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α-Glucosidase in Evaluation and Impact of Anti-Diabetic Retinopathy Activity of Ethanolic Extract of Tinospora Cordifolia Leaves

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ABSTRACT

In the ethanolic extract of Tinosporacardifolialeaves, the current study amply confirmed the existence of tinosporine, beriberine, glycosidase A to E, alkaloids, and triterpenoids. Among the active components identified by the phytochemical screening procedures were alkaloids, triterpenoids, steroids, glycosides, tannins, polysaccharides, and phenols. These phytochemical elements would be responsible for the diverse pharmacological effects of Tinosporacardifolia. The evaluation of flavonoid content and in vitro methods of anti-diabetic activity of α-Glucosidase inhibition method also show the efficacy of the ethanolic extract of Tinosporacardifoloia leaves in treating diabetes. The anti-oxidant action is carried out via the hydrogen peroxide scavenging method, which produces a 69% inhibition. By using the protein denaturing process, the Tinosporacardifolia leaf extract shows a 43% decrease of the anti-inflammatory effect. The retina was separated from the eyeballs as a result of the anti-diabetic retinopathy effect of goat eyeballs. The retinas are separated into reference and test samples for the ethanol extract. The Tinosporacardifolialeaves extract do not compare well to the benchmark. The pharmacological uses of Tinosporacardifolia are numerous. These include treatments for histamine, diabetes, high blood pressure, stress, arthritis, Parkinson's illness, HIV, and cancer.

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INTRODUCTION

A hyperglycemia issue that affects that whole sight is named retinopathy. A vascular system of a light-sensitive epithelium within back of a retinal are now being effected (retina). Orginally, retinopathy may well not express such illnesses and may solely lead to slight perspective difficulties. A microvascular condition called diabetic retinopathy is a long-term effect of diabetes mellitus that causes vision loss and blindness. Even though persistent hyperglycemia causes increasing malfunction of the retinal blood vessel, it is first asymptomatic if untreated. Blindness and poor vision are the results. In the Western world, diabetes retinopathy is a common cause of significant vision loss in persons of working age groups. Around the world, 30% of people have diabetic retinopathy. Clinically speaking, it is the retina's vascular anomalies manifesting themselves [Figure 1] [1].

There are two distinct forms of diabetic retinopa-

thy. Proliferative diabetic retinopathy and non-proliferative diabetic retinopathy are the two types. In 2050, there will be around 16.0 million DR patients in America, with 3.4 million of them expected to have difficulties related to vision treatment. The early stages of DR, known as NPDR (non-proliferative diabetic retinopathy), are characterised by increased vascular permeability and capillary blockage in the retina. Fundus photography can identify retinal disorders including microaneurysm and retinal haemorrhage at this stage, even if the patients may be asymptomatic [2]. PDR (proliferative diabetic retinopathy) is defined by the development of new blood vessels on the surface of the retina or the optic disc and develops as a result of further retinal ischemia. These aberrant blood vessels have a tendency to bleed, which can cause vitreous haemorrhage, follow-up fibrosis, and fractional retinal detachment. Neovascularization is a feature of PDR, a more advanced stage of DR. When the new aberrant arteries bleed into the vitreous at this point, the patient may have severe vision impairment. When a patient has diabetic macular edema, the most typical reason for visual loss [3].

Uncontrolled diabetes can cause a variety of visual problems, including diabetic retinopathy, the most common and serious ocular complication, cataracts, glaucoma, ocular surface abnormalities, recurrent style, and non-arteritic anterior ischemic optic neuropathy. The progression of diabetic retinopathy is linked to poor glycemic control, uncontrolled hypertension, dyslipidemia, nephropathy, male sex, and obesity. Recent research shows that the retinal neovascular unit and its interconnected vascular, neuronal, glial, and immunological cell are significantly impacted by diabetes [4]. Aloe, Tulasi, Neem, Turmeric, and Ginger are medicinal plants that treat a variety of common illnesses. These are commonly utilised as home remedies for illnesses across the nation. In a conventional system, tinosporacardifolia is an effective medicinal agent. Amrithavalli, Guduchi, Madhuparni, Giloya, Tippateega, Heart leaf, Moonseed, Gulancha, and other common names are among them. Anti-diabetic, antioxidant, antiviral, anti-microbial, anti-spasmodic, anti-cancer, and Parkinsonism treatments are all made with tinosporacardifolia. Additionally, it is used to treat fever, jaundice, chronic illnesses, dangerous organisms, diarrhoea, diabetes, skin conditions, poisonous bug bites, and eye problems. The research has primarily concentrated on naturally occurring anti-diabetic retinopathy, and many crude extracts and entirely natural substances have been shown to have positive effects on endothelial vascular growth factors as such. The primary goal of this study was to use accepted techniques to ascertain the plant leaf extract of Tinosporacordifolia's anti-diabetic retinopathy effectiveness.

MATERIALS AND METHODS

Collection and Authentication

The leaves of the Tinospora cordifolia plant was procured from Tirupati, Andhra Pradesh, India, which is close by. Dr K. Madhava Chetty, Assistant Professor, Department of Botany, Sri Venkateswara University, Tirupati, identified and verified the plant materials.

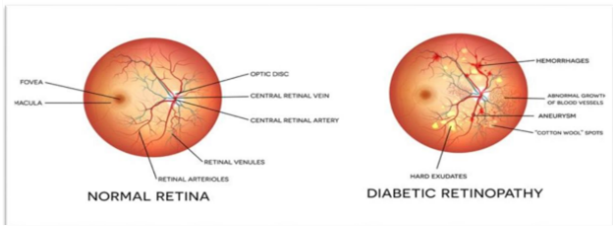

Figure 1: Difference between normal retina and diabetic retina

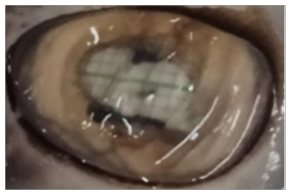

Figure 2: Normal retina

Figure 3: Retina with diabetes

Figure 4: Retina treated with Tinospora cardifolium

Figure 5: Retina with treatment

Plant Extraction

After being shade-dried for seven to ten days, the leaves of Tinopsora cordifolia was ground in an electric grinder. First, the dried material was extracted in the Soxhlet apparatus using ethanol and acetone in a 70:30 ratio (4000 ml x 4 cycles) at 40 for 16

Table 1: The percentage yield of crude drug of Tinosporsa cordifolia leaves extract and characteristics

S.NO	Solvent	Color and consistency	% Yield
1	Ethanolic extract of leaves	Thick green, non-sticky	30%

Table 2: Preliminary phytochemical analysis of Ethanolic extract of Tinospora cordifolia leaves

S.NO	Phytochemical screening tests		Ethanolic extract of Tinospora cordifolia leaves
1	Alkaloids	Mayer test	Present
		Dragendroff test	Present
		Wagners test	Present
2	Carbohydrates	Benedicts test	Present
		Fehling's test	Present
		Molish's test	Present
3	Flavonoids	Alkaline test	Absent
		Lead test	Absent
4	Tri terpenoids	Cooper acetate test	Present
		Salkowski test	Present
5	Protein and Amino acids	Biuret's test	Absent
		Xanthoproteic test	Absent
		Ninhydrin	Absent
6	Saponins	Forth test	Present
		Foam test	Present
7	Steroids	Liberman –burchard's test	Present
		Salkowski test	Present
8	Tannins	Gelatin test	Absent
9	Glycosides	Modified Bortrager's test	Present
		Legal's test	Present
10	Phenols	Ferric chloride test	Present

Table 3: Absorbance and % inhibition of anti-oxidant activity by hydrogen-peroxide scavenging assay method

S.No	Concentrations (μg/ml)	Absorbance of Ethanolic extract	Standard Ascorbic absorbance	% Inhibition
1	20 (μg/ml)	1.428	1.671	15.14%
2	40 (μg/ml)	1.321	1.613	19%
3	60 (μg/ml)	1.243	1.622	26%
4	80 (μg/ml)	0.867	1.389	36%
5	100 (μg/ml)	0.848	1.487	65%

Table 4: Total flavonoid content of Tinospora cardifolia leaves extract and standard Rutin absorbance

S.No	Concentrations (μg/ml)	Absorbance of Ethanolic extract	Absorbance of Standard Rutin
1	10 (μg/ml)	0.31	0.48
2	20 (μg/ml)	0.38	0.56
3	30 (μg/ml)	0.40	0.61
4	40 (μg/ml)	0.43	0.67
5	50 (μg/ml)	0.46	0.78

Table 5: The Tinospora cordifolia leaves ethanol extract and the standard acarbose absorbance

S.No	Concentrations of Ethanolic extract (μg/ml)	Absorbance of Standard	Absorbance of Ethanolic extract
1	50 (μg/ml)	0.783	0.56
2	200 (μg/ml)	0.654	0.47
3	300 (μg/ml)	0.684	0.35
4	400 (μg/ml)	0.497	0.28
5	500 (μg/ml)	0.361	0.14

Table 6: Anti-inflammatory activity of Tinospora cordifolia leaves ethanol extract and the standard Aspirin absorbance and % of inhibition

S.No	Concentrations (μg/ml)	Absorbance of Ethanolic extract	Standard Aspirin absorbance	% Inhibition
1	100 (μg/ml)	0.327	0.51	36.4%
2	200 (μg/ml)	0.368	0.56	36.42 %
3	300 (μg/ml)	0.374	0.59	37.17 %
4	400 (μg/ml)	0.383	0.62	39.89%
5	500 (μg/ml)	0.396	0.68	43.04 %

hours. A rotary vacuum evaporator was used to dry the residue under pressure [5].

Preliminary screening of Phytochemical

The phytochemical analysis studies have been performed such as assessing the various synthetic groups displaying an opioid, 10% (w/v) remedy like extracts were obtained except as otherwise did mention inside based on the respective exam. Overall assessment of varied samples of such plant matter has been done again for subjective persistence of a class of organic substances [6].

Test for Alkaloids

Preparation like sample solution: an experiment solution has been made by dissolving harvests through dilute acid, but also a solution has been filtrated. Its supernatant then was confined towards the following techniques again for identification of existence like alkaloids [7, 8].

Dragendroff's test: filtrates were treated with Dragendroff's reagents (solution of potassium Bismuth iodine).

Wagner's test: A tiny handful falls like wagner's reagent (iodine through potassium iodide have been decided to add through 3 ml supernatant. a creation like red-brown painted, whitish yellow, but rather precipitate demonstrates its existence of alkaloids.

Mayer's test: Add few of falls like Mayer's solvent of about 1ml like acidic aqueous leaf extract of an opioid. A pale yellow precipitate has been shaped.

Test for Carbohydrates: Preparation of test a solution: Extract was dissolved and usually in 5ml of distilled water and filtered. The filtrates were used to test for the presence of carbohydrates.

Benedict's test: Add a few drops of Benedict's solution to extract and heat gently. The Formation of the brick red color red precipitate is due to the presence of carbohydrates.

Fehling's test: To 2 ml like aqueous leaf extract of

an opioid 1ml of such a mishmash like portions like Fehling's solutions "a" as well as Fehling "b" but also boil its components of a test tube in a few min. A rose but rather reddish brown precipitate has been founded.

Molish's test: Within a tube containing 2 ml of such an aqueous leaf extract of an opioid add two drops like freshly made 20% alcoholic solution like β – naphthol and blend, flow 2 ml con. Sulfuric aspects are a covering underneath the weird mix. Carbs, if existing, generate a red-violet circle, where it fades away also with addition of such an extra of such an alkaline solutions.

Test for Flavonoids

Preparation of test solution: To a small amount of extract equal amount of 2 M Hydrochloric acid was added and heated for about 30-40 min at 100 C. The extract was cooled down and again extracted with ethyl acetate which was further concentrated in the dryness and ready to be used in the test sample [9, 10].

Alkaline: The extract was treated with some falls like sodium hydroxide. forming like golden yellow colouring. It becomes translucent also with addi - tion like dilute acid, confirms the presence like flavonoids.

Lead acetate: The extract was treated with a few falls like lead acetic acid solution. Yellow coloured precipitate identifies the existence like flavones.

Tests for Triterpenoids

Preparation of the test sample was prepared by dissolving the extract in chloroform and subjected to the following test.

Copper acetate test: extract was in the water and treated with 3-4 drops of copper acetate solution formation of Greencolor, Presence of terpenoids.

Salkowski's test: A few drops of concentrated sulfuric acid were to the test solution and allowed to stand for some time. The formation of red color in the lower layer indicates the presence of steroids and the formation of yellow color in the lower layer indicate the presence of triterpenoids.

Tests for Proteins and Amino acids

Preparation of test solution: it was prepared by dissolving it in water and making an aqueous extract [11].

Biuret's test: Of about 1ml like hot aqueous extricate of an opioid add 5-8 falls like 10%w/v sodium hydroxide accompanied through 1 and 2 falls like 3%w/v copper sulfate. A red but rather purple colouring has been acquired.

Xanthoproteic test: The test extract was treated with a few drops of conc. Nitric acid. The Yellow color indicates the proteins.

Ninhydrin test: 0.25% of the Ninhydrin reagent was added and boiled few mints. Blue color presence of amino acids.

Tests for Saponins

It was prepared by dissolving extract in water & making an aqueous extract. In a test containing about 5ml of an aqueous extract of the drug add a drop of sodium bio carbonate solution, shake the mixtures vigorously add for 3 minutes. Honeycomb-like fourth is formed [12].

Forth test: The extract was diluted with distilled water to 20 ml and this was shaken in a graduated cylinder for 15 min.

Foam test: 0.5 gm of extract was shaken with 2ml of water if the foam produced persisted for 10 minutes. Foam is produced by saponins present.

Tests for Steroids

Preparation of test sample was prepared by dissolving the extract in chloroform.

Liebermann-Burchard's test: Add 2 ml like acetic anhydride solution of about 1 ml like petroleum ether extricate of an opioid through chloroform accompanied by 1 ml conc. Sulfuric acid. A greenish color is developed which turns to blue.

Salkowski reactions: Add 1 ml of conc. Sulfuric acid to 2ml of chloroform extract of the drug carefully, form the side of the test tube. The red color is produced in the chloroform layer.

Test for Tannins

It was prepared with alcoholic extract and aqueous extract.

Gelatin test: To its extricate, 1% like gelatin solution usually contains sodium chloride has been got to add. A white precipitate existence like tannins.

Tests for Glycoside:

Modified Borntrager's test: Ethanol extracts are used in this test, extracts were with ferric chloride solution and immersed in boiling water for about 5 minutes. The extracts were cooled and extracted with equal volumes of benzene. The enzyme layer was separated with ammonium solution, rose pink color formed. It indicates glycosides presence.

Legal's test: Extracts were treated with sodium Nitroprusside in pyridine and sodium hydroxide.

1. Test for Phenols: Ferric chloride experiment: extract was mixed as for 3-4 falls of fe(oh)3

solution. A blue-black color represents its existence of total phenol.

2. Total Flavonoid Content: Total flavonoid content of the sample was determined by using methods described by faud, A. Flwi, A., and Zulkifly, with slight modifications. A stock rutin standard solution of 10 mg/ml was prepared.10μg/ml, 20μg/ml, 30μg/ml, 40μg/ml with different concentrations of sample extracts. 1ml of sample from the stock solution was mixed with 3 ml of methanol, 0.2 ml of 1 M potassium acetate and 0.2 mL of 10% aluminum chloride 5.6 ml of distilled water was added. The solution has been cultured such as 30 min there as room temp. 200 microlitres like specimen extracts have been transmitted it into a 96 - well plate out from centrifuge tube as well as the absorbance was recorded about as 415 nm using just a spectrometer. Its outcome has been demonstrated even though mg like rutin equal per gram of sample [13].

In Vitro Anti-Diabetic Activity

Alpha glucose oxidase inhibitory activity through diabetic action: inhibitory activity like α-glucosidase activity was measured utilising lactobacillus α-glucosidase but also p-nitrophenyl-α-d-glucopyranoside (pNPG) just like characterized once. nutrient extricate but rather acarbose (100μl of 2 to 20 mg/ml) has been got to add of about 50μl like α-glucosidase (1 U/ml) able to prepare through 0.1 m phosphate-buffered (ph 6.9), but also 250μl like 0.1 m phosphate-buffered to just get 0.5 to 5.0 mg/ml final concentration. a mix has been pre-incubated there as 37 °c such as 20 min. upon pre-incubation, 10μl of 10 mm pnpg ready through 0.1 m phosphate-buffered (pH 6.9) has been added, but also overnight at 37 °c such as 30 min. Its responses have been paused through introducing 650μl of 1 ml na2co3, as well as the absorbance was recorded in such a spectrometer (AMERSHAM BIOSCIENCES, USA) about as 405 nm. Proportion like inhibitory activity of enzymatic reaction has been estimated as

% Inhibition= [(A405Control-A405Treatment)/A405Control] x 100

Where in A405 regulate has been absorption at 405 nm in control specimen rather than nutrients extricate but also A405 diagnosis has been absorption at 405 nm through diagnosis as for nutrients extricate. An IC50 value was resolute through using linear regression equation i.e., Y = mx+c. Here, y = 50, m and c values were derived from the viability graph [14].

In Vitro Anti-Oxidant Activity:

Hydrogen Peroxide Scavenging Assay

Aliquot of 0.1 ml of extract (25-400μg/ml) was transferred into the Eppendroff tubes. The volume was made up to 0.4 ml with 50 mm phosphate buffer (PH 7.4). To the above solution add 0.6 of Hydrogen peroxide solution (2mm). The sample was placed in 10 mints. After 10 mints the absorbance was measured at 230 nm. Ascorbic acid was used as the positive control; Hydrogen peroxide scavenging activity in percentage was measured by using [15].

= $[(A0 - A1)/A0] \times 100$

Where, A0 = Absorbance of control, and A1= Absorbance of sample

In Vitro Anti-Inflammatory Activity:

Protein Denaturating Assay

The reaction of mixer contains 5ml of solution which was consists of 0.2 ml of freshly prepared 1% of bovine albumin solution, phosphate buffer saline of PH and Ethanol extract of 0.002 ml with different concentrations of 12.5mg/ ml, 6.25 mg/ ml 3.125mg/ml. The reaction was heated in water bath at 37ºC for 15 minutes and the temperature gradually increases up to 70^o C for 5 minutes. Then the sample are collected and cooled down at room temperature the turbidity formed and measured at 660nm using spectrophotometer, phosphate buffer saline used as a negative control, Aspirin used as a positive control [16].

% Inhibition of denaturation = 100 $\times$ [1-$A2/A1$]

Where, A1= Absorption of control sample and A2 = Absorption of test sample

Anti-Diabetic Retiinopathy Activity

Collection of eye balls: goat eyeballs have been used in the current research. Those that have been acquired as from slaughter house instantaneously transferred to research lab at 0-4 degrees c [17, 18].

Preparation of Retina culture

The retina have been excluded through additional capsular excavation but also sub cultured through artificial aqueous humor. The artificial humourous is ready just like follows

1. NaCl: 140mM
2. Kcl: 5mM
3. $Mgcl_2$: 2mM
4. $NaHCO_3$: 0.5mM
5. $NaH(PO_4)_2$: 0.5mM

6. $CaCl_2$: 0.4mM
7. Glucose: 5.5mM

Aqueous humor is ready about as room temp but also ph is 7.8 such as 72 hours. An amoxicillin 32mg% but also streptomycin- 250mg% have been got to add towards the pure culture to avoid microbial contamination. α- Glucose oxidase but also glycemic in such a concentration like 55mm has been used to stimulate cataract. Experiment on goat retina: The fresh goat eyeballs were collected and separated retina from the goat eyeball. It is placed in already prepared (10% of 1 ml α-Glucosidase and with 2 gm of glucose) aqueous humor for 24 hours for induced diabetes. After 24 hrs the retina has small damage with brown color strikes and dots, swelling of the tissue and white milky appearance. These symptoms indicate induced diabetes. The retinas were separated for the treatment of ethanol extract of Tinospora cordifolia leaves by using the standard as Metformin. Two retinas are placed separately in test sample and standard sample. A test sample is ethanolic extract of Tinospora cordifolia leaves (10 mg/ml is taken for treatment. And the standard sample metformin (1 mg/ml) is taken with aqueous humor. After 24 hours absorbing the small changes in the retina, the white color retina changed normal white color and some strikers lightly disappear. The standard and Tinospora cordifolia leaves extract shows action on the retina. It is estimated by some biochemical parameters [Figures 2, 3, 4 and 5].

1. Estimation of malondialdehyde
2. Estimation of lipid hydroperoxide
3. Estimation of carcinogenicity

RESULTS AND DISCUSSION

Tinospora cordifolia leaves hydroalcoholic extract has undergone preliminary phytochemical investigation. Alkaloids, triterpenoids, steroids, as well as sugars, glycosides, and phenols, are the main chemicals found in the ethanol extract [Tables 1 and 2].

Observation

In the ethanol extract Alkaloids, triterpenoids, steroids are major compounds, carbohydrates, glycosides, and phenols are present.

Using a hydrogen peroxide radical scavenging assay, Tinospora cordifolia ethanolic extract was found to have anti-oxidant properties. the UV absorption was measured below 230 nm. The test sample's absorbance is inferior to that of the standard sample. Increased concentrations result in higher inhibition percentages, lower absorbance, and higher inhibition percentages. Using a hydrogen peroxide radical scavenging assay, Tinospora cordifolia ethanolic extract was found to have anti-oxidant properties. the UV absorption was measured below 230 nm. The test sample's absorbance is inferior to that of the standard sample. Increased concentrations result in higher inhibition percentages, lower absorbance, and higher inhibition percentages. The standard acarbose, ethanolic extract absorbance at 430 nm, and the anti-diabetic activity by alpha-Glucosidase inhibition technique for induced diabetes. As the concentrations rise, the absorbance falls while the concentration rises conversely, the percentage of inhibition rises. The concentration, absorbance, and percentage of inhibition of the anti-inflammatory action by Protein denaturing assay for Tinospora cordifolia ethanol extract and the standard Aspirin are considerably increased.

Anti-oxidant activity

The anti-oxidant activity by Hydrogen peroxide radical scavenging assay of ethanolic extract of Tinospora cordifolia leaves. The absorbance was measured under the 230 nm in UV. The standard sample absorbance is better than the test sample. When the concentration is increased the % of inhibition is increased, absorbance is decreased, % of inhibition is increased [Table 3].

Flavonoid content

The total flavonoid content in sample of Tiinosporacardifolia leaves extract. The studies conducted by rutin as standard. The concentration of total flavonoid content is expressed by mg/gm of rutin equivalent. Maximum absorbance depends on the concentration of flavonoids [Table 4].

Anti-diabetic activity

The anti-diabetic activity by α-Glucosidase inhibition method for induced the diabetes, the absorbance is measured at 430nm for ethanolic extract, and the standard acarbose. As the concentrations increased the absorbances are decreased, the concentration is increased alternatively % of inhibition increased [Table 5].

Anti-inflammatory activity

The anti-inflammatory activity by Protein denaturation assay for Tinospora cordifolia ethanol extract concentration and the standard Aspirin absorbance and % of inhibition are relatively increased [Table 6].

CONCLUSION

The current investigation amply demonstrated the presence of tinosporine, beriberine, glycosidase A to E, alkaloids, and triterpenoids in the ethanolic extract of Tinosporacardifolia leaves. Alkaloids, triterpenoids, steroids, glycosides, tannins, polysaccharides, and phenols were among the active ingredients that were found in the phytochemical screening processes' results. The various pharmacological effects of Tinosporacardifolia would be caused by the presence of these phytochemical components. The effectiveness of the Tinosporacardifoloia leaves ethanol extract in treating diabetes is demonstrated by measurement of flavonoid content and in vitro methods of anti-diabetic activity of alpha-Glucosidase inhibition method. The Hydrogen Peroxide Scavenging method is used to perform the anti-oxidant action, and it results in a 69% inhibition. The Tinosporacardifolia leaf extract exhibits a 43% suppression of the anti-inflammatory action as measured by protein denaturing technique. Goat eyeballs performed anti-diabetic retinopathy action, and the retina was detached from the eyeballs. For the reference and test samples of the ethanol extract, the retinas are divided. The extract from Tinosporacardifolia leaves do not compare favourably to the standard. There are various pharmacological functions for Tinosporacardifolia. These include medications for diabetes, hypertension, stress, arthritis, Parkinson's disease, HIV, cancer, and histamine.

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Conflict of Interest

The authors attest that they have no conflict of interest in this study.

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