



EVALUATION OF TWO PHENOLIPIDS (QUERCETIN-ENRICHED LECITHIN) AND (RUTIN -ENRICHED LECITHIN) *IN VITRO* AND *In vivo*

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ABSTRACT: The present study was designed to investigate the lecithin and mixtures of quercetin and lecithin (1:1, *W/W*) and mixtures of rutin and lecithin (1:1, *W/W*) in the protection of triolein models stored under oxidative conditions for 15 days in the dark at 60°C. The progress of oxidation was tested by measuring the peroxide value (PV). The factors influencing the oxidative stability (OS) of different triolein models were also discussed. Inverse relationships were noticed between peroxide values and oxidative stabilities at termination of the storage. Also a biological study was carried out to evaluate the hypolipidemic effects of all previously studied additives. Oral administration of lecithin and mixtures of quercetin and lecithin and mixtures of rutin and lecithin to hyperlipidemic rats induced a highly decreasing effect on the levels of serum total cholesterol (TC), triacylglycerols (TG), low-density lipoprotein cholesterol (LDL) and increasing the levels of serum high-density lipoprotein-cholesterol (HDL). It is suggest that, phenolipids (quercetin-enriched lecithin) and (rutin -enriched lecithin) has a significant health benefits and can be explored as a potentially promising food additive for the prevention of hyperlipidemia diseases.

Key words: Lecithin, quercetin, rutin, lipid oxidation, oxidative stability, phenolipids, natural antioxidants, hypercholesterolemic, lipid profile.

INTRODUCTION

Phospholipids are constituents of all cell membranes and are present in food from plant and animal sources. Soy lecithin is a mixture of naturally occurring phospholipids, phosphatidylcholine (13-18g/100g), phosphatidyl ethanol amine (10-15g/100g), and phosphatidylinositol (10-15g/100g), and is utilized in a wide variety of food and industrial applications. This excellent source of choline (essential nutrient) is also used as a nutritional supplement. Lecithin helps to smooth the texture of food and serves as an emulsifying agent in margarine, chocolate, caramels, coatings (to control viscosity, crystallization, and sticking) and chewing gum (as a softening and plasticizing agent). Some baked goods, confections, infant formulas, cheese products, and instant products contain lecithin as an emulsifier, dispersant, viscosity modifier, or

wetting agent. Industrial applications of lecithin include paints, waxes, polishes, wood coating, plastic, magnetic-type media, paper and printing (Tanno, 1990 ; Joshi *et al.*, 2006). Production of cosmetics is an example of another still growing field of lecithin application. Refined grade lecithin (containing up to 99.7 g/100g of phospholipids) is used for pharmaceutical applications and research.

Antioxidative properties of phospholipids have been demonstrated through their addition to processed vegetable oils and animal fats (List and Friedrich, 1989; King *et al.*, 1992). These properties have been proposed to be a consequence in (i) synergism between phospholipids and tocopherol (Koga and Terao, 1995 ; Judde *et al.*, 2003); (ii) chelating of pro-oxidant metals by phosphate groups (Privett and Quackenbush, 1954; Jewell and Nawar, 1980); (iii) formation of Maillard-type products between amino phospholipids and oxidation

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products (Husain *et al.*, 1984); and (iv) action as an oxygen barrier between oil and air interfaces (Porter, 1980). Two crucial polyunsaturated fatty components of phospholipids (especially soy lecithin) are linoleic acid (C18:2) and linolenic acid (C18:3), and their carbon chains are the most sensitive sites for autoxidation. Thus, phospholipids are also very easily oxidized (Passi *et al.*, 2004; Undeland *et al.*, 2004), and these oxidative processes are likely to modify some of the phospholipids' properties responsible for their antioxidative activities.

Dietary phenolic substances have received much attention due to their biological activity. They have positive properties such as antimutagenic and anticancerogenic effects as well as being antioxidants. The structure, classification, and distribution of phenolic compounds in plant foods, their chemistry, and signification with regard to food processing and storage as well as their physiological effects have recently been reviewed and summarized (Kroll *et al.*, 2003 a, b). Flavonoids are a class of phenolics that exhibit powerful antioxidant effects in biological systems, including free radical scavenging and metal ion sequestering, but their effectiveness greatly depends on particular chemical features. However, it is widely accepted that phenols have complex pro- and antioxidant effects *in vitro*, depending on their structure and the assay system used, and it is often hard to predict their actual action (Rice-Evans *et al.*, 1995; Acker *et al.*, 1996; Rice-Evans and Miller, 1996). Quercetin is one of the most abundant of the flavonoids and occurs in food as aglycone (attached to a sugar molecule). It is found in many common foods including apple, tea, onion, nuts and berries. Quercetin has many health promoting effects, including anti-inflammatory and anti-allergic effects as well as improvement of cardiovascular health and reducing risk for cancer. All these activities are caused by the strong antioxidant activity of quercetin. It will help to combat free radicals, which can damage cells. As many other flavonoids, quercetin prevents the oxidation of LDL cholesterol (Makris and Rossiter, 2001 and Kroll *et al.*, 2003 a, b).

Several investigations have been carried out to study the synergism of phospholipids and tocopherols (Koga and Terao, 1995; Judde *et al.*,

2003). To best of knowledge, no attempts have been made to determine the changes produced in phospholipid antioxidant properties following mixing with phenolic compounds like quercetin or rutin, which found mostly in many food products. In an attempt to study the interaction between lecithin and quercetin or rutin, the present study examined the antioxidative activities of the native soybean lecithin as well as quercetin-enriched or rutin-enriched lecithin when added to triolein during accelerated oxidation test. For the first time, the oxidative stability (OS) of quercetin-enriched or rutin-enriched lecithin is reported. The objectives were (a) to assess OS of quercetin-enriched or rutin-enriched lecithin in triolein models by monitoring oxidative products and (b) to evaluate the biological activity of a combination of quercetin, rutin with lecithin. The results give an opportunity to gain new knowledge about the differences in oxidative behaviours of simple and complex lipids. Moreover, the results may be applied to increase the antioxidative activity and health impact of commercial lecithin in different food applications.

MATERIALS AND METHODS

Materials

Triolein (99 g/100g, Fluka, Buchs, Switzerland) and Soy lecithin (Lucas Meyer GmbH, Hamburg, and Germany) were used without further purification. Quercetin and rutin were purchased from Sigma-Aldrich (St. Louis, MO, USA). All solvents and reagents from various suppliers were of the highest purity needed for each application and used without further purification.

Preparation of Triolein Models

Quercetin or rutin was mixed with lecithin (1:1, *W/W*) and the mixture was dissolved in ethyl acetate (1:10, *W/V*) at $40 \pm 2^\circ\text{C}$. The mixtures were left for 60 min with continuous shaking then the solvent was rotary evaporated at 25°C . The mixtures were lyophilized to obtain yellow powder of quercetin or rutin-enriched lecithin formulations. To study the effect of different doses on the Oxidative Stability of triolein, two concentrations (0.25 g/100g and 0.50 g/100g) of quercetin-lecithin or rutin-

enriched mixtures were added to triolein. The quercetin-lecithin or rutin-lecithin mixture in ethyl acetate was added to triolein then the solvent was evaporated using a rotary evaporator at $50 \pm 2^\circ\text{C}$. To study the impact of individual components, each of quercetin and lecithin was added individually at the same concentrations (0.25 g/100g and 0.50 g/100g) to triolein as mentioned previously. Triolein with no additives was compared with models containing different concentrations of quercetin and lecithin.

Analytical Procedures for Monitoring Oxidative Stability

The progress of the oxidative deterioration of the oils during storage was followed by measuring at regular intervals changes in peroxide levels according to the official methods of AOCS (1995).

Biological Experiment

Animals and treatments

In this experiment 42 male albino rats weighing 120-140 g rats purchased from Faculty of Veterinary Medicine, Zagazig University. Rats were kept in wire-bottomed stainless steel cages, which were environmentally controlled (25°C , 12 hr., light dark cycle); with free access to water and food, the duration of the experiment was extended for two months. Ten days before the beginning of the experiment all rats were fed on basal diet American institute of nutrition standard reference diet (AIN-93M) (Reeves *et al.*, 1993) as an adaptation period after that the rats were divided into 7 groups each one contained 6 rats. Treatments used in this study were as shown in Table 1. The full composition of the high cholesterol diet (HCD) and cholesterol free diet (CFD) is detailed in Table 2.

Blood sampling

From the plexuses of eye in the present of diethyl ether anaesthesia, the samples of blood were taken after 15, 30, 45 and 60 days from the beginning into tubes with heparin as anti-coagulant and then centrifuged at 3000 rpm for about 25 min.

Biochemical analyses

The methods used for the determination Total cholesterol, low-density lipoprotein (LDL), triglycerides and high-density lipoproteins (HDL) (mg/dl) were calculated according to Friedewald *et al.* (1972), Richmond (1973), Demacker *et al.* (1984) and Fossati and Prencipe (1982).

Statistical Analysis

All studied data were statistically analyzed using SPSS Computer Program (Co- Stat Software Computer Program) using analysis of variance ANOVA "one way".

RESULTS AND DISCUSSION

The characterization of Oxidative Stability of triolein models enriched with different levels of mixed quercetin-lecithin and rutin-lecithin was done. Different solvents were tested to dissolve quercetin or rutin and lecithin, wherein the best results were achieved with ethyl acetate which was able to dissolve both compounds at $40 \pm 2^\circ\text{C}$. The objective was based on that the monitoring of antioxidant or pro-oxidant factors would add to the knowledge of interactions occurring between phenolic compounds and phospholipids. To evaluate the OS of triolein model, Peroxide Value was determined as indices of oxidation.

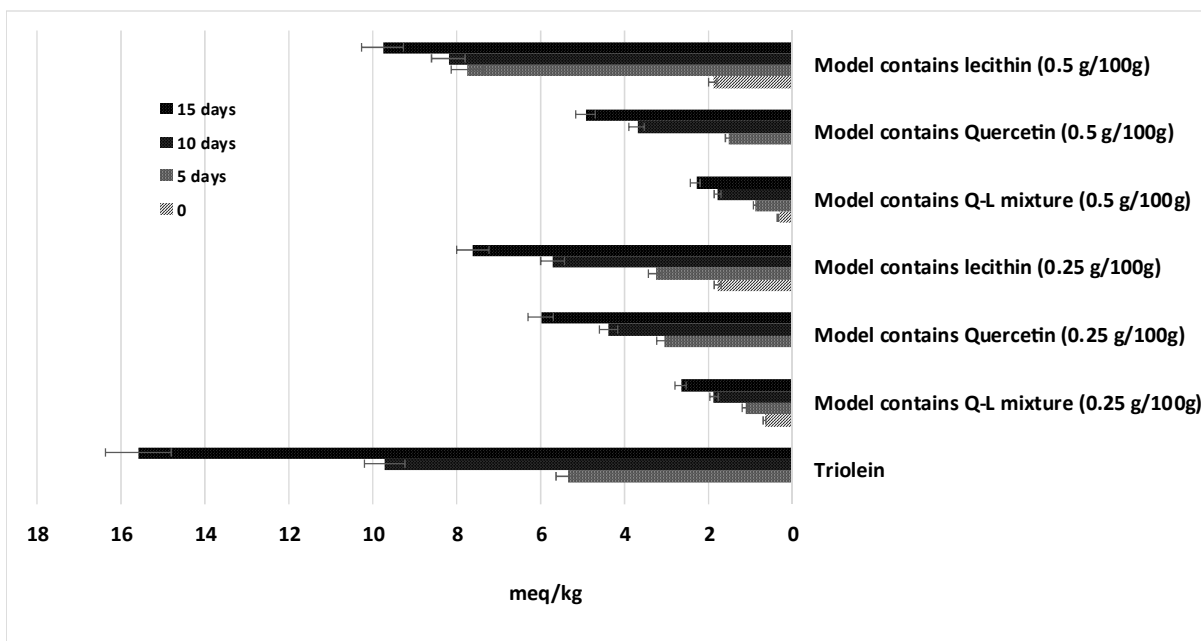
Hydroperoxide is the primary product of lipid oxidation; therefore, determination of PV can be used as oxidative index during the early stage of lipid oxidation. The PV calculated for different triolein models assayed are given in Figure 1. On the basis of PV, the OS of triolein models varied significantly, with the model enriched with the highest levels of quercetin-lecithin mixture (0.5 g/100g) being most stable followed by rutin-lecithin mixture (0.5 g/100g). The PV clearly showed that as the storage time increased the OS of the triolein models decreased (Fig. 1). Generally both quercetin-lecithin and rutin-lecithin enriched models had a much lower PV than that of models enriched with individual lecithin and quercetin over the entire storage period. PV in quercetin-lecithin and rutin-lecithin enriched models increased at a low level over 15 days, whereas the peroxides

Table 1. Treatments used in the experiment

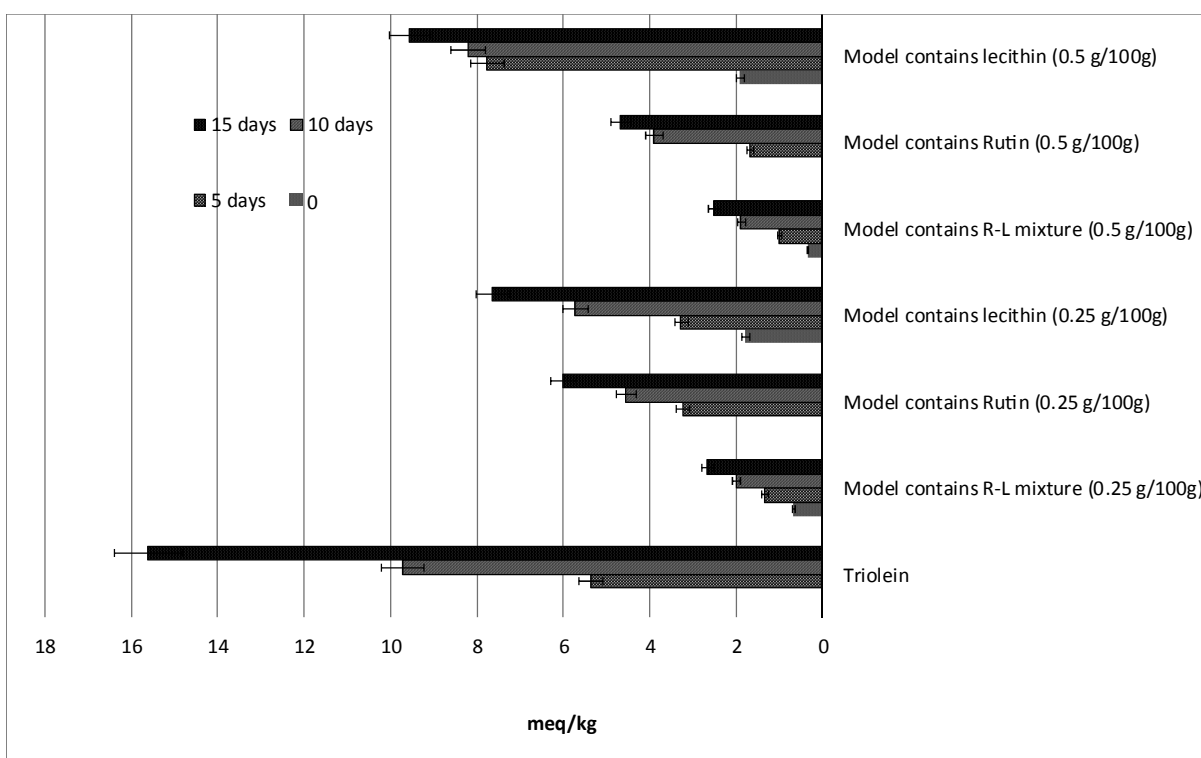
Group No	Treatment
G1	Received high cholesterol diet and daily doses of 1ml oil /200g B.W, oral (0.5% quercetin-enriched lecithin)
G2	Received high cholesterol diet and daily doses of 1ml oil /200g B.W, oral (0.5% rutin-enriched lecithin)
G3	Received high cholesterol diet and daily doses of 1ml oil /200g B.W, oral (0.5% lecithin)
G4	Received high cholesterol diet and daily doses of 1ml oil /200g B.W, oral (0.5% quercetin)
G5	Received high cholesterol diet and daily doses of 1ml oil /200g B.W, oral (0.5% rutin)
G6	Positive control received high cholesterol diet and daily doses of 1ml oil /200g B.W, oral
G7	Negative group (normal control) fed on basal diet and daily doses of 1ml oil /200g B.W, oral

Table 2. Diet composition g/100g diet of investigated groups fed high cholesterol diets (HCD) and cholesterol free diet (CFD)

	HCD						CFD
	1	2	7	4	5	6	
Casein	15	15	15	15	15	15	15
Starch	58.75	58.75	58.75	58.75	58.75	58.75	65
Oil	15	15	15	15	15	15	10
Salt mixture	4	4	4	4	4	4	4
Vitamin mixture	1	1	1	1	1	1	1
Cellulose	5	5	5	5	5	5	5
Cholic acid	0.25	0.25	0.25	0.25	0.25	0.25	-
Cholesterol	1	1	1	1	1	1	-



A



B

Fig. 1. Changes in PV of triolein models enriched with quercetin (A) and models enriched with rutin (B) during oven test. Values given are the mean of three replicates and error bars show the variations of three determinations in terms of standard deviation

accumulated in lecithin enriched models to high levels. The peroxide values after 15 days were 2.66 and 2.30 meq/kg in sample of models containing 0.25 g/100g and 0.50 g/100g quercetin-lecithin mixtures, respectively. Lecithin-contained models had the highest PV (9.55 and 9.77 meq/kg) and were oxidized rapidly. The results demonstrated that quercetin-lecithin enriched triolein models had a potent OS higher than models containing individual compounds. Meanwhile, adding lecithin caused significant increase in PV levels during incubation for 15 days.

The exact mechanism of action of phospholipids is still not fully established, four postulates have been proposed to explain their antioxidant activity: i) synergism between phospholipids and phenolic compound; ii) chelating of pro-oxidant metals by phosphate groups; iii) formation of Maillard-type products between phospholipids and oxidation products and iv) action as an oxygen barrier between oil/air interfaces (Hudson and Ghavami, 1984; Gordon and Kourimska, 1995). On the other side, phenolic compounds in vegetable oils are important factors when evaluating the quality of the oil because these compounds have been correlated with sensory quality, the shelf life of oil and in particular, its resistance to oxidation (Ramadan and Moersel, 2004). The OS of the vegetable oils, however, does not correlate directly with the amount of phenolics. It has been mentioned that oil stability is correlated not only with the total amount of phenolics, but also with the presence of selected phenols (Tovar *et al.*, 2001).

The addition of individual lecithin and quercetin or rutin as well as their mixtures to triolein increases the OS of different models when heated under air in the dark. Although different additions protected triolein, this protection was more effective for lecithin-quercetin mixtures while quercetin and lecithin alone exhibited only a minor protection. Furthermore, it was observed that in all cases lecithin was a less powerful antioxidant when compared to quercetin and lecithin-quercetin mixture. It was also indicated that, increasing the levels of lecithin or rutin (from 0.25 g/100g to 0.50 g/100g) as single additive reduces the OS of triolein. Generally, it is accepted that the

high degree of unsaturation of lecithin makes it susceptible to oxidative deterioration. Aside from fatty acid profile, factors, such as oxygen concentration, metal contaminants, lipid hydroxy compounds, enzymes and light may also influence the OS of lecithin.

Lipid peroxidation is initiated by the attack on a fatty acid or fatty acyl side chain of any chemical species that has sufficient reactivity to abstract a hydrogen atom from a methylene carbon in the side chain (Porter *et al.*, 1995). Therefore, in such system, rapid donation of hydrogen atom or scavenging of free radicals would be crucial to avoid chain reactions which result in lipid peroxidation. It is well known that quercetin has strong antiradical action.

Impact of Phenolipids on the Plasma Lipid Profile

Results presented in Table 3 show the levels of plasma triglyceride contents in albino rats receiving diet containing the treatments. Normal levels of serum triglycerides were recorded for group 7 (negative control) receiving (CFD) all over the experimental period, while levels rapidly increased to maximum in group 6 (positive control) fed on (HCD) throughout the assay.

After 60 days, it was noticed that Triglyceride level in hypercholesterolemic groups treated with phenolipids (quercetin + lecithin) or (rutin + lecithin) had the highest decrease compared with the hypercholesterolemic groups. Group 1 possessed the lowest level of TG when compared with the other groups. From the lowest to the highest level of TG the experimental groups can arranged as follow: 1 > 2 > 5 > 4 > 3.

From the obtained results, it is clear that the greatest reduction in TG was detected in group 1 that was fed on phenolipids (quercetin + lecithin) followed by group 2 which fed on (rutin + lecithin) compared with HCD group.

Levels of plasma TC in hyperlipidemic rats (Table 3) were directly affected in all rats receiving HCD and treated with different mixture of phenolipids and at different periods whereas it reached to its maximum values in group 6 (positive control) receiving HCD for the whole experimental period. After 60 days of feeding, results showed that group 1 had the lowest

Table 3. Impact of feeding different blends of phenolipids on the levels of triglycerides and total cholesterol (mg/dl)

Treatment	Plasma triglyceride (mg/dl)				Plasma total cholesterol (mg/dl)				
	15	30	45	60	15	30	45	60	
Hypercholesterolemic groups	Group 1	54.3	49.3	71.0	73.2	70.1	78.7	86.1	82.2
	(quercetin + lecithin)	± 6.5	± 2.2	± 5.4	± 1.9	± 3.1	± 6.5	± 4.3	± 3.7
	Group 2	49.3	62.2	78.2	81.3	85.3	88.2	90.1	85.3
	(rutin + lecithin)	± 3.3	± 6.6	± 6.7	± 4.3	± 0.5	± 3.6	± 3.1	± 2.3
	Group 3	66.3	84.0	102.1	120.0	82.5	90.81	95.09	120.1
	(lecithin)	± 12.6	± 7.8	± 18.3	± 5.4	± 1.83	± 9.5	± 11.1	± 5.9
	Group 4	77.9	69.2	82.3	112.8	75.2	86.3	89.3	116.0
	(quercetin)	± 3.4	± 6.5	± 5.01	± 10.5	± 6.8	± 1.45	± 2.5	± 6.4
	Group 5	76.07	74.35	78.47	105.8	89.4	71.3	88.5	105.3
	(rutin)	± 2.5	± 10.4	± 3.9	± 12.07	± 12.1	± 2.2	± 5.6	± 12.05
Group 6	65.9	77.4	135.1	188.83	112.0	135.0	179.0	198.38	
(hypercholesterolemic group)	± 3.7	± 9.1	± 12.04	± 9.6	± 6.8	± 3.19	± 18.3	± 21.7	
Group 7 (normal control)	47.3	49.9	59.4	57.6	62.3	75.2	69.3	67.2	
	± 12.8	± 3.5	± 3.5	± 10.8	± 4.8	± 8.4	± 1.99	± 2.64	

Results are given as mean ± SD from triplicate estimations

serum TC followed by group 2. Groups can be arranged due to the effect of treatments in reducing TC in the following order: 1 > 2 > 5 > 4 > 3. Treatments rats with blend 1 or 2 caused decreasing in TC in comparison with HCD group.

Accumulation of HDL (good cholesterol) within the arterial wall appears to play a crucial role in the initiation and progression of atherosclerotic plaque. Results in Table 4 show the levels of plasma HDL of hyperlipidemic rats fed on different treatments at different periods. At the end of the experiment results showed that all treatments had a highest increment of HDL compared to control group HCD.

The inverse association between the incidence of coronary heart diseases and HDL-cholesterol levels has been known. HDL is an important scavenger of surplus cholesterol transporting it from cell membrane to the liver

where it is metabolised or converted into the bile acids. However, the mechanism of the increase or maintenance of HDL-cholesterol is not at all clear.

Accumulation of LDL (bad cholesterol) within the arterial wall appears to play essential role in the initiation and progression of atherosclerotic plaque. Low-density lipoprotein, which included very low-density lipoprotein (VLDL + LDL) and known traditionally as LDL. Table 4 represents the levels of LDL during the experimental periods. Taking the periods of feeding into consideration results indicated that all groups treated had a lower LDL cholesterol level compared with control HCD, which was increased, to the maximum level. The highest decrease in LDL levels was in group 1 fed on blend containing (quercetin + lecithin) followed by group 2 which was fed (rutin + lecithin).

Table 4. Impact of feeding different blends of phenolipids on the levels of LDL- cholesterol, and HDL- cholesterol (mg/dl).

Treatment	HDL-cholesterol (mg/dl)				LDL-cholesterol (mg/dl)				
	15	30	45	60	15	30	45	60	
Hypercholesterolemic groups	Group 1	34.04	41.91	44.7	43.34	25.2	26.929	27.2	24.22
	(quercetin + lecithin)	± 4.3	±8.7	±7.8	±3.1	±2.19	±2.3	±2.96	±1.1
	Group 2	39.44	45.22	41.26	42.84	36.0	30.534	33.2	26.2
	(rutin + lecithin)	±4.4	±5.7	±5.7	±4.0	±5.34	±2.5	±4.7	±1.5
	Group 3	30.14	33.81	30.47	53.9	39.1	40.2	44.2	61.2
	(lecithin)	±4.4	±4.2	±8.3	±4.2	±3.4	±5.4	±2.4	±4.06
	Group 4	31.52	39.17	35.81	46.24	28.1	33.282	37.022	47.2
	(quercetin)	±6.7	±1.33	±7.2	±9.6	±4.08	±2.1	±5.8	±5.35
	Group 5	40.78	12.35	30.90	38.94	35.2	44.073	40.1	45.2
	(rutin)	±12.2	± 3.12	±4.2	±5.8	±9.7	±1.7	±2.4	±2.4
Group 6	38.62	37.22	39.78	33.434	60.2	82.3	112.2	139.2	
	(hypercholesterolemic group)	±5.8	±8.5	±5.9	±10.4	±2.2	±6.2	±11.2	±5.1
Group 7 (normal control)	30.84	30.97	29.22	31.45	22.0	23.24	28.2	26.12	
		±6.5	±9.3	±5.4	±4.9	±0.84	±2.4	±2.8	±6.19

Results are given as mean ± SD from triplicate estimations

Most of cholesterol is an essential structure element of biological membranes, the rests is transited through blood or functions as the starting material for the synthesis of bile acid, steroid hormones, and vitamin D. However, increased concentration of serum cholesterol increases the risk of developing coronary heart disease (Libby *et al.*, 2000).

Phenolic compounds have an anti-obesity effect through the suppression of dyslipidemia, hepatosteatosis and oxidative stress in obese rats (Smith *et al.*, 2004; Kals *et al.*, 2006).

Recently, epidemiological studies suggest that flavonoids have a positive influence on various cardiovascular diseases. These effects were not only attributed to their direct antioxidant properties, which include direct reactive oxygen species scavenging activity and transient metal chelation, but also their direct inhibition of some radical-forming enzymes (*i.e.* xanthine oxidase, NADPH oxidase, and

lipoxigenases), reduction in LDL cholesterol and improvement in vascular reactivity (Psotova *et al.*, 2004; Sanchez *et al.*, 2006; Loke *et al.*, 2008).

To evaluate the anti-hyperlipidemic effect of the quercetin, rutin, lecithin and the two phenolipids (quercetin-enriched lecithin) and (rutin -enriched lecithin), rats were fed with a high-fat diet for eight weeks. The quercetin, rutin, lecithin and the two phenolipids were orally administered. After four weeks of induction, prolonged administration of a high cholesterol diet to animals will accelerate the synthesis of triglycerides, inhibit the metabolism of fatty acids and diminish the secretion of triglycerides from the liver to blood by decreasing the β -oxidation of fatty acids. It leads to the accumulation of excess triglycerides in the liver and the content of total cholesterol in serum increased significantly, compared with the normal control rats (Luo *et al.*, 2009).

As shown in Table 3, the levels of total cholesterol and triglycerides in the high cholesterol diet group were significantly higher than those in the normal control group, which indicated that the model was successful in inducing hyperlipidemia in rats. Over a period of four weeks, compared with the high cholesterol diet group, the levels of serum total cholesterol and triglycerides were suppressed significantly by quercetin, rutin, lecithin and the two phenolipids administration. At the end of the experiment the degree of suppression of total cholesterol and triglycerides levels induced by phenolipids had led to attenuate to normal control levels, suggesting that phenolipids had a potent lipid lowering effect in the hyperlipidemia rats this result are in the same line with (Xin-Rong *et al.*, 2016) who stated that quercetin has poor water solubility and lipid solubility, making it difficult to penetrate cell membrane and leading to low bioavailability. Mixture with phospholipids may considerably improve the physicochemical properties of active components derived from herbal medicines, meanwhile enhance their absorption and pharmacological activities, and reduce adverse reactions. This method has been widely utilized in pharmaceutical sciences. Furthermore, it was also observed that consumption of dietary fat could induce anomalous changes of the levels of serum LDL and HDL. Compared with the normal control group, there was a significant increase in the levels of the serum LDL in the high cholesterol diet group of rats. However, after 30-days treatment with quercetin, rutin, lecithin and the two phenolipids, showed a significant decrease in the levels of serum LDL, and a significant increase in the level of HDL compared with the high cholesterol diet group (Table 4).

Conclusions

In the current study, ethyl acetate was used to dissolve lecithin and quercetin or rutin; however, in order to increase the stability. Currently available information indicates that flavonoids are relatively stable compounds with respect to various modes of processing (*e.g.*, boiling, frying, *etc.*). It means that this synergism can be employed, for example, to increase the antioxidative activity of lecithin that

is usually employed as food additives. Also phenolipids (quercetin-enriched lecithin) and (rutin -enriched lecithin) had significant health benefits and could be explored as a potentially promising food additive for the prevention of hyperlipidemia diseases.

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تقييم اثنين من الفينوليبيدات (الليسيثين مع الكورسيتين) و (الليسيثين مع الروتين) معمليا وحيويا

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أجريت هذه الدراسة بغرض اختبار الليسيثين وخليط من الليسيثين مع الكورسيتين بنسبة ١:١ وخليط من الليسيثين مع الروتين بنسبة ١:١ في حماية نموذج الترولين المخزن تحت ظروف الأكسدة لمدة ١٥ يوما في الظلام عند ٦٠ درجة مئوية، تم اختبار تقدم الأكسدة عن طريق قياس رقم البيروكسيد، كما نوقشت العوامل المؤثرة على استقرار الأكسدة لنموذج الترولين، وقد لوحظت علاقات عكسية بين رقم البيروكسيد واستقرار الأكسدة عند إنهاء التخزين، أيضا تم إجراء دراسة بيولوجية لتقييم تأثير كل من الليسيثين وخليط من الليسيثين مع الكورسيتين بنسبة ١:١ وخليط من الليسيثين مع الروتين بنسبة ١:١ على الجرزان المغذاة على عليقة عالية في محتواها من الدهون (hyperlipidemic rats) وقد كان فعالا للغاية في خفض مستويات الكوليسترول الكلي في الدم، والدهون الثلاثية، الليبوبروتينات منخفضة الكثافة، كما أدى إلى زيادة مستويات مصل الليبوبروتينات عالية الكثافة في الدم، وقد أوضحت النتائج أن الفينوليبيدات (الليسيثين مع الكورسيتين) و(الليسيثين مع الروتين) له فوائد صحية كبيرة ويمكن استخدامها كإضافات غذائية للوقاية من أمراض أكسدة الدهون.

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