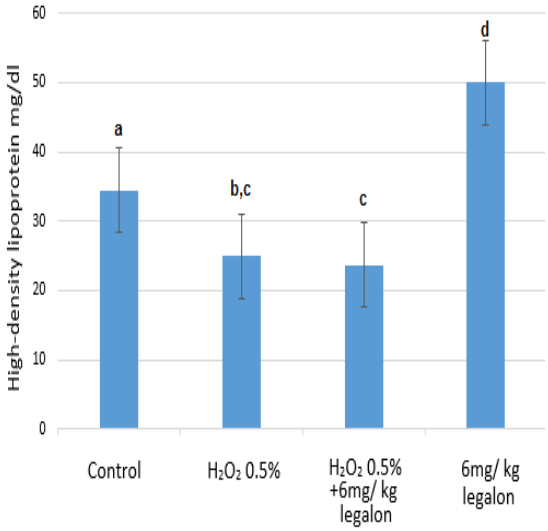

Figure 5: Effect of Legalon 70 on serum HDL.C

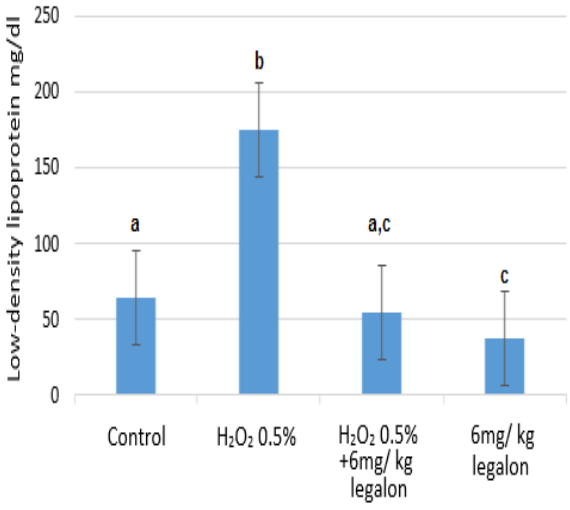

Figure 6: Effect of Legalon 70 on serum LDL.C

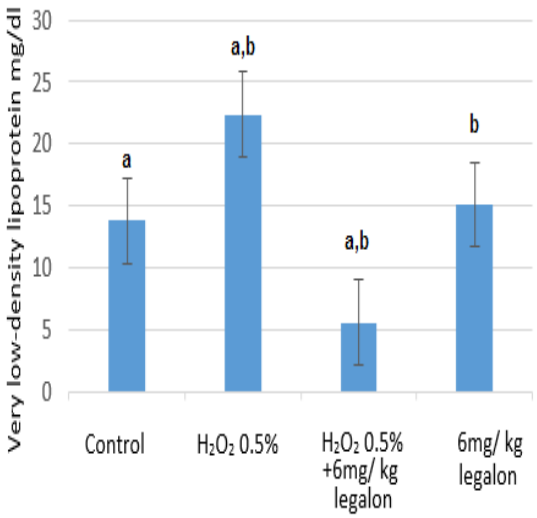

Figure 7: Effect of Legalon 70 on serum VLDL.C

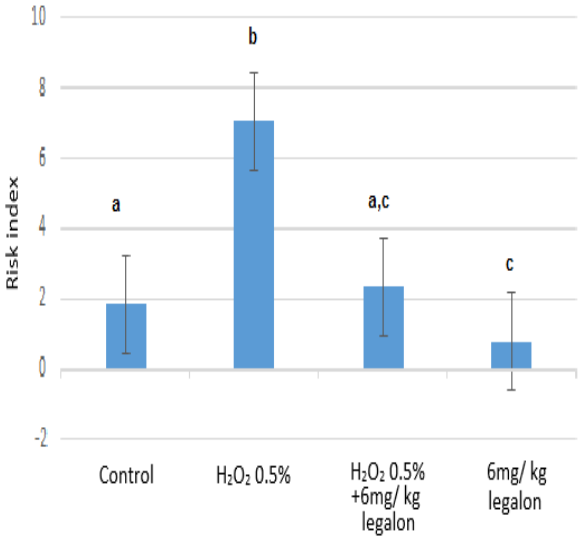

Figure 8: Effect of Legalon 70 on Risk index