Antioxidant and hepatoprotective activities of *Ocimum basilicum* Linn. and *Trigonella foenum-graecum* Linn. against H$_2$O$_2$ and CCl$_4$ induced hepatotoxicity in goat liver

R Meera*1, P Devi2, B Kameswari1, B Madhumitha4 & N J Merlin5

Department of 1Pharmaceutical Chemistry, 2 Pharmacognosy, 3 Biochemistry, 4 Pharmaceutics, K.M.College of Pharmacy, Uthangudi, Madurai-625 107, India

5Department of Pharmacology, Annamalai University, Department of Pharmacy, Annamalai nagar, Chidamabaram, India

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Significant hepatoprotective effects were obtained by ethanolic extract of leaves of *O. basilicum* and *T. foenum-graecum* against liver damage induced by H$_2$O$_2$ and CCl$_4$, as evidenced by decreased levels of antioxidant enzymes (enzymatic and non enzymatic). The extract also showed significant anti lipid peroxidation effects in vitro, besides exhibiting significant activity in superoxide radical and nitric oxide radical scavenging, indicating their potent antioxidant effects.

**Keywords**: Hepatoprotection, Lipid peroxidation, Nitric oxide scavenging, *Ocimum basilicum*, Superoxide scavenging, *Trigonella foenum-graecum*

Reactive oxygen species (ROS) are ubiquitous and occur naturally in all aerobic organisms, arising from both endogenous and exogenous sources. They are normally produced as a by product of cellular metabolism. They are capable of damaging biomolecules, provoking immune response, activating oncogenes and enhancing aging process. ROS metabolites can be generated by stepwise reduction of oxygen leading to the production of a series of oxidant molecules such as superoxide (O$_2^-$) and other reactive nitrogen species like nitric oxide (NO). Compounds with antioxidant activity are categorized into three groups namely, excellent, good and moderate. Excellent ones are those that perfectly quench the excited state and ground state radicals. Good antioxidants strongly inhibit the peroxide formation but are less effective in quenching excited states. Moderate antioxidants fail to excel in both reactivities. Antioxidants from plant products may fall under any of these three categories. The antioxidant activity may also depend on the type and polarity of the solvent.

The plant is extracted in ethanol. Lipid peroxidation alters the membrane permeability and causes tissue damage. Since, the liver is involved in various biochemical reactions, it is prone to be attacked by free radicals, resulting in and cell necrosis. However, inbuilt antioxidant systems like superoxide dismutase (SOD), tissue glutathione (GSH) etc. protect the tissues from free radical attack. The present study has been planned to further probe the antioxidant property of the leaves of *Ocimum basilicum* Linn. and *Trigonella foenum-graecum* Linn. In the present study, the extracts of the leaves were administered to different *in vitro* models of oxidative stress and the antioxidant status analysed in *in vitro* systems in goat liver slices exposed to oxidative stress from H$_2$O$_2$ and CCl$_4$. The antioxidant potential of the leaf extracts of *O. basilicum* and *T. foenum-graecum* was studied by co-incubation with the free radical generators.

*Ocimum basilicum* and *Trigonella foenum-graecum* are found growing abundantly in and around Madurai. *Ocimum basilicum* (Lamiaceae) Sweet Basil, *Thiruneetruppachchhai* in Tamil grows to a height of 50-80 cm, leaves are oval and slightly toothed and flowers are white in colour. The leaves and their oils have insecticidal, nematicidal, fungistatic and antimicrobial activities. (*Fabaceae*) *Vendayam* in Tamil. The seeds and leaves of *Trigonella foenum-graecum* fenugreek, are

* Correspondent author
Telephone: 09894353277
E-mail : meeraharsa@yahoo.com.
antichloestrolemic, anti-inflammatory, antitumour, carminative, hypoglycemic, restorative and a uterine tonic. Taken internally, a decoction of the ground seeds serves to drain off the sweat ducts. Since both the plants are reported to contain Vitamin C, a known antioxidant it is hypothesized that the leaves may also contain antioxidant principles and hence, they have been selected for phytochemical screening and evaluation of antioxidant and hepatoprotective properties.

Materials and Methods

Plant material — The leaves of *O. basilicum* and *T. foenum-graecum* collected from Aritapatti village near Madurai during April were identified and authenticated by Prof. Chandrakanth, Microbiology Department, Sourashtra College, Madurai.

Preparation of extract — The coarse powder of shade dried *O. basilicum* and *T. foenum-graecum* was extracted with petroleum ether, chloroform, alcohol and water as per Kokate[7]. Similarly the ethanolic extract were also prepared after defatting of the plant drugs. The ethanolic extracts were dried under reduced pressure by using Rota flash evaporator. For the experiments they were reconstituted in 0.5% Tween–80 to desired concentrations. They were referred to as OB and TF respectively.

Preliminary phytochemical screening All extracts obtained were screened for the presence of phytocinstitutents[7,9].

Animals — Goat liver was bought locally. It was washed in saline and peeled of the fat layer; 0.25 g of liver was weighed, made into very thin slices (8-9mm rectangular shape) and washed in Hank’s balanced salt solution (HBSS) using appropriate buffer. The in vitro model was challenged with the oxidants H2O2 or CCl4, in presence or absence of different concentrations of (OB/TF) extracts. The components were analysed spectroscopically in homogenate as well as goat liver slices after one hour incubation at 37°C.

H2O2 induced hepatotoxicity[10] — Animals were divided into 5 groups. Group I was normal control. Group II was toxin control group, treated with H2O2 (2 ml/kg). Groups III and IV were administered OB/TF (100 mg/kg, po) . Group V was given Silymarin, the known hepatoprotective agent (100 mg/kg, po) for 6 days. H2O2 (2ml/kg) was administered to groups 2-6 on the fifth day.

CCl4 induced hepatotoxicity[11] — Animals were divided into 5 groups. Group I, the normal control group was administered a single daily dose of 0.5% Tween-80 (1ml), for 5 days and olive oil (1 ml/kg) on days 2 and 3. Group II, the CCl4 control group was administered a single daily dose of 0.5% Tween-80 (1ml) po, on all days and on the second and third day they were administered 2 ml/kg of CCl4:olive oil mixture (1:1). Groups III and IV were administered OB/TF (100 mg/kg, po) for all 5 days and a single dose of 2 ml/kg of CCl4:olive oil mixture (1:1) on days 2 and 3, 30 min after OB/TF administration. Group V was given Silymarin (100 mg/kg, po) for all 5 days and a single dose of 2 ml/kg of CCl4:olive oil mixture (1:1) on days 2 and 3, 30 min after Silymarin administration.

Biochemical and histopathological studies — After 48 hr of H2O2 or CCl4 intoxication, the animals were sacrificed by mild ether anaesthesia. Blood samples were collected for evaluating enzymic and non-enzymic antioxidants and liver tissue slices were collected for histopathological studies. Activites of catalase (CAT)[12], peroxidase (PEO)[13], superoxide dismutase (SOD)[14], glutathione reductase (GTR)[15], polyphenol oxidase (PPO)[16], glutathione s tranferase (GST)[17], ascorbic acid (Vit C)[18], tocopherol (Vit E)[19], vitamin A[20], total phenols[21], carotenoids and lycopenes[22] were assayed. The liver function marker enzymes like aspartate aminotransferase (ALT)[23], alanine aminotransferase (ALT)[23], alkaline phosphatase (ALP)[24], and gammaglutamyl transpeptidase (GGT)[25] were determined. The protein content was determined by the method of Lowry et al[26].

Antioxidant activity

Superoxide radical scavenging activity[27] — The measurement of superoxide radical scavenging activity of OB/TF was performed using the method of Winterbourn et al[27]. Various concentrations of OB/TF solutions were prepared in such a way that each 0.1 ml contains 25, 50, 100, 200 and 400 μg. The assay tubes contained test sample with ethylene diamine tetra acetic acid (EDTA), nitroblue tetrazolium (NBT) solution (1ml, 145 μM NBT in 100 mM phosphate buffer, pH 7.4), and Riboflavin were mixed with 0.1 ml of various concentrations of OB/TF. All the tubes were vortexed and the inital optical density was measured at 560 nm. These tubes were placed in an area where they received uniform illumination for 30 min. Again the optical density was measured at 560 nm. The difference in optical density
before and after illumination is the quantum of superoxide production and the percentage of inhibition by the test sample was calculated by comparing with the optical density of control.

**Nitric oxide radical scavenging activity** — The measurement of nitric oxide radical scavenging activity of OB/TF was performed using the method of Green et al. Sodium nitroprusside (5 mM) was prepared in phosphate-buffered saline and mixed with different concentrations of the drugs dissolved in the aqueous medium. The content was incubated at 25°C for 2.5 hr. The samples from the above were reacted with Greiss reagent (1% sulphanilamide, 2% H₃PO₄ and 0.1% napthylethylenediamine dihydrochloride). The absorbance of the chromophore formed during the diazotization of nitrite with sulphanilamide and subsequent coupling with napthylethylenediamine was read at 546 nm and referred to the absorbance of standard solutions of sodium nitrite in the same way with Griess reagent.

**Assessment of lipid peroxidation** — The measurement of thiobarbituric acid reactive substances (TBARS) was done as an index of lipid peroxidation by using the following method: Liver tissue (0.5g) was weighed accurately and homogenized in 10 ml of 150 mM KCl-Tris-HCl buffer (pH 7.2). The reaction mixture was composed of 50 µl liver homogenate, Tris-HCl buffer (pH 7.2), 3mM FeSO₄ and 0.05 ml of various concentrations of OB/TF extract. A blank containing no plant extract and no lipid source but only FeSO₄ and TBA to make a final volume of 500 µl was also prepared. An assay medium corresponding to 100 % oxidation was prepared by adding all the other constituents except the plant extracts. The experimental medium corresponding to auto oxidation contained only the liver homogenate preparation. All the tubes were incubated at 37°C for 1 hr. Following the incubation period 500 µl of 70 % ethanol was added to all the tubes to arrest the reaction. TBA (1%, 1 ml) was added to all the tubes followed by boiling in a water bath for 20 min. After cooling to room temperature, the tubes were centrifuged to clear the solution and the supernatants collected. To the supernatants 50 µl of acetone was added and TBARS measured at 535 nm in a spectrophotometer.

**Statistical analysis** — The results are presented as the mean of ± SD. The data obtained by one way ANOVA followed by Dunnett t-test. The level of significance was set at P<0.05.

**Results**

The preliminary phytochemical investigations showed the presence of sterols in the pet ether extract, saponins, cardiac glycosides, triterpenoids and bitters in the alcoholic extract and carbohydrates in water extract. The presence of saponin was confirmed by Foam test and Heamolysis test as reported by Kokate and Khandelwal.

Effect of O.basilicum and T. foenum-graecum on liver function marker enzymes and antioxidant enzymes — Both the hepatotoxins (H₂O₂ and CCl₄) significantly produced severe liver damage as indicated by a marked increase in all antioxidant enzymes levels of the toxin groups. Treatment with *O. basilicum* and *T. foenum-graecum* (100 mg/kg) caused significant reduction of these values in all the cases (Tables 1, 2) and the hepatoprotective effect was comparable to that of silymarin the known hepatoprotective agent. Data pertaining to the levels of AST, ALT, ALP, GGT and total protein are presented in Table 3. The biochemical parameters except for total protein content, registered a significant rise in serum of H₂O₂ or CCl₄ treated liver slices as compared to the control group. All these parameters were restored to near normal values in Group III, Group IV, Group VII and Group VIII treated animals. However the total protein content in serum showed a significant decline in Group II animals as compared to Group I animals. These diminished protien value was restored to near normal in Group III, Group IV and Group VII, Group VIII extract treated animals.

Effect of *O. basilicum* and *T. foenum-graecum* on super oxide and nitric oxide free radical scavenging activity — *O. basilicum* showed maximum inhibition (88.02%) of superoxide free radical at 400 µg/ml and nitric oxide free radical (86.47%) at 400 µg/ml. *T. foenum-graecum* showed maximum inhibition superoxide free radical (73.01%) at 400 µg/ml and nitric oxide free radical (79.24%) at 400 µg/ml; 25 µg/ml dose failed to evoke significant response and it was observed that the free radical was scavenged in a concentration dependent manner up to 400 µg/ml. (Table 4).

Effect of *O. basilicum* and *T. foenum-graecum* on in vitro lipid peroxidation and reduced glutathione — *O. basilicum* and *Trigonella foenum-graecum* showed very potent inhibition of goat liver lipid peroxidation in vitro at 100µg/ml compared to normal controls.
Reduced glutathione activities were reduced significantly ($P<0.05$) in the $H_2O_2$ and $CCl_4$ intoxicated goat liver, when compared with normal control group. (Table 5).

**Discussion**

Many studies have been undertaken with traditional medicines in an attempt to develop new drugs for hepatitis$^{33}$. In the present study, two mechanistically different models $H_2O_2$ and $CCl_4$ for liver damage induction were used to investigate whether the plant extract of *O. basilicum* and *T. foenum-graecum* could decrease efficiently the toxicity produced by these hepatotoxins.

Hydrogen peroxide can easily cross the cell membrane and attack different sites by converting into water$^{34}$. It can cause DNA damage in the form of both single and double strand breaks believed to be the initial step in the induction of cancer$^{35}$. Free radical is an atom or a molecule with one or more unpaired electrons in its outermost orbit$^{36}$. Oxidative stress is a general term used to describe a state of damage caused by reactive oxygen species$^{37}$. The present results indicated that pretreatment of rats with *O. basilicum* and *T. foenum-graecum* extract 100 mg/kg, po before $H_2O_2$ administration resulted in a significant protection of $H_2O_2$ induced elevation of antioxidant enzymes and liver function marker enzymes.

The mechanism of hepatic damage by $CCl_4$ is well documented. $CCl_4$ is metabolized by Cytochrome P-450 enzyme system to trichloromethyl radical (CCl$_3$). This in turn reacts with molecular oxygen and gets converted to trichloromethyl peroxy radical. This
radical forms covalent bonds with sulfhydryl groups of several membrane molecules like GSH leading to their depletion and causes lipid peroxidation. The lipid peroxidation initiates a cascade of reactions leading to liver necrosis. Liver damage is detected by measuring the activities of liver function marker enzymes like AST, ALT, ALP and GGT, which are released into the blood from damaged cells. They are also indicators of liver damage. The normalization of the above enzyme levels in goat liver with the plant drugs establishes the hepatoprotective effect of *O. basilicum* and *T. foenum-graecum* which may be

| Table 3 — Effect of ethanolic extract of *O. basilicum* and *T. foenum-graecum* leaves on activities of liver function marker enzymes in serum  
[Values are mean ± SD from 3 experiments] |
<table>
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<tbody>
<tr>
<td>Groups</td>
<td>AST (U/L)</td>
<td>ALT (U/L)</td>
<td>ALP (U/L)</td>
<td>GGT (U/L)</td>
</tr>
<tr>
<td>Normal (0.5%Tween80)</td>
<td>33.32±2.11</td>
<td>28.36±1.22</td>
<td>95.22±1.23</td>
<td>7.20±1.23</td>
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<tr>
<td>H2O2+control (2ml/kg)</td>
<td>160.22±1.23*</td>
<td>165.30±2.11*</td>
<td>210.10±2.65*</td>
<td>12.42±0.67*</td>
</tr>
<tr>
<td>H2O2+OB (100 mg/kg)</td>
<td>56.13±2.22*</td>
<td>36.20±1.26*</td>
<td>108.01±0.87*</td>
<td>5.26±1.87*</td>
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<tr>
<td>H2O2+ TF (100 mg/kg)</td>
<td>61.22±1.33*</td>
<td>39.16±2.01*</td>
<td>115.22±0.63*</td>
<td>7.33±0.22*</td>
</tr>
<tr>
<td>H2O2+silymarin (100mg/kg)</td>
<td>35.2±1.23*</td>
<td>29.03±1.28*</td>
<td>98.20±1.70*</td>
<td>6.11±1.20*</td>
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<td>Normal (0.5%Tween80)</td>
<td>35.36±0.08*</td>
<td>30.60±1.34*</td>
<td>92.60±1.28*</td>
<td>5.62±1.34*</td>
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<tr>
<td>CCl4control (2ml/kg)</td>
<td>135.5±1.20*</td>
<td>175.25±1.20*</td>
<td>201.20±1.22*</td>
<td>11.61±3.21*</td>
</tr>
<tr>
<td>CCl4+OB (100 mg/kg)</td>
<td>43.2±1.32*</td>
<td>33.36±2.63*</td>
<td>105.04±1.06*</td>
<td>5.13±2.60*</td>
</tr>
<tr>
<td>CCl4+ TF (100 mg/kg)</td>
<td>50.1±3.10*</td>
<td>38.41±1.22*</td>
<td>110.10±2.61*</td>
<td>6.34±5.62*</td>
</tr>
<tr>
<td>CCl4+silymarin (100mg/kg)</td>
<td>36.1±1.22*</td>
<td>31.77±0.23*</td>
<td>95.20±1.63*</td>
<td>5.05±0.32*</td>
</tr>
<tr>
<td>Normal (0.5%Tween80)</td>
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<td>30.60±1.34*</td>
<td>92.60±1.28*</td>
<td>5.62±1.34*</td>
</tr>
<tr>
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<td>6.34±5.62*</td>
</tr>
<tr>
<td>CCl4+silymarin (100mg/kg)</td>
<td>36.1±1.22*</td>
<td>31.77±0.23*</td>
<td>95.20±1.63*</td>
<td>5.05±0.32*</td>
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Statistical analysis ANOVA followed by Dunnett t-test  
*P <0.05 as compared with normal control

| Table 4 — Effect of ethanolic extract of *O. basilicum* and *T. foenum-graecum* leaves on super oxide and nitric oxide radical scavenging activity  
[Values are mean ± SD from 3 observations in each group] |
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<tr>
<td>Con (µg/ml)</td>
<td>Super oxide (% inhibition)</td>
<td>Nitric oxide (% inhibition)</td>
<td></td>
</tr>
<tr>
<td>O.basilicum</td>
<td>T.f.graecum</td>
<td>O.basilicum</td>
<td>T.f.graecum</td>
</tr>
<tr>
<td>1.25±0.01</td>
<td>1.23±0.02</td>
<td>1.35±0.01</td>
<td>1.29±0.02</td>
</tr>
<tr>
<td>25</td>
<td>42.64*</td>
<td>42.01*</td>
<td>40.64*</td>
</tr>
<tr>
<td>0.74±0.01</td>
<td>0.86±0.02</td>
<td>0.78±0.02</td>
<td></td>
</tr>
<tr>
<td>50</td>
<td>61.36*</td>
<td>50.76*</td>
<td>62.76*</td>
</tr>
<tr>
<td>0.89±0.01</td>
<td>0.76±0.01</td>
<td>0.81±0.02</td>
<td>0.85±0.02</td>
</tr>
<tr>
<td>100</td>
<td>70.01*</td>
<td>57.81*</td>
<td>68.42*</td>
</tr>
<tr>
<td>0.93±0.01</td>
<td>0.84±0.02</td>
<td>1.02±0.01</td>
<td>0.87±0.01</td>
</tr>
<tr>
<td>200</td>
<td>77.22*</td>
<td>63.50*</td>
<td>73.03*</td>
</tr>
<tr>
<td>1.23±0.02</td>
<td>0.89±0.01</td>
<td>1.29±0.01</td>
<td>0.88±0.01</td>
</tr>
<tr>
<td>400</td>
<td>88.02*</td>
<td>73.01*</td>
<td>86.47*</td>
</tr>
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Statistical analysis ANOVA followed by Dunnett t-test  
*P <0.05 as compared with normal control

| Table 5 — Effect of ethanolic extract of *O. basilicum* and *T. foenum-graecum* on antioxidant (lipid peroxidation and reduced glutathione) status of liver in goat.  
[Values are mean ± SD from 3 experiments]  |
<table>
<thead>
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<tbody>
<tr>
<td>Groups</td>
<td>TBARS (µmol/kg)</td>
<td>GSH (mg/g)</td>
</tr>
<tr>
<td>I Normal</td>
<td>81.2±1.1</td>
<td>0.13±0.005</td>
</tr>
<tr>
<td>II H2O2+ control</td>
<td>67.3±0.5*</td>
<td>0.053±0.005*</td>
</tr>
<tr>
<td>III H2O2+ <em>O. basilicum</em></td>
<td>81.2±1.0*</td>
<td>0.08±0.005*</td>
</tr>
<tr>
<td>IV H2O2+ <em>T. foenum-graecum</em></td>
<td>75.6±0.55*</td>
<td>0.09±0.005*</td>
</tr>
<tr>
<td>V H2O2+ silymarin</td>
<td>78.3±2.33*</td>
<td>0.11±0.005*</td>
</tr>
<tr>
<td>VI normal</td>
<td>95.0±1.65*</td>
<td>0.738±0.005*</td>
</tr>
<tr>
<td>VII CCl4+control</td>
<td>44.4±0.55*</td>
<td>0.485±0.005*</td>
</tr>
<tr>
<td>VIII CCl4+ <em>O. basilicum</em></td>
<td>676.3±0.55*</td>
<td>0.029±0.005*</td>
</tr>
<tr>
<td>IX CCl4+ <em>T. foenum-graecum</em></td>
<td>75.9±1.65*</td>
<td>0.03±0.002*</td>
</tr>
<tr>
<td>X CCl4+ silymarin</td>
<td>76.4±3.35*</td>
<td>0.630±1.35*</td>
</tr>
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</table>

Statistical analysis ANOVA followed by Dunnett t-test  
*P <0.05 as compared with normal control
able to induce accelerated regeneration of liver cells reducing the leakage of the above enzymes into the blood. The present results indicated that *O. basilicum* and *T. foenum-graecum* at all the doses tested significantly prevented the increased liver function marker enzyme activity induced by H2O2 and CCl4 indicating improvement of the functional status of the liver by these herbs which was also supported by histopathological studies.

The recovery towards normalization of antioxidant enzymes, liver function marker enzymes and histological architecture caused by *O. basilicum* and *T. foenum-graecum* was almost similar to that caused by silymarin in the present study. Silymarin is a known hepatoprotective compound, protecting the plasma membrane of hepatocytes.

Vivek *et al* reported that CCl4 caused significant increase in hepatic lipid peroxidation due to free radical injury in cirrhotic livers of rats. In the present study elevated levels of TBARS and GSH observed in CCl4 treated rats indicate excessive formation of free radicals and activation of lipid peroxidation system resulting in liver damage. GSH is a major non protein thiol in living organisms which plays a central role in coordinating the body’s antioxidant defence process. In the present study, *O. basilicum* and *T. foenum-graecum* prevented the rise of lipid peroxides, showing their significant antilipid peroxidant effects.

The first product of oxygen superoxide anion produced by either univalent reduction of oxygen or by univalent oxidation of H2O2 is a reactive one. Nitric oxide is the primary reactive nitrogen species, a key signaling molecule in physiological conditions. Peroxy nitrite with excess of NO, generates NO2 which can combine with more NO2 to form N2O3 and cause nitroative stress. High concentration of NO induces apoptosis. *O. basilicum* and *T. foenum-graecum* significantly quenched superoxide and nitric oxide radicals indicating their potent free radical scavenging activity in the present study.

*O. basilicum* and *T. foenum-graecum* are rich sources of flavonoids which have been shown to possess various biological properties related to antioxidant mechanisms. Perhaps the flavonoids and saponins present in *O. basilicum* and *T. foenum-graecum* extract were responsible for their hepatoprotective effects. The findings of the present study therefore support the reported therapeutic use of *O. basilicum* and *T. foenum-graecum* in tribal medicine of Tamilnadu for treating liver ailments.

**References**

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