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Effect of Oral Cinnamon on Histological Damage Caused by Alloxan-Induced Diabetes Mellitus

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ABSTRACT

Cinnamon has been widely recognized as hypoglycaemic agent against diabetes mellitus. This study aimed to investigate the potential histological repairing ability of damaged pancreatic tissue due to alloxan-induced diabetic rats. Diabetes was induced in 24 male Albino rats using alloxan (120mg/kg intraperitoneal). Four groups (n=6 each) received or not suspensions of cinnamon (50mg/ kg and 100mg/kg OD orally). Body weights, fasting blood glucose, and serum insulin levels were measured. All biochemical results were compared with cinnamon effects on pancreatic histological changes. Diabetes decreased serum insulin due to damaged Langerhans islet cells, however, treatment of diabetic rats with cinnamon up to 30 days, significantly increased serum insulin and reduced blood glucose level. Moreover, cinnamon-treated rats with a low dose showed that the shape of pancreatic islets cells relatively irregular with some normal cells. Meanwhile the cinnamon-treated with a high dose showed considerable repairing effects and islets cells looks like normal. The biochemical and histological findings suggested that cinnamon extract has therapeutic and protective ability against alloxan-induced diabetic rats. The hypoglycaemic effect observed could be due to high repairing ability on pancreatic tissues leading to increased insulin levels. Hence, cinnamon may be useful in the treatment of diabetics.

Keywords: Cinnamon, diabetes mellitus, alloxan, histology

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INTRODUCTION

Diabetes mellitus is a chronic metabolic disease characterized by hyperglycaemia due to insulin deficiency, or insulin resistance, or both. Hyperglycaemia occurs when cells become unable to utilize glucose and/or the liver and skeletal muscles cannot store glycogen [1].

The prevalent treatment of diabetes mellitus besides controlling food intake; treating obesity; proper exercise and changing life style includes administration of oral hypoglycaemic drugs and subcutaneous injection of insulin [2]. Despite the presence of known anti diabetic medicine in the pharmaceutical market, diabetes and the related complications continued to be a major medical problem [3].

There are some medicinal plants have been declared in diabetes treatment worldwide and have been used experimentally as antidiabetic remedies. Antihyperglycemic effects of these plants are attributed to their ability to restore the function of pancreatic tissues by causing an increase in insulin output or inhibit the intestinal absorption of glucose or to the facilitation of metabolites in insulin dependent processes [3-4]. Among these plants *Zizyphus spina christi* [5]; Fenugreek [6]; *Urtica dioica* [7]; *Balanites aegyptiaca* [8]; *Rhazya stricta* [9]; *Viscum album* [10] and *Urtica dioica* [11].

Alloxan (2,4,5,6-pyrimidinetetrone) is an oxygenated pyrimidine derivative. It is a toxic glucose analogue, which selectively destroys insulin-producing cells (ß cells) in the pancreas when administered to rodents including rats. This causes an insulin-dependent diabetes mellitus (called "alloxan diabetes") in these animals, with characteristics similar to type 1 diabetes in humans. Alloxan is selectively toxic to insulin-producing pancreatic ß cells because it preferentially accumulates in ß cells through uptake via the GLUT2 glucose transporter. Alloxan, in the presence of intracellular thiols, generates reactive oxygen species (ROS) in a cyclic reaction with its reduction product, dialuric acid. The beta cell toxic action of alloxan is initiated by free radicals formed in this redox reaction [12-15].

Cinnamon is a common spice used by different cultures around the world for several centuries. It is obtained from the inner bark of trees from the genus Cinnamomum, a tropical evergreen plant that has two main varieties; *Cinnamomum zeylanicum* (CZ) and Cinnamon cassia (CC) (also known as *Cinnamomum aromaticum*/ Chinese cinnamon). In addition to its culinary uses, in native Ayurvedic medicine Cinnamon is considered a remedy for respiratory, digestive and gynaecological ailments [16].

In-vitro and in-vivo studies in animals and humans from different parts of the world have demonstrated numerous beneficial health effects of CZ, such as anti-inflammatory properties, anti-microbial activity, reducing cardiovascular disease, boosting cognitive function and reducing risk of colonic cancer [16-17].

A meta-analysis by Ranasinghe, et al., 2012 [18] and a systematic review by Bandara et al., 2012 [19], on the effects of CZ extracts on diabetes demonstrates numerous beneficial effects both in-vitro and in-vivo. In-vitro CZ has demonstrated a potential for reducing post-prandial intestinal glucose absorption and stimulating glucose metabolism and glycogen synthesis, in addition to stimulate insulin release and potentiating insulin receptor activity [16, 18-19].

Other studies showed that aqueous extracts from cinnamon have been shown to increase *in vitro* glucose uptake and glycogen synthesis as well as to increase phosphorylation of the insulin receptor. Also cinnamon extracts are likely to aid in triggering the insulin cascade system. These data suggest that cinnamon prevents the development of insulin resistance and aqueous extract of cinnamon has also been shown to improve insulin sensitivity in humans [20-21] The present study was carried out to investigate the potential histological repairing ability of cinnamon on pancreatic tissue of alloxan- induced diabetic rats.

MATERIALS & METHODS

Animals

Twenty four male Albino rats were used in the study with an average weight of 150 – 250g and an average age of 12-16 weeks, obtained from Animal House Lab, University of Al-Mustansyria, Baghdad, Iraq.

The animals were fed with rodent pellet diet and tap water *ad-libitum* under strict hygienic conditions. Ethical clearance for performing the experiments on animals was obtained from relevant committee in the institute that conforms to the guide for the care and use of laboratory animals [22], and all efforts were made to minimize animal suffering. The rats were acclimatized for a period of 21 days. Standard environmental conditions such as temperature (20-22 ^{0}C), relative humidity (45-55%) and 12 hrs dark/light cycles were maintained in the quarantine.

Chemicals

Alloxan was purchased from Al-Hayat Medical Company (Sigma), in the form of white powder packed in tightly closed bottles each containing 25gm alloxan monohydrate. Cinnamon (C. zeylanicum, Family Lauraceae) dried bark were obtained from local market of Herbs and Medicinal plants. The method of plant extraction was described by Suhad A. Ahmaed et al., [23]. In this method, the dried powder of cinnamon (500 gm) was soaked in 2 liter of distilled water and gently heated for 30 minutes (about 45^{o}C) until extracted out. Then the solution was cleared with filter paper concentrated at 50 ^{o}C in oven. Extract was then prepared to 50mg/ml and 100mg/ml using distilled water and made ready for oral administration. To prevent contamination, the extracts were kept in the refrigerator at 4^{o}C [23].

Design of the Experiment

The experiment was conducted on 24 male Albino rats randomly distributed into 4 groups, of 6 rats each. Group 1 (G1; control group); rats were injected with vehicle (citrate buffer, 120 mg/ Kg body weight); Group 2 (G2; untreated alloxan-diabetic rats). Diabetes was induced by intraperitoneal injection of alloxan (120 mg/kg/day) for 5 days [12, 24]. The development of diabetes was confirmed by the presence of hyperglycemia with blood glucose above 13.9 mmol/L (250 mg/dL), which last for at least three days; Group 3 (G3; alloxan-diabetic rats treated with 50 mg⁄ kg cinnamon) ; Group 4 (G4; alloxan-diabetic rats treated with100 mg⁄kg cinnamon).

The treatment by cinnamon was started for a period of 30 days. During this period, control and alloxan-treated animals had free access to standard diet and water until 6pm. None of the rats was treated with insulin at any time during the experiment. Blood glucose levels were tested every morning (at 8 am). Blood was collected from the tail of fasting (14 h) animals. A drop of blood was used for the blood glucose test with the help of a One Touch Glucometer GX.

On the last day (30th day) and after completion of the experimental protocols, blood samples were collected from overnight fasting rats by sacrificing each control and diabetic rats. The animals were anesthetized in a chamber containing diethyl ether. Cardiac puncture was made using a heparin syringe and blood was collected into a heparin containing container. Immediately after collection, 2.0 ml of blood was transferred into fresh tube and centrifuged at 3000 rpm for 10 minutes. The serum was collected and stored at – 80°C until serological analysis. Serum was assayed for serum insulin using enzyme-linked immunosorbent assay (ELISA) using a commercially available kits (Sanofi, France). Also pancreatic tissues were collected for histological examination. Pancreatic tissues were harvested from the animals and they were fixed in 10% neutral formalin solution, embedded in paraffin, and then stained with haematoxylin and eosin (H&E). The preparations were evaluated by means of a bright-field microscope, and photographed (Nikon, Japan).

Statistical analysis

Statistical analysis was performed using the SPSS for Windows statistical package, version 22 (SPSS Inc. Chicago, IL, USA). Data were expressed as means ± S.E.M. The effects of drug treatments were evaluated statistically using the one-way analysis of variance (one-way ANOVA) followed by the Dunnett post-hoc test to correct for multiple comparison treatments. Statistical significance was set at the $p < 0.05$ level.

RESULTS

The fasting blood glucose (FBG) of the control group (G1) was significantly changed during the 30 days of the experiment (figure 1). Body weight (BW) of the G1 continued to rise during the time of experiment (figure 2), however, induction of diabetes with alloxan was associated with the characteristic development of a

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slower rate of body weight gain, and higher FBS levels than in the control rats. Compared with that in the cinnamon-treated diabetic groups (low dose and high dose; G1 and G2 respectively). The rate of body weight gain were significantly affected by treatment with cinnamon. The FBS of diabetic rats was also decreased by treatment with both doses of cinnamon (figures 1 &2).

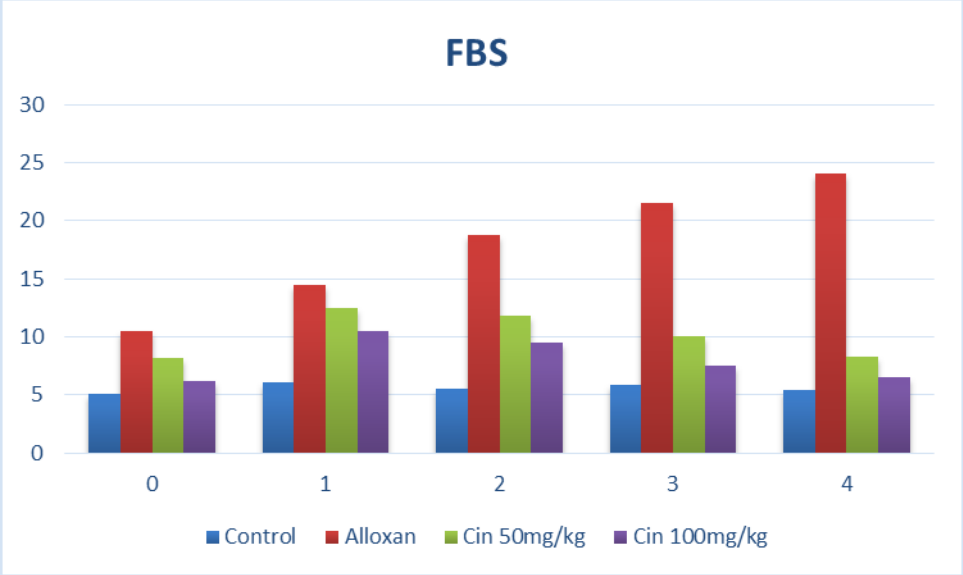

Figure1: Fasting blood sugar for all rat groups.

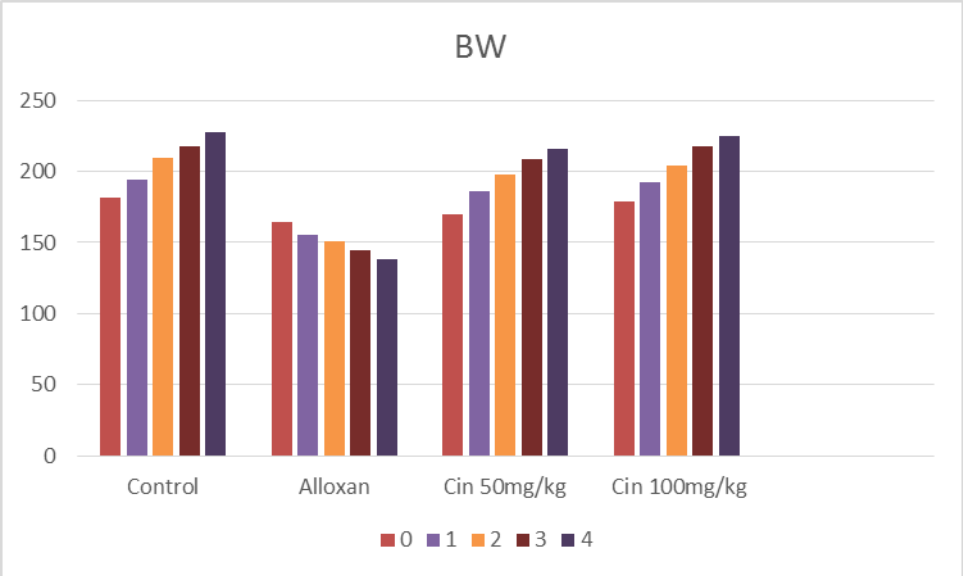

Figure2. Body weight for all rat groups.

Among diabetic rats group without treatment (G2), the serum insulin levels were significantly decreased when compared with control group (G1), meanwhile, treating rats with cinnamon specially in high dose (100mg/kg): levels of the insulin were significantly higher compared to those of the control groups at the end of the experiment (p=0.001). (Figure 3)

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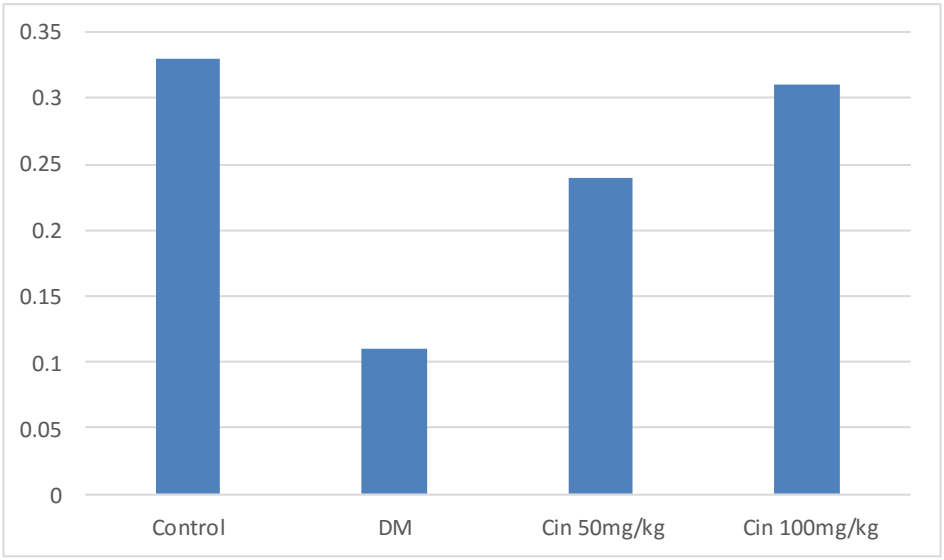
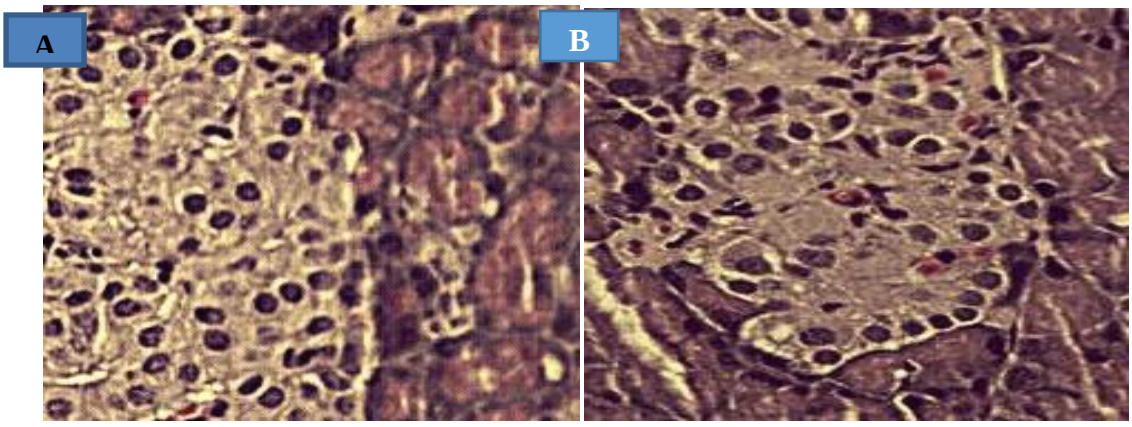


Figure 3. Effect of treatment of diabetic rats with cinnamon in low and high doses on serum insulin levels.

Histological findings

Pancreatic sections stained with haematoxylin and eosin (H &E) obtained from normal control rats (G1) showed normal histology (Figure 4A). Meanwhile, pancreatic tissue obtained from alloxan-induced diabetic rats without treatment by cinnamon (G2) showed severe degenerative changes of the pancreatic islets, particularly the cells in the centre of the islets, relative reduction in the size and number of the islets (Figure 4B).

Regarding microscopic examination of the cinnamon-treated rats with low dose (G3) showed that the shape of pancreatic islets cells, relatively irregular, with some normal cells and some others still appeared degenerated. (Figure 4C) Meanwhile, the last group (G4), which was treated by high dose of cinnamon, showed considerable repairing effects of cinnamon on the diabetic histological changes of the pancreas. There were noted for regeneration and more improved in islets cells and look like normal (Figure 4D).



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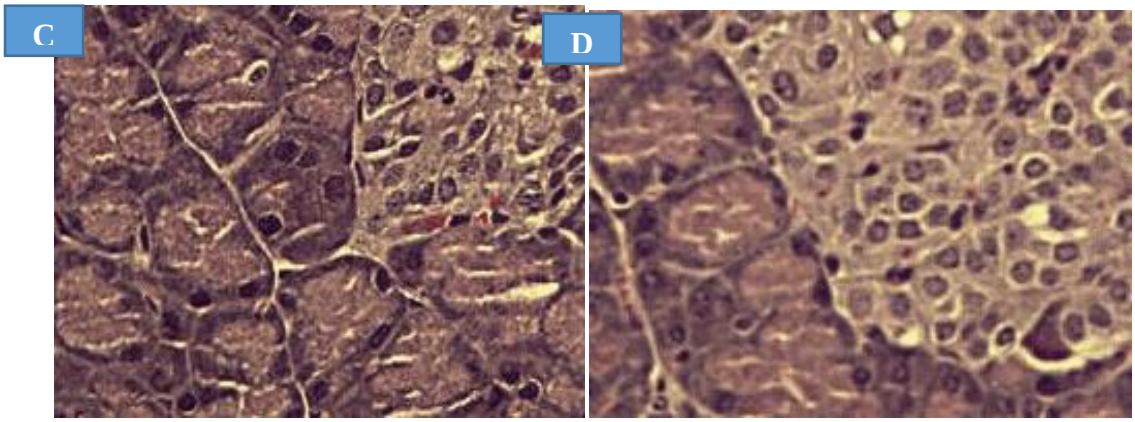

Figure 4. Microphotographs of pancreatic tissue. HE 420. (A) G1, normal control group (B) G2, alloxan-induced diabetic rats with no treatment; (C) G3, cinnamon-treated group (50 mg/kg); (D) G4, cinnamon-treated group 100 mg/kg.

DISCUSSION

Diabetes is a chronic metabolic disorder that affects approximately 3% of population worldwide [25]. It was reported that sustained reductions in hyperglycemia will decrease the risk of developing microvascular diseases and reduce diabetes complications. Usage of oral hypoglycemic drugs to treat diabetes has several limitations, such as adverse effects and high rates of secondary failure [26]. Those adverse effects forced the diabetic patients to use herbal medication that has a similar degree of efficiency without side effects [25-28]. This fact motivated us to carry out this study.

We have shown in this study that: 1) daily oral administration of cinnamon (either low dose of 50 mg/kg or high dose of 100 mg/kg) for up to 30 days to alloxan-induced diabetic rats effectively reduces the elevated levels of hyperglycaemia that is increased to due to the effect of alloxan, 2) reduced level of serum insulin due to damaged pancreatic Langerhans islet cell was significantly increased in the serum due to histological repairing tissue process due to cinnamon administration after it was destructed by the effect of alloxan.

The biochemical evidences about the effect of cinnamon indicates that cinnamon affects body weight, blood glucose and insulin level. The hyperglycaemia produced due to alloxan administration remained consistent in the G2. This is because of β-cell destruction (Fig 4B) which was most probably induced by oxidative stress of alloxan that was sufficient to decrease the levels of serum insulin. This is consistent with many other studies [4, 19-20, 23].

Current study showed that after orally administration of cinnamon, insulin levels were increased (G3 and G4) as compared to initial diabetic state (G2) which was statistical significant throughout the study period indicating its action like insulin secretagogue agent. These results was similar to other study which has demonstrated that cinnamon extract has insulin-like effect causing inhibition of hepatic glucose production and decreased the gene expression of enzymes involved in hepatic gluconeogenesis [29]. However, the objectives of our study didn't include the study of cinnamon components hence current study cannot rule out the possibility that effect seen with cinnamon groups (G3 and G4) was due to active agent that has dual action like insulin secretion enhancer and insulino-mimetic action.

These experimental findings of current study are similar to those reported by many clinical trials which were conducted on humans like the study of Khan *et al.* (2003) [30] who concluded that intake of oral cinnamon reduces the fasting serum glucose in people with type 2 diabetes. Stoecker et al., 2010, [31] have reported results of a larger (n = 137) cinnamon extract trial that found that after 2 months, FBG decreased ($P < .001$) in the cinnamon-supplemented group (from 8.85 – 0.36 to 8.19 – 0.29 mmol/L) compared with from 8.57 – 0.32 to 8.44 – 0.34 mmol/L in the placebo group ($P = .45$). In addition, Roussel et al., 2009 [32], have reported the results of a 4-month, double-blind, placebo-controlled trial using an aqueous extract of cinnamon in 22 subjects with impaired FBG. They reported a decline in fasting glucose accompanied by increases in plasma antioxidant markers [33].

Alloxan is selectively destroys insulin-producing cells (ß cells) in the pancreas when administered to rats. This results a type of diabetes characteristically similar to type 1 diabetes in humans [12-15]. Hence we examined the effects of cinnamon on cell damage in alloxan-induced rats in concordance with biochemical effects. Alloxan is cytotoxic to ß-cells [12-15]. Destruction of pancreatic ß cells by alloxan is thought to be mediated by the inhibition of free radical scavenger-enzymes, which enhances the production of superoxide radicals. The current study showed histopathological changes after alloxan injection represented by destructed ß-cells and vacuolated pancreatic acini (G2). These results are consistent with other studies [34-37]. Alloxan administration elicited significant morphological changes in diabetic rats with severe injury of pancreatic ß-cells, such as decreasing the islets cell numbers, cell damage, and cell death [fig 4B], this also similar to another study [37]. These effects of alloxan are may be due to that the thickened and hyalinised blood vessels causing not enough oxygen reach the tissue which resulted in degenerative changes and necrosis, these also similar with other studies [34-39].

Because almost all of the insulin-producing ß-cells were degenerated, or necrosed in the alloxan-treated rats (fig. 4B), this led to a decrease in insulin secretion and an increase in blood glucose levels. However, cinnamon treatment protected the majority of the Langerhans islet cells and prevented degeneration of ß-cells especially when the treatment of high dose (100mg/kg) (fig.4D). The diabetic rats after treatment (G3 and G4) showed pancreatic islet regeneration. The regenerative effect of the pancreatic cells by cinnamon via exocrine cells of pancreas may enlighten the positive effects of these agent on the production of insulin.

CONCLUSION

This study investigated the effect of alloxan on normal pancreatic tissue and the potential histological repairing ability of cinnamon on pancreatic tissue of diabetic rats. This effect of cinnamon may be used in clinical trials among humans to treat and prevent diabetes mellitus as the increasing demand by patients to use the natural products with antidiabetic activity, because insulin and oral hypoglycemic drugs possess undesirable side effects, although. We recommend more studies as until now the American Diabetes Association stated that cinnamon produces no benefit for people with diabetes [40].

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