

Research Article

Effects of Silimarin and Curcumin against LPS-Induced Hepatotoxicity in Rats

¹Sameh S. Gad, ²Ezzeddine El-Denshary and ¹Amany I. El-Brairy

¹Department of Pharmacology and Toxicology, Faculty of Pharmacy, MSA University, Cairo, Egypt

²Department of Pharmacology and Toxicology, Faculty of Pharmacy, Cairo University, Cairo, Egypt

Abstract: Hepatotoxicity is now receiving a supreme importance. LPS is the most potent activator of macrophages. The aim of this study is to demonstrate the prophylactic activity of silimarin and curcumin against LPS-induced hepatotoxicity in rats. The liver slices had been divided into 4 groups; normal, control (LPS), LPS+silimarin and LPS+curcumin (N = 12). After 24 h, analysis of ALT, GSH, TNF- α and IL-10 was carried out. ALT in the control group showed a high significant elevation in comparison to the normal group, while group 3 and 4 showed a high significant decrease compared with the control group. In control (LPS) group a significant reduction in GSH level was observed; where in group 3 and 4 a significant increase was noticed in comparison to the control group. Control (LPS) group showed a high significant elevation in TNF- α level in comparison to normal group, where in group 3 and 4 a high significant decrease was detected in comparison to the control group. Level of IL-10 in the control group indicated a high significant elevation compared to the normal group, while in group 3 and 4 a high significant decrease in the IL-10 levels were detected in comparison to the control group. In conclusion, silimarin and curcumin showed a hepatoprotective activity against hepatotoxicity induced by LPS.

Keywords: Alanine amino Transferase (ALT), Curcumin, Precision Cut Liver Slices (PCLS), hepatotoxicity, Lipopolysaccharide (LPS), prophylaxis, reduced glutathione (GSH), silymarin, Tumor Necrosis Factor alpha (TNF- α) lactate dehydrogenase (IL-10)

INTRODUCTION

Liver diseases and particularly hepatotoxicity are now receiving a supreme importance; due to the large population suffering of hepatic disorders all over the world. From the attempts to use the natural products to the intense use of drugs the patients are out there waiting the expected help so as to be well again. Depending on a wide research bases and a powerful huge advance in the medical field technology, hundreds of studies are made yearly for developing the art of using both the natural and the synthetic agents to enhance and enlarge the agents available to be used in such conditions. So, the current study is focusing on the use of the in-vitro method for the purpose of providing a new important tool to be added along the well documented and trusted studies to help minimizing the suffering of the patients especially that the use of that kind of studies could enable the researchers of the vast and wide screening of the new promising compounds, we thought in anew natural product to be screened.

Liver toxicity could be induced in the expermental field by usage of either chemicals such as (CCl₄, Alcohol) or biologically such as the use of (LPS) and the models used could be in-vivo, in-vitro in addition to the *ex-vivo*.

Precision-Cut Liver Slices (PCLS) from various species have been widely used to study the pathology of disease as well as the Absorption, Distribution, Metabolism, Elimination and toxicity (ADME-Tox) of drugs. Successful cryopreservation of PCLS would allow the creation of a tissue bank with PCLS from various species, which would be particularly valuable for enabling the use of human PCLS because of the scarcity of human material (Guan *et al.*, 2012).

Liver slices are used in various research fields, e.g., in toxicology, pharmacology and metabolism of xenobiotics. In these studies the incubation conditions (five incubation systems) of the liver slices are essential for maintenance of the viability and function of the cells in the slice (Olinga *et al.*, 1997).

Silymarin is a polyphenolic flavonoid derived from milk thistle (*Silybum marianum*), which has anti-inflammatory, cytoprotective and anticarcinogenic effects, that suppress the TNF-induced production of ROS and lipid peroxidation (Carini *et al.*, 1992; Manaa *et al.*, 1999) and improve the antioxidant status in blood and liver (Skottova' *et al.*, 2004).

Also reports about the use of silymarine as a liver protectant agent had been documented, Worldwide, researches continue to show interest in milk thistle derivatives as a potential treatment for several diseases

Corresponding Author: Sameh S. Gad, Department of Pharmacology and Toxicology, Faculty of Pharmacy, MSA University, Cairo, Egypt

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as evidenced by the over 12,000 related scientific publications produced on this subject within the last 10 years (Hackett *et al.*, 2013).

Curcumin is a bright yellow compound found in turmeric, which is derived from the rhizomes of the plant *Curcuma longa* Linn, a perennial herb of the Zingiberaceae family (Ammon and Wahl, 1991). Curcumin in various chronic illnesses in which inflammation is known to play a major role, curcumin has been shown to exhibit therapeutic potential (Aggarwal and Harikumar, 2009). The current study is focusing on the protective effect could be achieved on the stimulated PCLS by the two natural products Silymarin and Curcumin.

MATERIALS AND METHODS

Animals: Twelve adult male wistar rats (250 g) housed in individual cages in a humidity, temperature and light controlled room- (12 h dark/light cycle) - maintained on a standard laboratory diet and water ad libitum.

Liver slices preparation and work design: Animals sacrificed by decapitation and the whole liver quickly removed and placed on ice. The animals' debris treated according to safety and ethics guidelines (Frozen to be incinerated). Liver had been cut into transverse slices using Cryostat Macrotome.

Precision-cut liver slicing is performed -according to the principles stated by Olinga and Schuppan (2013) with Cryostat Macrotome (250 μ m) with a few modifications. Basically, the liver is removed from the animal and cores of tissue are removed using sharpened metal cylinders. The cores are placed in the tissue holders of the slicers, which contain the media Kreps ringer solution (10 mM D-glucose, 129 mM NaCl, 1.25 mM NaHPO₄, 22 mM NaHCO₃, KCl 3 mM, CaCl₂ 1.8 mM, MgSO₄ 1.8 mM, Hepes 5 mM, pH 7.4) (Olinga and Schuppan, 2013). Slices are produced by moving the cores across the Macrotome (Cryostat) and the freshly sectioned slices (5 slices each 50 μ m thickness) are swept away into the Six-Well Plates (submerged system). The thickness of the slices must be adjusted accurately. The success of the slicers is due to the slicing under physiological conditions and the uniform, reproducible thickness of the slices. Optimum thickness has been found to be 250 μ m (Gandolfi *et al.*, 1996).

The liver slices are incubated at 37°C in a humidified incubator in the presence of oxygen concentration varying between 95% oxygen/5% CO₂ and 20% oxygen (air)/5% CO₂. It was suggested that for short-term studies, incubation in 20% oxygen (air) /5% CO₂ is sufficient to retain slice viability, whereas oxygen concentration of at least 40% are essential for prolonged incubation liver slices.

A constant number of slices (5) had been selected each (50 μ m) and placed into the six-well plate and subjected to the different treatments.

The slices selected and placed in the six well plate and incubated in CO₂ incubator at 37°C and 20% humidity for 24 h as follow:

- A = Control group
- B = LPS treated group (Positive control) 100 μ g/mL
- C = Silymarin treated group 100 μ g/mL
- D = Curcumin treated group 100 μ g/mL

After the incubation time (24 h); the supernatant and/or tissue homogenate collected and send for the analysis and determination of different parameters (GSH, TNF- α , NO and LDH).

RESULTS

Effects of LPS, Silymarin + LPS and Curcumin + LPS on Alanin Amino Transferase Activity (ALT):

Measuring the liver transaminases in the normal group was as following; level of (ALT) ranged from (2 U/L) to (3 U/L) with the mean equal to (2.67 U/L \pm 0.51); Measuring the liver transaminases in the LPS group was as following; level of (ALT) ranged from (10 U/L) to (14 U/L) with the mean equal to (12.16 U/L \pm 1.8) indicating a high significant elevation in comparison to the control group (p<0.001). Using Silymarin in combination with (LPS) showed a range between (4U/L) and (6 U/L) a high significant decrease in the (ALT) levels were observed as the mean is (5 U/L \pm 0.9) in comparison to the use of (LPS) alone (p<0.001).

The use agent Curcumin in combination with (LPS) showed a range between (7 U/L) and (9 U/L) also a high significant decrease in the (ALT) levels were observed as the mean equals to (8.17 U/L \pm 0.7) in comparison to the use of (LPS) alone (p<0.001).

The results of the control, LPS, LPS+Silymarin and LPS+Curcumin on Alanin Amino Transferase activity levels are shown in Table 1, in addition to figure illustration (Fig. 1).

The results indicated that LPS induce significant elevation of ALT production (p<0.001). The increase induced by LPS had been significantly decreased by Silymarin and Curcumin (p<0.001).

Table 1: Effects of control, LPS, Silymarin + LPS and Curcumin + LPS on Alanin Amino Transferase Activity (ALT)

| Sample N = 6 | Control U/L | LPS U/L | Silymarin+LPS U/L | Curcumin+LPS U/L |
|--------------|-------------|----------|-------------------|------------------|
| 1 | 2 | 14 | 4 | 9 |
| 2 | 2 | 14 | 4 | 9 |
| 3 | 3 | 13 | 5 | 8 |
| 4 | 3 | 12 | 5 | 8 |
| 5 | 3 | 10 | 6 | 8 |
| 6 | 3 | 10 | 6 | 7 |
| MEAN | 2.66667 | 12.16667 | 5 | 8.16667 |
| SD | 0.5164 | 1.835 | 0.8944 | 0.7528 |
| SE | 0.2108 | 0.7491 | 0.3651 | 0.3073 |

Table 2: Effects of control, LPS, Silymarin + LPS and Curcumin + LPS on level of Reduced Glutathione (GSH)

| Sample (n = 6) | Control $\mu\text{mol/mL}$ | LPS $\mu\text{mol/mL}$ | Silymarin+LPS $\mu\text{mol/mL}$ | Curcumin+LPS $\mu\text{mol/mL}$ |
|----------------|----------------------------|------------------------|----------------------------------|---------------------------------|
| 1 | 1.19 | 0.28 | 0.78 | 0.39 |
| 2 | 1.18 | 0.27 | 0.76 | 0.41 |
| 3 | 1.19 | 0.29 | 0.89 | 0.53 |
| 4 | 1.21 | 0.33 | 0.88 | 0.55 |
| 5 | 0.91 | 0.37 | 0.81 | 0.6 |
| 6 | 0.96 | 0.38 | 0.75 | 0.62 |
| Mean | 1.066667 | 0.32 | 0.8116667 | 0.5166667 |
| SD | 0.1343 | 0.04733 | 0.06047 | 0.09626 |
| SE | 0.0548 | 0.0193 | 0.0246 | 0.0393 |

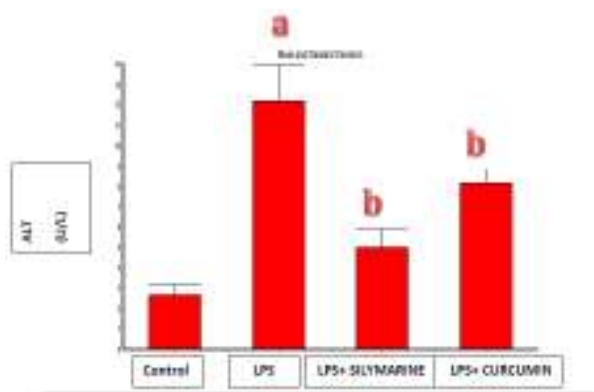


Fig. 1: Effects of LPS, Silymarin + LPS and Curcumin + LPS on level of Alanin Amino Transferase (ALT); (Mean \pm S.D.); a = significant in comparison to control, b = significant in comparison to LPS

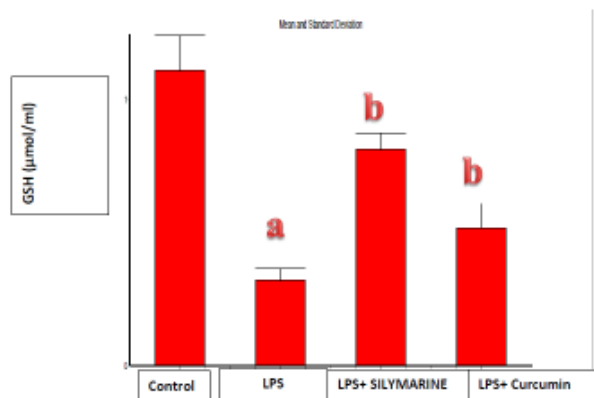


Fig. 2: Effects of LPS, Silymarin + LPS and Curcumin + LPS on level of Alanin Amino Transferase (ALT). (Mean \pm S.D.); a = significant in comparison to control, b = significant in comparison to LPS

Effects of LPS, Silymarin + LPS and Curcumin + LPS on level of Reduced Glutathione (GSH): In the current study, In the current study, the normal level of (GSH) in the tissue homogenate of the liver slices ranged from (0.91 $\mu\text{mol/mL}$) to (1.21 $\mu\text{mol/mL}$) with mean equal to (1.06 $\mu\text{mol/mL}\pm 0.13$); The use of bacterial lipopolysaccharide induced a significant reduction in the reduced glutathione level in the tissue

homogenate of the liver slices compared to the control level, ranging from (0.27 $\mu\text{mol/mL}$) to (0.38 $\mu\text{mol/mL}$) and the mean is (0.3 $\mu\text{mol/mL}\pm 0.13$) representing about 3 folds decrease of the normal level ($p < 0.001$).

In Silymarin+LPS group a significant increase in the (GSH) levels were noticed, ranging between (0.75 $\mu\text{mol/mL}$) to (0.89 $\mu\text{mol/mL}$) with mean (0.8 $\mu\text{mol/mL}\pm 0.06$) in comparison to the use of (LPS) alone ($p < 0.001$).

The use of the tested natural agent Curcumin in combination with (LPS) showed also a high significant increase in the (GSH) levels, ranging from (0.39 $\mu\text{mol/mL}$) to (0.62 $\mu\text{mol/mL}$) with mean (0.5 $\mu\text{mol/mL}\pm 0.09$) in comparison to the use of (LPS) alone ($p < 0.001$).

The results of the control, LPS, LPS+Silymarin and LPS+Curcumin on Glutathione level are shown in Table 2, in addition to figure illustration (Fig. 2).

Effects of LPS, Silymarin + LPS and Curcumin + LPS on level of Tumor Necrosis Factor Alpha (TNF- α): Normal level of (TNF- α) produced in the liver slices ranged from (0.79 Pg/mL) to (1.06 Pg/mL) with mean (0.93 Pg/mL ± 0.1); the use of bacterial lipopolysaccharide induced a high significant elevation of the (TNF- α) level ranged from (1.82 Pg/mL) to (2.47 Pg/mL) with mean (2.11 Pg/mL ± 0.1) ($p < 0.001$).

Using Silymarin in combination with (LPS) showed a high significant decrease in the (TNF- α) with mean (1.2 Pg/mL ± 0.13) in comparison to the use of (LPS) alone ($p < 0.001$) were the range was from (1.07 Pg/mL) to (1.44 Pg/mL); notably the decrease induced by the use of Silymarin is very intense; that there is no significant difference when compared with the control ($p > 0.05$).

The use of the tested natural agent Curcumin in combination with (LPS) showed also a high significant decrease in the (TNF- α) levels as the range found to be from (1.22 Pg/mL) to (1.79 Pg/mL) with mean equals to (1.6 Pg/mL ± 0.2) in comparison to the use of (LPS) alone ($p < 0.001$).

The results of the control, LPS, LPS+Silymarin and LPS+Curcumin on level of Tumor Necrosis Factor Alpha (TNF- α) are shown in Table 3, in addition to figure illustration (Fig. 3).

Effects of LPS, Silymarin + LPS and Curcumin + LPS on level of Interlukin ten (IL-10): The normal level of (IL-10) levels ranged from (0.49 pg/mL) to (0.88 pg/mL) with mean of (0.67 pg/mL ± 0.17); (IL-10) levels ranged from (1.85 pg/mL) to (2.74 pg/mL) with mean of (2.31 pg/mL \pm) indicating a high significant elevation compared to the normal group.

Using Silymarin in combination with (LPS) resulted in a range between (0.91 pg/mL) and (1.36 pg/mL) representing a high significant decrease in the (IL-10) levels with mean equals to (1.13 pg/mL ± 0.19) in comparison to the use of (LPS) alone ($p < 0.001$).

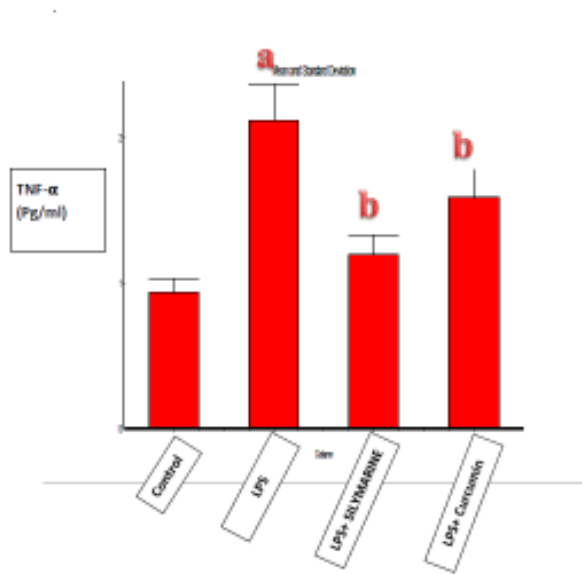


Fig. 3: Effects of LPS, Silymarin + LPS and Curcumin + LPS on level of Tumor Necrosis Factor Alpha (TNF- α). (Mean \pm S.D); a = significant in comparison to control, b = significant in comparison to LPS

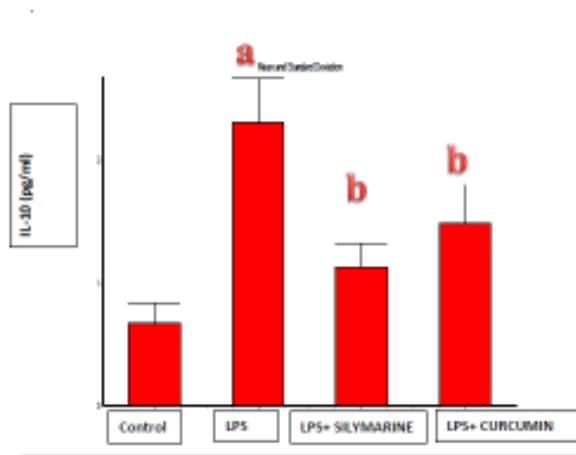


Fig. 4: Effects of LPS, Silymarin + LPS and Curcumin + LPS on level of Interlukin ten (IL-10). (Mean \pm S.D.); a = significant in comparison to control, b = significant in comparison to LPS

Table 4: Effects of control, LPS, Silymarin + LPS and Curcumin + LPS on level of Interlukin ten (IL-10)

| Sample N = 6 | Control pg/mL | LPS pg/mL | Silymarin+LPS pg/mL | Curcumin+LPS pg/mL |
|--------------|---------------|-----------|---------------------|--------------------|
| 1 | 0.66 | 2.74 | 0.91 | 1.17 |
| 2 | 0.59 | 2.56 | 0.93 | 1.18 |
| 3 | 0.49 | 2.44 | 1.11 | 1.41 |
| 4 | 0.53 | 2.39 | 1.14 | 1.39 |
| 5 | 0.87 | 1.87 | 1.33 | 1.91 |
| 6 | 0.88 | 1.85 | 1.36 | 1.84 |
| MEAN | 0.67 | 2.308333 | 1.13 | 1.483333 |
| SD | 0.1689 | 0.3677 | 0.1907 | 0.3205 |
| SE | 0.0689 | 0.1501 | 0.0778 | 0.1308 |

The use of the tested natural agent Curcumin in combination with (LPS) resulted in a range between (1.17 pg/mL) and (1.91 pg/mL) showed also a high significant decrease in the (IL-10) with mean (1.48 U/L \pm 0.13) in comparison to the use of (LPS) alone ($p < 0.001$).

The results of the control, LPS, LPS+Silymarin and LPS+Curcumin on (IL-10) are shown in Table 4, in addition to figure illustration (Fig. 4).

The results indicated that LPS induce significant elevation of IL-10 production ($p < 0.001$). The increase induced by LPS had been significantly decreased by Silymarine and Curcumin ($p < 0.001$).

DISCUSSION

Recently, great and promising results had been documented for the hepatotoxicity and hepatoprotective activity for different agents using the rat liver slices (Roos *et al.*, 2011).

The main advantage of PCLS can be summarized as follow:

- Preservation of a higher level of the tissue organization which are normally found in the intact organ and thus better reflection of the *in vivo* situation.
- Preservation of a differentiated state due to the maintenance of cell-cell and cell-matrix interactions.
- Preservation of different cell types, a critical issue since non-parenchymal cells such as Kupffer cells may significantly influence the viability and function of hepatocytes.
- Its facilitated histological and histochemical evaluation as an alternative, or as a complement, to biochemical tests.

Liver slices offer a system for evaluating whole or cryopreserved liver as well as regeneration of liver tissue after toxic insult. Liver slices have been shown to be a valid *in vitro* system for examining liver function and offer a bridge between *in vivo* and cell culture systems (Gandolfie *et al.*, 1996).

As the present study shows, Natural products represent great potentials in the protection and/or management of the different liver disease; such as

Table 3: Effects of control, LPS, Silymarin + LPS and Curcumin + LPS on level of Tumor Necrosis Factor Alpha (TNF- α)

| Sample N = 6 | Control Pg/mL | LPS Pg/mL | Silymarin+LPS Pg/mL | Curcumin+LPS Pg/mL |
|--------------|---------------|-----------|---------------------|--------------------|
| 1 | 0.86 | 2.11 | 1.07 | 1.22 |
| 2 | 0.89 | 2.31 | 1.11 | 1.79 |
| 3 | 0.79 | 2.47 | 1.44 | 1.57 |
| 4 | 0.93 | 2.14 | 1.15 | 1.6 |
| 5 | 1.04 | 1.84 | 1.19 | 1.77 |
| 6 | 1.06 | 1.82 | 1.21 | 1.54 |
| Mean | 0.928333 | 2.115 | 1.195 | 1.5816667 |
| SD | 0.105 | 0.2559 | 0.1305 | 0.2059 |
| SE | 0.0428 | 0.1045 | 0.0532 | 0.0840 |

Silymarine which protect and restore the normal function of liver cells. Recent studies proposed a protection activity for Curcumin against liver disease involving decreasing reactive oxygen species and enhancing anti-oxidant capacity (Robin *et al.*, 2012).

The current study showed that the use of Silymarine and Curcumin resulted in a protectant activity of the GSH level, that the decreased levels on GSH induced by LPS were greatly inhibited.

In a study by Evdokimova *et al.* (2001) the intracellular levels of GSH remained fairly constant (around 16 nmol/mg protein), regardless of the incubation medium used.

GSH is one of the most important tripeptide and a major non-enzymatic intracellular antioxidant (Morimoto *et al.*, 2008) and known to participate in the removal of free radicals such as H₂O₂, superoxide anions and alkoxy radicals and maintenance of membrane protein thiols. Depleted glutathione levels in target organs by the chemicals used in Naik *et al.* (2011) study were significantly restored with curcumin treatment like the current study. It is understood that the increased levels of GSH could either be due to its enhanced synthesis or due to improved glutathione reductase activity in the presence of curcumin (Naik *et al.*, 2011).

Lipopolysaccharide (LPS or endotoxin) a component of gram-negative bacteria cell walls is associated with tissue injury and sepsis. One of the major features of endotoxic shock is the induction of nitric oxide synthase in the liver. Inducible nitric oxide synthase (iNOS) induced by cytokines and LPS produces nitric oxide (NO) in large amounts. NO is known to be crucial factor in acute inflammation and sepsis. *In vivo*, NO has protective effects in inflammation and endotoxemia induced hepatic injury. In addition, in response to endotoxin, proinflammatory cytokines including interleukins and tumor necrosis factor α (TNF α) and anti-inflammatory cytokines such as IL-10 are produced by inflammatory cells.

In the liver LPS activates the resident macrophages, which results in cytokine release. Furthermore, LPS is cleared by the liver, mainly by kupffer cells. In the presence of LPS, the levels of LDH, GOT and GPT in the slice medium were increased during 24 h compared to control incubation (Olinga *et al.*, 2001).

Statistical analysis: All data were analyzed for statistical significant differences by use of Analysis of Variance (ANOVA) Followed by Tukey's t-test (Instat Biostatistics, V3.05 created Sep. 27, 2000. Graphpad software). Differences were considered statistically significant at $p < 0.05$.

CONCLUSION

In summary, the study concluded that the effectiveness of Curcumin is of a significant importance to be used effectively as hepatic supporting agent.

Offering a protection activity against LPS induced-hepatotoxicity by improving levels of GSH and decreasing the levels of deleterious parameters such as ALT, TNF- α and IL-10.

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