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Original Article

The Effects of Silymarin and N-Acetylcysteine on Liver and Kidney Dysfunction in Subjects with Severe Pre-eclampsia

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Abstract

Background: Silymarin and N-acetylcysteine are antioxidant supplements with protective effects on the liver and kidneys. The aim of this study was to investigate the effect of silymarin and N-acetylcysteine on liver and kidney disorders against severe pre-eclampsia.

Methods: In the present single-blind clinical trial, 60 mothers who underwent termination of pregnancy due to severe pre-eclampsia were divided into two groups. The first group received 70 *mg* of silymarin and the second group received 600 *mg* of N-acetylcysteine at 3 doses immediately, 12 and 24 hours after delivery. Patients were monitored for blood pressure, platelet and biochemical markers of liver injury and kidney function 12, 36 and 60 hours after drug administration.

Results: Over time, the mean Alanine Transaminase (ALT), Aspartate Transaminase (AST), Lactate Dehydrogenase (LDH) and Alkaline Phosphatase (ALP), levels in the two groups of silymarin and N-acetylcysteine significantly decreased ($p<0.001$). Silymarin and N-acetylcysteine were not significantly different in reducing the increased creatinine and BUN levels.

Conclusion: N-acetylcysteine and silymarin help patients with pre-eclampsia to improve kidney and hepatic dysfunction; however, silymarin was more effective in decreasing ALT, AST, ALP, and LDH levels than N-acetylcysteine. N-acetylcysteine was more effective in decreasing BUN and creatinine levels than silymarin.

Keywords: Kidney, N-acetylcysteine, Pre-eclampsia, Silymarin

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Introduction

Pre-eclampsia is one of the most common disorders during pregnancy, causing both maternal and fetal complications, and is the leading cause of maternal mortality especially in developing countries (1). The prevalence of pre-eclampsia is 5-8% in pregnancies, about 10% in first pregnancies, and 20-25% in women with a history of high blood pressure (2). Severe pre-eclampsia is defined by the presence of one of the following symptoms or signs based on the Guidelines of American College of Obstetricians and Gynecologists as stated below (3):

• SBP of 160 *mmHg* or higher or DBP of 110 *mmHg* or higher, on two occasions at least 4 hours apart, while the patient is on bed rest (unless antihypertensive therapy has previously been initiated).

• Impaired hepatic function as indicated by the abnormal elevation of blood concentrations of liver enzymes (two times higher than normal concentration), severe persistent upper quadrant or epigastric pain that does not respond to pharmacotherapy and is not accounted for by alternative diagnoses, or both.

• Progressive renal insufficiency (serum creatinine concentration >1.1 *mg/dL* or a doubled serum creatinine concentration in the absence of other renal diseases).

• New onset cerebral or visual disturbances.

• Pulmonary edema.

• Thrombocytopenia (Platelet count<100,000/μL).

Oxidative stress and increased production of free radicals can lead to vascular endothelial dysfunction, mismatch of immune and inflammatory responses, and development of diseases in pregnancy (4). Pre-eclampsia occurs from the 20th week of pregnancy till 7 days after delivery. Pre-eclampsia can be life-threatening and can lead to eclampsia, brain stroke, organ damage (liver and kidney) and even death (5). The oxidative stress in pre-eclampsia and the role of antioxidants in the treatment of its complications have been discussed recently (4).

Silybum marianum, the medicinal plant belonging to the family Asteraceae, is well known for its therapeutic effects in the treatment of various diseases including liver and kidney diseases. Silymarin is the extract of *Silybum marianum*. A standardized extract contains 70-80% of flavonolignans of silymarin (Silybin A and B, isosilybin A and B, silidianin, and silichristin), flavonoids (Taxifolin and quercetin), and 20-30% of oxidized polymeric and polyphenolic compounds (6). Silymarin has a wide range of biological and pharmacological effects, including antioxidant (7), anti-inflammatory (8) and anti-cancer (9) effects. Also, its protective effects on cardiovascular diseases (10), kidney (6) and liver (11), as well as fat reduction (12), immune system strengthening (13), and neuroprotection (14) have been reported.

N-acetylcysteine is a sulfhydry l compound, derived from the L-cysteine amino acid, and a naturally occurring antioxidant, a glutathione precursor, that counteracts intracellular free radical damage in the body (15). The drug has a variety of clinical applications due to its role and antioxidant effects on the nitric oxide system (16). It is used to prevent or lower the risk of acetaminophen- induced hepatotoxicity (15). In combination, it has a protective effect against kidney damage, the nervous system, and the genital system (16-18). Several studies have also shown the efficacy of silymarin (4,19) and N-acetylcysteine (20) in patients with pre-eclampsia; however, no comparison has yet been made between the two antioxidants.

Therefore, the purpose of the current study was to investigate the effect of silymarin and N-acetylcysteine on liver and kidney disorders in mothers with severe pre-eclampsia.

Materials and Methods

Study design

In the present single-blind clinical trial, 60 mothers, who underwent termination of pregnancy due to severe pre-eclampsia, were selected randomly and divided into two equal groups. The sample size was determined 30 in each group with considering type 1 error of 5% and power of 80% Inclusion criteria were the termination of pregnancy due to severe pre-eclampsia, being a nulliparous, history of single pregnancy and the exclusion criteria were history of overt hypertension, pregnancy-induced hypertension, diabetes, kidney disease, collagen vascular diseases or cardiovascular diseases, previous history of miscarriage or pregnancy, history of smoking or alcohol consumption, and eclampsia. After providing the necessary explanations for the mothers regarding the research objectives, informed consent was obtained from participants to ensure patients safety

during the study and to provide data (Justification) of subject appropriateness. Then, the selected samples were divided into two groups. The first group received 70 *mg* of silymarin with no adverse effects (18).

In our previous study, 70 *mg* of silymarin, twice a day, significantly decreased the pre-eclampsia- induced liver transaminases increments. The purpose in this study was to evaluate the effect of half of that dose in these patients and evaluate another anti-oxidant agent, N-acetylcysteine, as well. The second group received 600 *mg* of N-acetylcysteine at 3 doses, immediately, 12 and 24 hours after delivery (Figure 1). It was shown previously that this dosing schedule has an anti-inflammatory effect. The first dose of drugs was given after the first breastfeeding and it was recommended to discard the milk for the next 48 hours. Since the clinical symptoms and biochemical profile of these patients are available in the textbooks, the control group was removed in the study to reduce the risk of side effects for patients and health workers undertaking phlebotomy.

The drugs were provided in similar forms and were coded. In both groups, the patients underwent routine treatment for pre-eclampsia including receiving magnesium sulfate and hydralazine in cases of blood pressure (blood pressure greater than 160/110 *mmHg*) under close supervision (21). The patient's clinical signs and liver and kidney function tests were evaluated. Systolic and Diastolic Blood Pressure (SBP and DBP) of patients were measured every 3 hours with mercury sphygmomanometers and recorded in the checklists. Liver enzymes Alanine Transaminase (ALT), Aspartate Transaminase (AST), and Alkaline Phosphatase (ALP) and in serum samples and total

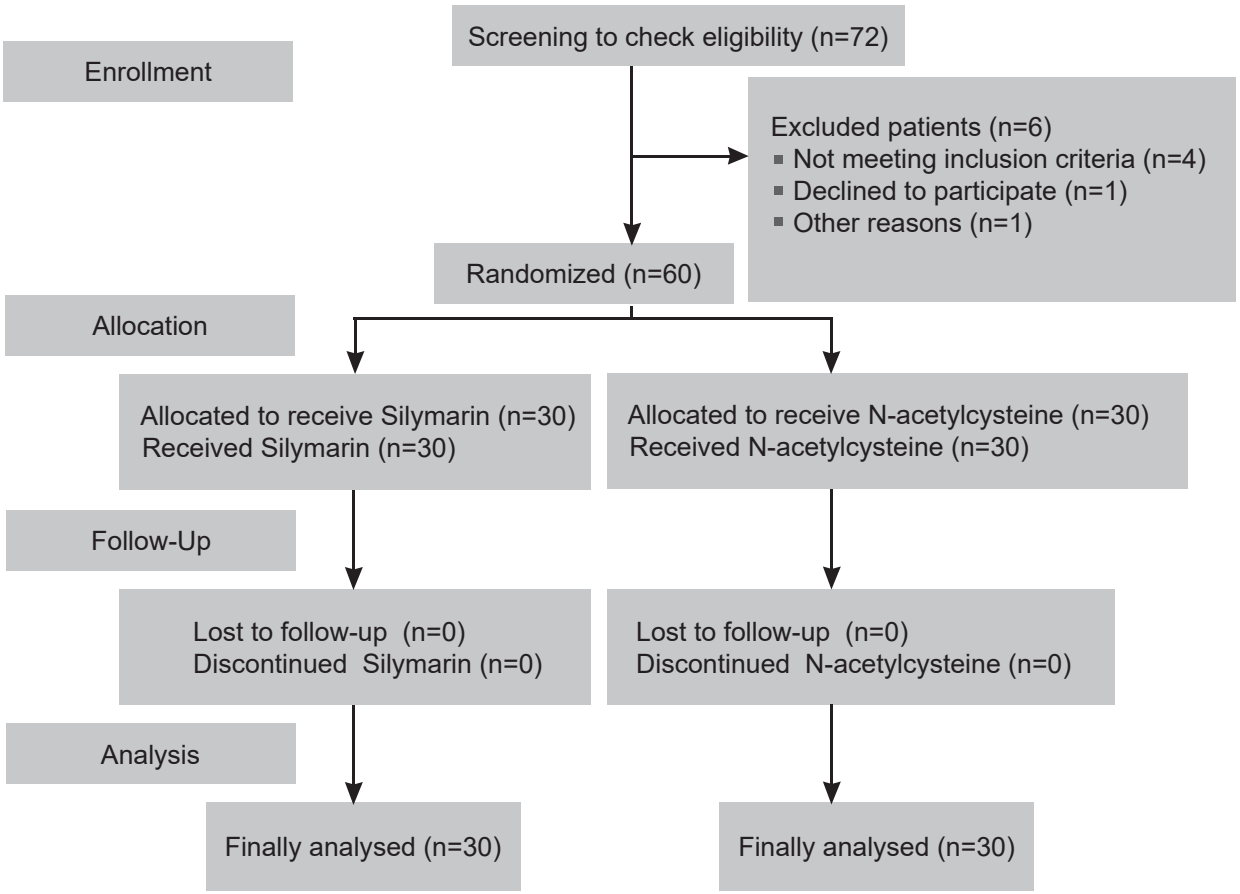

Figure 1. CONSORT flow diagram showing patients in the trial.

bilirubin (BilT), direct bilirubin (BilD), uric acid, Creatinine (Cr), Blood Urea Nitrogen (BUN) and proteinuria in urinary samples were measured along with Platelet (Plt) count 12, 36 and 60 hours after drug administration as recommended in reference books, and the results were recorded in each patient's checklist. Patients were also examined for seizures eclampsia and the results were recorded in the relevant forms. This study was approved by the ethics committee of Shahrekord University of Medical Sciences.

Statistical analysis

After entering the required data from the patient's records, they were analyzed using STATA, v14 (StataCorp, USA). Beside descriptive data for continuous variables such as mean and standard de*via*tion, qualitative data such as frequency and percentage were calculated. A Generalized Estimating Equation (GEE) was used to compare the patients and repeated measures. ANOVA was used to check the significant trend over time. The p-value of less than 0.05 was considered statistically significant to approve or reject the hypothesis of the study.

Results

In this study, the mean age, height and weight of the patients were 26.10±2.48 years, 163.40±3.85 *cm*, and 78.17±7.10 *kg*, respectively (without significant difference between the two groups, p>0.05) and no drug side effects were observed.

Over the time (four measurement points: before intervention, 12, 36 and 60 hours after intervention), the mean ALT, AST, ALP, and Lactic Dehydrogenase (LDH) in the two groups of silymarin and N-acetylcysteine significantly decreased which indicates the effect of the treatments (F=124.773, F=59.396, F=33.705 and F=65.330 with p<0.001, respectively) (Figures 2-5). Additionally, the analyzed data shows the decreasing trend of BUN and creatinine levels in the two groups (Figures 6 and 7); however, N-acetylcysteine showed better efficacy to increase the factors of interest than silymarin. According to the results of GEE, silymarin and N-acetylcysteine were not significantly different in reducing the increased creatinine (p=0.259) and BUN (p=0.140). Analyzed data showed that a one-year increase in age can affect creatinine levels (Coefficient: -0.0076, p=0.019). Also,

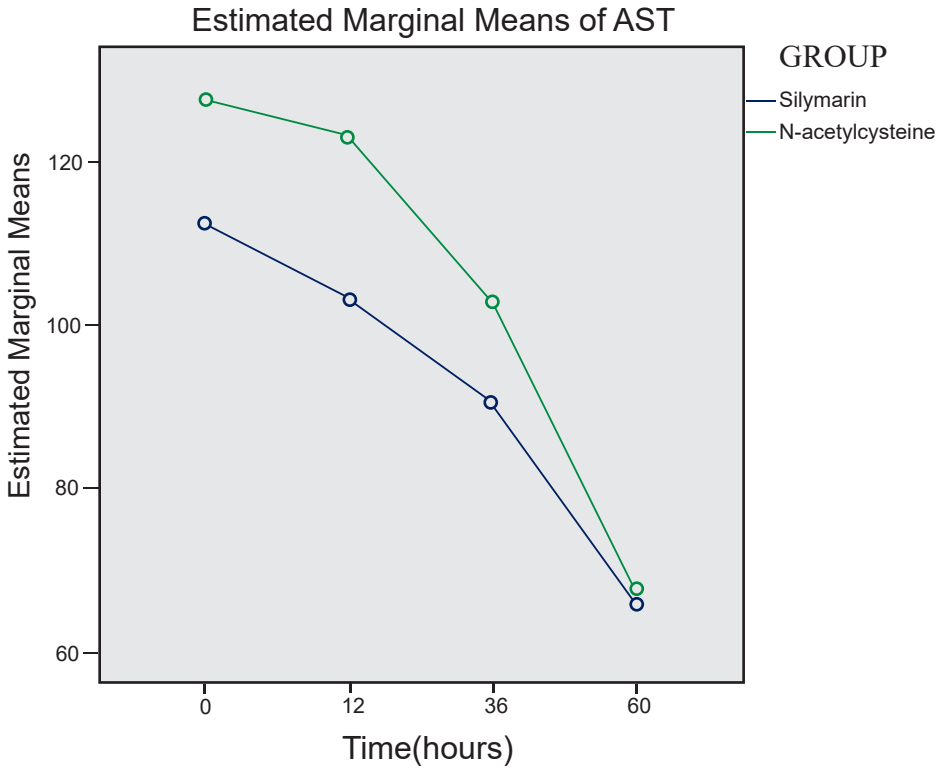

Figure 2. Trend of the mean and standard deviation of AST in the two groups of Silymarin and N-acetylcysteine (before intervention, 12, 36, and 60 hours after intervention).

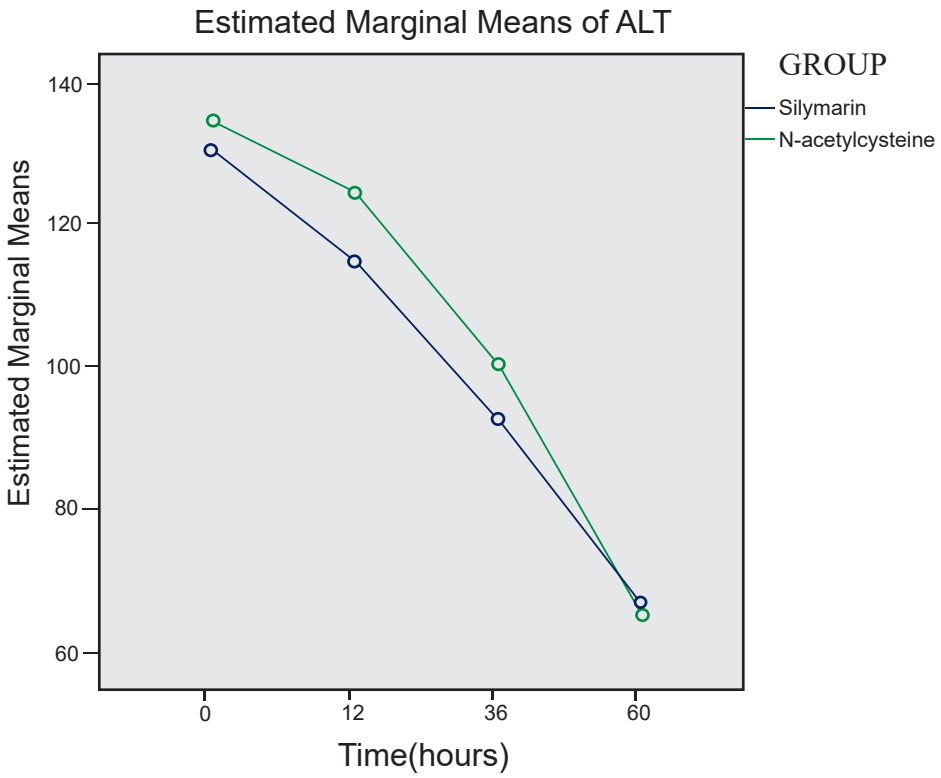

Figure 3. Trend of the mean and standard deviation of ALT in the two groups of silymarin and N-acetylcysteine (before intervention, 12, 36, and 60 hours after intervention).

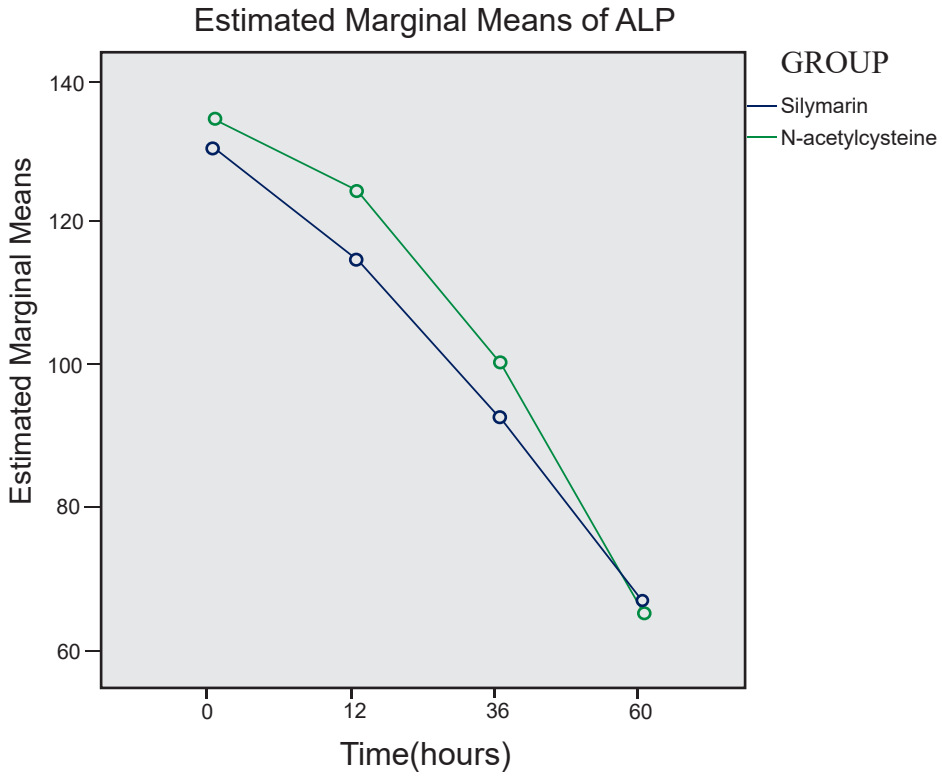

Figure 4. The mean and standard deviation of ALP in the two groups of silymarin and N-acetylcysteine (before intervention, 12, 36, and 60 hours after intervention).

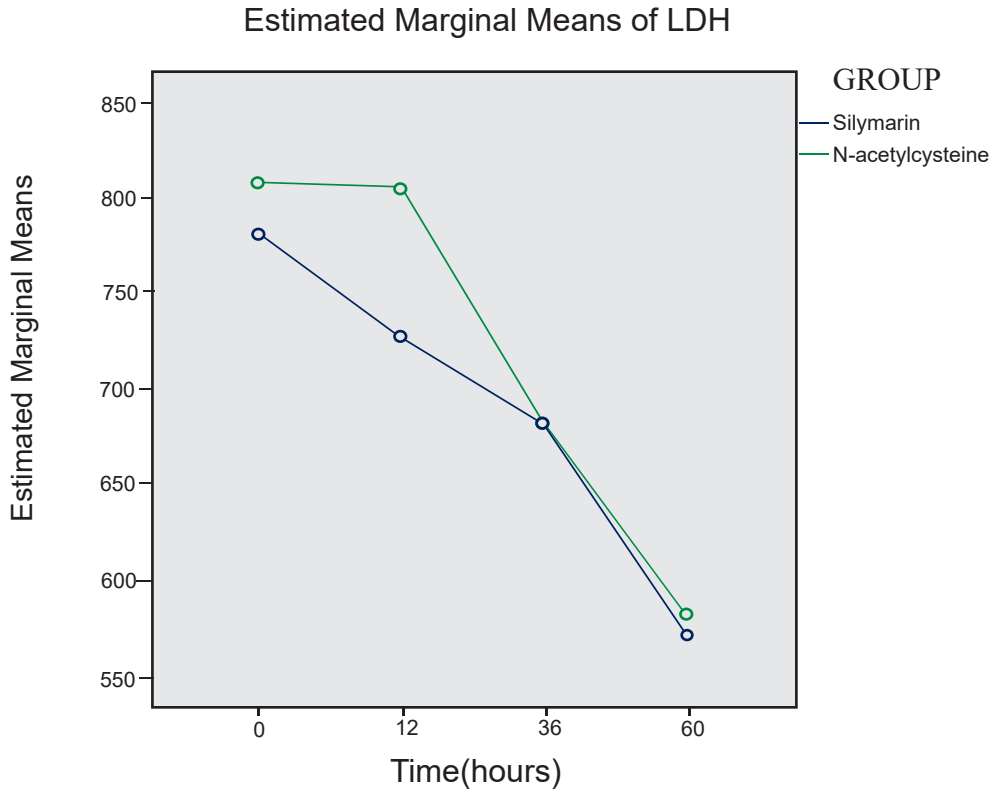

Figure 5. The mean and standard deviation of LDH in the two groups of silymarin and N-acetylcysteine.

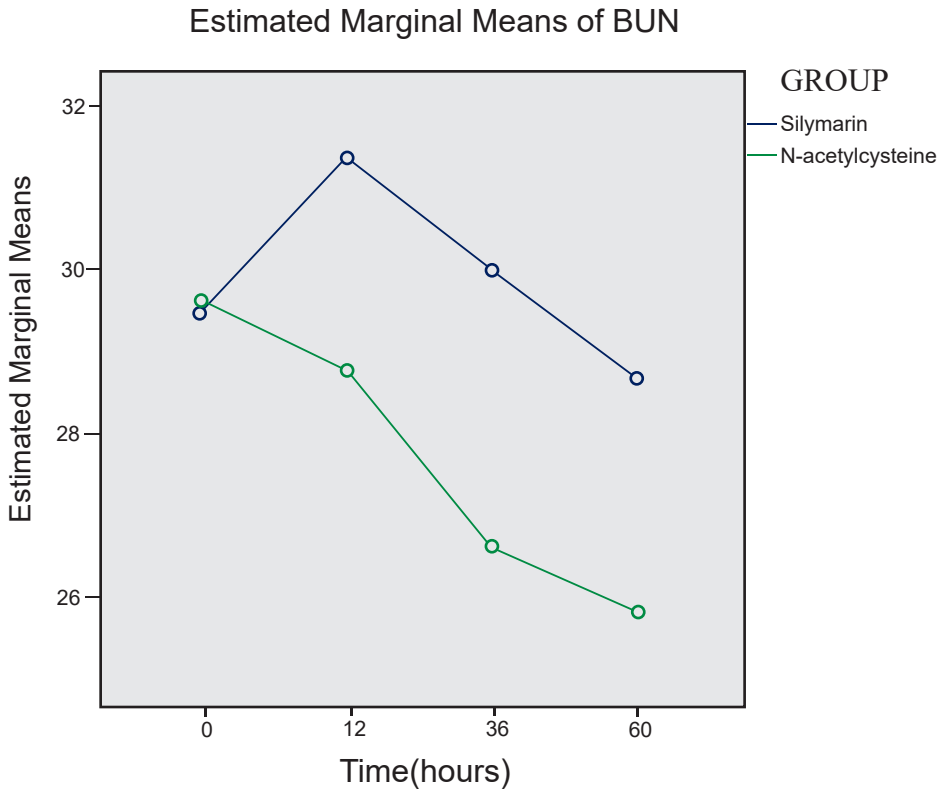

Figure 6. The mean and standard deviation of BUN in the two groups of silymarin and N-acetylcysteine (before intervention, 12, 36, and 60 hours after intervention).

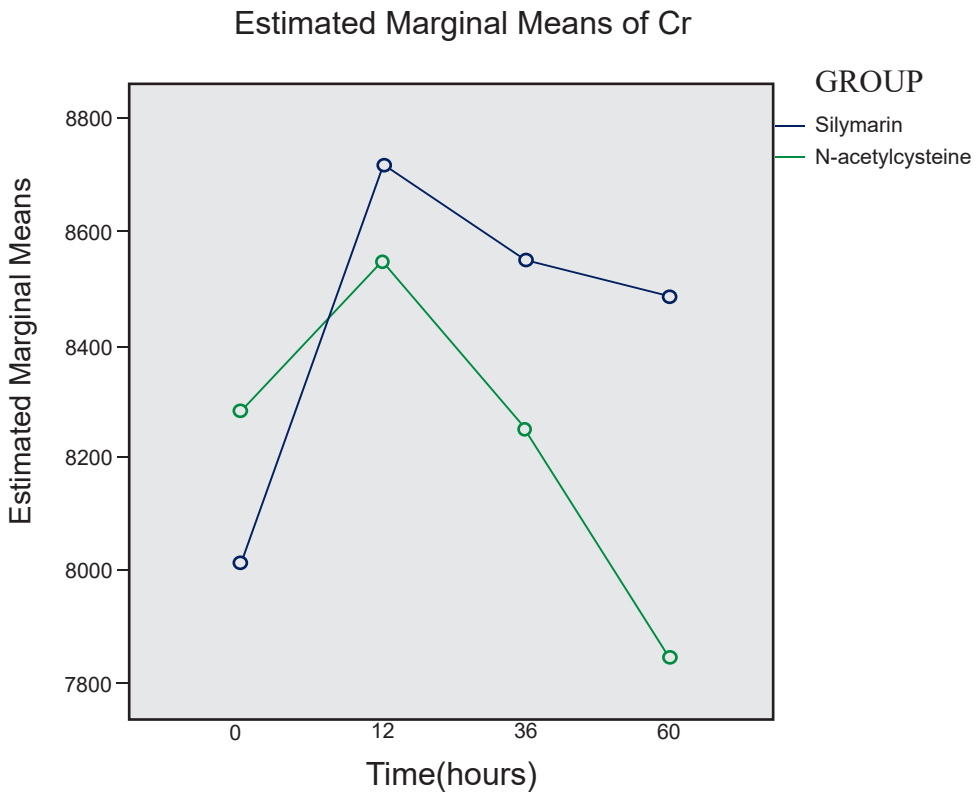

Figure 7. The mean and standard deviation of Cr in the two groups of silymarin and N-acetylcysteine (before intervention, 12, 36, and 60 hours after intervention).

BUN can be affected by the change of weight and height (Coefficient: 0.3075,p=0.021 and Coefficient: -0.4917, p=0.040, respectively) (data not shown).

According to the results of GEE, silymarin and N-acetylcysteine were not significantly different in reducing the increased ALP (p=0.360), AST (p=0.371), and ALT (p=0.994).However analyzed data showed that a one-unit increase in the LDH level can increase the ALP and AST levels (Coefficient: 0.07, 0.06, p=0.001 and p=0.003, respectively). Also, the AST value can be affected by the change of direct bilirubin level (Coefficient: 41.257, p=0.02 but the confidence interval is wide due to small sample size).

Discussion

The results of the present study showed that the treatment of women with severe pre-eclampsia by N-acetylcysteine and silymarin resulted in a significant decrease in liver enzymes and blood pressure; however, the efficacy of the two drugs in reducing the liver enzymes and blood pressure was almost similar without any significant differences. Moreover, the two drugs showed similar efficacy in reducing bilirubin, BUN and uric acid levels; however, in the N-acetylcysteine group compared to the silymarin group, the changes in creatinine (0.04±0.17 *vs*.–0.04±0.13) and platelet (19200.00±49907.77 *vs*.46800.00±54762.43) were significant, indicating the better efficacy of N-acetylcysteine in reducing creatinine and increasing platelet.

So far, several experimental studies have compared the hepatoprotective effects of silymarin and N-acetylcysteine; however, this study made a comparison in the clinical field for the first time. In a study conducted by Yorrmaz *et al*, the hepatoprotective effects of silymarin (10 *mg/kg*) and N-acetylcysteine (20 *mg/kg*) were investigated in rats under partial hepatectomy, and it was revealed that both drugs significantly reduced liver enzymes and significantly improved damaged liver tissue; however, these researchers stated that the rate of liver regeneration was higher in mice receiving silymarin (22). In another study conducted by Demiroren *et al*, the hepatoprotective activity of silymarin (100 *mg/kg*) and N-acetylcysteine (100 *mg/kg*) was evaluated in mice receiving CCl4 and it was shown that hepatoprotective activity of the two drugs did not differ significantly (23). Ustyol *et al* treated the

CCl4-receiving mice with silymarin (100 *mg/kg*, IP) and N-acetylcysteine (100 *mg/kg*, IP) for 4 weeks and finally found that the score of tubular injury, interstitial fibrosis as well as the creatinine, urea, and BUN levels did not differ significantly between the two groups (24). A study conducted by Singh *et al* showed that silymarin and N-acetylcysteine have similar efficacy in protecting HepG2 cells against the toxicity of isoniazid and rifampin (25). Our study findings are in line with the above-mentioned results regarding the efficacy of N-acetylcysteine and silymarin in reducing liver enzymes as well as renal function markers in comparison to creatinine.

In the present study, the treatment of patients with severe pre-eclampsia by silymarin (70 *mg*) at three doses (immediately, 3 and 24 hours after delivery) resulted in a significant reduction in liver enzymes and blood pressure. Baghbahadorani *et al* showed that the treatment of women with pre-eclampsia by silymarin (70 *mg*) at two doses resulted in a significant reduction in AST and ALT enzymes (19). In another study conducted by de Souza *et al*, the silibinin treatment of pre-eclampsia-like conditions induced by L-NAME in mice significantly reduced the systolic blood pressure, proteinuria, TNF-α, IL-1β, and IFN-γ levels and significantly increased platelet count (8). Martines *et al* studied pregnant women with pre-eclampsia and reported that silymarin, at a dose of 50 *mg* daily for three months, had no effect on reducing thrombophilia but reduced the chances of liver failure (26). The findings in this study are consistent with the above-mentioned results regarding the significant decrease in biomarkers related to liver dysfunction and blood pressure.

In the present study, N-acetylcysteine (70 *mg*), at three doses, significantly improved liver function and hypertension. A study conducted by Roes *et al* indicated that the treatment of patients with severe pre-eclampsia or HELLP syndrome by N-acetylcysteine caused a significant reduction in serum homocysteine, but had no effect on secondary outcomes (20). Chang *et al* stated that the treatment of pre-eclampsia in mice by N-acetylcysteine (100 *mg/kg*) significantly reduced systolic and diastolic blood pressure (27). Ryu *et al* also reported that the induction of pre-eclampsia in mice increased the leukocyte-endothelium adhesion, and the use of antioxidants like N-acetylcysteine, vitamins E and C inhibited this effect (28). Although the effect of N-acetylcysteine on markers of liver function in the pre-eclampsia has not been studied previously but its protective effects in other animal models have been shown against paraxone (18), cyclophosphamide (17), telephone waves (29), and CCl4-induced (23) liver damage.

It seems that N-acetylcysteine and silymarin reduce liver and kidney complications of pre-eclampsia by decreasing the oxidative stress markers and inflammation. Silymarin and N-acetylcysteine have been shown to enhance tissue antioxidant capacity (18,30) and prevent the synthesis of pre-inflammatory cytokines (31).

According to the results of the present study, N-acetylcysteine and silymarin had a similar effect on improving blood pressure, liver, and kidney functions in patients with pre-eclampsia. Therefore, it is of great necessity to find a more effective drug. The limitations of the present study were lack of a control group and follow-up of patients for a longer period due to limited budget and time of study.

Conclusion

The results of this study indicated that N-acetylcysteine and silymarin had similar effects in patients with pre-eclampsia; however, silymarin was more effective in decreasing ALT, AST, ALP and LDH levels (liver functions) than N-acetylcysteine. N-acetylcysteine was more effective in decreasing BUN and creatinine levels (renal functions) in comparison with silymarin. It seems that the effects of these two drugs on pregnancy must be investigated in future studies. Also, it is suggested to compare the two drugs in terms of cost and side effects to introduce a more effective, safer and affordable drug.

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The trial protocol was approved by the Iranian Registry of Clinical Trials (#IRCT registration number: IRCT20191231045965N1) and the ethics code (IR.SKUMS.REC.1397.181) was issued by Shahrekord University of Medical Sciences, Shahrekord, Iran.

Conflict of Interest

We wish to confirm that there are no known conflicts of interest associated with this publication.

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