



Effect of Avarai Kudineer on α - Amylase and α - Glucosidase Inhibition: A Preclinical Antidiabetic Study

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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ABSTRACT

In recent years there has been a mounting interest towards the traditional medicine globally for the treatment of type 2 diabetes. Avarai Kudineer (AK) is a Siddha classical polyherbal formulation that has been indicated for the management of Diabetes mellitus in Siddha literature. The goal of the present study is to provide an in-vitro evidence for the antidiabetic potential of Avarai Kudineer in terms of inhibiting the carbohydrate digesting enzymes alpha amylase and alpha glucosidase. Avarai Kudineer (1/4) was prepared by boiling the ingredients weighed 20g in 80ml and reduced to 20ml and filtered according to the decoction preparation method as indicated in the Siddha literature. The filtrate was dissolved in DMSO to make stock solution and serially diluted to make different concentrations ranging from 10,20,40,80 and 100 μ g/ml. The triplicates (n=3) were maintained. The invitro alpha amylase and alpha glucosidase enzyme inhibition of AK sample was compared with standard drug acarbose and the IC 50 value was calculated. The data was statistically analysed and expressed as Mean \pm SD (n=3). The results showed that AK had maximum activity towards the inhibition of the enzyme alpha amylase (59.83 \pm 7.10) and alpha glucosidase (71.94 \pm 1.22) when compared with the standard acarbose 81.42 \pm 5.51 and 91.59 \pm 12.79 respectively. The results reveal that the test drug AK has appreciable alpha amylase and alpha glucosidase inhibitory activity.

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1. INTRODUCTION

Diabetes mellitus (DM) continues to be as a major endocrine and metabolic disorder worldwide. It is characterized by high blood glucose levels and is a major worldwide concern due to its potential deleterious effects. The goal of therapy for diabetic patients, especially type 2, is the maintenance of normal blood glucose levels after meal [1]. While type 2 mellitus (T2DM) continues to be an increasing multifactorial endocrine disorder worldwide [2], postprandial hyperglycemia plays an important role in the earliest observable abnormalities of glucose homeostasis in the development of type 2 diabetes and associated chronic complications, such as micro- and macro-vascular disorders [3]. It has been established that postprandial hyperglycemia strongly depends on the absorbed monosaccharides and the velocity of absorption in the small intestine and it is mediated by carbohydrates hydrolyzing enzymes such as pancreatic α -amylase and intestinal α -glucosidase. Therefore inhibition of α -amylase and α -glucosidase that are carbohydrate hydrolyzing enzymes have been considered as significant therapeutic approaches for decreasing after meal blood glucose rise and to retard the glucose absorption [4].

Medicinal plants and the potential phytochemicals with ability to delay or prevent the glucose absorption is currently encountered as an area of immense research for the management of diabetes mellitus, a chronic metabolic disorder. Main source of glucose in diet is polysaccharide, starch and disaccharide which are acted upon by the enzymes alpha amylase and alpha glucosidases for their conversion into glucose [5].

Therefore the main therapeutic approach to treat diabetes mellitus is to decrease the postprandial hyperglycemia and is considered to be a source of developing novel anti-diabetic agents which can be achieved by the inhibition of carbohydrate degrading enzymes such as alpha amylase and alpha glucosidase [6].

Present day oral hypoglycemic agents such as sulfonyl ureas, biguanides, thiazolidinediones, alpha amylase inhibitors and alpha glucosidase inhibitors are synthetic molecules which are effective in Diabetes but they have their limitations and side effects. The phytochemical

polyphenol that is richly present in plants has been reported to cause effects that mimics insulin in the utilization of glucose and also act as inhibitors of key enzymes like alpha amylase and alpha glucosidase [7]. These natural enzyme inhibitors can be used effectively for the treatment of postprandial hyperglycemia with minimal side effects [6].

2. MATERIALS AND METHODS

2.1 Identification and Authentication

All the herbal ingredients of the study drug were purchased from local markets of Chennai and authenticated.

2.2 Preparation of the Study Drug – Aavirai kudineer [8]

2.2.1 Ingredients

1. Cassia auriculata (*Aavirai*)
2. Cassia fistula (*Kondrai*)
3. Syzygium jambos (*Naval*)
4. Olax scandens (*Kadalazhinjil*)
5. Saussurea lappa (*Koshtam*)
6. Terminalia arjuna (*Maruthampattai*)
7. Cyperus rotundus (*Korai kizhangu*)

The above ingredients were ground into coarse powder and the decoction was prepared by boiling the ingredients weighed 20g in 80ml and reduced to 20ml and filtered.

The alpha amylase and alpha glucosidase inhibitory action of AK has been owed to the presence of antidiabetic phytoconstituents present in them as shown in the Table 1.

2.3 In-vitro Alpha Amylase Inhibition Study [9]

2.3.1 Method adopted: The spectrophotometric assay method.

2.3.2 Reference standard: Acarbose

The enzyme α -amylase (0.5 U/ml) was prepared by mixing 3.24 mg of α -amylase in 100 ml of phosphate buffer (pH 6.9). Test Sample was prepared by boiling the ingredients weighed 20g in 80ml and reduced to 20ml and filtered according to the decoction preparation method

as indicted in the Siddha literature. The filtrate was dissolved in DMSO to make stock solution and serially diluted to make different concentrations ranging from 10,20,40,80 and 100 µg/ml. About 600µl of test and standard (acarbose) sample were added to 30µl of α-amylase enzyme solution and incubated at 37°C for 15 min. To this reaction mixture, 370 µl of substrate, 2-Chloro-4-Nitrophenyl-α-Maltotrioxide (CNP₃. 0.5 mg/ml) was added, mixed and for incubated 37°C for 10 min. Finally, absorbance was measured at 405 nm against blank in spectrophotometer. A control reaction was carried out without the test sample. Percentage inhibition was calculated by the following formula.

Percentage inhibition

$$\% \text{ Inhibition} = \frac{\text{Absorbance}_{\text{Control}} - \text{Absorbance}_{\text{Test}}}{\text{Absorbance}_{\text{Control}}}$$

2.4. In-vitro Alpha Glucosidase Inhibition Study

2.4.1 Procedure

A known (20 mg) quantity of Avarai kudineer was dissolved in DMSO to make stock solution and serially diluted to make different concentrations. Avarai Kudineer (1/4) was prepared by boiling the ingredients weighed 20g in 80ml and reduced to 20ml and filtered. The solvent DMSO control, positive acarbose drug control and (n=5) were also maintained. The concentration of α-glucosidase inhibitor required to inhibit 50% (IC₅₀) of the α-glucosidase activity under the assay conditions was calculated (based on the absorbance). The Bio-Rad microplate absorbance reader was used for the assay. [10].

2.5 Statistical analysis

The α-glucosidase inhibitory activity was statistically analyzed by one-way ANOVA followed by Tukey's test. Plasma glucose levels are expressed as the Mean ± Standard Error Mean.

3. RESULTS AND DISCUSSION

The enzyme inhibitors are present in plants as a defense mechanism to protect them from insects and are reported to alter the digestive action of these enzymes in the gut of insects thereby inhibiting their normal feeding behavior. Therefore the alpha amylase and alpha

glucosidase enzyme inhibitors have potential role in the management of hyperglycemia and crop protection [6,11].

The results in Table 2 and Table-3 showed the percentage inhibition of AK sample extracts and acarbose against α-amylase and demonstrated a dose-dependent α amylase activity as shown in Fig-1. The inhibitory activity of AK was found to be 59.83± 7.1 when compared to the standard drug acarbose (81.42± 5.51). The IC₅₀ Value (resulting in 50% inhibition of enzyme activity) of Alpha Amylase enzyme inhibition ± SD (µg /ml) of AK was 80.91± 14.18 when compared to standard drug acarbose 25.94± 3.67.

Similarly the concentration-dependent α-glucosidase inhibitory activities and the IC₅₀ values were estimated as indicated in Table 4 and Table 5 respectively. Percentage of invitro alpha glucosidase inhibitory activity of AK was maximum in the Sample AK at 100 µg/ml as shown in Fig-2. The inhibitory activity of AK was found to be 71.94 ± 1.22 when compared to the standard drug acarbose (91.59 ±12.79). According to a previous study, the β-glucosidase in human liver has a higher affinity for the flavonoid and isoflavonoid glycosides than for the other dietary compounds previously investigated and, therefore, these compounds could be substrates in vivo for this enzyme. The presence of glycosides in sample AK may be responsible for this action of invitro alpha glucose inhibitory action.

Regarding the antidiabetic effect of acarbose, it has been reported to be associated with gastrointestinal side effects as it causes increased pancreatic αamylase inhibition resulting in abnormal bacterial fermentation of undigested carbohydrates in the large intestine[20].

The test drug AK has been investigated to have antidiabetic activity almost percentage of inhibition closer to acarbose. Also the test drug sample AK has been found to have higher α-glucosidase activity than α-amylase. Previous studies support that any bioactive composed having lower inhibitory activity against α-amylase and stronger inhibitory activity against α-glucosidase a key enzyme for carbohydrate digestion and may be an effective therapeutic agent for the control of postprandial hyperglycaemia with fewer side effects than acarbose [21].

Table 1. Antidiabetic action of phytoconstituents of AvaraiKudineer

| S.no | Botanical name / Parts used | Tamil name | Antidiabetic Phytoconstituents | Antidiabetic Action |
|-------------|---|-----------------------|---|--|
| 1. | <i>Cassia auriculata</i> (Whole plant) | <i>Avirai</i> | Tannins, flavanoids, saponin,terpenoids,alpha tocopherol | Alpha amylase and alpha glucosidase inhibition[12,13]. |
| 2. | <i>Cassia fistula</i> (bark, leaves) | <i>Kondrai</i> | Flavanoid, Proanthocyanidin, Lupeol, saponin, Tannin,Triterpenoid | Alpha amylase inhibition[14]. |
| 3. | <i>Eugenia jambolana</i> (seeds) | <i>Naaval</i> | Flavanoid, myricetin, ellagic acid, Tannin, Jambosine, Jamboline . | Alpha-glucosidase inhibitory activities[15] |
| 4. | <i>Salacia reticulata</i> (Wholeplant) | <i>Kadalazhinjil</i> | Salacinol, Kotanalol, Salaretin, quercetin | Alpha glucosidase inhibition[16]. |
| 5. | <i>Cyperusrotundus</i> (Rhizome) | <i>Korai kizhangu</i> | Flavanoids,Tannins, Saponins, Cyperene, Nor cyperone, beta sitosterol . | Inhibits alpha amylase and alpha glucosidase[17]. |
| 6. | <i>Costusspeciosus</i> (Rhizome) | <i>Koshtam</i> | Eremanthin, Saponins, Triterpenes, betasitosterol. | Invitro alpha glucosidase formation[18]. |
| 7. | <i>Terminalia arjuna</i> (Bark) | <i>Marutham</i> | Flavanoids,Tannins , Triterpenoid saponinsacid,Arjungenin,luteolin, Arjungenin, Arjunicacid . | Alpha amylase inhibitionand alpha glucosidase inhibition[19]. |

Table 2. Percentage inhibition of test drug AK on alpha amylase inhibition study

| Concentration (µg/ml) | % Inhibition of AK | % Inhibition of Acarbose |
|------------------------------|---------------------------|---------------------------------|
| 10 µg/ml | 15.25±8.32 | 30.92± 3.51 |
| 20 µg/ml | 22.42± 5.49 | 53.92± 2.36 |
| 40 µg/ml | 35.58± 4.85 | 66.5± 1.80 |
| 80 µg/ml | 48.08± 7.91 | 70.67± 1.18 |
| 100 µg/ml | 59.83± 7.1 | 81.42± 5.51 |

Data are given as Mean ± SD (n=3)

Table 3. IC50 Values for standard and AK for alpha amylase inhibition

| Test Drug / Standard | IC50 Value of Alpha Amylase enzyme inhibition ± SD (µg /ml) |
|-----------------------------|--|
| AK | 80.91± 14.18 |
| Acarbose | 25.94± 3.67 |

Data are given as Mean ± SD (n=3)

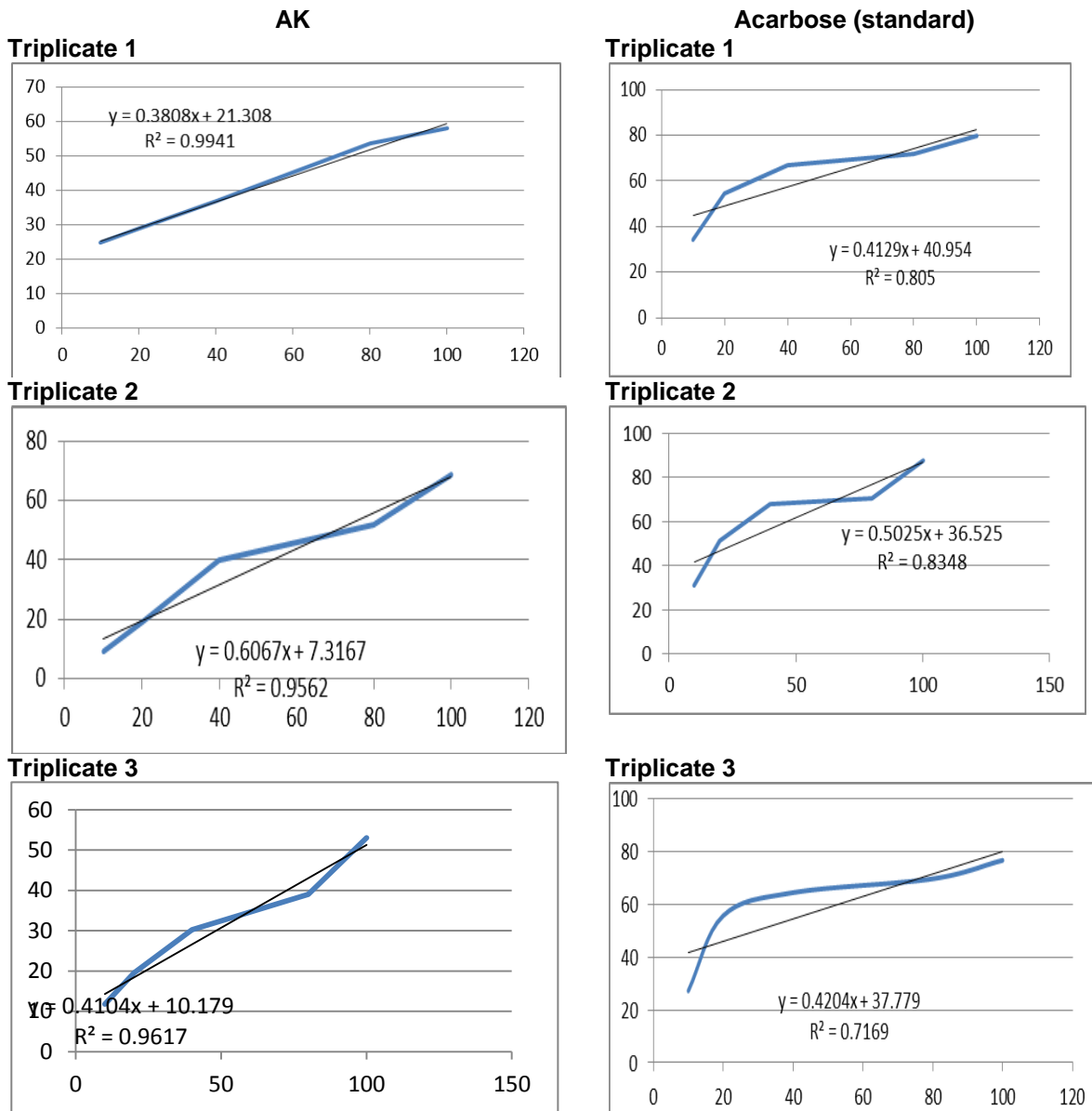


Fig. 1. Percentage inhibition of AK in Alpha Amylase Inhibition Study

Table-4. Percentage inhibition of test drug AK on α -Glucosidase Enzyme inhibition Study

| Concentration ($\mu\text{g/ml}$) | % Inhibition of AK | % Inhibition of Acarbose |
|------------------------------------|--------------------|--------------------------|
| 10 $\mu\text{g/ml}$ | 23.53 \pm 1.24 | 41.43 \pm 9.51 |
| 20 $\mu\text{g/ml}$ | 35.43 \pm 3.55 | 60.19 \pm 10.01 |
| 40 $\mu\text{g/ml}$ | 49.46 \pm 1.81 | 68.76 \pm 9.99 |
| 80 $\mu\text{g/ml}$ | 57.83 \pm 1.11 | 81.01 \pm 16.4 |
| 100 $\mu\text{g/ml}$ | 71.94 \pm 1.22 | 91.59 \pm 12.79 |

Data are given as Mean \pm SD (n=3)

Table-5. IC₅₀ Values for α -Glucosidase Enzyme inhibition by AK and standard

| Test Drug / Standard | IC ₅₀ Value of Alpha Glucosidase enzyme inhibition \pm SD ($\mu\text{g/ml}$) |
|----------------------|---|
| AK | 55.01 \pm 0.63 |
| Acarbose | 11.17 \pm 2.69 |

Data are given as Mean \pm SD (n=3)

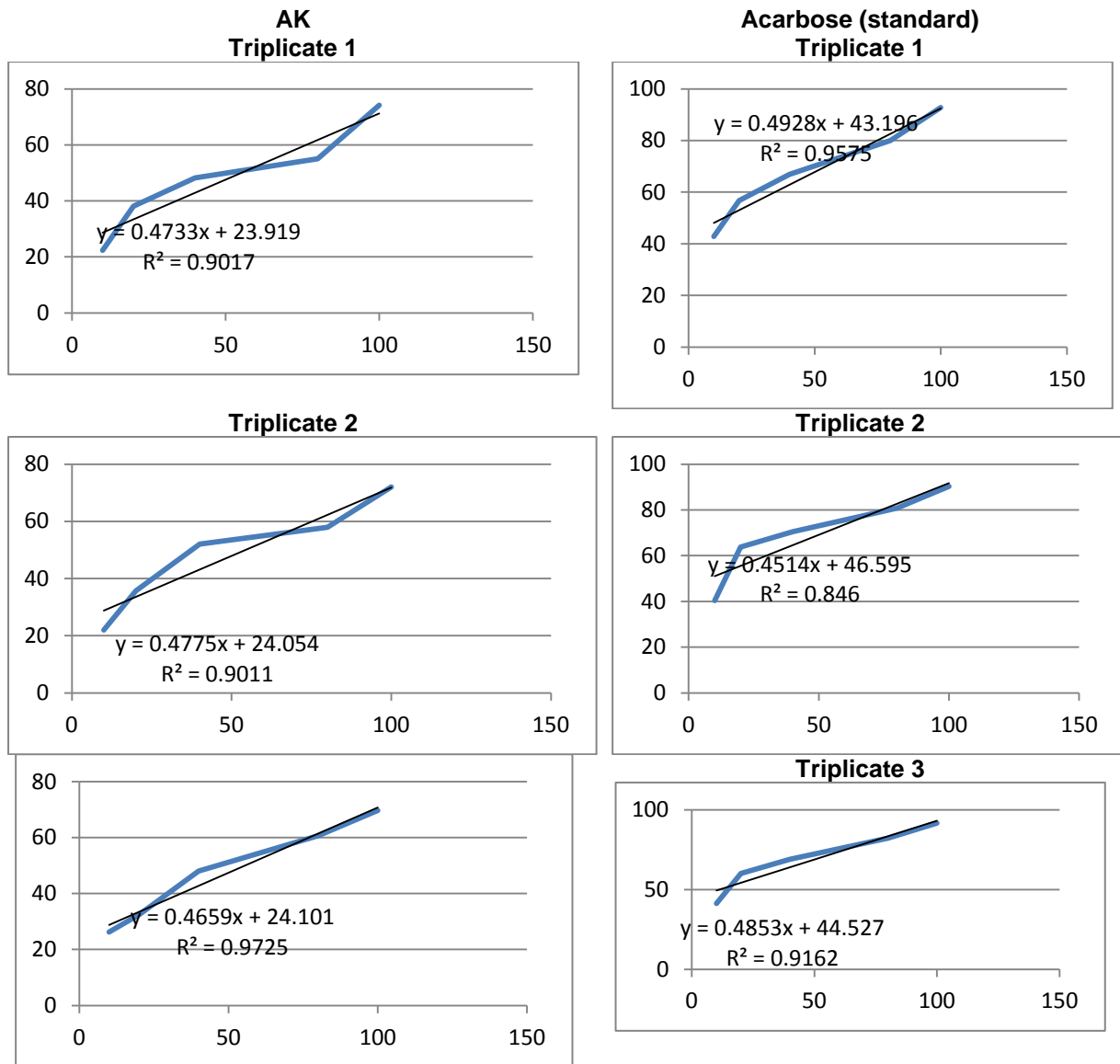


Fig. 2. Percentage inhibition of AK in Alpha glucosidase Inhibition Study

Phytochemicals have the ability to delay or prevent glucose absorption. [22] A study by Tundis.,2010, stated that natural alpha-glucosidase and alpha-amylase inhibitors from plant sources offer an attractive strategy for the control of hyperglycaemia. [23] Several in vitro studies that *Cassia auriculata*, *Cassia fistula*, *Salacia reticulata*, *Cyperus rotundus* and *Terminalia arjuna* which are the ingredients of AK has either or both alpha-amylase and alpha-glucosidase inhibitory activity. The other ingredients such as *Syzygium cumini* and *Costus speciosus* shows inhibition of intestinal glucose uptake and stimulates insulin secretion and tissue glucose uptake respectively. Thus all of

them act synergistically as effective anti-diabetic agents as shown in Table-1.[12-19] This cumulative action of all these ingredients has contributed to the results of this preclinical study.

In this aspect the test drug *Aavirai Kudineer* as indicated by the test results of the present study, can act as an effective antidiabetic agent for the control of Type 2 Diabetes mellitus that can decrease postprandial hyperglycemia by inhibiting carbohydrate digesting enzymes resulting in a delay of carbohydrate digestion to absorbable monosaccharide.

4. CONCLUSION

Medicinal plants constitute an important source of potential therapeutic agents for Type 2 Diabetes Mellitus. In this preliminary work we intended to evaluate the α -amylase and α -glucosidase inhibitory activities of *Aavirai Kudineer* to validate its traditional use indicated in the Siddha literature. The results were obtained for α -amylase and α -glucosidase enzyme inhibition activity in a dose-dependent manner. The test drug AK has been exhibited to have appreciable alpha amylase and alpha glucosidase inhibiting activity and can be clinically effective for Type 2 Diabetes Mellitus.

DISCLAIMER

The products used for this research are commonly and predominantly use products in our area of research and country. There is absolutely no conflict of interest between the authors and producers of the products because we do not intend to use these products as an avenue for any litigation but for the advancement of knowledge.

CONSENT

It is not applicable.

ETHICAL APPROVAL

It is not applicable.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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