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ORIGINAL PAPER

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A prospective, interventional clinical study to evaluate the safety and efficacy of Liv.52 DS in the management of non-alcoholic fatty liver disease

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ABSTRACT

Introduction. Non-alcoholic fatty liver disease (NAFLD) is excessive fat build-up in the liver due to causes other than alcohol use.
Aim. To evaluate the clinical efficacy and safety of Liv.52 DS tablets in the management of NAFLD.
Material and methods. Prospective, interventional clinical study conducted on 60 patients of both sex, aged between 18-65 years, confirmed with NAFLD from clinical examination, laboratory test, ultrasound findings and those willing to give informed consent. All patients received Liv.52 DS at a dose of 2 tablets twice daily for 2 months. All patients were evaluated at baseline, end of 1st month, and end of 2nd month for liver function tests, hepatomegaly by ultrasound, NAFLD Fibrosis Score, lipid profile, hematology and biochemical investigations.
Results. Study data was analyzed with GraphPad Prism Software Version 6.07. Data of those patients who completed the study was considered for analysis. Significant improvement in hepatomegaly, liver enzymes was observed. NAFLD fibrosis score revealed no progression of liver fibrosis due to NAFLD during the study period. No abnormal lab values were recorded and there were no adverse events reported during the study.
Conclusion. Study concludes that Liv.52 DS is safe and beneficial in individuals suffering from NAFLD.
Keywords. ALT/AST, fatty liver, fibrosis, non-alcoholic fatty liver disease

Introduction

Non-alcoholic fatty liver disease (NAFLD) is excessive fat build-up in the liver due to causes other than alcohol use.[1] NAFLD, encompassing both simple steatosis and non-alcoholic steatohepatitis (NASH), is the most common cause of liver disease.[2-3] It may lead to complications such as cirrhosis, liver cancer, liver failure, or cardiovascular disease.[4]

NAFLD is the commonest cause of liver disease in Western countries; NAFLD is strongly associated with

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obesity, insulin resistance, hypertension and dyslipidemia and is now regarded as the liver manifestation of the metabolic syndrome. Rapid spread of the obesity 'pandemic' in adults and children, coupled with the realization that the outcomes of obesity-related liver disease are not entirely benign, has led to rapid growth in clinical and basic studies in NAFLD.[5]

Many patients with NAFLD remain undiagnosed and recognizing those at risk is the first step. Clinicians overly rely on abnormal liver enzymes to identify patients with NAFLD, so patients with significant liver disease can be overlooked, potentially missing opportunities for intervention. Although liver biopsy is the gold standard method for diagnosing and staging NAFLD, most patients can be effectively diagnosed non-invasively with tests that are routinely available in the clinic today.[6]

In terms of epidemiology, several studies have tried to quantify the true worldwide incidence of NAFL/NASH; however, due to extreme variations in study parameters and available testing, a clear and reliable occurrence rate is not currently available.[7] With that being said, estimates have been posited suggesting the incidence of NAFLD to be 20%-30% in Western countries and 5%-18% in Asia.[7] It is no surprise that the prevalence of NAFLD is increasing worldwide with each passing year, given the current trends in dietary irresponsibility and preponderance of a sedentary lifestyle.[7] Additionally, there has been a linear rise of NAFLD with that of diabetes and metabolic syndrome.[8]

In one study from the United States, it was shown that the incidence of NAFLD was 10% higher in overweight individuals compared to lean persons.[9] At present, the high prevalence and negative pathological consequences of NAFLD represent a significant economic burden for many countries. However, up to now, there is no effective procedure to treat the disease.[10-11]

The primary therapeutic approach is to recommend healthy lifestyle strategies that are focused on reducing body weight and increasing insulin sensitivity, including dietary and exercise Regimens.[12]

Traditional medicines are abundant sources of biologically active substances that can be applied to prevent human diseases.[13-14] Currently, an increasing number of studies have focused on herbal extracts or natural products, and many of these studies have discovered herbal products with potent effects against NAFLD.[15-16] Thus, herbal medicines are promising candidate drugs for the treatment of NAFLD. There are no specific reliable treatment options available for NAFLD. During clinical studies with Liv.52 DS, in various liver disorders, some of the patients with NAFLD were benefitted with Liv.52 DS.

Maity SG et al. evaluated the clinical efficacy and safety of Liv.52 DS tablets with UDCA in patients with Non-Alcoholic Steatohepatitis in a randomized, comparative clinical study, Liv.52 DS appear promising in the management of Non-Alcoholic Steatohepatitis.[17] In a clinical study conducted by Gosh S et al., inferred that Liv.52 DS appear promising in the management of Non-Alcoholic Steatohepatitis.[18]

In an *in vitro* by Vidyashankar to evaluate the quercetin effectively reversed NAFLD symptoms by decreased triacyl glycerol accumulation, insulin resistance, inflammatory cytokine secretion and increased cellular antioxidants in OA induced hepatic steatosis in HepG2 cells. Hence quercetin is promising to carry out more experimental and clinical studies to understand the molecular mechanism to overcome NAFLD symptoms.[19] In a study conducted by Vidyashankar to evaluate LHAE which could effectively reverse the molecular perturbations underlying NAFLD symptoms suggesting its importance to ameliorate OA induced hepatic steatosis in HepG2 cells. Hence treatment with LHAE could be a new perspective to carry out more experimental and clinical studies to understand the molecular mechanism to overcome NAFLD symptoms.[20]

Liv.52 DS Tablet is a polyherbal formulation consisting of extracts of *Capparis spinosa, Cichorium intybus,* Mandura bhasma, *Solanum nigrum, Terminalia arjuna, Cassia occidentalis, Achillea millefolium and Tamarix gallica.* Some preliminary studies demonstrated leads in NAFLD. Therefore, a clinical study was planned and carried out to further evaluate role of Liv.52 DS in the management of NAFLD.

Aim

To evaluate the safety and efficacy of Liv.52 DS in the management of Non-Alcoholic Fatty Liver Disease

Material and methods

This study was a prospective interventional clinical study conducted at Department of Internal Medicine, Faculty of Medicine, University of Sumatera Utara, Medan, Indonesia. The essential study documents were submitted to the Health Research Ethical Committee. All the study related documents were reviewed and approved with the vide approval number No.74/TGL/KEPK FK USU-RSUP HAM/2017. All the patients gave their informed consent for their active participation in the study.

A total of 60 male and female patients aged between 18-65 years with NAFLD from clinical examination, laboratory test, and ultrasound findings; and those who were willing to give informed consent were included in the study.

Alcoholic subjects were excluded in the study. Patients with severe metabolic disorders, carcinoma of liver or pancreas. Subjects with a known history of present condition of allergic response to similar pharmaceutical products, its components or ingredients in the test products, Subjects with pre-existing systemic disease necessitating long-term medications, genetic disorders, Subjects who has partic-

ipated in a similar clinical investigation in the past four weeks and those who refused to sign the informed consent form were excluded. Women of child bearing age and lactating women were also excluded from the trial.

At the initial visit, a detailed medical history and symptomatic evaluation was done. In addition, examination specific to the steatohepatitis with Hepatomegaly (enlarged liver) was done. The Subjects were instructed to take Liv.52 DS tablets 2 tablets twice daily for a period of 2 months. Subjects were evaluated at baseline, at the end of 1st month, and at the end of 2ndmonth for liver function tests including AST, ALT, ALP, GGT, serum bilirubin, albumin, hepatomegaly (by ultrasound) and NAFLD Score. All subjects underwent complete blood count, and biochemical investigations, blood sugar levels and lipid profile at baseline, Month 1 and Month 2.

Non-invasive NAFLD fibrosis score was calculated at each assessment visits to assess the severity of fibrosis due to NAFLD. NAFLD fibrosis score is calculated by a formula -1.675 + 0.037 × age (year) + 0.094 × BMI (kg/m^2) + 1.13 × IFG/diabetes (yes =1, no = 0) + 0.99 × AST/ALT ratio - 0.013 ×platelet count (×10^9/L) - 0.66 × albumin. NAFLD score was evaluated with a score of NAFLD Score < -1.455 = F0-F2, NAFLD Score -1.455 to 0.675 = indeterminate score and NAFLD Score > 0.675 = F3-F4.

All data were analyzed with GraphPad Prism for Windows version 6.07. A $p<0.05$ was considered as statistically significant. Respective statistical tests are mentioned with the summary table. A subgroup analysis was carried out to evaluate the role of Liv.52 DS in the Management of Non-Alcoholic Fatty Liver Disease in Diabetes Mellitus Subjects among the total included subjects.

All the patients were provided with a chart and requested to write down the date of taking the drug as well as record the occurrence of adverse effects, if any. At the time of follow-up visits at the end of 1st month, and at the end of 2ndmonth, the participants were requested to bring back the empty boxes of the study medication to ensure that they had consumed it.

Statistical analysis

Statistical analysis was carried out using GraphPad Prism, Version 6.07 for windows, GraphPad Software, San Diego, California, USA. Liver function tests, biochemical parameters, NAFLD score were analyzed using ANOVA followed by Tukey's multiple comparisons test. Hepatomegaly was analyzed by Repeated measures ANOVA followed by Dunnett's multiple comparisons test. The values are expressed as Mean ± SD.

Results

Sixty patients (34 females and 26 males) with a mean age of 48.2±12.3 years, with a mean weight of 77.03±9.24 kgs participated in this clinical study (Table 1). All the patients completed the study and their data was available for analysis. In the present study, there were total 17 patients who were diabetics and they have not been considered for statistical analyses.

Table 1. Demographic details

No. of Subjects	60
Age in years	48.2±12.3
Weight in kgs	77.03±9.24
Height in cms	159.3±7.7
Gender (Female: Male)	(34:26)

Table 2 explains the effect of Liv.52 DS on liver fibrosis as evaluated by NAFLD Fibrosis score. The interpretation is as compared to baseline. In the score F0-F2, there is a trend towards reduction in the fibrosis score at month 1, and further a trend towards reduction in the fibrosis score by month 2. Similarly people with indeterminate score also demonstrated that fibrosis score reduced with Liv.52 DS treatment. There is an trend towards reduction in the fibrosis but was not statistically significant. This signifies that Liv.52 DS demonstrates a trend towards reduction of the liver fibrosis associated with NAFLD (Table 2).

Table 2. Effect of Liv.52 DS on liver fibrosis (NAFLD fibrosis score)

Scale	N		Baseline	Month 1	Month 2
NAFLD Score <-1.455 = F0-F2	33	Mean	-2.886	-2.911	-2.915
		SD	1.044	1.061	0.8617
NAFLD Score -1.455 – 0.675 = indeterminate score	27	Mean	-0.650	-0.684	-0.849
		SD	0.484	0.543	0.667
NAFLD Score > 0.675 = F3-F4	0		0	0	0

Statistical test: ANOVA followed by Tukey's multiple comparisons test, Value in: Mean±SD

Formula for NAFLD Score: -1.675 + 0.037 × age (years) + 0.094 × BMI (kg/m^2) + 1.13 × IFG/diabetes (yes = 1, no = 0) + 0.99 × AST/ALT ratio – 0.013 × platelet (×109/l) – 0.66 × albumin (g/dl)

NAFLD score was evaluated:

NAFLD Score< -1.455 = F0-F2

NAFLD Score -1.455 to 0.675 = indeterminate score

NAFLD Score > 0.675 = F3-F4

Table 3. Effect of Liv.52 DS on Hepatomegaly (number and %)

Liver size on Ultrasound	Baseline	Month 1	Month 2
	No. of Subjects (%)	No. of Subjects (%)	No. of Subjects (%)
Hepatomegaly > 16 cm	45 (75%)	43 (72%)	25 (42%)
No Hepatomegaly < 16 cm	15 (25%)	17 (28%)	35 (58%)

Effect of Liv.52 DS on number and percentage of subjects with hepatomegaly is given in Table 3 and Figure 1. At Baseline, 75% of subjects demonstrated hepatomegaly (Liver size >16 cm) and 25% subjects showed no hepatomegaly (liver size <16). After 2 months treatments with Liv.52 DS, only 42% people showed hepatomegaly and 58% subjects showed no hepatomegaly.

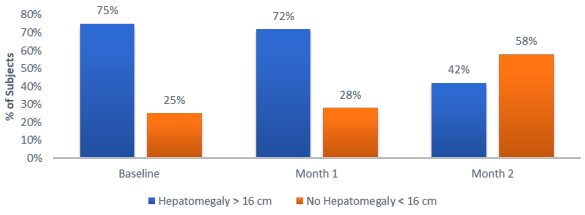

Fig. 1. Effect of Liv.52 DS on liver size by ultrasound measurement (expressed as % of individuals with hepatomegaly and no hepatomegaly)

Effect of Liv.52 DS on liver size is explained in Table 4 and Figure 2. At baseline liver size (cms) was 17.44 ± 1.9 reduced to 17.29 ± 1.77 at month 1 which further reduced 15.87 at the end of month 2 with statistical significance of p<0.0001.

Table 4. Effect of Liv.52 DS on Liver size (cms) expressed in mean ± SD

Liver size on Ultrasound	Baseline	Month 1	Month 2
Mean (cms)	17.44	17.29	15.87
SD	1.9	1.77	1.79
Std. Error of Mean	0.24	0.23	0.23
Significance		ns	p<0.0001[a]

Repeated measures ANOVA followed by Dunnett's multiple comparisons test
significance was fixed at <0.05
a: as compared to baseline
software: GraphPad Prism 6.07

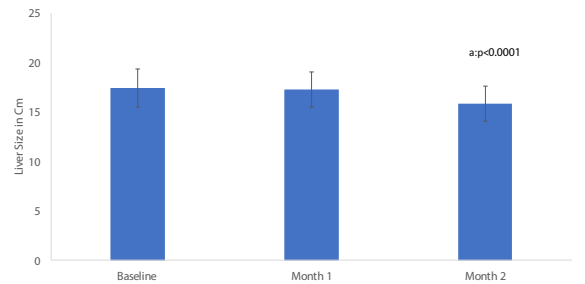

Fig. 2. Effect of Liv.52 DS on liver size by ultrasound measurement (expressed as cms (mean±SD) of individuals with hepatomegaly)

SGPT which was 60.33±13.04 at baseline reduced to 56.43±15.47 at month 1, which further reduced to 54.12±17.26 with a significance of p<0.0339 at the end of 2 months treatment of Liv.52 DS. SGPT which was 70.68±18.49 at baseline decreased to 64.13±22.07 at 1st month with a significance of p<0.0215 and further reduced to 61.9±22.7 with a significance of p<0.0022 at the end of 2nd month with Liv.52 DS (Table 5). There were no patients identified with hepatitis; although, ALT and AST levels were elevated at screening and was found to be reduced at the end of the study.

Table 5. Effect of Liv.52 DS on LFT (SGOT and SGPT)

Parameters	Baseline	Month 1	Month 2
SGOT (UI/L)	60.33±13.04	56.43±15.47	54.12±17.26 p<0.0339[a]
SGPT (UI/L)	70.68±18.49	64.13±22.07 p<0.0215[a]	61.9±22.7 p<0.0022[a]

Statistical test: ANOVA followed by Tukey's multiple comparisons test, significance was fixed at p<0.05, value in: mean±SD, a: as compared to baseline, SGOT – Serum glutamic-oxaloacetic transaminase, SGPT – Serum glutamic pyruvic transaminase

The hematological and biochemical investigations demonstrated that Liv.52 DS is a safe formulation. Although; high density lipoprotein, low density lipoprotein levels were within the normal range, total serum cholesterol and serum triglyceride levels were towards borderline high at screening and remained almost at same levels even at the end of the study (Table 6). There were no clinically significant adverse events either reported or observed during the entire study period. The overall compliance to the treatment was good and no treatment discontinuations were reported.

Table 6. Hematology and biochemical investigations

Parameters	Baseline	Month 1	Month 2	p value
Hemoglobin (g/dL)	13.07±2.15	12.96±2.25	13.09±2.15	ns
White Blood Count (/mm^3)	8445±1645	8455±2143	8229±1444	ns
Platelet count (/mm^3)	314950±77105	308002±65593	316150±51555	ns
Fasting glucose (mg/dL)	149.7±45.35	150.6±44.55	148.5±44.64	ns
Random Glucose (mg/dL)	182.8±61.42	184.1±43.64	192.4±29.26	ns
Gamma glutamyl transferase (UI/L)	40.52±14.05	40.68±11.69	38.42±10.42	ns
Alkaline Phosphatase (UI/L)	117.3±42.84	117.5±42.03	119±40.79	ns
Serum albumin (g/dL)	3.58±0.54	3.45±0.44	3.48±0.55	ns
Total bilirubin (g/dL)	0.68±0.22	0.60±0.27	0.77±0.25	ns
Direct bilirubin (g/dL)	0.35±0.13	0.31±0.15	0.36±0.15	ns
Total serum cholesterol (mg/dL)	233.3±29.33	235.3±28.46	228.9±33.91	ns
Serum triglycerides (mg/dL)	166.3±30.75	162.7±37.1	168±26.48	ns
High Density Lipoprotein (mg/dL)	41.23±5.82	41.22±4.99	42.32±4.78	ns
Low Density Lipoprotein (mg/dL)	160.5±30.27	162.3±30.8	160.3±30.1	ns

Statistical test: ANOVA followed by Tukey's multiple comparisons test, significance was fixed at p<0.05, value in: Mean ± SD. ns = Not significant

Discussion

Liv.52 DS Tablet is a hepatospecific formulation, designed for the management of liver disorders. It has a wide spectrum of therapeutic applications in liver disorders. It restores the metabolic efficiency of the liver

in various etiological forms of hepatocellular jaundice like infective and chronic active hepatitis, drug-induced hepatitis and alcohol-induced hepatic damage. It increases appetite. It corrects the hepatitis, and cirrhotic conditions, and in any hepatotoxic drug regimen. It is a supportive treatment as an adjuvant with hepatotoxic drugs. Therapy options include weight reduction in obese, good control in diabetics and exercise.[21]

Although there is no consensus for the treatment for NASH, effort needs to be made to prevent development of fibrosis, which results in cirrhosis and portal hypertension. As the pathogenesis of this condition is not clear, treatment has been largely empirical.[22]

Treatment should be focused on correction of the underlying metabolic syndrome. The role of specific pharmacologic treatment continues to evolve. Several large clinical trials using a variety of agents are currently under way and should provide additional treatment option for those with nonalcoholic steatohepatitis.[23]

In a study by Eguchi et al., who conducted pilot study of liraglutide effects in non-alcoholic steatohepatitis and non-alcoholic fatty liver disease with glucose intolerance in Japanese patients(LEAN-J); was aimed to evaluate the effect and action of liraglutide for biopsy-proven NASH. Subjects whose hemoglobin A1c levels failed to improve to less than 6.0% and/or whose alanine aminotransferase levels were not lower than baseline, received liraglutide at 0.9 mg/body per day for 24 weeks, after lifestyle modification intervention for 24 weeks. Study concluded that treatment with liraglutide had a good safety profile and significantly improved liver function and histological features in NASH patients with glucose intolerance.[24]

In a study by Belfort R et al., conducted placebo-controlled trial of pioglitazone in subjects with nonalcoholic steatohepatitis. They randomly assigned 55 patients with impaired glucose tolerance or type 2 diabetes and liver biopsy-confirmed nonalcoholic steatohepatitis to 6 months of treatment with a hypocaloric diet (a reduction of 500 kcal per day in relation to the calculated daily intake required to maintain body weight) plus pioglitazone (45 mg daily) or a hypocaloric diet plus placebo. Before and after treatment, they assessed hepatic histologic features, hepatic fat content by means of magnetic resonance spectroscopy, and glucose turnover during an oral glucose tolerance test ([14C] glucose given with the oral glucose load and [3H] glucose given by intravenous infusion). The study concluded that the administration of pioglitazone led to metabolic and histologic improvement in subjects with nonalcoholic steatohepatitis. Larger controlled trials of longer duration are warranted to assess the long-term clinical benefit of pioglitazone.[25]

In a study by Cusi K et al., which is RCT with Long-Term Pioglitazone Treatment for Patients with Nonalcoholic Steatohepatitis and Prediabetes or Type 2 Diabetes Mellitus; all patients were prescribed a hypocaloric diet (500-kcal/d deficit from weight-maintaining caloric intake) and then randomly assigned to pioglitazone 45 mg or placebo for 18 months, followed by an 18-month open-label phase with pioglitazone treatment. Study concluded that long-term pioglitazone treatment is safe and effective in patients with prediabetes or T2DM and NASH.[26]

Eight active medicinal herbs viz., *Capparis spinosa, Cichorium intybus, Mandura bhasma, Solanum nigrum, Terminalia arjuna, Cassia occidentalis, Achillea millefolium* and *Tamarix gallica* were carefully selected during the product development. These herbs possess significant hepatoprotective activity and the results of the present study might be possibly due to the synergistic potential of these polyherbal actives.

Capparis spinosa

P-Methoxy benzoic acid from *Capparis spinosa* has potent hepatoprotective activity against chemically-induced hepatotoxicity, prevents elevation of malondialdehyde levels (plasma and hepatic) and enzyme levels (AST and ALT).[27-29] It improves the functional efficiency of the liver and spleen, with protective action on the histological architecture of the liver, and a salutary effect on liver glycogen and serum proteins.[30] Flavonoids of *Capparis spinosa* have significant antioxidant activity, as demonstrated by lipid peroxidation, bleaching of free radicals, and auto-oxidation of iron ions.[31]

Cichorium intybus

Cichorium intybus protects the liver against alcohol toxicity. It increases circulating leukocytes, splenic plaque-forming cells, hemagglutination titers, secondary IgG antibody response, phagocytic activity, natural killer cell activity, cell proliferation, and interferon gamma secretion.[32,33] Its hepatoprotective activity suppresses the oxidative degradation of DNA in tissue debris. It also has potent antioxidant action, as evident by its free radical scavenging effects, inhibition of hydrogen peroxide and iron chelation.[34]

Solanum nigrum

Solanum nigrum protects DNA against oxidative damage[35], and also acts as a potent scavenger of hydroxyl and diphenylpicrylhydrazyl radicals.[36] The cytoprotective effect of *Solanum nigrum* against gentamicin-induced toxicity showed a significant inhibition of cytotoxicity, and hydroxyl radical scavenging potential.[37]

Terminalia arjuna

Terminalia arjuna reduces cholesterol levels and is also useful in liver disorders.[38-39] It has potent antioxidant activity, which is due to its effects on lipid peroxidation.[40] Arjunaphthanoloside from *Terminalia arjuna* inhibits nitric oxide production, and terminoside A decreases inducible nitric oxide synthase levels in lipopolysaccharide-stimulated peritoneal macrophages[41]. It has strong antiviral activity, inhibiting viral attachment and penetration.[42] It also has supportive antibacterial activity.[43]

Cassia occidentalis

Cassia occidentalis has significant hepatoprotective effects in chemically-induced liver damage.[44] It modulates hepatic enzymes, which provides hepatoprotection.[45]

Achillea millefolium

Achillea millefolium is beneficial in chronic hepatitis[46] and has anti-hepatoma activity.[47]

Tamarix gallica

Tamarix gallica is a hepatic stimulant, digestive and hepatoprotective, and has a salutary effect on liver glycogen and serum proteins.[48]

Mandura bhasma

Mandura bhasma has hepatoprotective property and is beneficial in chemically-induced hepatotoxicity as it prevents changes in liver enzyme activities.[49] Mandura bhasma is a powerful hematinic and tonic.[50]

Prospective, randomized, double-blind, placebo-controlled clinical studies with large sample size (which we can consider as a drawback in the present study) will be helpful in drawing further conclusions related to the action of Liv.52 DS in non-alcoholic steatohepatitis.

Conclusion

The present study demonstrates that with Liv.52 DS treatment, there was a significant improvement in hepatomegaly, liver function parameters namely SGPT and SGOT. There was a trend towards improvement in NAFLD score which signifies improvement in liver fibrosis due to NAFLD. Hematology and biochemistry investigation results are within normal limits and there were no clinically significant adverse effects were reported during the clinical study. Subgroup analysis carried out in diabetic subjects further demonstrated beneficial effects in those populations suffering from NAFLD with respect to hepatomegaly and LFT levels. From the results of the study, it can be summarised that Liv.52 DS is safe and beneficial in individuals suffering from NAFLD. Further conclusions related to action of Liv.52 DS in non-alcoholic steatohepatitis can be drawn only with the conduct of randomized, double-blind, placebo-controlled clinical studies with larger sample size.

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