PHYTOCHEMICAL AND PHARMACOLOGICAL INVESTIGATION ON GLINUS LOTOIDES. L AND MOLLUGO CERVIANA (L.) SER. FOR ANTI-DIABETIC ACTIVITY

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Abstract: Treatment for diabetes mellitus, a complicated metabolic illness, depends on blood glucose control, and appropriate eating practises. The two main enzymes that are involved in the digestion of carbohydrates are α-glucosidase and α-amylase (CHE) Therefore, inhibitors of these enzymes can prolong the absorption of absorbable monosaccharides from dietary complex carbohydrates into the bloodstream, limiting any abrupt spike in blood glucose level caused by a meal. Natural substance acarbose is a therapeutically used α-glucosidase inhibitor, but it has drawbacks. As a result, novel leads with CHE inhibitory capabilities are needed.
Glinus oppositifolius (L.) Aug. DC. is locally known as Pita bhaji in Chhattisgarh and taken as a food by peoples of Chhattisgarh, India. The whole plant is traditionally used in wound healing, anti-diabetes, fiver, inflammations, diarrhea, intestinal and skin problems. This study made highlights on this folk herbal medicine which will help in the identification of crude samples of this plants part. The plants that includes in the study are Mollugo cerviana (Linn.) and Glinus Lotoide Ser, locally referred to as thread stem carpet weed. It is a member of the Molluginaceae family. Both plants are found in marshy wet land as a weed.
A brief literature survey revealed that both plants have got immense pharmacological properties bcause of their rich ness in flavonoids class of secondary metabolites. Both the plant materials were reported to have anti-diabetic components in it.

Keywords: anti-diabetic, Glinus Lotoides,

INTRODUCTION

As the old adage goes, if you only have one disease, make it diabetes because it is the only one over which you have control. "Diabetes is a terrific illustration of how, by providing the patient the tools, you could manage yourself very well," Clayton M. Christensen stated. The statement "eradicating the excess" is thought to date back to 1500BC in the Egyptian writings Ebers Papyrus. Around the same time, Indian physicians recognised the condition and classified it as madhumeha, or "honey urine," since the urine attracted ants. Sushruta and Charaka, two Indian physicians, defined two forms of diabetes for the first time in 400–500 CE, one of which was linked to age and the other to being overweight [2]. Despite the fact that modern medicine and therapeutic agent development have progressed a long way from insulin therapy to stem cell therapy or various oral antihyperglycaemic drugs such as insulin secretagogues to the recently introduced incretins, the World Health Organization still lists diabetes as one of the top ten causes of death worldwide.

2. MATERIALS AND METHODS

2.1 Plant Collection and Preparation of Various Solvent Extracts

2.2 Chemicals and Reagents

SRL PVT LTD, Mumbai, India, provided chemicals such as -amylase, soluble starch, -glucosidase, p-nitro phenyl glucopyranoside, DNSA, and acarbose. Carrageenin (Sigma Chemical Co., St. Louis, USA), diclofenac injection (Novartis India Ltd., Bombay), formalin (Rankem, Ranbaxy), Vernier calliper (Percision India Ltd.). Biovision Amylase Activity Colorimetric Assay Kit Instructions in commercially available kits from Span Diagnostics Ltd, Surat, Gujarat, India, were used to determine biochemical parameters. Nicotinamide (100g, n-3376, sigma-aldrich), sodium citrate (mw: 294.10), citric acid (mw: 210.10), sugar 10%, sodium chloride (Nacl 0.9 percent).

2.3 Plant Material Procurement Collection

During the month of August 2019, fresh areal portions of Glinus lotoides (L.) & Mollugo cerviana (L.) Ser. was randomly gathered from marshy areas and dry ponds in Usilampetty, Tamilnadu.

Botanist Dr Raju Antony, Scientist B, Jawaharlal Nehru Tropical Botanical Garden, authenticated the plant samples, as well as a voucher herbaria specimen No.38234 and 38235 were lodged in the department.

3. Extract preparation

Both plants' shade-dried plant material was crushed into a pulverised condition, sieved to generate a homogenous coarse powder, and stored in airtight containers.A measured quantity including both drug samples was then defatted with petroleum ether for 72 hours using the cold maceration procedure.

a. Both plant concentrates evaluation of in vitro for anti-diabetic activity.

1. Inhibition of -amylase assay

When a starch substrate is treated with the alpha amylase enzyme, glucose is liberated. By reacting it with 3,5 dinitrosalicylic reagent to produce a coloured complex, the amount of released glucose may be determined. The enzyme-inhibiting chemical inhibits the alpha amylase enzyme, resulting in a decrease in glucose synthesis. The Amylase Activity Colorimetric Assay Kit was used to perform the test, which followed the normal screening technique. At 405nm, the optical density of the standard and samples were measured.

2. In nicotinamide and streptozotocin-induced type 2 diabetic rats, the extract's antihyperglycemic action was assessed.

I. Animals:

Male Albino rats, 6 weeks old and weighing between 150 and 200 grammes, were employed in the study. The animals were kept in clean polypropylene cages and fed a normal food. The temperature and humidity in the cages were maintained on a 12 hour day and night cycle. All of the tests followed CPCSEA rules (IAEC Approval number KAHE/IAEC/2020/16-10/002).

3. Solution of nicotinamide.

A weighed amount of Nicotinamide was placed in an Eppendorf tube, and 1-2mL of isotonic NaCl solution was added.

4. Streptozotocin solution.

In a vortex machine, a solution of weighed streptozotocin and 1-2 ml sodium citrate buffer was thoroughly mixed. Because STZ is cytotoxic in nature, it must be prepared immediately before use and administered within 10-15 minutes with extreme caution. Keep the solution out of direct sunlight.

5. Diabetes mellitus induction

The animals were placed into seven groups, each with six animals. The rats are fasted overnight and the first fasting blood glucose is measured at the tip of the rat tail vein. A single intra-peritoneal injection of 60 mg/kg Streptozotocin, 15 minutes after the i.p administration of 120 mg/kg nicotinamide, was used to produce non-insulin dependent diabetes mellitus in overnight fasted rats. The presence of hyperglycemia was confirmed after 72 hours when blood glucose levels were measured. The study will involve animals having a blood glucose content of greater than 250 mg/dl. [91]

The animals in each group received a vehicle (saline), conventional glibenclamide, and Plant extract extracts (Oral gavage not exceeding 1.5mL per day) for 28 days. Glibenclamide extract was freshly dispersed in normal saline and distilled water before delivery throughout the study period. On the 0th, 7th, 14th, and 21st days, the fasting animal body wt. and blood glucose level were measured from the tip of the rat tail vein.

Table 1.: Grouping of animals for STZ- NIC induced diabetic mode

Groups	Sample size	Group specification
Group I	6	Only normal saline
Group II	6	STZ 65 mg/kg/b.w. (i.p) +NIC 120mg/kg (i.p)
Group III	6	STZ(65 mg/kg) NIC 120mg/kg (i.p) rats treated with Glibenclamide 20 mg/kg (p.o)
Group IV	6	STZ (65 mg/kg) +NIC 120mg/kg (i.p) rats treated with Extract I Low Dose 250 mg/kg po(Glinus lotoides)
Group V	6	STZ (65 mg/kg) +NIC 120mg/kg (i.p) rats treated with Extract I High dose 500 mg/kg po(Glinus Lotoies)

Group VI	6	STZ (65 mg/kg) +NIC 120mg/kg (i.p) rats treated with Extract II Low Dose 250 mg/kg po (Mollugo cerviana)
Group VIII	6	STZ (65 mg/kg) +NIC 120mg/kg (i.p) rats treated with Extract II Low Dose 500 mg/kg po (Mollugo cerviana)

1.**Tissue homogenate preparation.**

To get a 10% homogenate, the dissected liver and kidneys were homogenised using a motorised Teflon covered homogenizer and 0.1 M Tris-HCl buffer (pH 7.4). At 5°C, the homogenate was centrifuged for 10 minutes at 10000 rpm. The decanted supernatant was employed in the experiments.

2. Haematological parameter estimation

RBC Enumeration

RBC diluting fluid was drawn to mark II and well mixed blood were drawn up to the 0.5 level utilising haemocytometer's RBC pipette. Before being placed in the counting chamber, the fluid blood mixture was shaken. For 2 minutes, the cells were allowed to settle to the bottom of the chamber. The fluid should not be allowed to dry out. Using a 45X or high power objective, RBCs are counted equally in the bigger corner square.

Enumeration of WBC

It was given as a number of cells x1012/l. Using a white blood cell pipette from a haemocytometer, well mixed blood was drawn up to a 0.5 mark as well as WBC diluting fluid is drawn up to mark II. Before being placed in the counting chamber, the fluid blood solution was stirred. The cells were permitted to stay to the bottom of the chamber for 2 minutes. I don't think the fluid really dries out. Using a 10X or low power objective, WBCs were counted equally in the blood. The cells were counted and expressed as a number of cells per tenth of a millimetre.

Differential leucocyte count

It is a method of determining the number of leucocytes. Using a blood film stained with Leishmann's stain and viewed under oil immersion, the distinct types of WBCs were identified. %distribution of these cells was then calculated. Leishmann's dye was used to generate smears of anticoagulant blood samples. No. of lymphocytes & neutrophils in each 100 cells was counted on the slides. Absolute lymphocyte & neutrophil counts were estimated using various Leukocyte and WBC counts.

$$\text{Absolute neutrophil count} = \frac{\text{Number of neutrophils}}{100} \times \text{TWBC}$$

$$\text{Absolute lymphocyte count} = \frac{\text{Number of lymphocytes}}{100} \times \text{TWBC}$$

Haemoglobin estimation using Sahli's acid haematin method:

Using a pipette, fill the Haemoglobinometer to the lowest marker with 0.1 N HCl. 20 litres of blood were extracted without any bubbles in the sahli's pipette. Wipe the extra blood from the pipette's edges with a dry piece of cloth. After that, the blood was blasted into the acid solution in the graduated tube, as well as the pipette was properly cleaned. Allow 10 minutes for the reaction to settle at room temperature. The solution was diluted by carefully adding a small amount of distilled water to the reaction mixture & mixing it till the colour matched the colour in the comparator. The lower meniscus of the fluid was observed, and the reading in g/100ml was collected.

RESULTS AND DISCUSSION

1. Percentage yield of various Plant Extracts

The percentage yield of various extracts of both the plants are tabulated. All the extracts were analyzed for secondary metabolites.

Table2. : percentage yield of various extracts

Mollugo Cerviana Ser. (L)	
Percentage yield of Pet ether extract	2.8% w/w
Percentage yield of Ethanolic Extract	8.9% w/w
Glinus Lotoides (L)	
Percentage yield of Pet ether Extract	3.4% w/w
Percentage yield of Hydro alcoholic extract	12.1% w/w

2. Preliminary Phytochemical screening

The results of preliminary phytochemical screening are depicted in Table No 5.2

Table 3.: Phytochemical Evaluation of Plant Extracts:

Phytochemical Constituent	Mollugo cerviana Ser. (L.) (Ethanolic extract)		Glinus Lotoides (L.) (Hydro--Alcoholic Extract)	
	Pet ether Extract	Pet ether Extract	Hydro--alcoholic extract	Ethanolic extract
Carbohydrates	++	++	++	++
Proteins	++	++	++	++
Alkaloids	--	--	--	++
Tannins	--	--	--	++
Phenols	++	--	--	++
Flavonoids	--	--	++	++
Saponins	--	--	++	++
Glycosides	--	++	++	++
Terpenoides	--	--	--	++
Steroids	++	++	--	++

The phyto-constituents in the petroleum ether extracts of both plants were lower. The ethanolic extract of Mollugo cerviana was found to contain the highest concentration of powerful phyto components such as alkaloids, flavanoids, steroid glysosides, while hydro-alocholic extract of Glinus lotoides was found to contain mostly flavanoids and saponins.

3 .Anti- Inflammatory activity of Glinus Lotoides

5.3.1 Invitro Anti- Inflammatory activity

5.3.1.1 Inhibition of protein denaturation

The hydro-alcoholic extract of Glinus lotoides was analysed for Inhibition of protein denaturation using salicylic acid as standard.

Table 4.: Inhibition of protein denaturation

Concentration of Plant extract (µg/mL)	Percentage inhibition of protein denaturation (%)
50	32±2.4
100	44±0.76
250	62±0.67
500	94±1.3

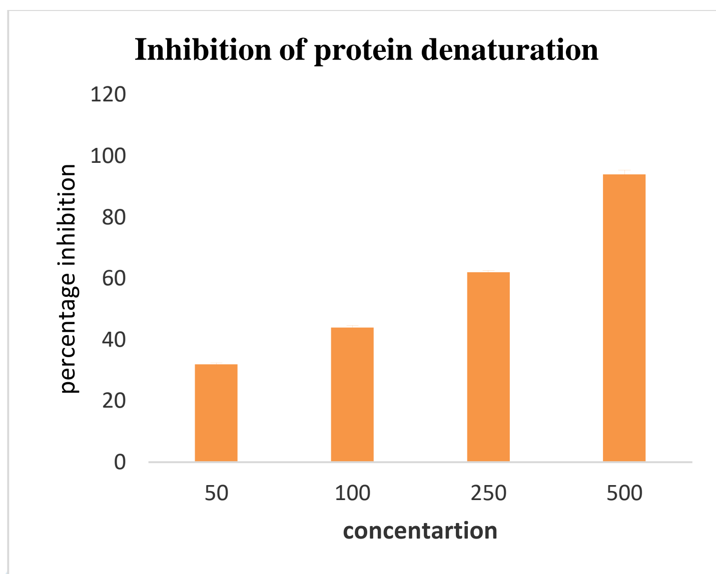

Figure 1: Inhibition of protein denaturation

Inflammation is primarily caused by protein denaturation. As part of the inquiry into the mechanism of anti-inflammatory effect, the extract's ability to prevent protein denaturation was tested. A few extracts substantially reduced heat-induced albumin denaturation. The IC50 of Glinus Lotoides was found to be 101.3 g/mL. The standard anti-inflammatory medicine was aspirin. [115]

In-vivo anti-inflammatory activity

Carrageenan-induced paw oedema model

Table 0.: Anti-inflammatory activity of Glinus lotoides-Carrageenan induced paw method

Groups	% Inhibition 1/2 hr	%Inhibition 1 hr	% Inhibition 1 1/2 hr	%Inhibition 2 hr
CRGN+Hydo Alc extract 250	24.93±0.12	30.44±3.45	31.36±2.95	39.0±2.6
CRGN+Hydro Alc. Extract 500	25.6±0.99	45.1±4.17	50.9±3.67	56.34±1.5
CRGN + Diclofenac sodium	65.97±0.05	86.24±0.67	78.82±0.88	82.13±1.33

Values are expressed as means of standard deviation of 6 Rats

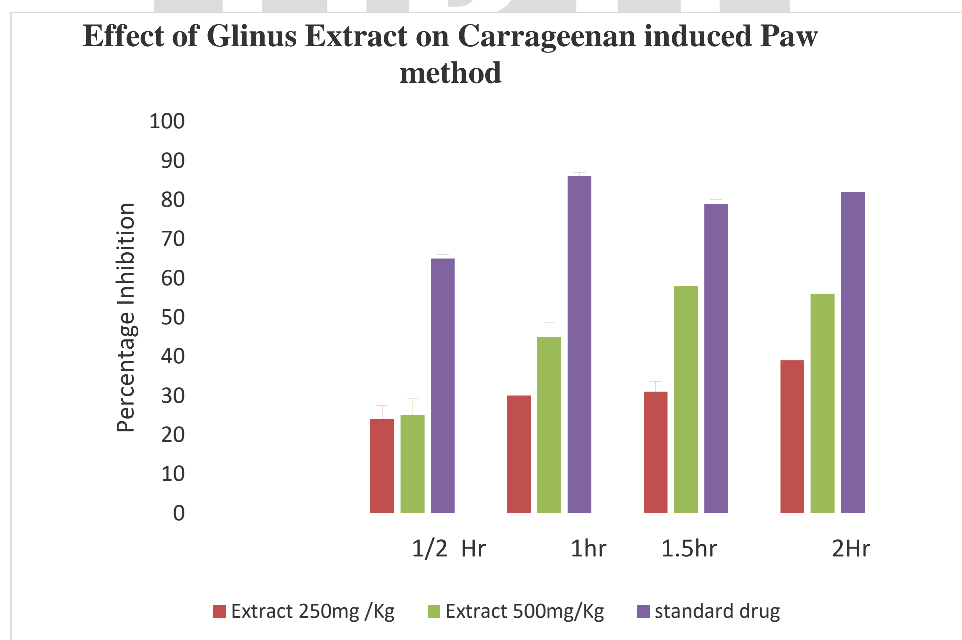

Figure 2: Carrageenan induced acute inflammation

5.4 In-Vitro Anti diabetic activity Studies
α-Amylase inhibition Assay
5.4.1 : α-Amylase inhibitory of Ethanolic extract of Mollugo cerviana (Ser) L

Table 6 .: α-Amylase inhibitory of Molllugo cerviana

Concentration	Percentage inhibition of Extract	Percentage inhibition of Acarbose standard
10	45±1.19	56±1.08
20	52±1.45	68±1.67
40	63±1.23	76±1.08
60	70±1.12	84±1.34
80	76±1.09	88±1.06
100	84±1.34	94±1.79

% inhibition value is mean ±SD

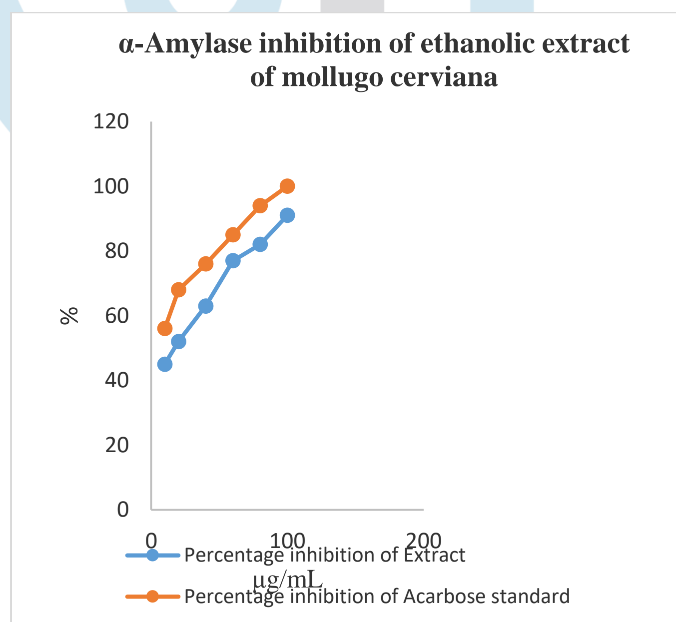

Figure 3: plot of concentration Vs Percentage inhibition

The rate hindrance of α-amylase compound by ethanolic extrace shows a dynamic nature as, inhibitory action increments with the expansions in focus. IC50 values were determined utilizing (Graph cushion programming, Inc, USA.) factual programming as 13.5µg/mL for mollugo cerviana ethanolic concentrate and 10 µg/mL for Acarbose standard. From the diagram it is obvious that the home grown extract has huge inhibitory action contrasted with acarbose standard. [120]

5.4.2 : α-Amylase inhibitory of Hydro-alcoholic extract of Glinus Lotoides (L)

Table7. : α-Amylase inhibitory of GLL

Concentration µg/mL	Percentage inhibition of Extract	Percentage inhibition of Acarbose standard
10	37±0.99	56±1.08
20	45.5±0.04	68±1.67
40	57.5±0.43	76±1.08
60	62±0.94	84±1.34
80	77±0.45	88±1.06
100	88±1.08	94±1.79

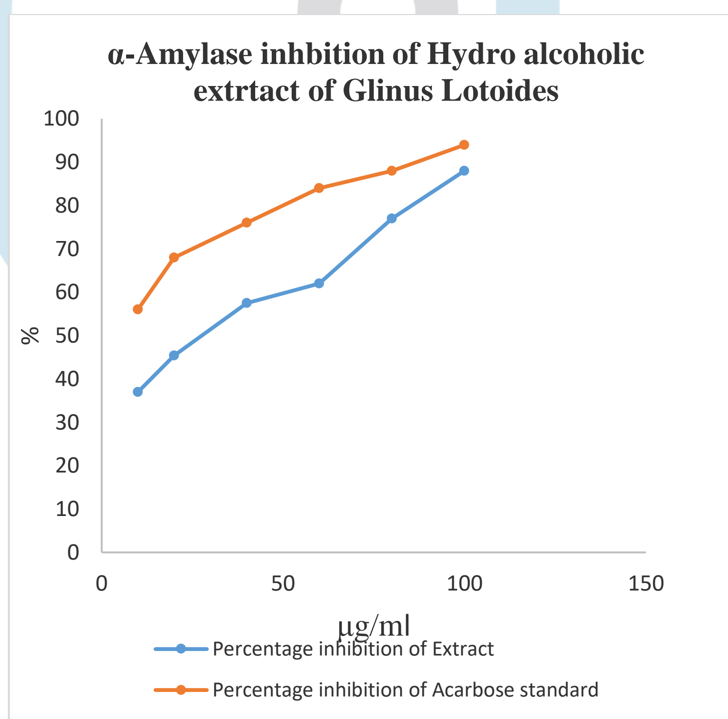

Figure 4: plot of concentration Vs Percentage inhibition

Table 5.6 shows the % inhibition of the hydro alcoholic extract. The findings of the experiment revealed that the percentage inhibitory activity against the -amylase enzyme increased in a dose-dependent manner. In Fig. 5.4, the outcomes are presented to a conventional plot of Acarbose. IC50 values of 12.5g/mL for hydro alcohlolic extract of glinus lotoides and 10g/mL for Acarbose standard were computed using (Graph pad software, Inc, USA.) statistical programme.

5.5 Evaluation of Anti-diabetic Activity of Plant extracts by In vivo Methods

When STZ and nicotinamide are given together, mild and stable hyperglycemia occurs, along with a reduction in pancreatic insulin reserves. Streptozotocin enters the pancreatic cell via the glucose transporter GLUT2 and damages the cell by alkylating its DNA. Damage to DNA causes polymerase activity to rise in order to repair it. Excessive activity

of this enzyme, on the other hand, results in a loss of intracellular NAD(+) and ATP, causing insulin-secreting cells to die. STZ may cause kidney damage, oxidative stress, inflammation, and endothelial dysfunction, in addition to pancreatic cell destruction. Thus, by inhibiting PARP-activity, concurrent administration of Nicotianmide helps to limit tissue necrosis and leads to a controlled hyperglycemia that mimics the diabetic state. This method is effective in terms of insulin sensitivity to glucose. [127]

5.5.1 Effect of extract on body weight analysis in normal and experimental rats

Due to increasing muscle wasting and tissue protein loss, the body weights of streptozotocin-nicotinamide injected rats tend to decline, with concomitant symptoms such as polyuria, polydipsia, and polyphagia. Diabetic animals' inability to gain weight over time is due to chronic glucose excretion induced by a decrease in the liver's ability to synthesise glycogen and an impairment in peripheral glucose absorption. All of the animals' body weights were measured on the first day and at the conclusion of the first, second, third, and fourth weeks, and the findings are shown in table 5.7.

When STZ +NIC injected diabetic rats were compared to a normal control group, they showed a substantial (p0.001) decrease in body weight. When compared to the diabetic control group, administration of Molllugo cerivana (MCR) ethanolic extract, Hydroalcohlolic extract of Glinus at dose levels of 250 mg/kg, 500 mg/kg each, and a standard drug glibenclamide (20 mg/kg body weight) significantly (p<0.001, p<0.05, p<0.01) improved body weight from the second to fourth week.

Group Before Induction of STZ After Induction of STZ Body Weight on diabetes animals with standard and extract

Initial Body weight Body weight on 1st week Body weight on 2nd week Body weight on 3rd week Body weight on 4th week

Control

160±0.65*

166±.93

168±0.799

175.8±0.85

178±1.648

ONLY STZ

162±1.12

162±1.63

90.8±32.1 111.5±39.47

105±37.4

STZ+STD

160±.93

164±1.41

143±1.7 164.8±1.702

169±1.25

STZ +

MCR EXT 250mg/kg

163±0.32

161±0.98

112±5.28 122±14.17

125.9±41.4

STZ + MCREXT 500mg/kg
161±2.02*

155±1.48
153±15.3 161±12.049

163±11.9

STZ +
GLL EXT 250mg/kg
160±1.42

158±1.08

130±35.9
142±39.17

144±41.4

STZ +
GLL EXT 500mg/kg
163±2.33*

156±2.74

150±2.9
2
.159±5.339

124±41.3

Table.1: Body weight analysis of STZ induced rats after treating with plant extract

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