THE EVALUATION OF HEPATOPROTECTIVE EFFECT OF ISILYMARIN, PHYLLANTHUS NIRURI EXTRACT AND CHOLINE COMBINATION

Florica Năftănăilă-Mali¹*, Ioana Alexandra Negoiță², Diana Elena Soloman Năftănăilă-Mali², Georgiana Robu²
1. National Institute of Aeronautical and Space Medicine “Gen. Dr. Aviator Victor Anastasiu”, Bucharest, Romania
2. Central University Emergency Military Hospital “Dr. Carol Davila”, Bucharest, Romania
*Corresponding author. E-mail: naftanaila_florica@yahoo.com

THE EVALUATION OF HEPATOPROTECTIVE EFFECT OF ISILYMARIN, PHYLLANTHUS NIRURI EXTRACT AND CHOLINE COMBINATION (Abstract): The high incidence of liver diseases due to the use of xenobiotics and unhealthy lifestyle is associated with increased need for hepatoprotective product administration. Aim: The hepatoprotective effect of the combination silymarin / Phyllanthus niruri extract / choline was investigated in comparison with silymarin alone in patients with hepatic steatosis, in an open clinical study. Material and methods: 101 patients with non-alcoholic hepatic steatosis confirmed by high values of serum transaminases and ultrasonography were included in the study. The patients were divided into two groups, group A received 150 mg silymarin three times daily, and group B a hepatoprotective complex (120 mg silymarin / 225 mg Phyllanthus niruri extract / 60 mg choline) three times daily, for 6 months. The hepatoprotective effects were assessed through the evolution of the following laboratory parameters: aspartate transaminase (AST), alanine transaminase (ALT), gamma-glutamyl transferase (GGT), alkaline phosphatase, serum bilirubin and serum cholesterol at 3 and 6 months after the initiation of the hepatoprotective treatment. Results: Both products were effective in the normalization of liver function, but the combination silymarin / Phyllanthus niruri extract / choline showed significantly better results than silymarin alone, considering the normalization of the hepatic parameter values after 3 and 6 months. The risk for hepatoprotective failure was significantly lower with the combination silymarin / Phyllanthus niruri extract / choline versus silymarin alone, the number needed to treat (NNT) being 3.7 after 3 months and 2.5 after 6 months. Conclusions: The combination silymarin / Phyllanthus niruri extract / choline induced a better liver protection than silymarin alone. Keywords: PHYLLANTHUS NIRURI, SILYMARIN, CHOLINE, HEPATIC STEATOSIS, HEPATOPROTECTIVE.

Liver diseases register an increasing prevalence in Europe. The increased intake of alcohol, xenobiotics, junk-food combined with an unhealthy lifestyle and, finally, the infections caused by hepatitis viruses contribute to this status quo (1, 2). In Romania, non-alcoholic hepatic steatosis alone is diagnosed in 20% of the patients presenting for an internal medicine consultation, according to a survey published in 2008 (3, 4). The warnings about the complications associated with non-alcoholic steatosis (5) indicate the need for treatment of patients and the correction of risk factors.
Florica Năftănăilă-Mali et al.

The patients with liver diseases are advised to use a large variety of hepatoprotective products, apart from the specific antiviral treatment in case of viral hepatitis. Of the herbal products, those containing silymarin standardized extracts from *Cardui mariae fructus* have a privileged position among the hepatoprotective products. A recent systematic review with meta-analysis of randomized and controlled clinical trials evaluating the effect of silymarin on biochemical indicators in patients with liver disease have found a statistical significant effect of silymarin in decreasing aspartate transaminase (AST) and alanine transaminase (ALT), but not gamma-glutamyl transferase (GGT) (8). The decrease in hepatocyte oxidative stress and lipid metabolism regulation are considered the main effects of silymarin in steatosis (9).

The aim of this study was to investigate the hepatoprotective effect of the combination silymarin / *Phyllanthus niruri* extract / choline in comparison with silymarin alone, to assess the contribution of *Phyllanthus niruri* (an ayurvedic herb) extract and choline (an essential nutrient for the optimal liver functioning) to the hepatoprotective effect of silymarin.

**MATERIALS AND METHODS**

*Study population.* Patients aged 18 to 80 years old, diagnosed with non-alcoholic hepatic steatosis confirmed by high values of serum transaminases and ultrasonography, were enrolled in this clinical study at “Gen. Dr. Aviator Victor Anastasiu” National Institute of Aeronautical and Space Medicine, Bucharest, Romania. The inclusion criteria were: patients with abnormally elevated level of at least one of the enzyme AST, ALT or GGT, according to the method used by the laboratory of the medical unit. Abnormal were considered the levels of AST above 36 IU / L in women and 59 IU / L in men, ALT above 52 IU / UL in women and 72 IU / L in men and of GGT higher than 73 IU / L in both genders. The exclusion criteria were patients who followed antiviral therapy or candidates for specific antiviral therapy for hepatic viral infections.

The patients randomly received either silymarin 150 mg three times / day for 6 months (food supplement that contains 150 mg silymarin / tablet) (group A) or a combination of 120 mg silymarin / 225 mg *Phyllanthus niruri* extract / 60 mg choline three times daily (food supplement) (group B). All the patients were advised to eat a healthy diet and avoid alcoholic (ethanolic) beverages and highly processed foods. Physical exercise programs were indicated for the overweight and obese patients. The patients were assessed at baseline and after 3 and 6 months.

*Evaluation criteria and outcome measures.* At every time points, the patients were assessed for: AST, ALT, GGT, alkaline phosphatase, serum bilirubin, and serum cholesterol. In addition, blood sugar level and blood pressure were measured to investigate the impact of 225 mg of *Phyllanthus niruri* extract / dose on these parameters. Blood sugar and blood pressure were considered because non-clinical studies described for *Phyllanthus niruri* a decrease in blood sugar, comparable with that induced by glibenclamide (10, 11, 12) and blood pressure, comparable with the one induced by hydrochlorothiazide (13); but the experience from well-organized studies in human subjects is sparse. Side effects that could occur following the administration of both products have been monitored.
The evaluation of hepatoprotective effect of silymarin, *Phyllanthus niruri* extract and choline combination

**Statistical analysis.**
The results were subjected to statistically interpretation using *EpiInfo* software; Z-test was used to determine the significance of the differences between the results in the two groups. The relative risks (RR) of not obtaining the normalization of laboratory parameters with silymarin / *Phyllanthus niruri* extract / choline versus silymarin administration and the NNT value were calculated.

**RESULTS**
The clinical study was conducted between March 2015 and December 2015. One hundred and one patients met the inclusion criteria and have completed the 6-month hepatoprotective treatment. The patients were randomly divided into 2 groups: 50 patients (28 M / 22 F) in group A (silymarin group) and 51 patients (35 M / 16 F) in group B (silymarin / *Phyllanthus niruri* extract / choline). The initial characteristics of the groups (demographic data and laboratory parameters) are summarized in first table. Initially, the groups were generally homogenous. Significant statistical differences were recorded only for ALT, which was significantly higher in the group B (p<0.001) and patient age which was significantly greater in group A (p=0.04).

**TABLE I**
Demographic data and laboratory parameters at baseline

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Group A (n = 50)</th>
<th>Group B (n = 51)</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Demographic data</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sex, Male / Female (%Male / %Female)</td>
<td>28 / 22 (56 / 44)</td>
<td>35 / 16 (68.63 / 31.37)</td>
<td>0.19</td>
</tr>
<tr>
<td>Age, years mean ± SD (range)</td>
<td>58.72 ± 10.46 (37 - 81)</td>
<td>53.71 ± 12.45 (25 - 77)</td>
<td>0.04</td>
</tr>
<tr>
<td>Body weight, Kg mean ± SD (range)</td>
<td>84.11 ± 11.94 (58 - 114)</td>
<td>82.29 ± 1 2.74 (68 - 118)</td>
<td>0.39</td>
</tr>
<tr>
<td><strong>Para-clinical parameters</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>AST, IU / L mean ± SD (range)</td>
<td>68.76 ± 30.26 (30 - 208)</td>
<td>68.94 ± 29.93 (39 - 180)</td>
<td>0.98</td>
</tr>
<tr>
<td>ALT, IU / L mean ± SD(range)</td>
<td>99.52 ± 26.72 (64 - 152)</td>
<td>126.92 ± 47.62 (68 - 280)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>GGT, IU / L mean ± SD (range)</td>
<td>73.72 ± 30.03 (28 - 152)</td>
<td>72.75 ± 34.09 (30 - 180)</td>
<td>0.88</td>
</tr>
<tr>
<td>Alkaline Phosphatase, U / L mean ± SD (range)</td>
<td>70.94 ± 23.33 (37 - 165)</td>
<td>67.20 ± 22.20 (37 - 145)</td>
<td>0.41</td>
</tr>
<tr>
<td>Serum Bilirubin, mg / dL mean ± SD (range)</td>
<td>1.11± 0.28 (0.8 - 2.2)</td>
<td>1.06 ± 0.18 (0.7 - 1.5)</td>
<td>0.35</td>
</tr>
<tr>
<td>Serum cholesterol, mg / dL mean ± SD (range)</td>
<td>223.54 ± 33.40 (164 - 295)</td>
<td>230.39 ± 31.20 (175 - 311)</td>
<td>0.29</td>
</tr>
<tr>
<td>Systolic Blood Pressure, mm Hg mean ± SD (range)</td>
<td>134.72 ± 13.22 (110 - 170)</td>
<td>130.92 ± 9.72 (112 - 148)</td>
<td>0.10</td>
</tr>
<tr>
<td>Diastolic Blood Pressure, mm Hg mean ± SD (range)</td>
<td>82.08 ± 9.37 (60 - 105)</td>
<td>81.75 ± 9.37 (60 - 100)</td>
<td>0.86</td>
</tr>
<tr>
<td>Blood Glucose, mg / dL mean ± SD (range)</td>
<td>108.62 ± 21.14 (82 - 197)</td>
<td>103.71 ± 15.74 (65 - 142)</td>
<td>0.19</td>
</tr>
</tbody>
</table>

n – number of patients, SD – standard deviation
Comparing to the baseline, at 3 months, significantly lower levels were recorded in group B than in group A for serum ALT, AST, bilirubin, alkaline phosphatase and cholesterol. Only for GGT was found no significant decrease in group B versus group A.

At 6 months, all the monitored parameters of the liver function were significantly lower in group B versus group A (fig. 1-6, tab. II).
The evaluation of hepatoprotective effect of silymarin, *Phyllanthus niruri* extract and choline combination

TABLE II
The evaluation of laboratory parameters after 3 and 6 months of hepatoprotective product administration

<table>
<thead>
<tr>
<th>Laboratory parameter</th>
<th>3 months</th>
<th>6 months</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Group A (n=50) mean ± SD (range)</td>
<td>Group B (n=51) mean ± SD (range)</td>
</tr>
<tr>
<td>AST, UI / L</td>
<td>54.02 ± 14.8 (30 - 112)</td>
<td>48.74 ± 9.82 (30 - 75)</td>
</tr>
<tr>
<td>ALT, UI / L</td>
<td>69.7 ± 16.57 (32 - 110)</td>
<td>67.36 ± 12.80 (43 - 108)</td>
</tr>
<tr>
<td>GGT, UI / L</td>
<td>60.12 ± 17.04 (25 - 92)</td>
<td>57.76 ± 16.80 (21 - 98)</td>
</tr>
<tr>
<td>Alkaline phosphatase, U / L</td>
<td>64.38 ± 18.46 (29 - 98)</td>
<td>57.55 ± 16.28 (30 - 102)</td>
</tr>
<tr>
<td>Serum bilirubin, mg / dL</td>
<td>1.11 ± 0.25 (0.8-2.1)</td>
<td>1.01 ± 0.13 (0.8-1.4)</td>
</tr>
<tr>
<td>Serum cholesterol, mg / dL</td>
<td>210.41 ± 20.10 (172-241)</td>
<td>211.33 ± 16.87 (185-258)</td>
</tr>
<tr>
<td>Systolic Blood Pressure, mg Hg</td>
<td>129.36 ± 7.95 (115-145)</td>
<td>128.06 ± 6.51 (115-140)</td>
</tr>
<tr>
<td>Diastolic Blood Pressure, mg Hg</td>
<td>80.20 ± 5.80 (70-90)</td>
<td>80.06 ± 6.53 (60-95)</td>
</tr>
<tr>
<td>Blood sugar, mg / dL</td>
<td>104.12 ± 11.71 (82-139)</td>
<td>100.88 ± 10.07 (80-131)</td>
</tr>
</tbody>
</table>

n – number of patients, SD – standard deviation

The results are more relevant when considering the rate of hepatic enzyme normalization. At baseline, in both groups, all patients had abnormal level of at least one of the AST, ALT or GGT enzymes, but after 3 months 10 (20%) patients in group A and 24 (47.1%) patients in group B had normal hepatic enzyme levels. Thirteen (26%) patients in group A and 34 (66.7%) patients in group B had normal hepatic enzyme levels after 6 months (fig. 7, tab. III).

![Hepatic enzymes normalization with hepatoprotective products administration](image-url)

**Fig. 7.** The rate of hepatic enzyme normalization after 3 and 6 months of treatment with hepatoprotective products
No statistically significant differences in blood sugar level and blood pressure were recorded between the two groups (tab. II) and no side effects were reported in both groups.

**DISCUSSION**

Silymarin standardized dry extracts from *Cardui mariae fructus* are used in Europe at least since 1974 in liver diseases such as toxic liver damage, chronic inflammatory liver disease, cirrhosis and are also clinically proven to be effective in non-alcoholic steatosis (14, 15). Silibinin, the main component of flavonolignan complex silymarin, stimulates the protein biosynthesis and induces stabilization of the hepatocyte membranes, exerts antifibrotic effect and prevents the cellular damage and the secretory failure associated with various hepatotoxins (15).

*Phyllanthus niruri* is an ayurvedic herb, traditionally used for the effect on kidney stones and hepatoprotective effect. Aerial parts are rich in lignanic compounds such as: phyllanthin and hypophyllantin responsible for hepatic tissue regenerating effects (16). *Phyllanthus niruri* has an efficient antitoxic effect, confirmed by acetaminophen, carbon tetrachloride, and thioacetamide-experimentally induced hepatotoxicity models (16, 17, 18, 19). Similar hepatoprotective activities both for silymarin and *Phyllanthus niruri* have been recorded in paracetamol-induced liver toxicity (20). The hepatoprotective effect of *Phyllanthus niruri* was clinically demonstrated and explained by the increase in physiological antioxidants, reduced lipid peroxidation in hepatocyte membrane and counteracting of the destructive effects of free oxygen radicals in the liver (21). The combination between silymarin and *Phyllanthus niruri* demonstrated synergistic hepatoprotective effect in a non-clinical study in rats with carbon tetrachloride-induced hepatotoxicity. A superior effect in normalizing the blood biochemical parameters was recorded for the combination of silymarin with *Phyllanthus* in comparison with silymarin or *Phyllanthus* alone (22). Another non-clinical research (23) supported the beneficial effects of the combination of silymarin and *Phyllanthus*; more efficient hepatoprotective effects were recorded for this combination compared with silymarin alone. The study considered the prevention of liver injury and the normalization of transaminase values in carbon tetrachloride - and paracetamol-induced hepatotoxicity models.

Choline is a source of methyl for the hepatic transmethylation reactions and it is also an essential component of cell membranes and plasma lipoproteins. Choline deficiency is associated with liver injuries (hepatic steatosis and high level of alanine...
aminotransferase) that have been recorded both in choline-free parenterally fed patients (24) and in the choline-poor diet in post-menopausal women (25); these effects are reverted by choline dietary supplementation. Currently, choline is the single nutrient with specific liver health claim approved by the European Food Safety Authority “maintenance of normal liver function” (26).

The current study investigated the hepatoprotective effect of the combination silymarin / \textit{Phyllanthus niruri} extract / choline in comparison with silymarin alone.

The study confirmed the hepatoprotective effect assessed by the normalizing effect on serum transaminases for silymarin alone and the combination silymarin / \textit{Phyllanthus niruri} extract / choline. However, the combination silymarin / \textit{Phyllanthus niruri} / choline was significantly more efficient than silymarin alone in normalizing the AST, ALT and GGT levels after 3 and 6 months of administration. A brief assessment revealed that the results obtained after 6 months with silymarin alone have been already obtained after 3 months with the combination silymarin / \textit{Phyllanthus niruri} extract / choline, even though a lower dose of silymarin was used in the combination product. Results with statistical significance favorable for silymarin / \textit{Phyllanthus niruri} / choline combination were also obtained for alkaline phosphatase, serum cholesterol and serum bilirubin.

The study also aimed to investigate if \textit{Phyllanthus niruri} extract influences the glycemic profile and blood pressure. In both groups, a slight decrease of blood sugar and blood pressure was recorded. No statistically significant difference between groups in the levels of these parameters was recorded. On the other side, most patients had quite normal levels of these parameters at the inclusion in study: higher rate of abnormal levels at inclusion was observed for the blood sugar levels. Twenty-one (42%) patients in group A and 16 (31.4%) patients in group B presented blood sugar levels above normal ranges at baseline. After 6 months of hepatoprotective treatment, 12 (24%) patients in group A and 6 (11.8%) patients in group B presented higher blood sugar levels. No significant difference in blood sugar levels was found between groups (p=0.52) and no influence of \textit{Phyllanthus niruri} on blood sugar levels could be demonstrated. The results are not in agreement with the results of non-clinical studies that demonstrated the hypoglycemic effect (10, 11, 12) and the hypotensive effect mediated by the diuretic effect of \textit{Phyllanthus niruri} (13, 27). In our study, no statistically significant differences were recorded between group B, receiving silymarin / \textit{Phyllanthus niruri} extract / choline combination and group A, receiving silymarin alone.

No side effects were reported, and this study confirmed the safety use of the combination silymarin / \textit{Phyllanthus niruri} / choline.

**CONCLUSIONS**

The results of our clinical study indicate that the combination silymarin / \textit{Phyllanthus niruri} extract / choline achieved a better liver protection than silymarin alone in patients with hepatic steatosis. Significantly better results were also obtained after 6 months of administration.

The combination silymarin / \textit{Phyllanthus niruri} / choline showed a safety profile like silymarin alone since no adverse reactions were recorded.
REFERENCES


The evaluation of hepatoprotective effect of silymarin, *Phyllanthus niruri* extract and choline combination


---

**PLACENTA ACCRETA FOLLOWING HYSTEROSCOPIC LYSIS OF ADHESIONS CAUSED BY ASHERMAN’S SYNDROME: A CASE REPORT AND LITERATURE REVIEW**

Asherman’s syndrome is defined as partial or complete obstruction of the uterine cavity primarily caused by intrauterine procedures and infections. Hysteroscopic adhesiolysis is commonly used to treat Asherman’s syndrome. Although the frequency of placenta accreta is known to increase with pregnancy after hysteroscopic adhesiolysis, precise data remain unknown. We report a case of placenta accreta following hysteroscopic lysis of adhesions caused by Asherman’s syndrome and IVF treatment and review the literature on placenta accreta following hysteroscopic adhesiolysis. It is necessary to consider placenta accreta as a complication of pregnancies after hysteroscopic adhesiolysis for Asherman’s syndrome, particularly in those conceived using IVF. The incidence of placenta accreta is significantly higher in IVF pregnancies than in spontaneous pregnancies [16]. Esh-Broder et al. reported that the rate of placenta accreta in spontaneous pregnancies was 12 / 752 (1.2 / 1000), while that in IVF pregnancies was 30 / 24,441 (16 / 1,000). The higher rate of placenta accreta in IVF pregnancies may be due to the change in endometrial environment and morphological and structural changes to the endometrium due to the IVF treatment protocol (stimulation protocol). Intrauterine adhesions (IUA) severity is associated with greater reduction in fertility thereby increasing the likelihood that affected patients will need to undergo IVF to have a successful pregnancy. The extent of endometrial loss increases with IUA severity, which likely confers a greater risk of placenta accreta. For this reason, doctors should be aware that IVF pregnancy following hysteroscopic adhesiolysis involves a greater risk of placenta accreta (Yuko Sonan, Shigeru Aoki, Kimiko Enomoto, Kazuo Seki, Etsuko Miyagi. Placenta Accreta following Hysteroscopic Lysis of Adhesions Caused by Asherman’s Syndrome: A Case Report and Literature Review. *Case Reports in Obstetrics and Gynecology*, 2018, https://doi.org /10.1155/2018/6968382).