

RESEARCH ARTICLE

Comparison between ursodeoxycholic acid and silymarin in anticonvulsive drugs induced hypertransaminasemia

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Liver is the central organ to metabolize almost all drugs and foreign substances in body and liver injury is a potential complication of nearly every drug that is prescribed. In this randomized open-label clinical trial we have compared Ursodeoxycholic acid (UDCA) versus Silymarin effectiveness and tolerability in anticonvulsant induced hypertransaminasemia in children. Silymarin is an antioxidant and UDCA is a primary bile acid with hepatoprotective effects. 54 children aged between 4mo-14yr with anticonvulsant induced hypertransaminasemia were randomized based on block randomization in two groups; they were recruited over two year (2012 to 2014) from Valiasr hospital pediatric neurology clinic, a referral and public educational center. Other common causes of hepatitis and anatomic anomalies were excluded before randomization. Any patient with viral, autoimmune or metabolic evidences or transaminases levels under twice the upper normal limits or without parents' consent was excluded from study. None of patients were suffered from functional liver failure. We used UDCA (with commercial name of Ursobil) capsule 250 mg in dose of 10-15 mg/kg/day once a day and silymarin (with commercial name of Livertgol) 70 mg tablet in dose of 5 mg/kg/day once a day for one month and followed our patients for another month. 46 patients (25 boys and 21 girls) completed two months trial and follow up. Pre intervention transaminases quantities were similar in both groups. After one month trial transaminases decreased in both groups significantly ($P < 0.05$) except for γ GT in UDCA group. Normalization of transaminases (AST and ALT less than 40 IU/l) was occurred in 3 patients in silymarin group and 5 patients in UDCA group. Comparing between UDCA and silymarin, ALT changes were better in silymarin group ($P = 0.017$). Both of them were tolerated well and no known side effects of them seen.

Keywords: Drug induced hepatitis; Silymarin; Ursodeoxycholic acid

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Introduction

Liver is the central organ to metabolize almost all substances entering body and drug -induced injury in liver is a potential complication of almost every medication that is prescribed [1].

"Drug induced hepatotoxicity is a common concern in

anticonvulsive therapy especially when multiple drugs are used. In liver metabolism, activation and detoxification are two stages should be passed in turn to activate the medicine showing the pharmacologic effects and then evacuate its metabolite safely through the urine or bile [2]." Any imbalance between these two successive processes can lead to hepatic injury. In the activation phase; oxidation or demethylation occurs, mediated by cytochrome P450, a gene

superfamily with nearly 300 members [1]. In multi drugs regimen the mechanism of injury is believed to be the induction of cytochrome P450 by one agent, and increase the toxic metabolite formed from the other.

"Ursodeoxycholic acid (UDCA) is a primary bile-acid that's synthesized lightly in human body [2]."

UDCA is a choleric agent with direct Cytoprotective effect; it can improve mitochondrial oxidative phosphorylation and stabilize cell membrane. Also it has immunomodulatory effects through reducing HLA Class I antigens expression on hepatocytes and anti-apoptotic effects. [3-6] UDCA may have direct antioxidant effects in vivo specially by blocking the biomolecular oxidative damage dependent to Fe^{3+} and OH^- radicals [7-9].

Many studies have shown that UDCA improves liver function by three major mechanisms of action, including cell permeability prevention, stimulation of bile secretion, and inhibition of liver cell death [9].

There are trials of UDCA in hepatitis C patients to improve transaminases response [10, 11].

In drug-induced liver disease steroid and UDCA may be beneficial [12].

UDCA has been tried in chronic active hepatitis, hyperbilirubinemia and cholestasis in PICU patients, acute hepatitis, autoimmune hepatitis and nonalcoholic steatohepatitis [13-17].

There are few case reports of beneficial effects of UDCA therapy in toxic hepatitis [18-22].

There are also experimental trials indicating preventive effects of UDCA in drug induced liver damage based on its hepatoprotective and antioxidant properties [23, 24].

It has been recommended to prescribe UDCA in treatment of chronic hepatitis and drug induced cholestasis because of its efficacy and tolerability [12].

Silymarin is a flavonoid extracted from the seeds of *silybum marianum* or milk thistle. Silymarin shows hepatoprotection properties through antioxidant action (regulation of glutathione), cell membrane stabilization and ribosomal RNA repair facilitation. Silymarin acts as inhibitor of myofibroblast transformation of stellate cell too. The main mechanism of its action is free radical scavenging (through antioxidant property). Its toxicity is rare [25].

"Anticonvulsants are one of the most important and

popular drugs that might show their adverse effects on liver (mostly in form of Cytotoxicity on the hepatocytes or cholangiocytes) during hepatic metabolism [2]. "

"During the first phase of drugs hepatic metabolism (Activation) mostly Cytochrome P450 - the mono oxygenase enzymes are playing an important role. Active metabolite is hydrophobe and will be toxic for cells thus must be changed to hydrophil and nontoxic molecule during the second phase (Detoxification) to be excreted with the urine or bile" [2].

There are suggestive evidences of silymarin effectiveness and tolerance for use in hepatotoxicity produced by a number of toxins as *Amanita Phalloides*, ethanol, paracetamol, carbon tetrachloride, psychotropic drugs, etc, and its activity against lipid peroxidation and stimulation of liver regeneration with antifibrotic effects. There are clinical trials of silymarin in acute viral hepatitis, NASH and alcoholic related liver disease and in chronic hepatitis C [25-34].

There isn't any clinical trial of UDCA or silymarin in drug induced hepatitis in human.

In this randomized open-label clinical trial we have evaluated ursodeoxy cholic acid versus silymarin effectiveness and tolerability in anticonvulsant induced hypertransaminasemia in children.

Materials and Methods

This is an open-label, randomized clinical trial. Based on available evidences at a power of 80% and two sided $\alpha=0.05$, we needed 20 patients in each group. We recruited 54 children aged between 4mo-14yr with anticonvulsant induced hypertransaminasemia in study, they were selected from 60 patients with hypertransaminasemia and anticonvulsive therapy, 6 of them were shown other causes of hepatitis or their hypertransaminasemia were controlled during evaluation period, thus were excluded. They were randomized based on block randomization in two groups; ursodeoxy cholic acid and silymarin. They were recruited over two year (2012 to 2014) from pediatric neurology clinic of Valiasr hospital in Imam Khomeini Hospital Complex, a referral and educational center. The used anticonvulsive drugs were Primidone, Valperic acid, Levetiracetam and Phenobarbital or any combination of them. Duration of anticonvulsive therapy before aminotransferases rises was between 3 months to 10 years with mean of 6 months. There were no significant differences between groups in anticonvulsive drugs or duration of seizure treatment. All of these 54 patients were suffered from hepatitis (more than two times rises of the upper limits of normal range for transaminases). They were checked by complete liver

function tests including AST (Aspartate aminotransferase)-ALT (Alanine aminotransferase) - γ GT (γ -Glutamyl transpeptidase) - Bilirubin (total and conjugated)-Albumin-PT and INR (Prothrombin time and International normalization ratio)-PTT (Partial thromboplastin time) with one standard kit (Pars Azmoon co.) and at one laboratory department. In Pars Azmoon Co. Kits, the normal ranges have been defined as following:

ALT: 0-37 IU/l

AST: 0-41 IU/l

$$\gamma\text{GT: } \begin{cases} 2 - 4^{\text{mo}}: 8 - 90 \text{ IU/l} \\ 4^{\text{mo}} - 10^{\text{yr}}: 5 - 32 \text{ IU/l} \end{cases}$$

After rule out of other common causes of liver damage such as viral hepatitis -autoimmune hepatitis -genetic errors of metabolism and anatomical causes (with serologic tests including HAV IgM, HBS Ag, HBC IgM, HCV Ab, EBV VC IgM, CMV IgM, ANA, AMA, ASMA, ANCA and metabolic screening by measurement of blood sugar in ortho and glucose oxidase manner - serum amino acids chromatography, blood ammonia and lactate level, ABG and Urine analysis for Ketone, reducing substances and organic acids and performing complete abdominal ultrasound by the same specialist and Siemens G40 system with CH₅ -2 prob), Also after trial of reducing dose of anticonvulsant drug to minimal effective dose or if possible substituting drug with a safer one when they were ineffective, patients allocated randomly to receive Ursodeoxy cholic acid or Silymarin for one month. Before trial starting two months' timeout for complete investigation was spent and if significant decrease was seen in transaminases, patients were excluded.

Any patient with viral, autoimmune or metabolic evidences or transaminases under twice the upper normal limits or spontaneous decline in enzymes during two month of primary evaluation was excluded from study. Exclusion of other 8 patients was done based on parents' desire during trial too. In UDCA group 24 recruited patients completed trial and follow up period. In silymarin group 22 of primary recruited patients completed intervention period.

Fortunately none of our patients were suffered from functional liver failure (Icterus, coagulopathy or encephalopathy). We used UDCA 250 mg capsules made with commercial name of Ursobil by ABC Farmaceutici of Spain in dose of 10-15mg/kg/day once a day and silymarin with commercial name of Livergol 70 mg tablet (Goldaru company) in dose of 5 mg/kg/day once a day for one month. In children under 5 years old, the desired dose of drugs as

suspension in water was used.

All patients showed hypertransaminasemia for more than two months before trial and all parents gave written informed consent.

Complete liver function tests were checked before intervention, every two weeks during trial and one month after end of it. Our study primary outcome was decrease or normalization of aminotransferases.

Expected side effects of UDCA like gastrointestinal disturbances, rash, arthralgia, anxiety and headache were monitored.

Side effects of silymarin including allergic reactions like urticaria- mild laxative effect- nausea- epigastric discomfort- arthralgia- pruritus- headache were monitored too.

Data were registered by SPSS v.18. Descriptive statistics were evaluated as frequencies, percentiles and mean \pm SD (standard deviation). Analytical statistics and comparison between groups were evaluated by ANOVA measures, chi - square and multivariate general linear model- repeated measures analysis. According to the Helsinki Declaration this study was approved by the Medical Ethics Committee of Medicine School-Tehran University of Medical Sciences, as a medical student thesis with registration ID: 1391-1075. Also this study is registered in IRCT with ID number of 2015010711392N2.

Results

46 patients (25 boys and 21 girls) aged 4 month to 14 year completed one month intervention and one month follow up period.

Mean ages were 61.043 \pm 37.863 months in UDCA group and 53.636 \pm 42.071 months in silymarin group. Demographic data of patients have been shown in table 1. Sex and age range are homogeneous between two groups (P value=0.971 and 0.168 respectively) (Table 1).

First transaminases quantities (before intervention and after rule out of other common causes of hypertransaminasemia) were similar in two groups too (P value=0.448, 0.071 and 0.105 for ALT, AST and γ GT respectively) (Table 2).

After one month trial transaminases levels decreased in both groups significantly (P< 0.05) except for γ GT in UDCA group (p=0.552) (Table 3).

Decrements differences were not significant between groups after one month intervention for AST and γ GT (p=0.297 and 0.257 respectively) but decrements of ALT was

Table 1. Patients' demographic data. Chi-square and T-test- P<0.05 significant

variable	Group(UDCA)	Group(Silymarin)	Pvalue
Sex			
Male	15	10	0.971
female	9	12	
Age			
Mean+SD	61.043±37.863	53.636±42.071	0.168
Minimum	4mo	5mo	
Maximum	14yr	13yr	

Table 2. Pretreatment transaminases. ANOVA- P<0.05 significant

group	ALT (mean)	AST (mean)	γGT (mean)
silymarin	131.090 u/l	117.969 u/l	73.333 u/l
SD	54.152	67.201	30.099
UDCA	115.782 u/l	88.434u/l	122.521u/l
SD	37.222	19.528	170.928
P value	0.448	0.071	0.105

Table 3. Before and after trial trends of transaminases in each group. paired T test P<0.05 significant

group	silymarin			UDCA		
before	ALT(131.090)	AST(117.969)	γGT (73.333)	ALT(115.782)	AST (88.434)	γGT(122.521)
intervention						
after one month	ALT(87.636)	AST (86.545)	γGT (52.727)	ALT (65.173)	AST (57.304)	γGT (57.782)
P value	0.0001	0.0001	0.0001	0.047	0.030	0.552

Table 4. Comparison of transaminases changes after one month intervention in each group. T-test p≤0.05 significant

group	ALT change	AST change	γGT change
silymarin	-68.260	-31.424	-20.606
UDCA	-43.454	-42.000	-80.304
p value	0.017	0.297	0.257

Table 5. Comparison of Transaminases trends one month after intervention cessation in groups T-test p≤0.05 significant

group	ALT change	AST change	γGT change
silymarin	-64.333	-54.606	-37.212
UDCA	-17.652	-10.869	-15.565
P value	0.017	0.003	0.038

better in silymarin group (p=0.017) (Table 4)

Normalization of transaminases (AST and ALT less than 40 IU/l) was occurred in 3 patients in silymarin group (13.6%) and 5 patients in UDCA group (20.8%).

All of participated patients were followed one month after intervention cessation for rebound rising. Decreasing trend continued in both groups but was better in silymarin group than UDCA. (p=0.017, 0.003 and 0.038 for ALT, AST and γGT respectively)

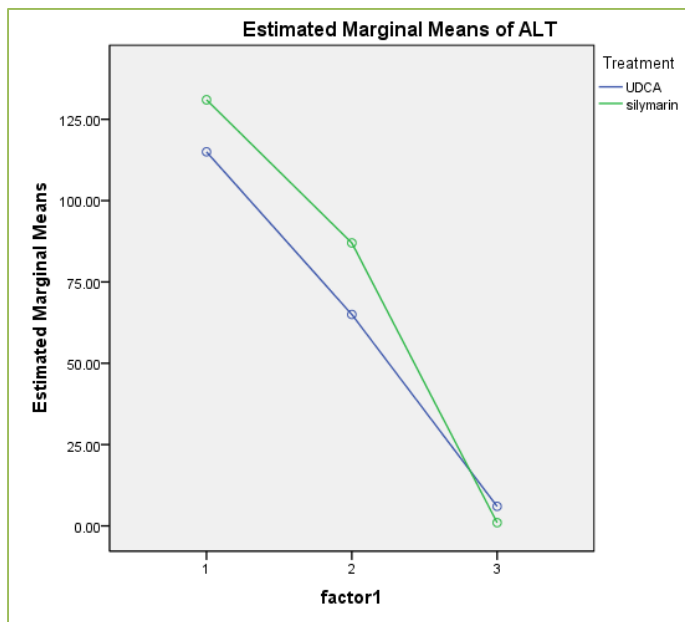
Figures 1, 2 and 3 are showing trend of liver enzymes in both groups. All of recruited patients were remained on their anticonvulsive drugs during intervention and follow up period.

No known side effect of UDCA or Silymarin was seen in patients.

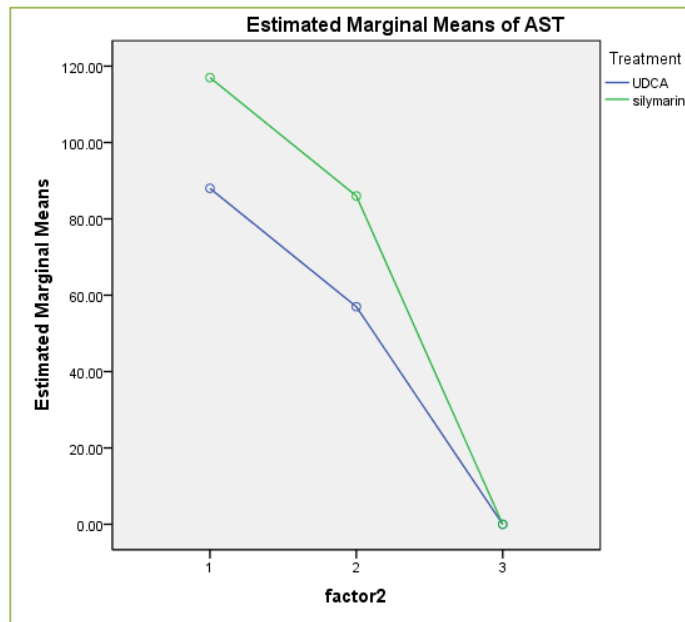
Discussion

Drug induced hepatotoxicity is a common concern in Anticonvulsive therapy. Anticonvulsive drugs are commonly enzyme inducer for cytochrome P450 system in liver and especially when multi drugs are used they can dispose liver to injury through the induction of cytochrome P450 by one agent, which increases the quantity of the toxic metabolite formed from the other^[1].

"During the first phase in hepatic metabolism of drugs (Activation) mostly Cytochrome P450 - the mono oxygenase enzymes are playing an important role. Active metabolite is hydrophobe and will be toxic for cells thus must be changed



Figures 1. Trend of ALT in both groups.



Figures 2. Trend of AST in both groups.

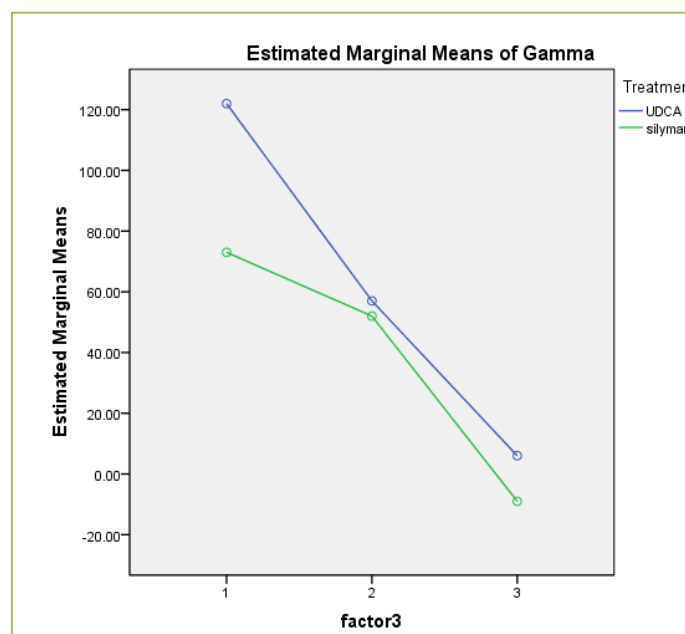
to hydrophil and nontoxic molecule during the second phase (Detoxification) to be excreted with the urine or bile. Imbalance between activation and detoxification phases of liver metabolism can produce liver damage" [2].

Liver damage shows as hepatocellular injury (transaminases raise) and liver functional defects (coagulopathy, jaundice, encephalopathy and homeostatic defects). Transaminases rising without functional defects can be not disturbing but can be early sign of liver damage too [1].

Fortunately none of our patients showed liver functional defects. In this randomized clinical trial we tried silymarin as a hepatoprotective antioxidant compared UDCA (a primary bile acid with hepatoprotective properties) to evaluate their efficacy in drug induced hypertransaminasemia.

Silymarin has been tried in nonalcoholic fatty liver disease, drug-induced hepatic injury in animals, acute and chronic hepatitis and there is preclinical evidence for silymarin's hepatoprotective and anticarcinogenic effects [25-34].

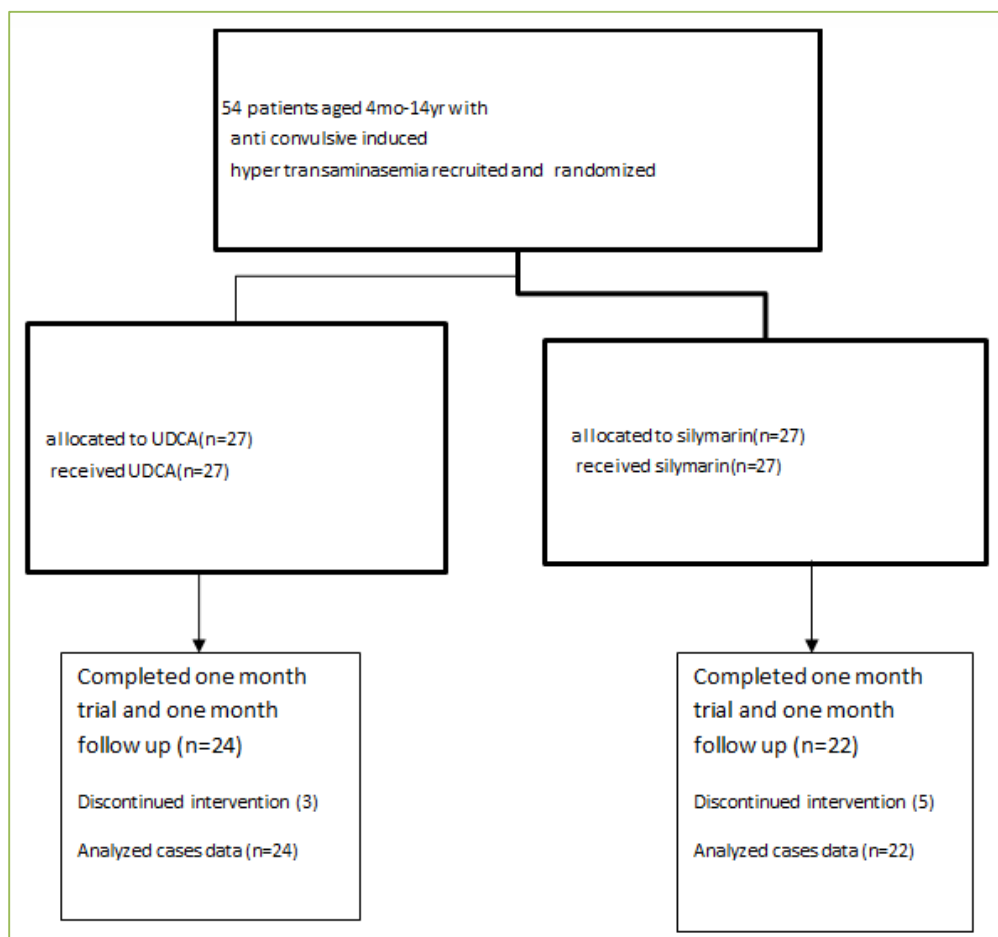
The flavonoid silymarin and one of its structural components, silibinin, are substances with documented hepatoprotective properties that act in four different ways: as antioxidants, as cell membrane stabilizers, as promoters of ribosomal RNA synthesis and as inhibitors of the transformation of stellate hepatocytes into myofibroblasts. The main mechanism of their action is free radical scavenging. Anti-inflammatory and anticarcinogenic properties have also been documented [25].



Figures 3. Trend of γ GT in both groups.

Hashemi et al tried silymarin for six months in patients with nonalcoholic fatty liver disease in a placebo-controlled trial and found significant decrease in transaminases after treatment with silymarin and significant normalization rate of transaminases in silymarin comparing placebo [27].

UDCA is a primary and nontoxic bile acid which obviously has direct cytoprotective and antioxidant effects in vivo. It is the major bile acid of the black bear and has been used for centuries in traditional Chinese and Japanese medicine for the treatment of gallbladder and liver diseases [3-9].



Consort chart

Its therapeutic effects have been shown in Cholestatic Liver disease, alcohol induced Liver damage, non-alcoholic steatohepatitis (NASH), cholesterol gallstone, acute and chronic viral hepatitis, autoimmune hepatitis and in cholestasis in critically ill patients. It also has been tried in drug induced hepatitis (as case reports) [10, 11, 13-22].

It has been suggested by Teschke for drug induced liver disease [12].

There are five case reports by Sabariah *et al*, Soza *et al*, Dandakis *et al*, Manolakopoulos *et al* and Cicognani *et al* and based on therapeutic effects of UDCA in management of drug-induced hepatitis arising from Carbamazepine, Amoxicillin- Clavulanic acid, Flutamide and Cyproterone acetate sequential administration and Flutamide which show its potential efficacy in toxic hepatitis [18-22].

Also the preventive effect of UDCA has been experimentally proven in Amoxicillin + Clavulanic acid and in Methotrexate induced Liver toxicity [23, 24].

The therapeutic effects of UDCA in drug induced and

toxic hepatitis could be as a result of its cytoprotective and antioxidant properties, which have been proven in two in vivo investigations [8, 9].

ALT and AST decreased in our patients similarly in silymarin and UDCA groups in one month intervention (P values < 0.05) but not for γ GT that its change was significant in silymarin group and no significant in UDCA group. (P value= 0.001 and 0.552 respectively). Decrement quantities were better in ALT in silymarin group ($p=0.017$). In one another month of follow up after discontinuation of drugs transaminases declines were better in silymarin group than UDCA (P value= 0.017, 0.003 and 0.038 for ALT, AST and γ GT respectively). Overall silymarin seems more effective than UDCA in transaminases decrease.

None of our patients suffered from hepatic functional defects (icterus, coagulopathy or mental deterioration) and none of them showed systemic hypersensitivity signs (that may be seen as some anticonvulsive drugs side effects).

Fortunately no known side effects of UDCA and silymarin were seen in our patients. It seems that silymarin and UDCA

can be safe and worthy choices in drug induced hepatitis. Further studies particularly on patients with functional liver compromise due to drug hepatitis and with placebo-controlled trials are recommended.

We designed this trial as a placebo-controlled trial and selected Folic acid as placebo, but we noticed some reports of efficacy of Folic acid and Vitamin B6 complex in drug induced hepatitis in animals, thus we excluded placebo group from our study and lack of placebo group is our study weakness. By the way our study is the first clinical trial in human to compare UDCA and silymarin in drug induced hypertransaminasemia and their safety and tolerance were shown in this trial is a promising omen to use them in drug hepatotoxicity. More studies with placebo- controlled design are suggested.

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Conflict of interest

Authors have no conflict of interest.

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