The Clinical Outcome of the Extract of *Ginkgo biloba* in Egyptian Chronic Hepatitis C Patients.

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ABSTRACT: This study was designed to evaluate the clinical efficacy and safety of *Ginkgo biloba* (EGb) extract, ursodeoxycholic acid (UDCA) and their combination in patients with chronic hepatitis C.

This is a prospective 3-arm randomized controlled pilot study performed on 67 patients diagnosed with chronic HCV, who were randomly assigned to receive either EGb, UDCA or EGb+UDCA for a period of 4 weeks. Patients were monitored for liver transaminases, albumin, total bilirubin, International normalized ratio (INR), serum total antioxidant capacity (TAC), serum transforming growth factor beta-1 (TGF-β1) and serum hyaluronic acid (HA) at baseline and after 4 weeks. Assessment of quality of life was performed using the RAND SF-36 questionnaire. Safety was evaluated by adverse effect reporting and monitoring the complete blood count.

After treatment, no change in liver transaminases was observed in the EGb-group, while the UDCA-group and the EGb+UDCA-group showed significant decrease in the transaminases level ($p<0.05$). No significant changes in the levels of total bilirubin, albumin nor INR were observed. The serum TAC level was significantly increased, while the serum levels of TGF-beta1 and HA were significantly declined in all the three groups ($p<0.001$). Regarding quality of life, EGb-group showed no change in the component summary scores of the RAND SF-36 questionnaire. On the other hand, the UDCA-group showed a significant increase in both the physical and mental component summary scores, while the EGb+UDCA group showed a significant increase in only the mental component summary score. All treatment regimens were well tolerated by all patients.

In conclusion, the usage of *Ginkgo biloba* extract in the studied HCV-cohorts proved to exhibit antioxidant efficacy and potential to lower fibrosis markers and profibrotic growth factors; yet, this hepatoprotective action failed to ameliorate the liver transaminases. The combined administration of EGb and UDCA showed no additional clinical benefit.

Keywords: Hyaluronic acid; Oxidative stress; Total antioxidant capacity and Transforming growth factor beta-1.

INTRODUCTION:
According to The World Health Organization estimates, about 150 million people are chronically infected with hepatitis C virus and more than 350,000 people die every year from hepatitis C-related liver disease (WHO, 2014). In Egypt, Hepatitis C virus is considered a major public health crisis with a prevalence of 14.7% of the Egyptian population with the predominance of genotype 4 (Hnatsyszyn, 2004; El-Zanaty and Way, 2009). The incidence of HCV in Egypt seems to be continuing at a rate of ≈6.9/1,000 persons per year, indicative of possibly ongoing hyperepidemic transmission (Miller and Abu-Raddad, 2010).

To a great extent, the pathologic consequences of HCV infection are probably due to the direct effect of the expression of viral proteins, in addition to the active, but ultimately ineffective immune response to the virus that causes liver injury, but fails to eliminate the infection. Oxidative stress is also an important aspect of HCV pathogenesis that plays a deleterious contributing role, leading to the development of fibrosis and liver cancer (Neumann-Haefelin and Thimme, 2013; Yamane et al., 2013). The standard of care for genotype 4 is a combination treatment of pegylated interferon alpha by subcutaneous injection once weekly and oral ribavirin daily (Lever and Nash, 2011). Unfortunately, for genotype 4, the sustained virological response is achieved in only approximately 60% of the patients (Kamal et al., 2007), although this percentage may change in the coming years with the introduction of the new agent, sofosbuvir, an HCV viral polymerase nucleotide inhibitor (WHO, 2014). Moreover, the increased rate of adverse events, the high cost and the
many contraindications to these drugs (Modi and Liang, 2008) may possibly preclude their use in clinical practice. Individuals who fail to respond, or who are unable to tolerate treatment, can benefit from the use of complementary and alternative medications instead of, or in addition to, the standard therapy (Verma and Thuluvath, 2007).

Ginkgo biloba extract (EGb) has a reported hepatoprotective activity, that could be due to its radical scavenging action and antioxidant activity (Li et al., 1995; Feng et al., 2009). Moreover, it improved hepatic microcirculation and protected the liver through an immunomodulatory effect by inhibiting expression of transforming growth factor β1 (TGF-β1), thus can inhibit hepatic stellate cell activation and expression of extracellular matrix (Li et al., 1995; Zhang et al., 2006; Zhang et al., 2008). Therefore, EGB can be therapeutically used to prevent and treat hepatic damages induced by various hepatopathologies.

Ursodeoxycholic acid (UDCA) has been previously used in patients with chronic hepatitis C to decrease serum liver enzymes despite having no effect on the viral load (Omata et al., 2007). The mechanism involved in the aminotransferase lowering effect of UDCA is likely related to its anti-apoptotic property rather than an antagonising effect towards toxic bile acids at a biophysical level (Solá et al., 2006). To this end, the present study was designed to evaluate the clinical safety and efficacy of EGb and its combination with UDCA in Egyptian patients with chronic hepatitis C.

MATERIAL AND METHODS:

Patients:

This study was conducted on 67 patients aged 18-65 with proven chronic hepatitis C virus infection according to standard diagnostic criteria. Patients were recruited from the outpatient clinic of the Tropical Medicine Department, El-Demer dash Hospital, Ain Shams University and Yassin Abdel Gaffar Charity Center for Liver Diseases and Research, Cairo, Egypt from December 2011 to February 2014. Inclusion criteria included: (1) positive serum anti-HCV Ab and HCV RNA; (2) clinical and ultrasonographic criteria suggestive of chronic hepatitis C; (3) Elevation of ALT at least 1.5 times the upper limit of normal (but <5 times the normal limit); (4) Child Pugh Grade A. Patients were not eligible if their Child Pugh Grade was B or C, had other causes of liver disease or had evidence of advanced liver disease e.g. history or presence of ascits, bleeding esophageal varices, and hepatic encephalopathy. Patients were also excluded if they had portal vein thrombosis, hepatocellular carcinoma, seizure disorders, body mass index >40 or advanced systemic disease like heart failure or any debilitating disease that will affect life expectancy, in addition to patients using oral contraceptive pills, selective serotonin reuptake inhibitors, narcotic drugs, blood thinning drugs except for low dose aspirin ≤325 mg/day or those who received a treatment of antiviral agents (interferon with or without ribavirin) in the previous 2 months. Other exclusions included: pregnancy or lactation, patients with platelet level <50,000/µL and patients with sensitivity to UDCA or EGb.

Study design:

This pilot study was conducted through a prospective parallel open label 3-arm randomized controlled trial. The study design was approved by the local Ethics Committee Review Board at Faculty of Pharmacy, Ain Shams University. Patients, who satisfied the inclusion criteria, were randomly assigned after providing written informed consents to one of three groups: EGb-group, UDCA-group or EGb+UDCA-group. Patients in the EGb-group were given one capsule of 260 mg EGb 761 (Ginkgo biloba®, EMA Pharm Pharmaceuticals, Egypt) once daily. Patients in UDCA-group were given one capsule of 300 mg ursodeoxycholic acid (Ursogall® 300, Sigma, Egypt) three times daily, while patients in EGb+UDCA-group were given one capsule of 260 mg EGb 761 (Ginkgo biloba®) once daily in addition to one capsule of 300 mg ursodeoxycholic acid (Ursogall®) three times daily. Treatment duration was 4 weeks in all the 3 groups.

Follow up and laboratory testing:

The following parameters are baseline and monitoring clinical indicators:

1-Clinical examination with special stress on the general clinical manifestations of chronic liver disease and careful abdominal examination on each visit.

2-Laboratory investigation: Liver profile tests including [ALT, AST, albumin and total bilirubin], international normalized ratio (INR) and complete blood counts were done to all patients at baseline and at the end of the treatment period.

3-Specific investigations:

To assess the effect of the treatment on the oxidative stress, serum total antioxidant capacity (TAC) level was detected at baseline and after 4 weeks using a commercial kit purchased from BioAssay Systems, Hayward, CA, USA. Also, serum transforming growth factor beta1 (TGF- beta1) (RayBiotech, Inc., Norcross, GA, USA) and serum hyaluronic acid (HA) (Corgenix Inc., Westminster, Colorado, USA) were measured at baseline and after 4 weeks following the manufacturers’ protocols.

4-Quality of life assessment: through a questionnaire filled by the patients at the beginning and at the end of the treatment period. The questionnaire was translated by EL-Monefia Liver Institute to Arabic from the Rand SF 36- item health survey.

Statistical analysis:

Statistical analysis was performed using SPSS software (statistical package for the social sciences, version 16, SPSS Inc., Chicago, IL, USA) and GraphPad Prism® (version 5, Graphpad Software Inc., CA, USA). Data were analyzed by Shapiro-wilk test to test the normality of distribution. Wilcoxon Signed Rank test was used to assess any significant difference between each group before and after treatment. Kruskal-Wallis test was used to assess any significant difference among the results of the three groups all together. Dunn’s Multiple comparison test was used for multiple comparisons between groups. Categorical data were
analyzed using the Chi-square test. Data were presented as median (Interquartile range) (IQR) for continuous, non-normally distributed variables and percentages for categorical data. The values of \( P < 0.05 \) were considered to indicate statistical significance.

**RESULTS:**
A total of 67 patients were enrolled in this prospective study, 22 were randomly assigned to the EGb-group, 23 were assigned to the UDCA-group and 22 were assigned to EGb+UDCA group. Out of the 67 patients assigned, only 60 patients (34 males and 26 females) continued the study to the end; 20 in each group. The reason for dropout of 7 of the patients was voluntarily discontinuation of the assigned treatment without any adverse effect. The three groups were statistically similar with respect to age and gender distribution, as shown in table (1). Baseline biochemical parameters were also similar among the three groups, as presented in tables (2 and 3).

As shown in table (2) and figure (1), there were no significant changes in the serum ALT or AST levels in the EGb-group after 4 weeks of the intervention. On the other hand, the UDCA-group showed 30.5% and 16.7% significant decrease in the median value of ALT and AST levels, respectively, while the combined administration of EGb+UDCA-group showed 32.3% and 16.3% significant decrease in the median value of ALT and AST levels, respectively \( (p<0.05) \). No statistically significant changes in the serum total bilirubin, serum albumin or INR levels were observed in the three groups, as illustrated in table (2).

As shown in table (3) and figure (2a), there were significant elevations in the median value of serum total antioxidant capacity level in the EGb-group, the UDCA-group and the EGb+UDCA-group, \((56.7\%, 45.5\% \text{ and } 36.1\% \text{; respectively at } p<0.001)\). Table (3) and figures (2b and 2c) show significant decrease in the median serum Hyaluronic acid level \((51.6\%, 44.1\% \text{ and } 54.1\%)\), as well as significant decrease in the median serum TGF-\(\beta1\) level \((44.6\%, 49.7\% \text{ and } 41.9\%)\) in the EGb-group, the UDCA-group and the EGb+UDCA-group, respectively \( (p<0.001) \).

Results of the RAND SF-36 questionnaire were derived before and after treatment. Results were calculated to generate the eight health domains, with a potential score of 0 to 100. Higher scores indicated better health.

As illustrated in figure (3) and table (4), the physical component summary score showed a 16.7% significant increase in the UDCA-group \( (p=0.031) \), while the EGb-group and the EGb+UDCA-group showed no significant changes. The Mental component summary score showed 28.8% and 23.5% significant increases in both the UDCA-group and the EGb+UDCA-group, respectively \( (p<0.05) \), while the EGb-group showed no significant changes.

Regarding safety, both EGb and UDCA were well-tolerated. Severe complications attributable to this treatment were not observed. Side effects reported during the study are presented in table (5). No significant changes in the complete blood count after 4 weeks of treatment except for 1.4% decrease in Hemoglobin levels in the EGb+UDCA-group that was statistically significant \( (p=0.032) \).

**DISCUSSION:**
To the best of the authors’ knowledge, this study is the first clinical pilot trial to address the effect of *Ginkgo biloba* extract (EGb 761) alone and in combination with ursodeoxycholic acid on liver functions and health-related quality of life in patients with chronic hepatitis C. There are published articles studying the effect of EGb on chronic hepatitis B (Li et al., 1995; He et al., 2002; Zhang et al., 2008); however, those on HCV-infected patients are scarce. The current approach was conducted in an attempt to improve the quality of life of chronic hepatitis C patients who are facing situations of contraindication, intolerability or non-response to the current gold standard therapy. *Ginkgo biloba* has been studied for being a potential hepatoprotective drug in the treatment of liver diseases (Li et al., 1995, Feng et al., 2009). The standardized EGb 761 consists of 22–27% flavone glycosides, 5–7% terpene lactones and less than 5 ppm ginkgolic acid (Van Beek, 2005). The flavonoid constituents are responsible for the immunomodulatory modulation, free radicals scavenging and antioxidant potential, while the terpenoid constituents improve microcirculation, inhibit platelet activation factor, lower blood triglycerides and prevent vascular sclerosis (Zhang et al., 2008).

In chronic hepatitis C, the pathologic consequences of the disease cause liver injury that leads to the leakage of ALT and AST, the liver enzymes which are originally present in high concentrations in the cytoplasm, into the blood stream in conformity with the extent of liver damage. Assessment of liver function can be performed by estimating the activity of these enzymes (Naik and Panda, 2007).

Data presented in our study showed that EGb failed to induce a significant change in the liver transaminases, total bilirubin and serum albumin. These results are consistent with the results of Zhang et al. (2008) who investigated the effect of EGb on the microcirculation of chronic hepatitis B patients. They attributed the decrease in liver transaminases to the effect of the control drug alone but not the EGb. In contrast to our results, some experimental studies showed a significant decrease in liver transaminases after supplementing the animals with EGb suggesting a hepatoprotective action of the EGb due to its antioxidant and antifibrotic abilities (El Mesallamy et al., 2011; Al-Attar, 2012).

Interestingly, our data revealed that the UDCA-group and the combined UDCA+ EGb-group showed a significant decrease in ALT and AST with no significant changes in serum albumin and total bilirubin. These findings are in agreement with Omata et al. (2007) and Sato et al. (2009) who investigated the effect of ursodeoxycholic acid in patients with chronic hepatitis C. This hepatoprotective effect was explained to be the result of the anti-inflammatory mechanism of UDCA causing either a reduction in the cytotoxicity of hydrophobic bile acids, stimulation of
hepatobiliary secretion, suppression of NF-κB-dependent transcription by binding to the glucocorticoid receptor, or a decrease in proinflammatory cytokine-induced transcription of phospholipase A2 (Sato et al., 2009).

Conflicting results about the tendency of EGB to cause bleeding have been discussed in many studies. Accordingly, a meta-analysis of 18 randomized controlled trials was done by Kellermann and Kloft (2011). They found no evidence of any significant effect of EGB on Adenosine diphosphate-induced platelet aggregation, fibrinogen concentration, activated partial thromboplastin time (aPTT), and prothrombin time. Although subgroup analyses revealed a statistically significant reduction in aPTT for subgroups receiving high-dose EGB of 240 mg/day or more, findings were not clinically relevant. So, they concluded, in consistency with our study, that EGB doesn’t cause a higher bleeding risk.

Oxidative stress plays an important role in HCV pathogenesis. It may result directly from the expression of viral proteins, as well as, from inflammation related to immune recognition of the virus leading to the formation of liver fibrosis via increasing the stellate cell activation and collagen synthesis (Al-Attar, 2012; Neumann-Haefelin and Thimme, 2013; Yamane et al., 2013).

Measurement of TAC has been commonly done in biomedicine with the aim of having an indication of oxidative status in body fluids or tissues (Ahmed et al., 2013). Generally, TAC is decreased in conditions associated with oxidative stress, and increased with the administration of antioxidants (Young, 2001). Patients with chronic hepatitis C were reported to have low serum total antioxidant capacity level compared to the control group (Ahmed et al., 2013). In this study, EGB, UDCA and EGB +UDCA showed significant increase in the level of total antioxidant capacity proving the antioxidant action of these drugs. In several clinical trials, the Ginkgo biloba extract was reported to have antioxidant activity in oral doses of 120 mg once daily and up to 270 mg of EGB twice daily (Kudolo et al., 2005; Suter et al., 2011). This antioxidant action is attributed to the polyphenol structure of its flavonoid constituents (Rodriguez et al., 2007; Shi et al., 2010). It was reported that EGB could decrease the oxidative stress through directly scavenging free radicals and indirectly inhibiting the formation of free radicals (Mahadevan and Park, 2008). The Ginkgo leaf extract had shown significant potential to scavenge reactive oxygen species (ROS) and nitric oxide radicals (NO'). Moreover, it was reported to be able to inhibit lipid peroxidation and lower malondialdehyde (MDA) (Li et al., 1995; Kudolo et al., 2005; Yang et al., 2011), as well as, enhance the activities of antioxidant enzymes such as superoxide dismutase (SOD), glutathione peroxidase, catalase, thereby indirectly contributing as an antioxidant (Li et al., 1995; Feng et al., 2009).

According to the study of El-Sherbiny et al. (2009), UDCA acted as a ROS scavenger molecule itself and protected the components of the mitochondrial electron chain against the uncoupling effect of endogenous hydrophobic bile salts. Moreover, UDCA was reported to be able to enhance the endogenous antioxidant defenses by improving glutathione synthesis or preventing its consumption in non-enzymatic removal of oxygen radicals (El-Sherbiny et al., 2009).

Persistent HCV infection is known to be associated with chronic inflammatory changes within the liver that lead to an unresolved wound healing response that includes abnormal production of extracellular matrix proteins and progressive fibrosis (Young, 2001). The perisinusoidal hepatic stellate cell (HSC) plays a central role in fibrosis as they undergo transformation to become proliferative and contractile myofibroblasts. The activation of HSCs is both positively and negatively regulated via multiple growth factors, cytokines, and chemokines, including transforming growth factor β (TGF-β) which were thought to act positively to enhance fibrogenesis (Friedman, 2011).

Hyaluronic acid is reported to be an accurate tool to predict significant fibrosis, severe fibrosis and cirrhosis. The level of HA increases in relation with the increase in severity of fibrosis and cirrhosis (Halfon et al., 2005). Thus, the study supports the clinical use of HA as a non-invasive marker for liver fibrosis and/or cirrhosis. Serpaggi et al. (2006) proved that liver cirrhosis could be reversed by curative therapies, and since patients with cirrhosis obviously need an antifibrotic therapy most urgently (Popov and Schuppan, 2009), efforts should be done to find new and effective antifibrotic medications. According to our findings, EGB, UDCA and Egb+UDCA caused a significant decrease in serum TGF-β1 and serum HA, yet the antifibrotic action of these drugs couldn’t be proved due to the absence of post-treatment biopsies. Supporting our results with EGB, Zhang et al. (2008) observed that the EGB 761 can inhibit expression of TGF-β1, thus, can inhibit HSC activation and expression of extracellular matrix, such as type I and III collagen. This could be attributed to the hepatoprotective effect of EGB as it could improve sinusoidal microcirculation and alleviate inflammation; causing inhibition of fibrosis.

Concerning UDCA, our findings are in agreement with the results of Neuman et al. (2002) who showed a significant decrease in serum TGF-beta level compared to baseline level in patients with primary biliary cirrhosis treated with UDCA. Moreover, Liang et al. (2009) found that UDCA could significantly inhibit the expression of TGF-β1 mRNA, as well as, the expression of protein TGFβ1, indicating that UDCA plays an important role in the inhibition of hepatic fibrosis. Wu et al. (2012) also investigated the effect of UDCA in patients with primary biliary cirrhosis. They observed a decrease in serum levels of HA and attributed that to the beneficial effects of UDCA therapy.

In contrast to the aforementioned findings, Voumvaraki et al. (2011) and Pan et al., (2012) found no change in the serum level of hyaluronic acid after 6 month administration of UDCA attributing that to the short duration of the study.
Chronic hepatitis C has been associated with low quality of life (Alves et al., 2012). In this study, quality of life was assessed using the RAND SF-36 questionnaire. Quality of life in the EGb-group didn’t show improvement during the treatment period. On the other hand, our study showed a significant improvement in both the Physical and Mental Component summary scores in the UDCA-group and an improvement in only the Mental Component summary score in the EGb+UDCA-group. These findings were not addressed in the literature, as the studies focusing on the quality of life of the investigated drugs in HCV are very scarce. Concerning safety, both EGb and UDCA were well-tolerated. Severe complications were not observed. These findings are in agreement with a previous clinical trial (Zhang et al., 2008) that addressed the effect of EGb on patients with chronic hepatitis B.

The main limitation of the current study is the inability to follow up patients for a longer duration and absence of post-treatment biopsies. We designed this relatively short term study to answer a simple research question; is there any therapeutically feasible potential of Ginkgo biloba extract in chronic HCV patients, that is worthy to conduct a larger study with longer follow up period?

**Conclusion:** The extract of *Ginkgo biloba* may be used, complementary to standard therapy, in patients with chronic hepatitis C, as it exhibits antioxidant actions and ability to lower fibrosis markers and profibrotic growth factors; yet, this hepatoprotective action of EGb was not reflected on the liver transaminases or the quality of life. We also conclude that UDCA has a hepatoprotective action in chronic hepatitis C patients, evidenced in improvement in liver transaminases, oxidative stress, fibrosis and a significant improvement in the quality of life variables. This study also showed that adding EGb to UDCA failed to provide a clinical ameliorative effect. However, administration of EGb and UDCA was clinically safe and tolerable with no evident complications.

**Acknowledgements:** The authors want to thank the Staff of Dr Yassin Abd El-Gaffar Charity Center and Dr. Inas El-Attar, the statistician, for their contribution to accomplish this study.

**Conflict of interest:** The authors, Dina El-Gindy, Mona Schaalan, Runia El-Folly, and Ayman Abdelkader declare that they have no conflict of interest.

Table (1): Age and gender distribution among the EGB-group (*Ginkgo biloba*; 260 mg once daily, p.o. for 4 weeks), UDCA-group (*Ursodeoxycholic acid*, 300 mg three times daily, p.o. for 4 weeks) and EGb+UDCA-group

<table>
<thead>
<tr>
<th>Parameter</th>
<th>EGb-group (n=20)</th>
<th>UDCA-group (n=20)</th>
<th>EGb+UDCA-group (n=20) Combined therapy</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age(years) Median (IQR)</td>
<td>53 (50-60.75)</td>
<td>52 (44.25-56)</td>
<td>54 (45-58)</td>
<td>p1=0.414</td>
</tr>
<tr>
<td>Sex: Male/Female</td>
<td>13/7</td>
<td>10/10</td>
<td>11/9</td>
<td>p2=0.622</td>
</tr>
</tbody>
</table>

Data are presented as Median (IQR).

**p1:** p-value when comparing the three groups at the same time using Kruskal-Wallis test.

**p2:** p-value when using Chi-square test.

Significant difference at p-value <0.05.
Table (2): Hepatoprotective effect of the daily administration of the extract of *Ginkgo biloba* (EGb; 260 mg once daily, p.o. for 4 weeks), Ursodeoxycholic acid (UDCA, 300 mg three times daily, p.o. for 4 weeks) and their combination on Liver function tests.

<table>
<thead>
<tr>
<th>Groups Parameter</th>
<th>EGb-group</th>
<th>UDCA-group</th>
<th>EGb+UDCA-group</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>ALT (U/L), Median(IQR)</td>
<td>Pre 86 (74.5-136.3)</td>
<td>98.5 (78.3-151.5)</td>
<td>102 (85.3-131.0)</td>
<td>NS</td>
</tr>
<tr>
<td></td>
<td>Post 85.5 (63.0-122.8)</td>
<td>68.5 (51.3-95.3)</td>
<td>69.0 (53.3-100.5)</td>
<td>NS</td>
</tr>
<tr>
<td>AST (U/L), Median(IQR)</td>
<td>Pre 72.5 (67.3-97.0)</td>
<td>89 (70.5-120.5)</td>
<td>92.5 (77.5-121.8)</td>
<td>NS</td>
</tr>
<tr>
<td></td>
<td>Post 71.0 (64.3-92.5)</td>
<td>74.5 (57-123.5)</td>
<td>77.0 (51-96.5)</td>
<td>NS</td>
</tr>
<tr>
<td>T. Bil (mg/dl), Median(IQR)</td>
<td>Pre 0.95 (0.70-1.10)</td>
<td>0.65 (0.43-0.98)</td>
<td>0.75 (0.33-1.00)</td>
<td>NS</td>
</tr>
<tr>
<td></td>
<td>Post 1.0 (0.7-1.1)</td>
<td>0.6 (0.4-0.8)</td>
<td>0.8 (0.4-1.1)</td>
<td>NS</td>
</tr>
</tbody>
</table>

Data are presented as Median (IQR).

- a: significant difference between post-treatment and pre-treatment medians using Wilcoxon Signed Ranks Test at p-value <0.05.
- p-value: p-value when comparing the three groups at the same time using Kruskal-Wallis test.
- p1: p-value when comparing EGb-group with UDCA-group using Dunn's Multiple Comparison Test.
- p2: p-value when comparing EGb-group with EGb+UDCA-group using Dunn's Multiple Comparison Test.
- p3: p-value when comparing UDCA-group with EGb+UDCA-group using Dunn's Multiple Comparison Test.
- *: significant difference when comparing the three groups at the same time at p <0.05.
- #: significant difference when comparing EGb-group with UDCA-group at p <0.05.

Abbreviations: ALT, alanine aminotransferase; AST, aspartate aminotransferase; T. Bil, total bilirubin; Alb, Albumin; INR, International Normalized Ratio.

Table (3): Hepatoprotective effect of the daily administration of the extract of *Ginkgo biloba* (EGb; 260 mg once daily, p.o. for 4 weeks), Ursodeoxycholic acid (UDCA, 300 mg three times daily, p.o. for 4 weeks) and their combination on serum levels of total antioxidant capacity, hyaluronic acid and transforming growth factor Beta-1.

<table>
<thead>
<tr>
<th>Groups Parameter</th>
<th>EGb-group</th>
<th>UDCA-group</th>
<th>EGb+UDCA-group</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>TAC (µM), Median(IQR)</td>
<td>Pre 22.6 (21.53-23.68)</td>
<td>23.3 (21.95-25.82)</td>
<td>23.9 (22.4-26.3)</td>
<td>NS</td>
</tr>
<tr>
<td></td>
<td>Post 35.4 (32.3-38.2)</td>
<td>33.9 (31.5-37.0)</td>
<td>32.6 (30.8-34.2)</td>
<td>p=0.035*</td>
</tr>
<tr>
<td>HA (pg/ml), Median(IQR)</td>
<td>Pre 283.8 (261.9-311.0)</td>
<td>290.4 (265.4-326.1)</td>
<td>315.4 (286.8-372.4)</td>
<td>NS</td>
</tr>
<tr>
<td></td>
<td>Post 137.3 (116-159)</td>
<td>162.4 (135.0-193.9)</td>
<td>144.6 (122.6-162.1)</td>
<td>NS</td>
</tr>
<tr>
<td>TGF-β1 (pg/ml), Median(IQR)</td>
<td>Pre 224.9 (193.9-250.4)</td>
<td>208.7 (193.1-245.7)</td>
<td>228.1 (198.4-276.4)</td>
<td>NS</td>
</tr>
<tr>
<td></td>
<td>Post 124.5 (104.3-144.9)</td>
<td>104.9 (84.9-157.4)</td>
<td>132.4 (103.5-179.8)</td>
<td>NS</td>
</tr>
</tbody>
</table>

Data are presented as Median (IQR).

- a: significant difference between post-treatment and pre-treatment medians using Wilcoxon Signed Ranks Test at p-value <0.05.
- p-value: p-value when comparing the three groups at the same time using Kruskal-Wallis test.
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- p2: p-value when comparing EGb-group with EGb+UDCA-group using Dunn's Multiple Comparison Test.
- p3: p-value when comparing UDCA-group with EGb+UDCA-group using Dunn's Multiple Comparison Test.
- *: significant difference when comparing the three groups at the same time at p <0.05.
- #: significant difference when comparing EGb-group with UDCA-group at p <0.05.

Abbreviations: TAC, Total Antioxidant Capacity; HA, Hyaluronic acid; TGF-β1, Transforming Growth factor Beta-1.
Table (4): Effect of the daily administration of the extract of *Ginkgo biloba* (EGb; 260 mg once daily, p.o. for 4 weeks), Ursodeoxycholic acid (UDCA, 300 mg three times daily, p.o. for 4 weeks) and their combination on the Physical and Mental Summary Components of the RAND SF-36 questionnaire.

<table>
<thead>
<tr>
<th>Groups</th>
<th>Parameter</th>
<th>EGb-group</th>
<th>UDCA-group</th>
<th>EGb+UDCA-group</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Pre</td>
<td>53.8(41.6-71.6)</td>
<td>52.5(34.2-70.0)</td>
<td>57.5(43.1-68.9)</td>
<td>NS</td>
</tr>
<tr>
<td>Physical component</td>
<td>Post</td>
<td>55.0(47.5-74.2)</td>
<td>61.3 (41.6-87.0)*</td>
<td>65.9 (39.7-77.0)</td>
<td>NS</td>
</tr>
<tr>
<td>Mental component</td>
<td>Pre</td>
<td>59.9(53.5-76.5)</td>
<td>58.2(33.3-77.9)</td>
<td>64.2 (45.3-76.9)</td>
<td>NS</td>
</tr>
<tr>
<td></td>
<td>Post</td>
<td>62.0(52.9-78.3)</td>
<td>74.9 (59.2-83.9)*</td>
<td>79.3 (53.6-86.8)*</td>
<td>NS</td>
</tr>
</tbody>
</table>

Data are presented as Median (IQR).

*NS*: non-significant

*Significant difference between post-treatment and pre-treatment medians using Wilcoxon Signed Ranks Test at *p*-value <0.05.

*p*-value: *p*-value when comparing the three groups at the same time using Kruskal-Wallis test.

Table (5): Frequency of side effects reported after the administration of the extract of *Ginkgo biloba* (EGb; 260 mg once daily, p.o. for 4 weeks), Ursodeoxycholic acid (UDCA, 300 mg three times daily, p.o. for 4 weeks) and their combination

<table>
<thead>
<tr>
<th>Side effects</th>
<th>EGb-group (n=20)</th>
<th>UDCA-group (n=20)</th>
<th>EGb+UDCA-group (n=20)</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Abdominal distension</td>
<td>1 (5%)</td>
<td>1 (5%)</td>
<td>0 (0%)</td>
<td></td>
</tr>
<tr>
<td>Abdominal Pain</td>
<td>0 (0%)</td>
<td>1 (5%)</td>
<td>0 (0%)</td>
<td></td>
</tr>
<tr>
<td>Constipation</td>
<td>0 (0%)</td>
<td>0 (0%)</td>
<td>1 (5%)</td>
<td></td>
</tr>
<tr>
<td>Fatigue</td>
<td>0 (0%)</td>
<td>2 (10%)</td>
<td>1 (5%)</td>
<td></td>
</tr>
<tr>
<td>Headache</td>
<td>2 (10%)</td>
<td>0 (0%)</td>
<td>1 (5%)</td>
<td></td>
</tr>
<tr>
<td>Nausea</td>
<td>0 (0%)</td>
<td>0 (0%)</td>
<td>1 (5%)</td>
<td></td>
</tr>
<tr>
<td>Flatulence</td>
<td>1 (5%)</td>
<td>0 (0%)</td>
<td>1 (5%)</td>
<td></td>
</tr>
<tr>
<td>Bleeding gums</td>
<td>1 (5%)</td>
<td>0 (0%)</td>
<td>0 (0%)</td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>5 (25%)</td>
<td>4 (20%)</td>
<td>5 (25%)</td>
<td></td>
</tr>
</tbody>
</table>

*p*-value: *p*-value when comparing the three groups using Chi-square test at *p*-value <0.05.

Figure (1): Hepatoprotective effect of the daily administration of the extract of *Ginkgo biloba* (EGb; 260 mg once daily, p.o. for 4 weeks), Ursodeoxycholic acid (UDCA, 300 mg three times daily, p.o. for 4 weeks) and their combination on serum levels of Alanine Aminotransferase (ALT) (A) and Aspartate Aminotransferase (AST) level (B).

Data present median values.
Figure (2): Hepatoprotective effect of the daily administration of the extract of *Ginkgo biloba* (EGb; 260 mg once daily, p.o. for 4 weeks), Ursodeoxycholic acid (UDCA, 300 mg three times daily, p.o. for 4 weeks) and their combination on serum levels of total antioxidant capacity (TAC) (A), hyaluronic acid (HA) (B) and Transforming Growth Factor-beta1 (TGF-β1) (C).

Data present median values.

Figure (3): Effect of the daily administration of the extract of *Ginkgo biloba* (EGb; 260 mg once daily, p.o. for 4 weeks), Ursodeoxycholic acid (UDCA, 300 mg three times daily, p.o. for 4 weeks) and their combination on the Physical and Mental summary scores of the RAND SF-36 questionnaire.

Data present median values.

REFERENCES:


The use of Ginkgo biloba extract in the treatment of patients with chronic hepatitis B was evaluated. The study showed that Ginkgo biloba extract EGB 761 could alleviate hepatic fibrosis and sinusoidal microcirculation disturbance in patients with chronic hepatitis B. The results indicated that Ginkgo biloba extract EGB 761 has potential therapeutic effects on patients with chronic hepatitis B.