Comparative Effects of Silymarin and Vitamin E Supplementation on Oxidative Stress Markers, and Hemoglobin Levels among Patients on Hemodialysis

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Abstract

Background—The incidence of accelerated atherosclerosis among patients on hemodialysis is very high and oxidative stress is a potentially major contributor to their morbidity and mortality.

Objective—to evaluate the effects of Silymarin and/or vitamin E on oxidative stress markers and hemoglobin level in patients on hemodialysis.

Methods—Eighty patients on hemodialysis were randomized into 4 groups: Group 1 received Silymarin 140 mg 3 times daily; Group 2 received Vitamin E 400 IU/day; Group 3 received Silymarin 140 mg 3 times daily and Vitamin E 400 IU/day; Group 4 was the control. Samples
were obtained at baseline and on day 21 for measurement of malondialdehyde (MDA), RBC glutathione peroxidase (GPX), and hemoglobin.

Results—Combination of Silymarin and vitamin E led to a reduction in the MDA levels (7.84±1.84 vs. 9.20±2.74 nmol/ml; p=0.008). There was a significant increase in RBC GPX levels in all treatment groups compared to controls after 3 weeks. This was more pronounced in the group receiving combination compared to vitamin E or the control group (5.78±3.51, 4.22±1.63, and 3.16±1.89 Iu/gr-Hb respectively; p<0.001). There was also a significant increase in mean hemoglobin of all treatment groups compared to control.

Conclusions—Oral supplementation with Silymarin and vitamin E leads to reduction in MDA, increase in RBC GPX and increase in hemoglobin levels in patients with ESRD. Studies with larger sample sizes and longer follow-up are required to investigate the effect of Silymarin on cardiovascular outcomes, and erythropoietin requirement.

Introduction

Atherosclerotic cardiovascular disease is the most common cause of morbidity and mortality in patients on maintenance dialysis. A proportion of these patients do not have traditional risk factors of atherosclerosis such as hypertension, hyperlipidemia, diabetes, and smoking [1–3]. Recent clinical and experimental evidence suggests that oxidative stress (OS), chronic inflammation, malnutrition and endothelial dysfunction are increased in end stage renal disease. Oxidative stress may be implicated in the pathogenesis of atherosclerosis, malnutrition, anemia, and dialysis-induced amyloidosis [4–8].

There is ample evidence for increased oxidative stress in renal failure: chronic inflammation associated with uremia; presence of trace amounts of endotoxins in the dialysate; activation of oxidative metabolism in leukocytes by dialysis membrane; depletion of antioxidants such as vitamin E, flavonoids, superoxide dismutase, glutathione and glutathione peroxidase. Other evidences for increased OS in renal failure patients are increased levels of malondialdehyde (MDA) in serum, red blood cells, platelets and peripheral blood mononuclear cells; increased formation of advanced oxidation proteins, advanced glycosylation end-products (AGE), and increased oxidation of lipoproteins. As a result, supplementation with antioxidants has been proposed as a reasonable strategy to prevent the deleterious effects of oxidative stress in this population. Vitamin E, an effective lipid-soluble antioxidant in biologic membranes, has been widely used in the defense against oxidative stress in patients with end stage renal disease [9–11].

Silymarin, a polyphenolic flavonolignans from Silybum marianum plant consists of four flavonolignans isomers -Silybin, Isoisilybin, Silydianin and Silychristin. It is orally absorbed and excreted mainly through bile as sulfates and conjugates. While most of the evidence indicates a renal-protective impact of Silymarin in animal models of acute kidney injury, the effect is inconclusive [12–16]. Silymarin has also been demonstrated to lead to enhanced recuperation of renal tissue damage among alloxan-induced diabetic rats [17].

Silymarin has been used in alcoholic liver diseases, liver cirrhosis, Amanita mushroom poisoning, viral hepatitis, toxic and drug induced liver diseases and among diabetic patients. It has been claimed to promote protein synthesis, help regenerate liver tissue, control inflammation, enhance glucuronidation and protect against glutathione depletion [18, 19]. Clinical and experimental studies have shown Silymarin to act by antioxidative, anti-lipid peroxidative, antifibrotic, and anti-inflammatory, membrane stabilizing, and immunomodulatory mechanisms. In the kidney cells damaged in vitro by paracetamol, cisplatin, and vincristin Silybin can reduce or avoid nephrotoxic effects [18–20].
Silymarin has also been used in the treatment of patients suffering from end-stage diabetic nephropathy (ESDN). The molecular basis of the anti-inflammatory effect of Silybin/Silymarin is yet unknown. This might be related to inhibition of the nuclear factor NF-κB, which regulates and coordinates the expression of various genes involved in the inflammatory process, in cytoprotection. NF-κB seems to be subject to redox regulation, suggesting thus an important role of antioxidants in its inactivation. The treatment with Silybin or Silymarin led to a restoration of the thiol status both in vitro and in vivo. These data provide the rationale for using flavonolignans in ESDN to normalize immunoregulatory defects via restoration of the cellular thiol status [20, 21]. Considering the importance of this subject and lack of experience with Silymarin among hemodialysis patients, we investigated the effect of this flavonoid alone, and in combination with vitamin E, on biomarkers of oxidative stress in this patient population.

Subjects and Methods

This study is in conformity with the Declaration of Helsinki and was approved by the institutional ethics committee of Shiraz University of Medical Sciences. All patients completed the informed consent form. Subjects were recruited from among clinically stable hemodialysis patients (age range 18–60 years) in Ebrahimi Hemodialysis Center in Sadra City (Shiraz, Iran). All patients received four hour hemodialysis treatments, three times per week. The membrane and the general dialysis prescription were similar for all patients. Exclusion criteria consisted of history of a cardiovascular event within the previous 12 months, Vitamin E, levothyroxin, and oral contraceptives. Patients with any active infection including hepatitis B or C, New York Heart Association class III and IV heart failure were also excluded. All patients were instructed to maintain a renal prudent diet and were asked not to change their diet or level of physical activity throughout the study.

Eighty consenting patients were randomized into 4 equal groups: Group 1 received Silymarin 140 mg three times daily; Group 2 received vitamin E 400IU/day, while group 3 received both Silymarin 140mg three times daily and Vitamin E 400 IU/day. Group 4 was the control group and received no study-related intervention. The duration of the study was 3 weeks. Each 140 mg coated tablet of Silymarin (Livergol ®; Goldaru Herbal Products Pharmaceutical Company) contains the dried extracts of Silybum marianum equivalent to 140 mg Silymarin. Vitamin E (Zahravi Pharmaceutical Company) was administered as 400 mg pearls. Fasting blood samples (10 ml) were collected in EDTA-containing tubes from the arterial line immediately before a mid week dialysis session, before heparin administration, immediately before supplementation (baseline) and on day 21 after the intervention period. Samples were immediately centrifuged and frozen at −70°C.

Analytical procedures

Malondialdehyde, an indirect index of lipid peroxidation, was assayed as Thiobarbituric Acid Reactive Substances (TBARS) using a colorimetric method [22]. Briefly, 0.5 ml of diluted plasma (1:1, v/v) was mixed with 2 ml a TBA reagent containing trichloroacetic acid [15% (w/v)], thiobarbituric acid [0.375% (w/v)] and hydrochloric acid (0.25 N) and the mixture placed in a boiling water bath for 15 min. The samples were cooled and centrifuged at 3000 g for 15 min at 4 °C. The absorbance of the supernatant was measured at 532 nm. The TBARS concentration was calculated using 1, 1, 3, 3-tetraethoxy propane (TEP) as a standard. Results are expressed as nmol/ml.

The glutathione peroxidase (GPX) activity was measured in red blood cells hemolysate according to the method of Paglia and Valentine [23] by the decrease in absorption at 340 nm due to oxidation of NADPH to the NADP⁺, when oxidized glutathione was reduced by glutathione reductase (GR). Oxidized glutathione had been formed before by reaction of its
reduced form with \( t \)-butyl hydroperoxide (\( t \)-BuOOH) and GPX. To obtain erythrocyte hemolysate, 100 \( \mu \)l of packed erythrocytes was hemolyzed by adding 9 volumes of cold distilled water. The resulting suspension was centrifuged twice to eliminate all of the cell membranes. Each assay consisted of 0.25 mM GSH, 0.38 mM NaN\(_3\), 0.23 mM EDTA, 0.175 mM NADPH, 0.1 units of GR, 0.05 mM \( t \)-BuOOH in 37.6 mM phosphate buffer, pH 7.2, and an appropriate amount of hemolysate in a final volume of 600 \( \mu \)l. The units of enzymatic activity were calculated using an extinction coefficient of 6.22 mM\(^{-1}\) cm\(^{-1}\) for NADPH. One unit was equivalent to the oxidation of 1 \( \mu \)mol of NADPH per minute. GPX activity in RBC was expressed as IU/gr-Hb. The hemoglobin concentration was determined by using an autoanalyser SE-9000 (Sysmex KX-21N Instruments, Mundelein, Illinois).

Statistical analysis

All data were analyzed using SPSS statistical software (version 15.1) and degree of significance was set at 0.05. The ANOVA and Chi-square test were used for statistical comparisons of demographic variables among groups. For comparing the values of variables after 21 days of supplementations, adjusted for baseline value, ANCOVA test was used.

Results

The mean age was 46.2 ± 13.6. Only 8 (10%) patients were smokers. Fifty eight patients (72.5%) were male. Approximately 31% were diabetic, and 67.5% were hypertensive (n=25 and 54, respectively). Table 1 describes patient characteristics in each group. Mean age was not significantly different among the four groups (P=0.225). Similarly, there were no statistically significant differences among the groups regarding sex, hypertension (HTN), and diabetes (DM). All 80 participants successfully completed the study. Patients confirmed that no changes were made to their diet or level of physical activity during the study period. In addition, none of the subjects reported any adverse effects with Silymarin and vitamin E supplementation. Table 2 shows plasma hemoglobin and MDA, as well as RBC GPX levels, at baseline, and after 21 days of Silymarin and/or vitamin E supplementation.

The mean values of RBC GPX in all 3 treatment groups were significantly higher than the control group after 3 weeks of treatment, and adjusted with baseline values using ANCOVA and Least Significant Difference (LSD) tests. This effect was more pronounced when the supplements were used in combination, leading to a significantly higher value compared to the group receiving vitamin E alone (Table 2). Regarding plasma MDA level, a significantly decreased level was observed after 3 weeks, only in the group receiving the combination compared with control. Although not significant, MDA levels were also lower after 3 weeks in the groups receiving vitamin E or Silymarin alone.

As shown in Table 2 there is also a statistically significant increase in mean hemoglobin at 3 weeks in all three treatment groups.

Discussion

In the present study we showed that Silymarin alone, or in combination with vitamin E can reduce MDA levels and increase RBC GPX. The effect on RBC GPX was statistically significant in the groups treated with Silymarin, vitamin E and combination. To our knowledge this is the first study to evaluate the antioxidative and anti-lipid peroxidative effects of Silymarin alone or in combination with vitamin E in hemodialysis patients. One of the main results of our study was a decrease in the end product of lipid peroxidation, MDA, and an increase in the RBC glutathione peroxidase, an antioxidant enzyme, after supplementation with vitamin E for 3 weeks. The effects of vitamin E on oxidative markers has been evaluated by many studies with variable dose and duration of therapy among
hemodialysis patients. Vitamin E supplementation caused an increase in GPX and SOD activities and a decrease in TBARS concentrations and a decrease in MDA in this population [24–32]. Coating of the dialyzer membrane with vitamin E has been used to prevent neutrophil activation [33, 34]. Our study confirms the results of other studies regarding antioxidative and antiperoxidative effect of vitamin E in hemodialysis patients and extend these observations to show that administration of vitamin E at a dose of 400 mg/day for 3 weeks causes a reduction in MDA. This effect was further increased by dietary supplementation with Silymarin, which suggest that increasing the flavonoid content of the diet may counteract the increase in oxidative stress burden in hemodialysis patients. Compared with previous studies, this beneficial effect on oxidative stress markers occurred with relatively lower dose and shorter duration of therapy. This can be attributed to potentiating and synergistic effect of combination supplements, implying that Silymarin as a potent antioxidant intensified this beneficial effect. Galli et al. showed that vitamin E, 800 mg/day for only 3 weeks led to a slight and non-significant decrease in TBARS levels in HD patients [34]. The treatment with complex of Silymarin and Silibinin by Dietzman et al. led to a restoration of cellular deficiency of thiol in patients with end stage diabetic nephropathy on hemodialysis. This was followed by down-regulation of TNF-α [20]. In contrast to our study, only patients with type 2 diabetes were included and plasma MDA, RBC GPX, and hemoglobin values were not evaluated.

The efficacy of Silymarin in type II diabetes mellitus was also evaluated in a randomized double blind study. A significant decrease in HbA1c, FBS, total cholesterol, LDL and triglyceride levels was shown in Silymarin treated patients compared with placebo. In addition, Silymarin and its major constituent (Silibinin) led to reversal of deficiency of cellular thiol in patients with ESDN and an improvement in functional key-elements of the T-cell system and a down regulation of TNF-α [35].

Lipid peroxidation of erythrocyte membranes by toxic oxygen-free radicals plays a major role in the anemia of patients on hemodialysis and administration of antioxidants contributes to an improvement of anemia independent of erythropoietin need [36–41]. The study by Cristol et al. in a group of hemodialysis patients revealed a significant increase in RBC and a reduction in erythropoietin dose with Vitamin E supplementation, while maintaining stable hemoglobin concentrations [27]. Usberti et al. reported that a combination of a vitamin E-bonded dialysis membrane and glutathione infusion achieved better control of anemia than a vitamin E-bounded membrane alone, with a significant reduction of erythropoietin requirement [42, 43]. In the present study, we demonstrated a statistically significant increase in the mean hemoglobin levels of all three treatment groups compared with control. Previously mentioned studies as well as our study, suggest that the oxidative stress could be one of the resistance factors to erythropoietin response among patients on hemodialysis and that Silymarin and/or vitamin E supplementation could have a sparing effect on erythropoietin dosage requirement. The two main limitations of the study (the small sample size and the short duration of follow-up) preclude us from drawing definite conclusions about the effect of the interventions on erythropoietin requirement.

Conclusions

The results of the present study demonstrate that oral supplementation with Silymarin alone or in combination with vitamin E leads to a decrease in plasma MDA levels, an increase in the level of RBC GPX, and an increase in hemoglobin levels in patients with end stage renal disease. Studies with larger sample sizes and a longer duration of follow-up are required to investigate the effect of Silymarin supplementation on a clinically meaningful reduction in cardiovascular morbidity and mortality, erythropoietin requirement and subsequent reduction in healthcare costs.
References


### Table 1

<table>
<thead>
<tr>
<th>Group characteristics</th>
<th>Control</th>
<th>Silymarin</th>
<th>Vit E</th>
<th>Silymarin + Vit E</th>
<th>P</th>
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<tbody>
<tr>
<td>Age*</td>
<td>44 ± 17</td>
<td>50 ± 10.3</td>
<td>43 ± 13.4</td>
<td>48.5 ± 13</td>
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<td>Sex (M/F)</td>
<td>16/4</td>
<td>18/2</td>
<td>11/9</td>
<td>13/7</td>
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<td>HTN (+/-)</td>
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<td>14/6</td>
<td>14/6</td>
<td>15/5</td>
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<td>DM (+/-)</td>
<td>4/16</td>
<td>7/13</td>
<td>9/11</td>
<td>5/15</td>
<td>0.330</td>
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</table>

*mean ± S.D
Table 2

Oxidative stress markers, and hemoglobin changes in four groups of study patients

<table>
<thead>
<tr>
<th>Study period</th>
<th>Baseline</th>
<th>3weeks</th>
<th>P*</th>
<th>Estimated means* (3weeks)</th>
<th>Pariwise significant results (LSD)</th>
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<tr>
<td>Hb(mg/dl)</td>
<td></td>
<td></td>
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<tr>
<td>1.control</td>
<td>11.01±2.58</td>
<td>10.92±1.68</td>
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<td>2.silymarin</td>
<td>10.93±1.53</td>
<td>12.55±1.79</td>
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<tr>
<td>3.vit E</td>
<td>11.29±2.46</td>
<td>12.61±2.18</td>
<td>12.60</td>
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<td>2,3,4 vs.1</td>
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<tr>
<td>4.combined</td>
<td>11.88±1.63</td>
<td>12.68±1.90</td>
<td>12.36</td>
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<tr>
<td>GPX(UU/gr-Hb)</td>
<td></td>
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<td></td>
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</tr>
<tr>
<td>1.control</td>
<td>5.62±2.85</td>
<td>3.16±1.89</td>
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<tr>
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<td>4.28±1.94</td>
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<tr>
<td>3.vit E</td>
<td>3.93±2.18</td>
<td>4.22±1.63</td>
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<td>2,3,4 vs.1 &amp; 4 vs.3</td>
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<td>4.combined</td>
<td>3.98±2.03</td>
<td>5.78±3.51</td>
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<td>MDA(nmol/ml)</td>
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<td>4 vs.1</td>
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<td>9.27±2.62</td>
<td>7.84±1.84</td>
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*Based on ANCOVA: 3 week measurement of each variable as “dependent” and “baseline” as “covariate” (significance level= 0.05)

GPX=Glutathione peroxidase, MDA=Malondialdehyde, Hb=Hemoglobin