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Dietary supplementation with the natural carotenoids curcumin and/or milk thistle seed improved the rats' liver and kidney functions and structure

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Abstract

Milk thistle (Silymarin) and curcumin are the most famous medical plants which have attracted the attention of many investigators for long time. The present study aimed to evaluate the possible effects of a partial replacement of soybean meal in control economic diet by milk thistle and/ or curcumin on the possible harmful changes in improvement the liver and kidney functions and lipid profiles in male rats. Feeding diet supplemented with milk thistle and/ or curcumin increased the food intake, body weight rate and relative organ weights of kidney, heart and testes and decrease the liver and kidney functions and lipid profiles. The used of milk thistle alone or either mixture with curcumin are good supplements for growing without any adverse effect on the functions of liver, kidney and testis in rats.

Keywords: milk thistle (silymarin), curcumin, feeding diet supplemented, liver and kidney functions, lipid profiles

Introduction

There are different factors affecting growth and development during the entire life cycle, all these factors could be categorized in two main groups; genetic and environmental factors. Because of increasing the cost of animal feed components, many researches were carried out to use local agricultural by products in animal feeding due to their participation as part in the solution of feed shortage problems and dramatic increases in prices of animal feed ingredients [1, 2]. The natural materials as alternative growth promoters such as medicinal plants are widely accepted, in addition to, the herbal plants are used as medicines in folk and traditional medicinal practice based on the use of plants and plant extracts [3]. Milk thistle (*Silybum marianum*) is native to the Mediterranean, but now widespread throughout the world [4]. Milk thistle appears to be safe and have multiple health benefits on various liver conditions; liver cirrhosis, alcoholic hepatitis, alcoholic fatty liver, liver poisoning and viral hepatitis [5]. Milk thistle seed contains 1.5-3% flavoneslignans, collectively referred to as silymarin; 20-30% fixed oil, of which approximately 60% is linoleic acid, approximately 30% is oleic acid and approximately 9% is palmitic acid; 25-30% protein; 0.038% tocopherol; 0.63% sterols, including cholesterol, campesterol, stigmasterol, and sitosterol and some mucilage. The three principle components of silymarin are the flavanolignans silybin, silychristin, and silidianin [6]. Mechanism of action for silymarin conducted mainly to the antioxidant, anti-inflammatory, antifibrotic and antilipdemic roles [7]. Curcumin has been identified as the major constituent of turmeric powder which is derived from the dried ground rhizome of curcuma long and is a perennial plant that is native to Southeast Asia [8, 9]. Curcumin is used as a spice and a coloring agent in curries [10]; it is not only a food coloring agent but is also a significant “cleanser of the body” used in Ayurvedic medicine. Curcumin exhibits anti-inflammatory, anticarcinogenic, antibacterial, antiprotozoal, antiviral, hypocholesterolemic and antioxidant properties [11, 12]. Curcumin was shown to be a potent scavenger of a variety of reactive oxygen species including hydroxyl radicals, nitrogen dioxide radicals, and superoxide radicals [13]. Many researchers shown the effectiveness of milk thistle and/or curcumin in growing diets on nutrients digestibility, feeding values, nitrogen balance and productive performance without had shown the different blood parameters analysis. Therefore, the present study was designed to investigate the possible changes in blood parameters after addition milk thistle and/or curcumin to the growing diets.

Material and methods

The experiments were performed on twenty male albino rats (*Rattus norvigicus*) weighing 130 g (±5g) and of two month age. They were obtained from Irbid - Jordan laboratory farms. The rats were kept in the laboratory for one week before the experimental work and maintained on a standard diet and water available *ad libitum*. The rats were equally divided into four groups; the 1st group was the control group in which rats was fed on control diet without any supplementation; the other three rations were the control diet supplemented with different sources of medicinal herbs (Table 1) as follow:

2nd Group (G2): Control diet supplemented with 0.25kg curcumin /ton.

3rd Group (G3): Control diet supplemented with 0.25kg milk thistle /ton.

4th Group (G4): Control diet supplemented with a mixture of 0.125kg milk thistle + 0.125kg curcumin /ton.

The composition of the experimental rations is presented in Table (1). Medicinal plants (milk thistle and curcumin) were collected in dry during winter 2016 till spring 2016, clean and ground form. Diets were manufactured in pellets shape 3mm diameter at El-Amel Factory, Irbid, Jordan.

Blood analysis

At the end of the experimental period, eight rats from each group were euthanized with intravenous injection with sodium pentobarbital and subjected to a complete necropsy. Blood samples were individually collected from each rat in non-

heparinized glass tubes to estimate different blood parameters. Blood serum was separated by centrifugation at 3000 rpm for 15 minutes and the collected serum was stored at -18 °C until analysis. Blood serum were analyzed to determine the concentration of liver functions {SGOT (AST), SGPT (ALT), total protein, albumin and Globulin}; kidney functions {creatinine, blood urea, sodium and potassium levels} and lipid profiles {triglycerides, cholesterol, LDL, HDL}.
Serum GOT and SGPT were spectrophotometrically determined on an autoanalyzer using commercial diagnostic kits (AMS, Italy) according to Reitman and Frankel [14]. Alkaline phosphatase activity in serum was assayed by using commercial kit that was supplied by BioMérieux Co, from France according to Belfield and Goldberge, [15]. Albumin was measured using diagnostic kit according to Drupt [16]. The serum globulin was calculated by subtracting the value of albumin from the value of total protein according to Doumas and Biggs [17]. Blood urea was measured using diagnostic kit according to Fawcett [18] and creatinine according to Bowers and Wong [19]. Serum sodium and potassium concentration were assayed by colorimetric method using commercial kit supplied by Diatek Company [20, 21]. Serum total cholesterol was determined according to the method of Allain *et al*. [22] and serum triglycerides were determined according to the method of Fossati and Prencipe [23] using kits supplied by Human. The HDL cholesterol was determined by enzymatic colorimetric according to the method of Lopes-Virella *et al*. [24]**,** LDL-cholesterol was assayed colorimetrically according to the method of Wieland and Seidel [25].

Statistical analyses

Results were analyzed using one-way analysis of variance (ANOVA) followed by the Least Significant Difference (LSD) tests to compare between different groups. Data were presented as the mean±SEM. P values less than 0.05 were considered significant. All statistical analyses were performed using SPSS statistical version 16 software package (SPSS® Inc., USA).

Table 1: The composition of the dietary treatments (Diets were manufactured in pellets shape 3mm diameter at El-Amel Factory, Irbid, Jordan).

Ingredients, %	Treatments			
	G1	G2	G3	G4
Yellow corn	12	12	12	12
Wheat bran	23	23	23	23
Barley grain	14.5	14.5	14.5	14.5
Soybean meal	15.5	14.75	14.75	14.75
Berseem hay	30	30	30	30
Molasses	3	3	3	3
Salt	0.5	0.5	0.5	0.5
Limestone	1	1	1	1
Premix	0.5	0.5	0.5	0.5
Milk Thistle	0	0.25	0	0
Curcumin	0	0	0.25	0
Milk Thistle & curcumin	0	0.125	0.125	0.25

Results

Table 2 showed that, the changes in the food intake, body weight gain and relative organ weights in male rats in different groups. The food intake and body weight rate was insignificantly increasing (P< 0.05) after addition of curcumin &/or milk thistle to the economic control diets (Table 2). The present results shows no clear differences were observed regarding relative organ weights (Liver, kidney, heart, spleen and testes) after the partial addition of curcumin to diet supplemented (Table 2). In contrast, the addition of curcumin & milk thistle to the economic control diets increase the relative organ weights of kidney, heart and testes and decrease the relative organ weights of liver and spleen (Table 2).

Table 2: Food intake (g/rat/day), body weight gain (g) and relative organ weights (g/100 g body weight) in male rats in different groups.

		Control	Curcumin	Milk Thistle	Milk Thistle & Curcumin
Food intake		13.4 ± 0.37^b	14.1 ± 0.38^dl	14.4 ± 0.58^d	14.6 ± 0.44
Body weight gain		19.5 ± 1.13^a	20.2 ± 1.09^djm	21.3 ± 1.60^dg	22.0±0.85
Relative organ weights	Liver	3.51 ± 0.15 ^d	3.58 ± 0.14 ^a	3.50 ± 0.09 ^d	3.53 ± 0.31^d
	Kidney	0.52 ± 0.08^a	0.52 ± 0.03^a	0.55 ± 0.01^a	0.57 ± 0.03^a
	Heart	0.43±0.02	0.43 ± 0.04 ^ac	0.39 ± 0.03^di	0.41±0.11
	spleen	0.383 ±0.02	0.372 ±0.05	0.367±0.04a	0.362±0.,01a
	Testes	1.08 ± 0.015	1.07 ± 0.029	1.11 ± 0.052	1.12 ± 0.035

Value represents mean ± SE of 10 rats, a, b, c: Superscripts with different letters in the same row differs significantly (p<0.05).

$$\text{Relative organ weight} = \frac{\text{Organ weight}}{\text{Body weight}} \times 100$$

The changes in the liver functions as affected by the different experimental diets are shown in Table (3). The addition of curcumin &/or milk thistle to the economic control diets improved GPT, GOT and ALP, where the levels of GPT, GOT and ALP were significantly decreased in G2, G3 and G4 when compared with G1 (Table 3). On the other hand; total bilirubin levels showed no significant differences between the different treatment groups. In contrast, the addition of curcumin only to the economic control diets improved GGT. Table 3 shows that serum total proteins and their fractions (albumin and globulin) as indicator of protein metabolism were affected by the addition of curcumin &/or milk thistle to economic control diets, the total proteins, albumin were significantly increased in milk thistle (G3) and milk thistle & curcumin (G4) when compared with control group (G1) while globulin levels showed no significant differences between the different treatment groups (Table 3).
The changes in the kidney functions as affected by the different experimental diets are shown in Table (4). On the other hand, the levels of creatinine, urea and potassium were significantly decreased in G2, G3 and G4 when compared with G1 (Table 4). The present results shows no clear differences were observed regarding sodium levels in rat blood serum after the partial addition of milk thistle &/or curcumin to diet supplemented (Table 4). The addition of curcumin &/or milk thistle to the economic control diets improved lipid profiles, where the levels of triglycerides, cholesterol and LDL were significantly decreased in G2, G3 and G4 when compared with G1 while the levels of HDL were significantly increased in G2, G3 and G4 when compared with G1 (Table 5).

Table 3: Changes in the activity of liver enzymes in serum of male rats in different groups.

	Control	Curcumin	Milk Thistle	Milk Thistle & Curcumin
GPT (U/l)	24.5 ± 1.1^{b}	20.5 ± 1.5^{a}	17.9 ± 0.86^{c}	20.0 ± 0.55^{a}
GOT (U/l)	38.5 ± 1.03^{b}	33.4 ± 0.6^{a}	26.4 ± 1.55^{c}	32.1 ± 1.05^{a}
G.G.T	25.3 ±0.58^{b}	18.3± 0.49^{a}	24.8 ± 0.8^{b}	24.0 ± 0.31^{b}
Bilirubin T. (mg/dl)	0.49 ± 0.013	0.49 ± 0.019	0.46 ± 0.003	0.47 ± 0.013
ALP (U/l)	143±2.18^{b}	140±3.85^{b}	129±4.25^{a}	130±3.98^{a}
Total Protein (g/dl)	5.47 ± 0.39^{b}	5.88 ± 0.23^{b}	6.25 ± 0.31^{a}	6.45 ± 0.81^{a}
Globulin (g/dl)	4.15±0.37	4.10±0.19	4.14±0.21	4.15±0.20
Albumin (g/dl)	4.05 ± 0.1^{b}	4.10 ± 0.18^{a}	4.28 ± 0.55^{c}	4.21 ± 0.28^{bc}

Value represents mean ± SE of 10 rats, a, b, c: Superscripts with different letters in the same row differs significantly ($p<0.05$).

Table 4: Changes in the kidney functions and electrolyte levels in different groups.

	Control	Curcumin	Milk Thistle	Milk Thistle &Curcumin
Creatinine (mg/dl)	0.75±0.07^{b}	0.44 ± 0.16^{a}	0.62 ± 0.03^{ab}	0.45 ± 0.10^{a}
Urea (mg/dl)	29.5 ± 0.73^{b}	25 ± 0.46^{ab}	23.1 ± 1.04^{b}	21 ± 0.55^{b}
Sodium (mEq/l)	133.4±3.75	135.5±5.55^{a}	136.5±4.62	137.1±3.65
Potassium (mEq/l)	4.89±0.18^{b}	4.52±0.82^{b}	4.59±0.39^{b}	4.35±0.25^{c}

Value represents mean ± SE of 10 rats, $^{a, b, c}$: Superscripts with different letters in the same row differs significantly ($p<0.01$).

Table 5: Changes in lipid profiles in different groups.

	Control	Curcumin	Milk Thistle	Milk Thistle & Curcumin
Cholesterol (mg/dl)	111.2±2.5^{b}	82.5 ±3.24^{a}	91.9 ± 2.4^{ab}	79.4±3.35^{a}
Triglyceride (mg/dl)	96 ± 3.1^{b}	63 ± 5.0^{a}	78.5 ± 1.9^{c}	65.4±2.07^{a}
HDL (mg/dl)	47 ± 3.1^{b}	46.1 ± 2.8^{a}	41.6 ± 2.4^{a}	39.5±2.1^{c}
LDL (mg/dl)	45± 2.5^{b}	23.8± 1.1^{a}	34.6± 1.3^{c}	26.8± 1.3^{a}
vLDL (mg/dl)	19.2± 0.44^{b}	12.6± 0.21^{a}	15.7± 03.69^{ab}	13.1± 0.37^{a}

Value represents mean ± SE of 10 rats, $^{a, b, c}$: Superscripts with different letters in the same row differs significantly ($p<0.05$).

Discussion

Phytochemicals, which are found in fruits, vegetables, and plant-derived beverages, may have important roles as dietary components as a result of their cytoprotective actions in many organs. Naturally occurring carotenoids and flavonoids possess free radical scavenging properties and offer cytoprotection from oxidative injury [26]. Flavonoids are large group naturally occuring antioxidant polyphenols and they have received considerable attention during the last decade [27]. In human the flavonoids contribute greatly to the dietary sources of antioxidants and their intake decreased the risk of coronary heart disease [28]. The present results showed that the supplementation of milk thistle to the diets of rats alone or mixed with curcumin did not have any adverse effect on the physiological and biochemical blood parameters. Many studies have shown that dietary curcumin has a wide range of pharmacological effects, such as elimination of free radicals [29], antioxidant [30], antibacterial and immunomodulatory functions [31-33]. In the current study; food intake, body weight rate and relative organ weights weights of kidney, heart and testes and decrease were insignificantly increasing ($P< 0.05$) after addition of curcumin &/or milk thistle to the economic control diets. This is may be because both milk thistle (silymarin) and curcumin increase the elimination of toxins directly from the intestines without absorption and perhaps because they prevent fat accumulation in the liver. Therefore, milk thistle seeds appear to be potential multipurpose feed growth promoter and may be promising in improving broiler performance, particularly feed efficiency, weight gain and immune system. Water is retained in the cytoplasm of hepatocytes leading to enlargement of liver cells, resulting in increased total liver mass and volume by the action of silymarin. This is compatible with results which obtained by Noorani *et al*. [34]. In contrast, Das and Vasudevan [35] reported that there were no significant differences in kidneys weights and that are compatible with our study.

In the current study; the addition of curcumin &/or milk thistle to the economic control diets improved the liver functions (GPT, GOT and ALP, where the levels of GPT, GOT and ALP) and kidney functions (Creatinine and urea) were significantly decreased in curcumin &/or milk thistle groups when compared with control group. These results indicate that treating diets with milk thistle alone or mixture with curcumin did not have any adverse effect on the function of liver and kidneys. In this respect, Abdel-Khalek [36] found that activity of SGOT and SGPT was affected by quality and quantity of dietary protein. Results of the present work are compatible with those reported by Saller *et al*. [37], Raja *et al*. [38] and El-Gazayerly *et al*. [39] who noted that the liver enzymes activities was significantly decreased in female rats exposed to silymarin drug and silybum marianum extract. The increase in concentration of total protein and their fractions within the normal range in curcumin &/or milk thistle groups may reflect an increase in the hepatic function when animals were fed on protein. These findings suggest that curcumin or milk thistle may increase the metabolic rate. It is known that the change in albumin level reflects the change in liver function and the presence of the fatty acids may affect muscle protein synthesis and protein deposition through a prostaglandin depend mechanism [40]. These results were in agreement with those of El-Shafeey *et al*. [41] and Noorani and Kale [34].

Also; treatments with the silymarin extract decreased the levels of urea and cratinine. These findings were in accordance with those reported by El-Shafeey *et al.* [41]. Recent evidence suggests that silymarin may be just as important for kidney health. Silymarin concentrates in kidney cells, where it aids in repair and regeneration by increasing protein and nucleic acid synthesis [42]. The current results indicate that treating diets with curcumin &/or milk thistle significantly decrease the lipid profiles. Our results are in agreement with Daghash *et al*. [43] who reported that the addition of NS seeds on rabbit diet display significant decrease of serum cholesterol, total lipids and triglyesrids. Omar [44] attributed the decreased cholesterol and triglyseride levels in diets containing high levels of NS to unsaturated fatty acids, which may stimulate the cholesterol excretion into the intestine, and the oxidation of cholesterol to bile acids. Similar results were reported by Ghazalah *et al*. [45] in ducks. Silymarin is used in supportive therapy of liver diseases and its cytoprotective activity is believed to be based on antioxidant properties. Škottová *et al*. [46] reported that silymarin (but not silibinin) prevents the development of dietary induced hypercholesterolemia in rats [47], and both silymarin and silibinin possess LDL antioxidant activity in vitro. However, in vivo effects of silibinin are limited by its low bioavailability, which can be improved by complexation of silibinin with phosphatidylcholine [48]. Also, some studies suggested that curcumin can act in several ways to lower serum LDL bound cholesterol. First, uptake of cholesterol in the gastrointestinal tract could be inhibited; second, LDL-C could be eliminated from the blood via LDL receptor and finally, the activity of cholesterol degrading enzyme namely cholesterol hydroxylase could be increased [49]. It has been that curcumin decreased total cholesterol and LDL-C, while increased HDL-C due to absorption, degradation or elimination of cholesterol.

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