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Changes in Proteins, Total Lipids and Phospholipids in Mice Liver Treated with *Urtica pilulifera* Extracts

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Abstract: *Urtica pilulifera* is classified as a popular plant used in remedy. The effect of petroleum ether and 20% methanol extracts for different parts (herb, root and seeds) of this plant were determined in albino mice liver pretreated with 200 ppm (0.25 mL/70-80 g mouce) on protein, lipid and phospholipids (PLs) concentrations as well as phospholipid compositions. Dendrogram for SDS-PAGE showed dissimilarity in mice groups between different extracts and parts compared with control group. Lipid and PL concentrations were decreased. Petroleum ether extracts were more effective in lipid concentration while aqueous methanol extracts were more effective for PL concentration. On the other hand, the two extracts increase phosphatidylcholine, phosphatidylethanolamine, phosphatidic acid and phosphatidylinositol concentrations. The increase in PLs composition was more pronounced in Pet E. extracts than aqueous methanol extracts. In conclusion, *Urtica pilulifera* can be used for many therapeutic goals. It can improve liver tissue by increasing protein concentration, reduce lipid in lipidemic liver and remodels phospholipids compositions which can be used in treatment of cancer diseases.

Key words: *Urtica pilulifera*, lipids, phospholipid compositions

Introduction

Characterization of protein isolates from liver depends on the source of proteins and the compounds affecting it (Zancani *et al*., 2004). Sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE) is currently one of the most commonly used techniques for the characterization and analysis of proteins. It has been extensively used to determine the molecular weights of polypeptide chains and to establish the homogenicity of protein fraction. This method is usually faster than DNA-hybridization method and elaborates phenotypic comparisons between different extracts.

Phospholipids are the major lipid components of cell membrane. Few human diseases have been described to be due to a defect in phospholipids biosynthesis. The benefits of PLs were referred to their dynamic role in the function of membrane protein modulating enzymatic activities which was proved to help normal function and to protect against atherosclerotic heart disease (Yokoyama, 2006). Phospholipids (PLs) are required for providing the essential components of biological membranes and they are important precursors of signaling molecules. One would anticipate that a null mutation in an enzyme required for the biosynthesis of a PL type would be compatible with life, particularly if that mutation resulted in complete elimination of that PL. Phospholipids bilayer does not only surround the protein membrane but it also controls their environments by regulating the fluidity of membranes; furthermore some other functions of biomembranes include their responses to hormones (Hirata and Axelrod, 1980; Prasad and Kumar, 2005). Cellular functions associated with membranes are expected to be modulated by any modifiction in Pls (Schroeder, 1981; Nakano *et al*.,1982; Dobrzynska *et al*., 2005). It was determined that Pls efficaciously prevented or reduced gastric cancer cell peritoneal adhesion formation in numerous studied animals (Jansen *et al*., 2004).

The objective of the present study is the characterization of proteins in mice liver treated with *Urtica pilulifera* crude extracts. Petroleum ether and methanol extracts from different parts of the plant were used in this study to determine the relationship between the whole cell protein fingerprints in control and treated livers. Furthermore, the effects of the two extracts on the amount of total lipid and phospholipids and their compositions in liver tissues were also studied. The most abundant species of PLs are phosphatidylcholine (PC), phosphatidylethanolamine (PE), phosphatidic acid (PA) and phosphatidylinositol (PI), so study in PL composition includes these four constituents.

Materials and Methods

All Chemicals are of analytical grade products of Sigma

Animals

Male albino mice (70-90 g).

Experimental Design

The mice were fed with normal diet. The animals were divided into 7 groups of 7 animals; each three groups were treated with petroleum ether extract from different parts of *Urtica pilulifera* (herb, root and seeds), the other three groups were treated with methanol extract from the same organs of the plant. The last group was served as control and was treated with saline (0.25 mL saline). The six treated groups were injected with 0.5 mL 200 ppm extract intraperitoneal for 10 days. Then the animals were sacrified and livers were removed for analysis.

Tissue Homogenate

The liver was homogenized in deionized distilled water (1:10) W/V by electric homogenizer and then centrifuged for 10 min at 3000 rpm in a cooling centrifuge. The supernatant was used for protein electrophoresis and for protein determination.

Protein Electrophoresis

Sodium dodecylsulphate polyacrylamide gel electrophoresis (SDS-PAGE) of the sample homogenates of the seven groups (one control and 6 treated groups) was carried out as described by Laemmli (1970) using prestained high molecular weight standered marker (Biored, USA). After electrophoresis, the gel was stained with coomassie brilliant blue and destained by acetic acid and methanol.

Dice index of similarity was determined in each isolate (Dice, 1945) and a dendrogram was then constructed (advanced American Biotechnology, UPGMA, USA).

Lipid Extracts

Total lipids were estimated in mice liver tissues by the method of Duncan *et al*. (1987).

Analysis of Phospholipid Compositions

HPTLC of PC, PE, PA and PI on silica gel was carried out by four fold automated multiple developments with chloroform-methanol-2-propanol-triethylamine-0.25% aqueous KCl 60:18:50:36:9, chamber precondition with 0.1 N NH_4OH. Visualization by spraying with 1) 1,6-diphenyl-1,3,5-hexatriene and after intermediate drying with 2) molybdenum blue reagent was carried out according to Dillmer and Lester. Quantification was performed by densitometry at 370/>460 nm. Visual detection limits of down to 10 ng were obtained (Muthing and Radloff, 1998).

Statistical Analysis

Data are expressed as mean±SD and statistically analyzed using analysis of variance (ANOVA) and Least Significant Difference (LSD) post hock test.

Results

Liver proteins in treated mice had revealed 7 to 9 protein bands in Pet E extracts in the different parts and 9 to 12 protein bands in ME of molecular weight varying from 192 to 7.1 KDa had also be obtained (Table 1). A protein band of 173.53 KDa was present only in herb petroleum ether extract treated mice liver tissue homogenates after 10 days of injection while 4 bands appeared in mice liver homogenates treated with root and seed extracts (192, 125.9, 115.36 and 9.26 KDa). In mice liver treated with herb methanol extract, there are four protein bands that are different from those obtained from control group; three bands look like those found in liver mice homogenates treated with root and seeds of the Pet E. extracts (125.9, 115.36 and 9.26 KDa) and only one band looks like that found in root Pet E. extract treated mice liver homogenates. Furthermore, there are other four protein bands that appeared in root and seed ME treated mice liver homogenates that are not found in control liver (148.5, 40.1, 22.6 and 9.26 KDa).

Figure 1 illustrates the cluster analysis on the basis of similarity coefficient values and average likage method of groups formed by SDS-PAGE protein patterns.

Effect of *U. pilulifera* extracts on lipid and phospholipid concentrations in mice liver tissue is presented in Table 2. It was found that all extracts decreased lipid and phospholipids concentrations except in seed ME where lipid concentration increased. The phospholipid compositions in liver tissues were represented in Table 3. The data showed that all determined types of PL, whish are more dominant in Pet E extract, increased.

For simplicity, it was of interest to present the obtained data of phospholipid compositions (mg/gt) in terms of percentage compositions (Table 4). The PL composition ratio R in the liver of mice is expressed by this equation:

$$R = \frac{\text{Change in concentration of PL composition}}{\text{Initial concentration of control PL}} \times 100 \quad (1)$$

Table 1: Effect of *Urtica pilulifera* extracts on mice liver protein profile

		Petroleum ether extracts			Methanol extracts		
M.Wt	Control	Leaves	Roots	Seeds	Leaves	Roots	Seeds
192			+	+			
181.6	+				+		
173.53		+					
156.91	+						
148.5						+	+
133.07							+
125.9			+	+	+	+	
118.14							+
115.36			+	+	+		
110.66	+	+	+	+	+	+	+
104.39							+
99.64	+	+	+	+	+	+	+
60.005						+	+
53.00	+	+	+	+	+		+
40.1						+	+
27.9	+	+	+	+	+	+	
22.6						+	+
18.2				+	+		
14.60	+	+					+
9.26			+	+	+	+	+
7.1		+	+	+			+
Total	8	7	9	9	9	10	12

Table 2: Effect of *Urtica pilulifera* extracts on lipid and phospholipid concentrations of mice liver tissues

Extract	Groups	Lipid Ggt^{-1} Mean±SE	Total phospholipids Ggt^{-1} Mean±SE
---------	1-Control	0.053±0.004	0.019±0.0005
	LSD	(2,3,4,5,6,7)	(2,3,4,5,6,7)
Petroleum-ether	2-Herb	0.028±0.002	0.014±0.0005
	LSD	(1,3,4,5,6,7)	(1,3,4,5,6,7)
	3-Root	0.035±0.003	0.015±0.0005
	LSD	(1,2,4,5,6,7)	(1,2,4,5,6,7)
	4-Seed	0.047±0.001	0.008±0.0001
	LSD	(1,2,3,5,6,7)	(1,2,3,7)
Methanol	5-Herb	0.048±0.001	0.007±0.0005
	LSD	(1,2,3,4,7)	(1,2,3,7)
	6-Root	0.044±0.001	0.006±0.0008
	LSD	(1,2,3,7)	(1,2,3,7)
	7-Seed	0.066±0.003	0.011±0.0009
	LSD	(1,2,3,4,5,6)	(1,2,3,4,5,6)

Table 3: Phospholipid composition levels (μg/g tissue) in mice liver tissues injected with *Urtica pilulifera* extracts

Extract	Groups	Phosphatidyl -choline	Phosphatidyl -ethanolamine	Phosphatidic acid	Phosphatidyl -insitol
---------	1-Control	16	11.6	16.1	15.8
Petroleum-ether	2-Herb	73	61.7	66.3	69.8
	3-Root	81	36.0	36.6	63.0
	4-Seed	59	38.0	36.8	42.0
Methanolic	5-Herb	28	18.4	20.3	30.3
	6-Root	18	17.4	15.4	23.1
	7-Seed	38	32.7	26.9	32.6

Each reading is mean of seven samples

Table 4: Phospholipid composition percents in mice liver tissues injected with *Urtica pilulifera* extracts

Extract	Groups	Phosphatidyl -choline	Phosphatidyl -ethanolamine	Phosphatidic acid	Phosphatidyl -insitol
---------	1-Control	26.94	19.53	27.1	26.6
Petroleum-ether	2-Herb	26.96	22.78	24.48	25.78
	3-Root	37.4	16.62	16.9	29.09
	4-Seed	33.56	21.62	20.93	23.89
Methanolic	5-Herb	28.87	18.97	20.93	31.24
	6-Root	24.36	23.55	20.84	31.26
	7-Seed	29.19	25.12	20.66	25.04

Discussion

The effect of *Urtica pilulifera* extracts on protein, lipid and phospholipids (PL) levels depend on two main factors that are; the type of the used solvent and the plant part which was extracted (herb, root or seeds). Injecting the animals with petroleum ether and methanol extracts of *Urtica* markedly affected all parameters in mice liver. The petroleum ether (Pet E) extracts induced higher change than that of the methanol extracts. This effect may be due to its higher selectivity towards certain biological components. Although methanol is more polar and hence it extracts the polar compounds from the plant tissue, some of these components antagonize with each other; consequently its effect is comparatively low.

SDS-PAGE analysis indicates the multiple differences in protein expression as mutants between different plant extracts which may lead to physiological change in the animal life (Plosch *et al*., 2004).

By cluster analysis of tissue homogenate pattern of the basic similarity coefficient values and average linkage method, the proteins analysis showed different similarities. The occasional appearance of protein fractions of high molecular masses (192, 173.53, 148.5 125.9 and 115.36 KDa) and others of lower masses (60, 40.1, 22.6, 18.2 and 9.26 KDa) in liver tissue homogenates of mice treated with *U pilulifera* extracts (Table 1) may be due to gene expression of

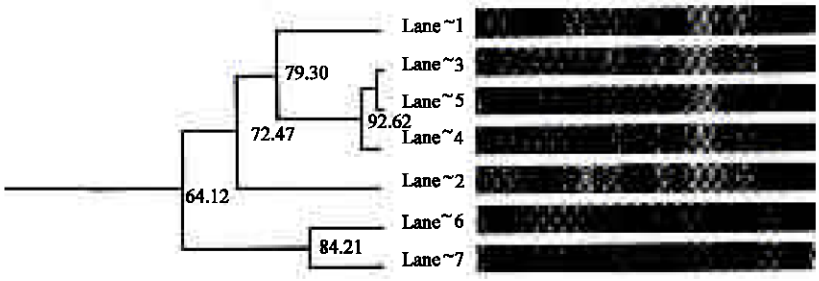

Fig. 1: Dendrogram showing similarity based on protein patterns lanes (1-7)

certain protein masses (Riehemann *et al*., 1999). These results were coincided with Mahmoud *et al*. (2006) who found that injection of the petroleum ether and hydro alcoholic extracts of *Urtica pilulifera* to mice showed a marked effect on protein synthesis, some hepatic biotransformation enzyme systems and antioxidant enzymes. Accordingly, there were alterations in the SDS-PAGE separated protein profile in mice liver homogenates ascertained through comparing the identity index of treated and control mice (Fig. 1).

The degree of dissimilarity of protein bands obtained from mice treated with different extracts showed variations in the type and/or the concentration of the compounds that occurred in the different extracts of each part of the plant as well as among the different solvent extracts. The identified compounds in the methanol *U. pilulifera* herb extracts are also present in Pet E extracts of root and seeds but of different concentrations (Saeed and Omer, 1994; Mahran *et al*.,1994). This can explain the high similarity of protein bands in liver mice treated with root and seeds Pet E extracts and those of herb methanol extract of the same plant.

The change of protein bands in liver homogenates of mice injected with petroleum ether extracts of roots and seeds may be due to high sterol components in these parts, whereas in herbs no change was detected since herbs contains low sterol components (Drsata, 2002; Durak *et al*., 2004).

Alteration in protein's localization might affect its function as well as lipid metabolism and phospholipids transferring and composition (Komatsu *et al*., 2004; Ma *et al*., 2004). It was observed that lipid concentration as well as phospholipids in mice liver of all groups are generally decreased (Table 2). The decrease in lipid concentration in liver tissue is correlated with the previous finding of different authors.

Fekete *et al*. (1990) found that when animal fat diet was mixed with Pet E extract of sunflower, the lipid concentration of the animal decreased. The authors concluded that vegetable oil modified the fatty acid composition of blob lipid due to the presence of polyunsaturated fatty acids.

Asai *et al*. (1999) found that spice extracts has the ability to prevent the deposition of triacylglycerol in the mice liver.

Han *et al*. (2005) found that *Z. offianale* Rosce inhibited the hydrolysis of triolein emulsified with phosphatidylcholine by pancreatic lipase *in vitro* and it reduced the elevation of plasma triacylglycerol levels after oral administration. They also suggested that this extract might inhibit intestinal absorption.

Most of proteins of PL biosynthesis seem to be constitutively expressed (Vance, 1989). Alteration in PL synthesis occurs mainly by changes in the supplied substrates in addition to changes in enzymes activity. This fact can be explained through a change in protein bands (Table 1). The decrease in phospholipids may be due to the presence of polysaccharides extracted with aqueous methanol (Yu and Zhang, 1995) and/or steroid compounds separated by Pet E (Jung *et al*., 2004); which differ in their concentrations according to the type of the plant parts and the used solvent. It was found that when the S180 cell treated with polysaccharide extracted from *Achyranthes bidentata*, the sialic acid increased and PL decreased after 24 h. It is also well known that sterol functions to

modulate the physical properties of membrane PLs. It is undoubted that there is an appropriate interaction between specific sterols and the particular phospholipids found in a given biological system and a small fraction of it may have an essential regulatory function distinct from PL's role modulating membrane lipid physical properties.

Injection with leaves and root Pet E extracts affect phosphatides concentration, in mice liver tissues, more than those of other extracts. This may be due to the possibility that minor components which are present in herb and root extract, decreased the PL concentrations more than other extract parts.

Furthermore, phospholipids composition levels in mice liver treated with Pet E extracts were higher than those treated with ME (Table 3). The presence of a parochial amount of active compound/s may enhance remodeling of PL constituents (Rujanavech and Silbert, 1986). Moreover, excess of choline, free and combined amino acids in plant extracts might help CDP- choline pathway for the production of PC and/or PE (Vance and Vance, 2005). Phosphatedylcholine plays a role in cholesterol clearance in blood being hydrolysable by PLase-A_2 controlling potent lipolysis. The increase level of it, in the liver tissues of treated mice, was in parallel with the absence of 30 KD proteins (Ishigami *et al*., 2004). This protein is responsible for translocating PC from liver to the luminal side of the canalicular membrane where bile salts extract the PC in the form of micelles into bile (Ruetz and Gros, 1995; Vance and Vance, 2005). The increase in PE also helps in synthesis of PC which in turn help in decrease lipid concentration (Muller *et al*., 2004).

All iLps exhibit a net negative charge that is determined by both the apoprotien and lipid constituents of the lipoprotein particle thus, their individual charge varies quantitavely (Sparks *et al*., 1992). The primary anionic lipid lipoprotein particle is PI (Chauhan *et al*., 1998). It normally affects lipoprotein metabolism both by controlling interfacial interactions and uniquely regulating intracellular signaling pathways. PI increased the remodeling ability of PL molecules provided for macrophage recognition and phagocytosis at any stress (Fabisiak *et al*., 2000). PE was further proved to be precognitive marker of appoptic cells preceding the development of other biochemical steps of apoptotic machinery (Shvedova *et al*., 2002; Xie *et al*., 2005). Increased Phosphatidic Acid (PA) may be due to enhance phospholipids enzymatic activity.

In conclusion, *Urtica pilulifera* can be used for many therapeutic goals. It can improve liver tissues by increasing protein concentration, reduce lipid level in lipidemic liver and remodels phospholipids compositions which may help in treatment of cancer diseases.

References

Asai, A., K. Nakagawa and T. Miyazawa, 1999. Antioxidative effects of turmeric, rosemary and capsicum extracts on membrane phospholipids peroxidation and liver lipid metabolism in mice, Biosci. Biotechnol. Biochem., 63: 2118-2122.

Chauhan, V., X. Wang, T. Ramsamy, R.W. Milne and D.L. Sparks, 1998. Evidence for lipid dependent structural changes in specific domains of apolipoprotein B100. Biochemistry, 37: 3735-3742 .

Dice, L.R., 1945. Measures of the amount of ecological association between species. Ecology, 26: 297-302.

Dobrzynska, I., B.S. Petelska, S. Sulkowski and Z. Figaszewski, 2005. Changes in electric charge and phospholipids composition in human colorectal cancer cells. Mol. Cel. Biochem., 276: 113-119.

Drsata, J., 2002. Enzyme inhibition in the drug therapy of benign prostatic hyperplasia. Cas. Lek. Cesk., 141: 630-635.

Duncan, M., F. Bernatd and S. Joseph, 1987. Lipid in fed and starved *Biomphalaria glabrata* (Gastropoda). Comp. Biochem. Physiol., 86A: 663-665.

Durak, I., H. Biri, E. Devrim, S. Sozen and A. Avci, 2004. Aqueous extract of Urtica dioica makes significant inhibition on adenosine deaminase activity in prostate tissue from patients with prostate cancer. Cancer Biol. Ther., 3: 855-857.

Fabisiak, J.P., V.A. Tyurin, Y.Y. Tyurina, A. Sedlov, J.S. Lazo and V.E. Kagan, 2000. Nitric oxide dissociates lipid peroxidation from apoptotsis and phosphatidylserine externalization during oxidative stress. Biochimistry, 39: 127-138.

Fekete, S., I. Hullar, H. Febal and J. Bokori, 1990. The effect of animal fat and vegetable oil supplementation of feeds of different energy concentration upon the digestibility of nutrients and some blood parameters in rabbits. Acta Vet. Hung., 38: 165-175.

Han, L.K., X.J. Gong, S. Kawano, M. Saito, Y. Kimura and H. Okuda, 2005. Antiobesity actions of Zingiber officinale Roscoe. Yakugaku Zasshi, 125: 213-217.

Hirata, F. and J. Axelrod, 1980. Phospholipid methylation and biological signal transmission. Sci., 209: 1082-1090.

Ishigami, A., Y. Kondo, R. Nanba, T. Ohsawa, S. Handa, S. Kubo, M. Akita and N. Maruyama, 2004: SMP30 deficiency in mice causes an accumulation of neutral lipids and phospholipids in the liver and shortens the life span. Biochem. Biophys. Res. Commun., 315: 575-580.

Jansen, M., K.H.Treutner, B. Schmitz, J. Otto, P.L. Jansen, S. Neuss and V. Schumpelick, 2004. Phospholipids reduce gastric cancer adhesion to extra cellular matrix *in vitro*. BMC Gastroenterology, 4: 33.

Jung, E., B. Lee, Y. Yun, J. Kang, I. Baek and J. Yon, 2004. Effect of exposure to genistein and estradiol on reproductive development in immature male mice weaned from dams adapted to a Soy- based commercial diet. J. Vet. Med. Sci., 66: 1347-1354.

Komatsu, H., J. Westerman, G.T. Snoek, T.F. Taraschi and N. Janes, 2004. Effect of D-Myo-inositol 1- phosphate on the transfer function phosphatidylinositol transfer protein alpha. J. Liposome Res., 14: 141-153.

Lamilli, U.K., 1970. Cleavage of structural proteins during the assembly of the head of bacteriophage T4. Nature, 227: 680-685.

Lerner, D.R. and N.V. Raikhel, 1992. The gene for stinging nettle leclin (Urtica dioica agglutinin) encodes both a lectin and achitinase. J. Biol. Chem., 267: 11085-11091.

Ma, D.W., L.A. Davidson, E.S. Callaway, Y.Y. Fan, J.R. Lupton and R.S. Chapkin, 2004. N-3 PUFA alter caveolae lipid composition and resident protein localization in mouse colon. FASB. J., 18: 1040-1042.

Mahmoud, A.H., H.M. Motawa, H.E. Wahba and A.Y. Ebrahim, 2006. Study of some antioxidant parameters in mice livers affected with *Urtica pilulifera* extracts. Asian J. Biochem.,1: 67-74.

Mahran, G.H., A.M. El-Fishawy and A.M. Hilal, 1994. Lipid content and larvicidal activity of the fixed oil of *Jacaranda mimosaefolia* D. Don. Growing in Egypt. Bull. Fac. Pharm. Cairo Univ., 32: 253-257.

Muller, H., L.I. Hellgren, E. Olsen and A. Skrede, 2004. Lipids rich in phosphatidylethanolamine from natural gas-utilizing bacteria reduce plasma cholesterol and classes of phospholipids: A comparison with soybean oil. Lipids, 39: 833-841.

Muthing, J. and M. Radloff, 1998. Nanogram detection of phospholipids on thin-layer chromatograms. Anal. Biochem., 257: 67-70.

Nakano, A., M. Maeda, M. Nishijim and Y. Akamatsu,1982. Phospholipid modification retards intracellular transport and secretion of immunologlobin G1 by mouse MOPC-31C plasmacytoma cells. Biochem. Biophys. Acta, 692: 177-186.

Plosch, T., V. W. Bloks, Y. Terasawa, S. Berdy, K. Siegler, F. Van Der Sluijs, I.P. Kema, A.K. Groen, B. Shan, F. Kuipers and M. Schwarz, 2004. Sitosterolemia in ABC-transporter G5-deficient mice is aggravated on activation of the liver-X receptor. Gastroenterolo.,126: 290-300.

Prasad, R. and V. Kumar, 2005. Thyroid hormones increase Na+-Pi co-transport activity in intestinal brush border membrane: Role of membrane lipid composition and fluidity. Mol. Cell. Biochem., 278: 195-202.

Riehemann, K., B. Behnke and K. Schulze-Osthoff, 1999. Plant extracts from stinging nettle (Urtica dioica), an antirheumatic remedy, inhibit the proinflammatory transcription factor NF-kappaB. FEBS Lett., 442: 89-94.

Ruetz, S. and P. Gros, 1995. Enhancement of Mdr2-mediated phosphatidylcholine translocation by the bile salt taurocholate. Implications for hepatic bile formation. J. Biol. Chem., 270: 15395-25388.

Rujanavech, C. and D.F. Silbert, 1986. LM cell growth and membrane lipid adaptation to sterol structure. J. Biol. Chem., 261: 7196-7203.

Saeed, A. and E. Omer, 1994. A study of the mucilage and free sugars of *Nymphaea hybrida* (Tach.V) and *Nelumbo nucifera* (Gaertn). Bull. Fac. Pharm., 32: 183-185.

Schroeder, F., 1981. Altered phospholipids composition affects endocytosis in cultured LM fibroblasts. Biochem. Bophys. Acta, 649: 162-174.

Sparks, D.L. S. Lund-Katz and M.C. Phillips, 1992. The change and structural stability of apolipopotein A-I in discoidal and spherical recombinant high density lipoprotein particales. J. Biol. Chem., 267: 25839-25847.

Shvedova, A.A., J.Y. Tyurina, K. Kawai, Tyurin, C. Kommineni, V. Castranova, J.P. Fabisiak and V.E. Kagan, 2002. Selective peroxidation and externalization of phosphatidylserine in normal human epidermal keratinocytes during oxidative stress induced by cumene hydroperoxide. J. Investig. Dermatol., 118: 1008-1018.

Vance, D.E., 1989. Regulatory and functional aspects of phosphatidylcholine metabolism. In: Phosphatidylcholine Metabolism, D.E. Vance, Ed. CRC Press, Boca Raton. FL., 225-239.

Vance, J.E. and D.E. Vance, 2005. Metabolic insights into phospholipids function using gene-targeted mice. J . Biol. Chem., 280: 10877-10880.

Xie, Y., Y.Q. Ding, Y. Hong, Z. Feng, S. Navarre, C.X. Xi, X.J. Zhu, C.L. Wang, S.L. Ackerman, D. Kozlowski, L. Mei and W.C. Xiong, 2005. Phosphatidylinositol transfer protein-alpha in netrin-1-induced PLC signaling and neurite outgrowth. Nat. Cell. Biol., 7: 1052-1053.

Yokoyama, S., 2006. Assembly of high-density lipoprotein. Arterioscler. Thromb. Vasc. Biol., 26: 20-27.

Yu, S. and Y. Zhang, 1995. Effect of Achyranthes Bidentata Polysaccharides (ABP) on antitumor activity and immune function of S180-bearing mice. Zhonghua Zhong Lui Za Zhi,17: 275-278.

Zancani, M., C. Peresson, A. Biroccio, G. Federici, A. Urbani, I. Murgia, C. Soave, F. Micali, A. Vianello and F. Macri 2004. Evidence for the presence of ferritin in plant mitochondria. Eur. J. Biochem., 271: 3657-3664.