Effect of Lecithin on D-Galactosamine Induced Hepatotoxicity Through Mitochondrial Pathway Involving Bcl-2 and Bax

P. Vasanth Raj · K. Nitesh · Jain Prateek ·
M. Neena Sankhe · J. Venkata Rao ·
C. Mallikarjuna Rao · N. Udupa

Received: 28 March 2011 / Accepted: 3 August 2011 / Published online: 23 August 2011
© Association of Clinical Biochemists of India 2011

Abstract Twenty four Wistar strain albino rats were used for the investigations. Lecithin 50 and 100 mg/kg b wt was administered for 1 week by oral route. Liver damage was induced by intra peritoneal administration of 400 mg/kg b wt D-galactosamine on the last day. At the end of the study animals were sacrificed and liver enzyme levels, histopathology, mitochondrial integrity, expression of p53, Bax and Bcl-2 mRNA levels were studied. Increases in the liver enzyme levels by D-GalN were significantly inhibited by pretreatment with lecithin. Histopathological observation further confirmed the hepatoprotective effect of lecithin. In addition, the disruption of mitochondrial membrane, up regulation of Bax and down regulation of Bcl-2 mRNA levels in the liver of D-GalN intoxicated rats were effectively prevented by pretreatment with lecithin. The results of the present study validate our conviction that D-GalN causes hepatic damage via mitochondrial pathway involving Bax and Bcl-2.

Keywords Lecithin · D-galactosamine · Mitochondria · Bax · Bcl-2

Introduction

Lecithin, an important phospholipid is found in the major organs in our body such as the heart, the liver, and the kidneys [1, 2]. Lecithin, a component of most cells, will

help in transport and responsible for overall health of the body. Though it is produced within our own bodies, we do not always consume enough of the nutrition needed to produce it in adequate amounts [1, 2]. As a result, lecithin supplementation is necessary for overall health and prevention of many conditions and diseases.

Lecithin is composed of phosphoric acid, choline, fatty acids, glycerol, glycolipids, triglycerides and phospholipids (e.g., phosphatidylcholine, phosphatidylethanolamine and phosphatidylinositol) [1, 2]. However, lecithin is sometimes used as a synonym for pure phosphatidylcholine a phospholipid that is the major component of its phosphatide fraction. It may be isolated either from egg yolk or soya beans.

Few studies have been carried out exploring different biological activities of lecithin. Soya-derived lecithin have significant effects on lowering cholesterol and triglyceride, while increasing HDL levels in the blood [1, 2]. Effect of lecithin on ethanol induced oxidative stress was reported [3]. Phosphatidylcholine synthesis in hepatocytes [4], lipoprotein secretion from hepatocytes [5, 6], role of phosphatidylcholine biosynthesis [7], The role of phosphatidylethanolamine methylation in hepatocytes [8] and role of lecithin and favorable changes in lipoprotein composition in hypercholesterolemic rats were studied [9, 10]. Also hepatic uptake and metabolism of phosphatidylcholine associated with high density lipoprotein was reported [11]. However, no investigation on the effect of lecithin on the expression of genes involved in liver cell apoptosis has been reported. Since antioxidant activity of lecithin is well known, it will be interesting to know its hepatoprotective nature and the expression pattern of important genes involved in liver before and after D-galactosamine (D-GalN) induced toxicity and apoptosis. Hence, in this study we report the activity of lecithin in D-galactosamine

P. V. Raj · K. Nitesh · J. Prateek · M. N. Sankhe ·
J. V. Rao · C. M. Rao (✉) · N. Udupa
Department of Pharmacology, Manipal College
of Pharmaceutical Sciences, Manipal University,
Manipal 576104, Karnataka, India
e-mail: mallikin123@gmail.com

(D-GalN) induced toxicity and the expression of Bax, Bcl-2 and p53 levels.

Materials and methods

Materials

All routine chemicals were obtained from SD-fine chemicals, Mumbai, India. Mitochondrial isolation and staining kit, Primers for Bax, Bcl-2 and GAPDH and D-galactosamine were purchased from Sigma-Aldrich Co., St. Louis, MO, USA. Primers for p53 was synthesized and purified by Bioserve, India. Cobas kits for ASAT, ALAT, ALP and LDH were purchased from Roche diagnostics, USA. Soy lecithin pure sample was a gift from Perfect Biotech, Nagpur, India. Trizol Reagent, dNTPs and 100 bp DNA ladder was purchased from Invitrogen Life Technologies Co, USA. RT PCR enzymes, RT PCR buffers, oligo dT primers and *Thermus thermophilus* DNA polymerase enzyme was procured from Merck, Germany.

Animals

Colony bred Wistar stain adult albino rats (150–200 g) of either sex were used for the investigations. All the animals were maintained under standard husbandry conditions with food and water ad libitum. The experimental procedures were approved by the Institutional Animal Ethics Committee (IAEC), KMC, Manipal, India (No. IAEC/KMC/06/2006-2007).

Lecithin administration and D-galactosamine (D-GalN) challenge

The animals were divided into four groups of six animals in each group. Liver damage was induced by intra peritoneal administration of 400 mg/kg b wt D-galactosamine (D-GalN). Group I received only the vehicle (sodium CMC 0.3%) and served as control. Group II served as toxicant D-GalN (400 mg/kg b wt) treated control. Group III received lecithin (50 mg/kg b wt). Group IV received lecithin (100 mg/kg b wt). The animals received these treatments by the oral route for a period of 7 days. On the seventh day except group I, all other groups received 400 mg/kg b wt of D-GalN by intra-peritoneal administration. Blood was withdrawn and collected in sterile centrifuge tubes and allowed to clot.

Tissue homogenate preparation and serum collection

After 24 h of intoxication, on the 8th day all animals were sacrificed and serum was collected by centrifugation of

blood at 3500×g (Eppendorf 5415R, Germany) for 10 min at 4°C and stored at –80°C (Sanyo, Japan). Rinsed with ice cold isotonic saline, dissected hepatic samples were quickly dried by blotting between two pieces of filter paper; one lobe is immersed into liquid nitrogen for over 10 min and then stored at –80°C used for Reverse transcriptase PCR. Other fresh liver sample is processed for histopathology and mitochondrial isolation.

Determination of serum ASAT, ALAT, ALP and LDH level

Serum Aspartase aminotransferases (ASAT), Alanine aminotransferases (ALAT), Alkaline Phosphatase (ALP) and lactate dehydrogenase (LDH) levels were measured with enzymatic kinetic method by automatic biochemical analyzer (Cobas, USA) using kits.

Histopathology

The fresh liver samples were processed according to the classical procedure using hematoxylin–eosin staining [12]. Briefly, liver tissues were cut into about 3-mm-thick slices and fixed with 10% phosphate-buffered formalin (pH 7.4). The tissue slices were dehydrated and embedded in paraffin. Tissue sections of 5–8 µm were stained by hematoxylin and eosin, and were observed with Olympus phase contrast microscope with Motic imaging system, China.

Isolation of total RNA and reverse transcription polymerase chain reaction (RT PCR)

Total RNA from hepatic tissues was extracted using trizol reagent following the manufacturer's instructions and quantified by DNA protein enzyme analyser (Shimadzu, Japan). cDNA synthesis and amplification was performed by PCR apparatus (Eppendorf Germany) in a volume of 50 µl comprising of 2 µl of 2 µg total RNA, 2 µl of 5 µM oligo(dT), 25 µl of RT PCR master mix (0.25 mM dNTP, 10 U of RNase Inhibitor, 100 U of reverse transcriptase), 3 µl of 50 mM Mn(0Ac)₂, 2 µl of respective forward and reverse primers and 16 µl of RNase free water. Polymerase activation was done at 90°C for 30 s and reverse transcription was performed at 60°C for 30 min. *Thermus thermophilus* DNA polymerase enzyme was used for cDNA synthesis step and PCR amplification step. The sequences of the PCR primers for Bax (NM_017059) were 5'-CCA AGA AGC TGA GCG AGT GTC TC-3' (forward) and 5'-AGT TGC CAT CAG CAA ACA TGT CA-3' (reverse), Bcl-2 (NM_016993) were 5'-GGA GCG TCA ACA GGG AGA TG-3' (forward) and 5'-GAT GCC GGT TCA GGT ACT CAG-3' (reverse), p53 (NM_030989) were 5'-CAG CTT TGA GGT TCG TGT TTG T-3' (forward) and 5'-ATG CTC TTC TTT TTT GCG GAA A-3'

(reverse) and the sequence for GAPDH (NM_017008) were 5'-CCA AGA AGC TGA GCG AGT GTC TC-3' (forward) and 5'-CCT GCT TCA CCA CCT TCT TG-3' (reverse). The cycle condition of PCR amplification process consisted of 40 cycles, including denaturation at 94°C for 1 min, annealing at 60°C for GAPDH, 51°C for Bax and Bcl-2 and 46°C for p53 for 30 s, and extension at 72°C for 1 min with 1 cycle of final extension at 60°C for 7 min. The predicted sizes of the amplified products of Bax, Bcl-2, p53 and GAPDH were 487, 127, 82 and 349 bp respectively. Equal amounts of corresponding products of Bax, Bcl-2, p53 and GAPDH were separated by 1.5% agarose gel electrophoresis (Bangalore Genei, India) and optical densities of ethidium bromide-stained DNA bands were quantified by Alpha Innotech software, USA.

Mitochondrial isolation

Mitochondria were isolated from rat liver as previously described [13]. Briefly, the tissue was manually homogenized by four strokes with a Teflon pestle in solution I containing 230 mM mannitol, 70 mM sucrose, 1 mM ethylene glycol tetraacetic acid (EGTA) and 5 mM HEPES (pH 7.4) on ice. After centrifugation [100×g for 80 s at 4°C], the supernatant was layered in solution II [460 mM mannitol, 14 mM sucrose, 1 mM EGTA and 10 mM HEPES (pH 7.4)] and centrifuged at 2000×g for 5 min at 4°C. The mitochondrial pellet was resuspended in 215 mM mannitol, 71 mM sucrose, 10 mM succinate and 10 mM HEPES (pH 7.4), and kept on ice until mitochondrial staining procedure.

Isolated mitochondrial staining

Isolated mitochondrial preparation was stained with help of JC-1 (5,5,6,6'-tetrachloro-1,1'-3,3'-tetra ethyl benzimidazolocarbo-cyanine iodide) dye. The concentration of mitochondrial preparation was diluted to 40 µg/ml and used for staining. Final concentration of JC-1 staining solution was 0.2 µg/ml. 90 µl of JC-1 staining solution was added to 10 µl of isolated mitochondrial sample and excitation wave

length of 490 nm and emission wave length of 590 nm was used to visualize the samples with help of Olympus inverted microscope with fluorescence attachment (Olympus, USA).

Statistical analysis

The statistical analysis was carried out by one way analysis of variance (ANOVA). The values are represented as mean ± S.E.M. Probability value of $P < 0.05$ was determined to be statistically significant.

Results

Determination of serum ASAT, ALAT, ALP and LDH level

All rats survived the experimental period until sacrifice. Intoxication of rats with D-GalN (400 mg/kg) significantly altered the biochemical parameters when compared with the normal control rats ($P < 0.001$ Table 1). A significant increase in the levels of ASAT, ALAT, ALP and LDH levels were observed in toxicant group. Treatment with lecithin at 50 and 100 mg/kg b wt significantly decreased the level of ASAT, ALAT, ALP and LDH levels towards normal. All these significant changes in the levels of biochemical parameters refer to the effect of lecithin in protecting the liver by restoring the altered levels in rats.

Histopathology

Normal histological structures of hepatic lobules were observed in normal liver (Fig. 1). Group treated with D-GalN showed complete damage to hepatocytes with hepatocellular vacuolization, focal hepatic necrosis and congestion of hepatic sinusoids (Fig. 2). Lecithin at a dose of 50 and 100 mg/kg b wt showed good improvement in hepatocytes as compared to that of D-GalN treated animals (Figs. 3, 4). There was slight congestion and mild vacuolization observed in lecithin treated groups.

Table 1 Effect of lecithin on the biochemical parameters of D-galactosamine intoxicated rats

Treatment group	Concentration	ASAT (U/l)	ALAT (U/l)	ALP (U/l)	LDH (U/l)
Control	—	66.60 ± 2.207	27.51 ± 1.15	325.30 ± 4.874	333.04 ± 6.399
D-Galactosamine (D-GalN)	400 mg/kg	103.20 ± 2.525 ^a	61.43 ± 0.5619 ^a	503.10 ± 1.881 ^a	655.80 ± 3.84 ^a
Lecithin	50 mg/kg	85.83 ± 1.254 ^{a, y}	43.87 ± 1.029 ^{b, y}	391.7 ± 1.409 ^{a, x}	393 ± 2.1 ^{a, x}
	100 mg/kg	78.79 ± 1.871 ^x	36.23 ± 1.517 ^x	350.7 ± 7.919 ^{a, x}	378.8 ± 9.22 ^{a, x}

Values are ± S.E.M of six animals. Superscripts a, b and c denote statistical significance in comparison to normal group $P < 0.001$, $P < 0.01$ and $P < 0.05$ respectively. Superscripts x and y denote statistical significance in comparison to D-galactosamine (D-GalN) group at $P < 0.001$ and $P < 0.01$ respectively

Fig. 1 Normal liver having histological structures of normal hepatic lobules

Fig. 3 Lecithin (50 mg/kg bt wt) treated group showing mild vacuolization

Fig. 2 D-GalN treated liver showing damage to hepatocytes with hepatocellular vacuolization, focal hepatic necrosis and congestion of hepatic sinusoids

Fig. 4 Lecithin (100 mg/kg bt wt) treated group showing apparently normal hepatocytes

Reverse transcriptase PCR analysis

p53 mRNA expression

Reverse transcriptase PCR was used to analyze the levels of p53 mRNA in livers of rats treated with D-GalN and their control group in comparison with lecithin (50 and 100 mg kg bt wt) pretreated groups. Amplification of p53 was found in cDNA prepared from livers, and the specificity was confirmed using agarose gel electrophoresis. D-GalN caused marginal increase in p53 levels in the livers when compared with the normal control livers. The treatment of lecithin (50 and 100 mg/kg bt wt) resulted in decrease in the level of p53 back to normal (Fig. 5).

Bax mRNA expression

The mRNA levels of Bax in livers are shown in Fig. 6. The levels of Bax in livers of D-GalN treated rats increased significantly compared with control group. Only a marginal increase was detected in lecithin pretreated groups when compared with the normal.

Bcl-2 mRNA expression

Figure 7 presents Bcl-2 mRNA levels in livers of rats exposed to D-GalN with and without lecithin pretreatment in comparison with control group. D-GalN at a dose of 400 mg/kg bt wt caused significant decrease of Bcl-2 mRNA levels when compared with that of the normal

Fig. 5 The mRNA expression of p53 by RT PCR. Gr. I: untreated, Gr. II: D-GalN treated, Gr. III: Lecithin 50 mg/kg bt wt + D-GalN treated, Gr. IV: Lecithin 100 mg/kg bt wt + D-GalN treated. The data is represented as the mean of $\pm$ S.E.M of five independent experiments. **Shows significant increase over untreated Gr. I, $P < 0.001$. *Shows significant decrease over Gr. II, $P < 0.001$

Fig. 6 The mRNA expression of Bax by RT PCR. Gr. I: untreated, Gr. II: D-GalN treated, Gr. III: Lecithin 50 mg/kg bt wt + D-GalN treated, Gr. IV: Lecithin 100 mg/kg bt wt + D-GalN treated. The data is represented as the mean of $\pm$ S.E.M of five independent experiments. **Shows significant increase over untreated Gr. I, $P < 0.001$. *Shows significant decrease over Gr. II, $P < 0.001$

group. Whereas no alteration in the expression of Bcl-2 levels was observed in livers from rats pretreated with lecithin (50 and 100 mg/kg bt wt).

Fig. 7 The mRNA expression of Bcl-2 by RT PCR. Gr. I: untreated, Gr. II: D-GalN treated, Gr. III: Lecithin 50 mg/kg bt wt + D-GalN treated, Gr. IV: Lecithin 100 mg/kg bt wt + D-GalN treated. The data is represented as the mean of $\pm$ S.E.M of five independent experiments. **Shows significant decrease over untreated Gr. I, $P < 0.001$. *Shows significant increase over Gr. II, $P < 0.05$

Isolated mitochondrial staining to study inner membrane potential

Mitochondrial fraction was prepared from the livers of rats from normal group, D-GalN groups and lecithin pretreated group (Fig. 8). Mitochondrial inner membrane potential was studied from the uptake of the cationic carbocyanine dye JC-1 into the mitochondrial matrix. In normal group, this dye concentrates in the matrix and bright red fluorescence was observed. In D-GalN treated group a shift from red to green fluorescence was observed which indicates damage to the inner membrane potential, hence prevents the accumulation of the JC-1 dye in the mitochondrial matrix. In lecithin (50 and 100 mg/kg bt wt) treated groups showed red fluorescence which indicate mitochondrial inner membrane integrity was maintained.

Discussion

Liver injuries induced by D-GalN are the best characterized system of xenobiotic-induced hepatotoxicity and commonly used models for the screening of anti-hepatotoxic and/or hepatoprotective activities of drugs. The changes associated with D-GalN induced liver damage are similar to that of acute viral hepatitis [14, 15]. Hence, D-GalN mediated hepatotoxicity was chosen as the experimental model. D-GalN acts directly or indirectly and alters antioxidant

Fig. 8 Isolated mitochondrial staining. **a** Normal liver, the dye JC-1 concentrates in the matrix and bright *red* fluorescence was observed. **b** D-GalN treated liver. Shift from *red* to *green* fluorescence was observed up on incubation with JC-1 dye, which indicates damage to the inner membrane potential. **c** Lecithin (100 mg/kg bt wt) + D-GalN treated liver show *red* fluorescence up on incubation with JC-1 dye, which indicate mitochondrial inner membrane integrity was maintained

status that makes certain organs, more susceptible to oxidative stress. Several studies have shown that D-GalN causes alteration of liver marker enzymes [16]. D-GalN is known to selectively block the transcription and indirectly hepatic protein synthesis and as a consequence of endotoxin toxicity, it causes fulminant hepatitis [15, 16]. The toxicity of D-GalN results from inhibition of RNA and protein synthesis in the liver. The metabolism of D-GalN may deplete several uracil nucleotides including UDP-glucose and UDP-galactose. Accumulation of UDP-sugar nucleotide may contribute to the change in the rough endoplasmic reticulum and to the disturbance of protein metabolism [14, 15]. Intense D-GalN of the membrane structures is thought to be responsible for loss in the activity of ionic pumps. The impairment in the calcium pumps, with consequent increase in the intracellular calcium is considered to be responsible for cell death [15, 16]. D-GalN has been found to induce extensive liver damage within a period of 24 h following intra-peritoneal administration. Hence we decided to study the levels of expression of important genes such as p53, Bax and Bcl-2 involved in regulation of apoptosis before and after D-GalN intoxication, further to study the effect of lecithin against D-GalN induced hepatotoxicity.

Apoptosis is programmed cell death characterized by cytoplasmic fragmentation and nuclear condensation [17]. Apoptosis signaling pathways fall under two categories: receptor-mediated apoptosis and mitochondrial-dependent apoptosis. Receptor mediated apoptosis involves the triggering of cell surface “death receptors” a specialized

subset of the tumor necrosis factor receptor superfamily that includes Fas (CD95/APO-1). Stimulation of Fas by its natural ligand, FasL, or by cross linking the receptor with agonistic anti-Fas antibodies results in activation of a series of cysteine proteases (caspases). This culminates in the cleavage of a variety of substrates, such as DNA repair enzymes, cellular and nuclear structural proteins, endonucleases, and many other cellular constituents. Mitochondrial-dependent apoptosis occurs in response to certain liver insults and to drug administration [18]. Bcl-2, an oncoprotein, functions as a suppressor of apoptosis, and its down regulation causes tumor regression [19]. On the other hand, predominance of Bax, a proapoptotic protein over Bcl-2 promotes apoptosis [20]. Studies have also shown that the ration of Bax to Bcl-2 proteins increases during apoptosis [21]. The process is initiated by the translocation or activation of proapoptotic genes to the mitochondrial membrane. Permeabilization of the outer mitochondrial membrane results in release of cytochrome c into the cytosol, where it combines with caspase-9 to activate the terminal caspase cascade, resulting in apoptosis. Hence the ratio of proapoptotic vs antiapoptotic molecules will determines the fate of the cell.

Further, p53 plays a key role in the process of apoptosis. p53 is a nuclear phosphoprotein induced in response to cellular stress, functioning as a transcriptional transactivator in DNA repair, apoptosis and tumor suppression pathways [22]. p53 mediated apoptosis involves multiple mechanisms including modulation of the expression of Bcl-2, Bax and other BH3 only proteins, amplification of

death signals and activation of caspases [23]. Hence in this present study more importance was given for the study of genes such as P53, Bax, Bcl-2 and mitochondrial integrity.

The main goal of this study was to obtain a better understanding of the mechanism responsible for the D-GalN induced hepatotoxicity and to study the effect of lecithin against D-GalN induced hepatotoxicity. The results of the present study validate our conviction that D-GalN causes hepatic damage via up regulation of Bax levels and down regulation of Bcl-2 levels and inducing mitochondrial damage. Further Lecithin at a dose of 50 and 100 mg/kg bw prevented the up regulation of Bax and maintained mitochondrial integrity hence, protected the liver from D-GalN induced toxicity in rats. These results substantiate our previous research work Raj et al. [24].

Conclusion

This present work is an expansion of our previous research work on in vitro models using hepatocytes which was published in Indian journal of clinical biochemistry [24]. Our current results clearly highlight the key role of Bax protein in triggering the mitochondrial mediated apoptotic pathway in D-GalN induced toxicity. mRNA levels of Bax increased significantly, while Bcl-2 mRNA levels decreased in livers of D-GalN treated rats. Further D-GalN intoxication caused loss of mitochondrial membrane integrity. Thus, these observations hold promise for further molecular target oriented studies. Since P53 expression was not very significant, it will be interesting to investigate the genes involved in p53 independent pathways leading to apoptosis.

Acknowledgments We thank All India Council of Technical Education (AICTE) for funding (200-62/FIN/04/05/1784/268). We also thank Perfect Biotech, Nagpur, India for providing the pure samples of soy lecithin.

References

- Iwata T, Kimura Y, Tsutsumi K, Furukawa Y, Kimura S. The effect of various phospholipids on plasma lipoproteins and liver lipids in hypercholesterolemic rats. *J Nutr Sci Vitaminol*. 1993;39:63–71.
- Jimenez MA, Scarino ML, Vignolini F, Mengheri E. Evidence that polyunsaturated lecithin induces a reduction in plasma cholesterol level and favorable changes in lipoprotein composition in hypercholesterolemic rats. *J Nutr*. 1990;120:659–67.
- Das SK, Vasudevan DM. Modulation of lecithin activity by vitamin-B complex to treat long term consumption of ethanol induced oxidative stress in liver. *Indian J Exp Biol*. 2006;44:791–801.
- Kulinski A, Vance DE, Vance JE. A choline-deficient diet in mice inhibits neither the CDP-choline pathway for phosphatidylcholine synthesis in hepatocytes nor apolipoprotein B secretion. *J Biol Chem*. 2004;279:23916–24.
- Vance JE, Vance DE. Specific pools of phospholipids are used for lipoprotein secretion by cultured rat hepatocytes. *J Biol Chem*. 1986;261:4486–91.
- Yao Zemin, Vance Dennis E. The active synthesis of phosphatidylcholine is required for very low density lipoprotein secretion from rat hepatocytes. *J Biol Chem*. 1988;263:2998–3004.
- Vance JE, Vance DE. The role of phosphatidylcholine biosynthesis in the secretion of lipoproteins from hepatocytes. *Can J Biochem Cell Biol*. 1985;63:870–81.
- Nishimaki-Mogami T, Suzuki K, Takahashi A. The role of phosphatidylethanolamine methylation in the secretion of very low density lipoproteins by cultured rat hepatocytes: rapid inhibition of phosphatidylethanolamine methylation by bezafibrate increases the density of apolipoprotein b48-containing lipoproteins. *Biochim Biophys Acta*. 1996;1304:21–31.
- Iwata T, Hoshi S, Takehisa F, Tsutsumi K, Furukawa Y, Kimura S. The effect of dietary safflower phospholipid and soyabean phospholipid on plasma and liver lipids in rats fed a hypercholesterolemic diet. *J Nutr Sci Vitaminol*. 1992;38:471–9.
- Iwata T, Hoshi S, Tsutsumi K, Furukawa Y, Kimura S. Effect of dietary safflower phospholipid on plasma and liver lipids in rats fed a hypercholesterolemic diet. *J Nutr Sci Vitaminol*. 1991;37:591–600.
- Robichaud JC, van der Veen JN, Yao Z, Trigatti B, Vance DE. Hepatic uptake and metabolism of phosphatidylcholine associated with high density lipoproteins. *Biochim Biophys Acta*. 2009;1790:538–51.
- Keegan A, Martini R, Batey R. Ethanol-related liver injury in the rat: a model of steatosis, inflammation and pericentral fibrosis. *J Hepatol*. 1995;23:591–600.
- Galindo MF, Jordan J, Gonzalez-Garcia C, Cena V. Chromaffin cell death induced by 6-hydroxydopamine is independent of mitochondrial swelling and caspase activation. *J Neurochem*. 2003;84:1066–73.
- Chaung SS, Lin CC, Lin J, Yu KH, Hsu YF, Yen MH. The hepatoprotective effects of *Limonium sinense* against carbon tetrachloride and beta-D-galactosamine intoxication in rats. *Phytother Res*. 2003;17:784–91.
- Nakagiri R, Hashizume E, Kavaishi S, Sakai Y, Kamiya T. Suppression by *Hydrangeae Dulcis* Folium of D-galactosamine-induced liver injury in vitro and in vivo. *Biosci Biotechnol Biochem*. 2003;67:2641–3.
- Tang XH, Gao L, Gao J, Fan YM, Xu LZ, Zhao XN, et al. Mechanisms of hepatoprotection of *Terminalia catappa* L. extract on D-galactosamine-induced liver damage. *Am J Chin Med*. 2004;32:509–19.
- Cummings MC, Winterford CM, Walker NI. Apoptosis. *Am J Surg Pathol*. 1997;21:88–101.
- Kromer G, Reed JC. Mitochondrial control of cell death. *Nat Med*. 2000;6:513–9.
- Kluck RM, Bossy-Wetzel E, Green DR, Newmeyer DD. The release of cytochrome c from mitochondria: a primary site for Bcl-2 regulation of apoptosis. *Science*. 1997;275:1132–6.
- Wolter KG, Hsu YT, Smith CL, Nechushtan A, Xi XG, Youle RJ. Movement of Bax from cytosol to mitochondria during apoptosis. *J Cell Biol*. 1997;139:1281–92.
- Green DR, Reed JC. Mitochondria and apoptosis. *Science*. 1998;281:1309–12.
- Yan Y, Shay JW, Wright WE, Mumby MC. Inhibition of protein phosphatase activity induces p53-dependent apoptosis in the absence of p53 transactivation. *J Biol Chem*. 1997;272:15220–6.
- Mihara M, Erster S, Zaika A, Petrenko O, Chittenden T, Pancoska P, et al. p53 has a direct apoptogenic role at the mitochondria. *Mol Cell*. 2003;11:577–90.
- Raj PV, Nitesh K, Chandrashekhara HR, Mallikarjuna Rao C, Venkata Rao J, Udupa N. Effect of lecithin and silymarin on D-galactosamine induced toxicity in isolated hepatocytes and rats. *Ind J Clin Biochem*. 2010;25:169–74.