RESEARCH PAPER

Assessment of antioxidants and metabolic enzyme activities of Desmidorchis indica stem extract in high fructose induced rats

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ABSTRACT

Objectives: The purpose of this study is to assess Desmidorchis indica stem extract in vivo antioxidant and metabolic enzymes activities in Wistar rats that have been made fat by a high fructose diet.

Methods: Group I: Normal rats fed with the control diet. Group II: High fructose diet-fed animals received a fructose enriched diet for a period of 8 weeks. Group III: High fructose diet fed animals co-administrated with hydro-ethanolic extract of Desmidorchis indica by oral gavage daily at a dose of 200 mg/kg body weight (Based on effective dosage fixation studies) for 8 weeks. Group IV: High fructose diet fed animals treated with standard drug Orlistat at a dose of 10 mg/kg body weight for 8 weeks. On completion of the experimental period, animals were anaesthetized with thiopentone sodium (50mg/kg). The blood was collected with and without EDTA as anticoagulant. Plasma and serum were separated for the estimation of various biochemical parameters.

Results: Over the course of the trial, the activities of antioxidants and metabolic enzymes investigated. When compared to rats fed a high-fructose diet, it was shown that the Desmidorchis indica hydro-ethanolic extract restored the plasma MDA and antioxidant enzymes, and the activities of carbohydrate and lipid metabolic enzymes.

Conclusion: These results demonstrate the antioxidant properties and metabolic regulatory enzymes activity by Desmidorchis indica extract, which warrant more research in clinical trials to support their therapeutic and pharmaceutical uses.

Keywords: Desmidorchis indica, anti-obesity activity, antioxidant activity, Metabolic enzymes, Oxidative stress

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INTRODUCTION

Obesity is a multifactorial condition characterized by an abnormal or excessive accumulation of body fat, commonly identified by a body mass index (BMI) of 30 or above. It develops when energy intake consistently surpasses energy expenditure, leading to fat storage in the body. This imbalance is often influenced by factors such as calorie-dense diets, reduced physical activity, and various environmental and lifestyle contributors. Importantly, obesity is associated with increased risk of several serious health conditions, making it a major public health concern (Alberti et al., 2005; Eckel et al., 2005). The obesity and dyslipidemia metabolic disorders such as hypertension, impaired glucose tolerance, being this last disorder a key factor in the etiology of the syndrome. MetS increases the incidence of cardiovascular diseases, type 2 diabetes (T2D) and nonalcoholic fatty liver disease (Misra et al., 2008; Ready et al., 2012). The widespread of MetS in actual society, mainly in developed countries, is becoming an important health problem and the need to develop new treatments against this pathology is increasing fast. To develop successful strategies to treat MetS, studies using animal models that adequately mimic all the aspects of human disease, developing all major alterations of the illness are needed. Regarding to this, as central obesity is a key factor in the development of MetS, Diet Induced Obesity (DIO) rodent models are frequently used to get better knowledge about the pathways implied in the development of MetS (Ready et al., 2012). Currently, diets high in both carbohydrates and fat, are the most used models to resemble the nowadays human diet, also called “Western Diet” (Misra et al., 2008).

Actual society has experimented important changes in global food distribution and bioavailability. Changes in physical

activities patterns together with the arrival of food processing centers producing energy-dense foods ready-to-eat, as well as the global presence of fast food establishments have carried to a westernized lifestyle in which sedentary and high fat-high carbohydrate dietary habits are alarmingly common (Gupta et al., 2018; Ramachandran and Snehalatha, 2003. Western diet is characterized by high caloric, energy-dense, take-away foods and sweetened beverages consumption. Carbohydrates present in this kind of diets are mainly sugars, being high-fructose corn syrup (HFCS) and sucrose the most commonly used sweeteners (Prasad et al., 2012). The increased use of HFCS and sucrose, together with the rising incidence of MetS in our society during last years, lead scientific community to postulate that high fructose consumption is related to the development of the pathology. Fructose, commonly added as HFCS or sucrose in processed food, is believed to promote less satiety than other sugars, thus increasing caloric intake, mainly through sweetened beverages (Grundy et al., 2004); its different metabolism pathways compared with glucose one, suggests that fructose is the main cause in the development of MetS, and it is been traditionally used to develop DIO rodent models for MetS research (Expert Panel, 2009).

However, it is important to note that sucrose, which contributes to more than 90% of caloric sweeteners globally, is a disaccharide containing equal amounts of fructose and glucose, while HFCS (main sweetener used) differs little in its fructose and glucose contents (42–55% fructose: 45–58% glucose), and acts metabolically identical to sucrose(Prasad et al., 2012). Therefore, fructose and glucose intakes are similar in United States dietary patterns. In addition, due to HFCS (also called Isoglucose in Europe) production quota, HFCS is not as available in Europe as it is in United States, being more common the use of sucrose or products derived from glucose syrups, such as dextrose, a simple sugar made from corn, which is chemically identical to glucose and highly used in processed foods due to its versatility and its multiple food applications (Mohan et al., 2006). Even so, the prevalence of MetS in European society is increasing fast, as well as in United States. However, few animal models to investigate the influence of glucose or its derived products in MetS development have been carried out. In order to investigate the antioxidant and metabolic enzymes activities of the hydro-ethanolic extract of the stem of *Desmidorchis indica*.

MATERIALS AND METHODS

Animals

In this investigation, male Wistar strain albino rats weighing between 170 and 190 grams were employed. They came from Sri Venkateswara Enterprises in Bangalore, India, and were healthy animals. The animals were kept in roomy polypropylene cages with rice husk bedding. Throughout the experiment, the animal room was kept in regular circumstances with a 12-hour light/dark cycle and a temperature of 27±2ºC. It was also adequately ventilated. Every animal was given a regular pellet meal and unlimited access to water. Before being used in the experiment, they were given a week to become used to the surroundings. The experiment was carried out according to the guidelines of the Committee for the Purpose of Control **(SU/CLATR/IAEC/XXIII/37/2024)** Supervision of Experiments on Animals (CPCSEA), New Delhi, India.

Chemicals

Casein, sucrose, 1-chloro-2,4-dintirobenzene (CDNB), 5,5'-dithio-bis (2-nitrobenzoic acid), ethylenediaminetetra acetic acid (EDTA), nitroblue tetrazolium (NBT), trichloro acetic acid (TCA), thiobarbituric acid (TBA), reduced and oxidized glutathione, nicotinamide adenine dinucleotide phosphate (NADP+/NADPH), and L-ascorbic acid were all acquired from Sigma Chemical Company. The remaining substances utilized in the study were all analytical grade and came from Sisco Research Laboratories and Glaxo Laboratories in Mumbai, India.

Preparation of control and high fructose diet

The control and high fructose diet were prepared by the method of Nandhini et al*.,* (2002).

Standard Orlistat preparation

Orlistat was bought from a medical store. It was freshly made by dissolving it in animal ghee to the necessary concentration (10 mg/kg body weight) (Saba Khaldoon Mohammed and Shihab Hattab Mutlag, 2023).

Experimental design

Body weight of the animals was recorded and they were divided into 4 groups of 6 animals each as follows.

Group I: Normal rats fed with the control diet

Group II: High fructose diet-fed animals received a fructose enriched diet for a period of 8 weeks (Yuan *et al*., 2020).

Group III: High fructose diet-fed animals co-administrated with hydro-ethanolic extract of *Desmidorchis indica* by oral gavage daily at a dose of 200 mg/kg body weight (Based on effective dosage fixation studies) for 8 weeks.

Group IV: High fructose diet fed animals treated with standard drug Orlistat at a dose of 10 mg/kg body weight for 8 weeks.

Collection of samples

On completion of the experimental period, animals were anaesthetized with thiopentone sodium (50mg/kg). The blood was collected with and without EDTA as anticoagulant. Plasma and serum were separated for the estimation of various biochemical parameters. Adipose tissue dissected out and used for histological study.

Antioxidant enzymes

Malondialdehyde was estimated by the thiobarbituric acid assay method of Beuge and Aust (1978). Reduced glutathione was estimated by method of Moron *et al.,* (1979). Superoxide dismutase activity was determined by the procedure of Kakkar *et al.,* (1984). Catalase was assayed by the method of Beers and Sizer (1952). Glutathione peroxidase was assayed by the method of Rotruck *et al* (1973). Ascorbic acid levels were estimated by the method developed by Omaye *et al.,* (1979). α-tocopherol was estimated by the method of Baker *et al.,* (1980).

Estimation of glucose metabolizing enzymes

Glycogen content was estimated by the method of Morales *et al.* (1973). Hexokinase activity was assayed by the method of Brandstrup *et al* (1957). Fructose-1, 6-diphosphatase was determined by the method of Gancedo and Gancedo (1971). Phospho gluco isomerase was determined by the method of Horrocks *et al.,* (1963). Glucose-6-phosphatase was determined according to the method of Harper (1965). Glucose-6-phosphate dehydrogenase was determined by the method of Beutler (1983). Pyruvate content was estimated by the method of Friedman and Hangen, (1942).

Lipid metabolizing enzymes

HMG CoA reductase was determined by the method of Rao and Ramakrishnan (1975) by determining the ratio of HMG CoA: Mevalonic acid. Total lipase was determined by the method of Beir (1955). Lecithin cholesterol acyl transferase (LCAT) in Plasma was assayed by the method of Hitz *et al.* (1983). Lipoprotein lipase (LPL) activity was assayed by the method of Korn (1955).

Statistical Analysis

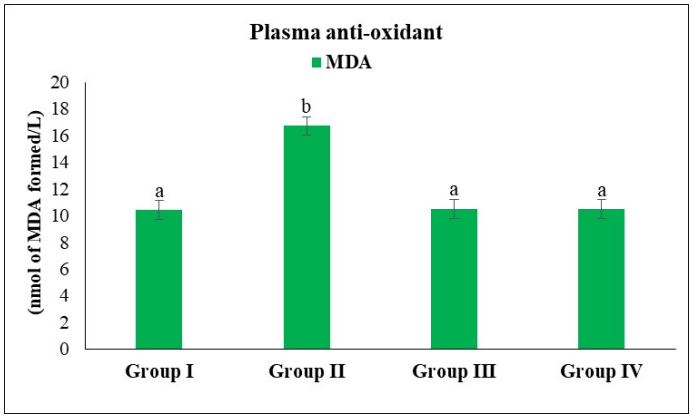
The results were analyzed by SPSS Software ver. 20. Values are expressed as Mean ± SD for six rats. Mean values within the row followed by different letters (Superscript) are statistically significant ($P<0.05$) from each other, and the same letter is non-significant ($P>0.05$). The analysis was an ANOVA followed by post-hoc Duncan's multiple range test (DMRT).

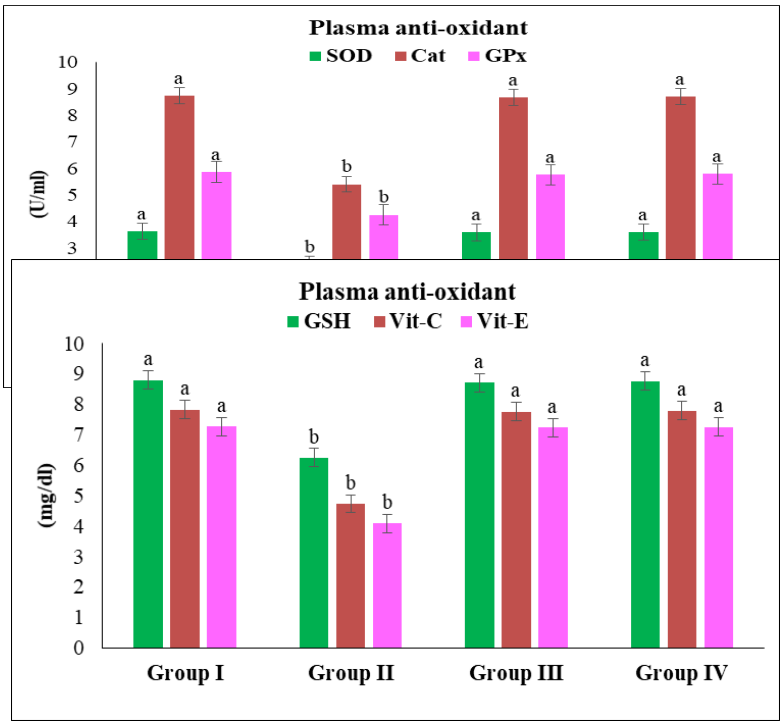
RESULTS

This study assesses the antioxidant and metabolic enzymes activities of the hydro-ethanolic extract of *Desmidorchis indica* in high fructose diet induced rats. Orlistat has been approved by the FDA as an anti-obesity agent used to compare the results.

Effect of *Desmidorchis indica* hydro-ethanolic extract on plasma antioxidant defence in experimental rats

The concentration of plasma MDA was significantly higher in HFD fed rats, as compared to control animals. MDA levels in plasma were found to be decreased in *Desmidorchis indica* extract treated rats. Conversely, plasma GSH, vitamin-C and vitamin-E content in HFD fed animals showed a significant decline when compared with control. Antioxidant enzymes activities are presented in Figure 1. The SOD, CAT and GPx activities were significantly declined in HFD fed rats when compared with normal control. In *Desmidorchis indica* extract treated rats the activities of these enzymes restored. In Orlistat treated animals also significant restoration of antioxidants were observed.



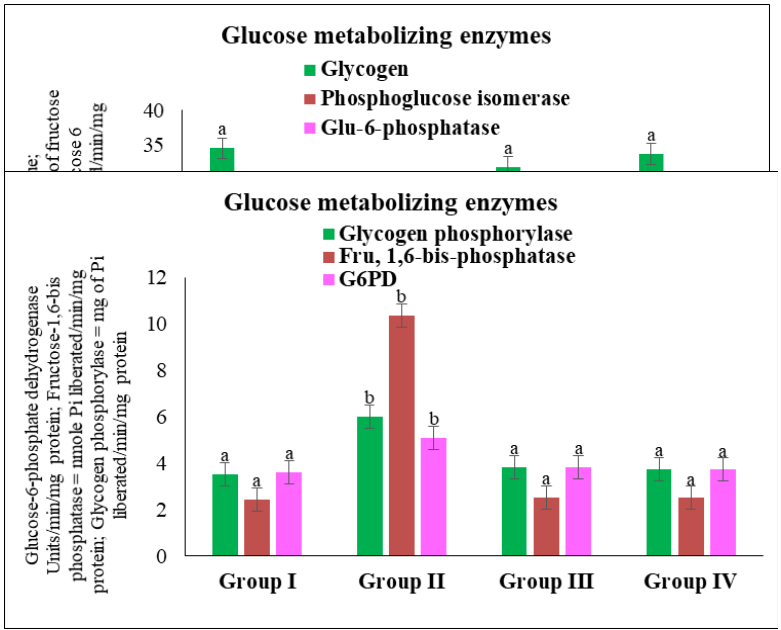


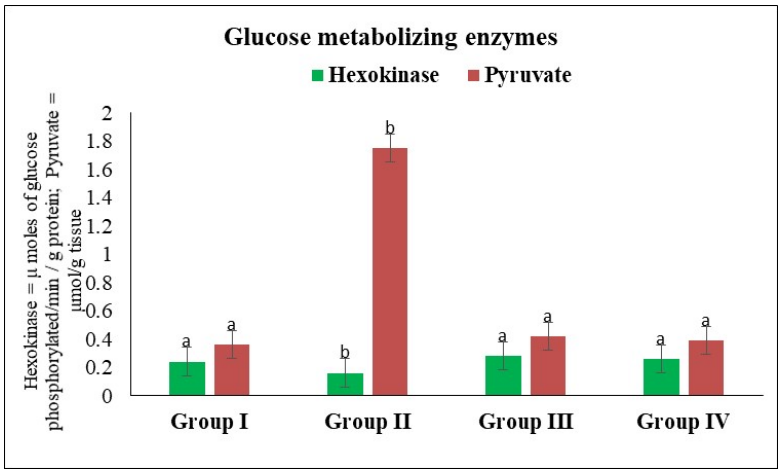
The values are Mean ± SD for six rats. Using SPSS version 20, the data were analyzed using a one-way ANOVA and a post-hoc DMRT test. Within the row, mean values are followed by various letters. At the significant level alpha 0.05, homogeneous subgroups denoted by superscripts are statistically significant (P<0.05), whereas the same letter indicates statistical non-significant (P>0.05) differences between the groups. Group II was significant in comparison to Group I, Group III, and Group IV, but Group I, Group III, and Group IV were not significant.

Figure 1: Effect of *Desmidorchis indica* extract on plasma antioxidants in experimental rats

Effect of *Desmidorchis indica* hydro-ethanolic extract on glucose metabolizing enzyme activities in experimental rats

The activities of hexokinase and the glycogen content were significantly decreased while that of Phosphoglucoisomerase, glucose 6-phosphatase, Fru-1,6-bis-phosphatase and glycogen phosphorylase activities were increased in fructose-fed rats as compared to control rats (Figure 5). *Desmidorchis indica* treatment ameliorated glycogen alterations and the enzyme activities were near normal in *Desmidorchis indica* supplemented HFD fed rats. The effect of *Desmidorchis indica* on the circulating levels of gluconeogenic substrates such as pyruvate in control and experimental animals were investigated. Fructose-fed rats showed significant increase in the concentrations of pyruvate as compared to the control rats. Administration of *Desmidorchis indica* to high fructose fed rats significantly reduced the levels as compared to the untreated high fructose fed rats.





The values are given as Mean ± SD for six rats. Using SPSS version 20, the data were analyzed using a one-way ANOVA and a post-hoc DMRT test. Within the row, mean values are followed by various letters. At the significant level alpha 0.05, homogeneous subgroups denoted by superscripts are statistically significant ($P<0.05$), whereas the same letter indicates statistical non-significant ($P>0.05$) differences between the groups. Group II was significant in comparison to Group I, Group III, and Group IV, but Group I, Group III, and Group IV were not significant.

Figure 2: Effect of *Desmidorchis indica* on glucose metabolism in experimental rats

DISCUSSION

In the current investigation, albino rats were made obese using a high fructose diet. Rats fed HFD acquired a notably greater amount of weight than rats fed the control diet. Using various high fructose diet formulae, several researchers were able to make rats obese (Kim et al., 2005; Milagro et al., 2006), and body weights fell when *Desmidorchis indica* was added. These findings indicated that rats given a high-fat diet were obese, as shown by the antioxidant and metabolic enzymes analysis of the rats. Our findings are consistent with those reported in the previous study conducted on obesity-induced rat models by Hemmat *et al*. (2011). In their study, obesity was associated with significant alterations in metabolic and inflammatory parameters, including increased oxidative stress, elevated lipid profiles, and impaired glucose metabolism. Similarly, our results demonstrate comparable trends, suggesting that obesity plays a critical role in disrupting normal physiological homeostasis.

Furthermore, Hemmat *et al*. (2011) observed structural and functional changes in various tissues, which were attributed to chronic inflammation and metabolic dysregulation. These observations align with our findings, reinforcing the hypothesis that obesity-induced biochemical and cellular alterations contribute to disease progression. The agreement between our study and previous literature strengthens the validity of our results and highlights the reproducibility of these pathological mechanisms across different experimental settings.

Oxidative stress plays an important role in the development of co-morbidities in obesity. Over the last few years, evidence of obesity-induced oxidative stress in humans has been reported in the literature (Vincent and Taylor *et al*., 2006). Obesity induces significant chronic, low-grade inflammation and increased oxidative stress, which typically results in depleted antioxidant defenses and elevated reactive oxygen species (ROS). Serum levels of antioxidants like Vitamin C, E, and enzymes (SOD, GPx) are often lower in obese individuals (Anaya-Morua et al., 2023). The enzymes catalase, superoxide dismutase, glutathione peroxidase, and

glutathione-S-transferase are among the natural antioxidant defense systems found in plasma. The detoxification of large volumes of H_2O_2 is carried out by CAT. Superoxide radicals (O2•-), which would otherwise harm cellular structures and membranes, are eliminated by SOD. In reality, GST is made up of a collection of isoenzymes that may conjugate with glutathione to detoxify a variety of endogenous and exogenous chemicals. These enzymes' decreased activity is linked to the buildup of very reactive free radicals, which can have harmful consequences such compromising the integrity and functionality of cell membranes. (Velavan, 2011)

Rats given a high-fat diet showed decreased plasma activity of the enzymes CAT, SOD, and GPx (Bhandari et al., 2013). According to Noeman et al. (2011), the decreased activity of these enzymes may be the result of oxidative protein inactivation or feed-back inhibition brought on by an excess of ROS production. In the current study, rats that were fed a high-fat diet (HFD) showed noticeably lower levels of these enzymes than rats who were not on the diet. Activities of the antioxidant enzymes, Cu-Zn superoxide dismutase (SOD) and glutathione peroxidase (GPx), were found to be lower in the erythrocytes of obese subjects compared to those of non-obese controls (Olusi et al., 2002).

Lipids and carbohydrates are two of the most important biomolecules in our body, both of which play vital roles in various physiological processes. While lipids are essential for energy storage, cell membrane structure, and signaling pathways, carbohydrates are the primary source of energy for our body, as well as providing structural support and serving as signaling molecules. The relationship between these two biomolecules is complex and important for maintaining a healthy metabolism. In this article, we will discuss the role of lipid and carbohydrate metabolism in the body, as well as their interactions and effects on health (Jacob, 2023)

The metabolic fate of dietary lipid and carbohydrate is governed by the body's need to (i) synthesize essential cellular components, (ii) provide energy to fuel essential processes and (iii) store carbon (primarily in the form of lipid) to meet future needs particularly during times of reduced nutrient intake or vigorous exercise. Directing metabolites away from storage and into other metabolic pathways therefore lies at the heart of triacylglycerol (TAG) accumulation and weight management. The contribution that dietary fat and de novo synthesized fats make towards stored lipid is dependent upon a number of factors including energy requirements, the caloric content and composition of the diet as well as the type of ingested fat and carbohydrate. Under typical conditions of Western style diets the stored lipid will mainly be representative of the dietary TAGs but when excess energy intake (i.e., a positive energy balance) is in the form of carbohydrate, more of that stored lipid will be derived from de novo lipogenesis (Jacob, 2023). Obesity induces significant metabolic dysfunction, characterized by insulin resistance, reduced lipid oxidation, and impaired glucose metabolism, leading to excessive white adipose tissue (WAT) expansion. Over nutrition causes fat to store as triglycerides (TAG) in adipose tissue, while high carbohydrate intake increases hepatic glucose production and promotes de novo lipogenesis (Roger Jeffcoat, 2007).

Ultimately, it was concluded that supplementing with *Desmidorchis indica* could assist rats given a high-fat diet overcome their dysregulation of lipid and glucose metabolism. The regulating action of *Desmidorchis indica* in this paradigm may be explained by a decrease in hepatic gluconeogenesis, an increase in oxidative glucose consumption, and the restoration of lipid-metabolizing enzymes by the hepatocytes. Additional *Desmidorchis indica's* hepatic metabolism-regulating actions suggest anti-obesity efficacy.

In comparison to the control group, which displayed normal adipocyte distribution and cells of regular sizes, histopathological analyses of group II epididymal white adipose tissue (WAT) at the end of the study revealed a large number of adipocytes tightly packed, clumped together, and an increase in adipocyte cell size (high fat accumulation) in the obese rats. However, *Desmidorchis indica* supplemented diet indicated histology similar to the control, suggesting inhibition of the hyperplasic growth of the adipocytes. In rats, adipose tissue develops in two stages: the first is stem cell differentiation, and the second is the increasing filling of the differentiated tiny cells with triacylglycerol (MacKellar et al., 2010). Animals fed with *Desmidorchis indica* showed reduced fat accumulation. Likewise, the standard-treated group was also noted. Adipose tissue histology supported the shifts in total body weight. This suggested that *Desmidorchis indica* supplemented food inhibited adipocyte formation in a manner similar to that described by Atangwho et al.

(2012), who found that obese rats given *Vernonia amygdalina* showed a reduction in adipose size and fat storage.

CONCLUSION

Overall, the current study suggests that *Desmidorchis indica* extract may have antioxidant properties, which may help control the enzymes involved in the metabolism of fats and carbohydrates. *Desmidorchis indica* may therefore be a useful dietary supplement for the treatment and prevention of obesity. The ingestion of *Desmidorchis indica* has scientific legitimacy because this study is the first to offer clear proof for its relevance and anti-obesity capabilities. To have a better understanding, more research is necessary to elucidate the molecular mechanism of the many intracellular signaling pathways. For the best outcome, clinical research and study on *Desmidorchis indica* will result in pharmacological treatments for obesity that are safer and more efficient.

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