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**Original Article**

# Comparative Assessment of *Portulaca oleracea*, *Omega-3* Fatty Acids, and Combination of Selenium Plus Vitamin E on Histopathology of Pancreas in Diabetic Rats

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**ABSTRACT**

**Introduction:** Antidiabetic effects of *Portulaca oleracea* (PO) plant, omega-3 and combination of Selenium and Vitamin E have been reported which could compensate defective insulin release and enhance antioxidant level. The purpose of the *present study* was *comparative* of serum glucose level and *histopathological effects* on PO, Omega-3 and combination of Selenium and Vitamin E in pancreas of adult male diabetic rats.

**Materials and methods:** 96 adults male *Wistar rats*, weighing approximately 220 g were used. The rats divided into four groups with 4 replicates for each group accidently. The rats were diabetic via single Injection of streptozotocin solution (60 mg/kg, IP injection). The groups contained the control group of diabetic rats, which received the standard ration daily, the second group of diabetic rats were fed standard ration plus *Portulaca oleracea* extract (1.5 mg/kg/day/orally), the third group of diabetic rats were fed standard ration plus omega 3 (500 mg/kg/day/orally), the fourth group of diabetic rats were fed standard ration plus Vitamin E (400 iu/kg/day/orally) and Selenium (0.5 mg/kg/day/orally) for a period of 14 days. *At the end* of the study, the samples *were taken* for histopathological investigation of pancreas and serum glucose levels. The mean *diameter of* pancreatic islets and percentage of beta and alpha cells were calculated in all groups.

**Results:** The percentage of alpha cells in treatment groups were higher than the control group. The percentage of beta cells in the third group was higher than the fourth group and control group. The mean *diameter of* pancreatic islets in *omega-3* treated rats was higher than other groups. The insulin level increased in treated rats in comparison with the rats were not treated significantly.

**Conclusion:** Diabetic male rats treated with omega-3 showed more positive effects on pancreatic islets and blood glucose compared to other treatment groups.

## 1. Introduction

Diabetes mellitus is a pathologic condition which causes extensive and non-physiological metabolic imbalance disorders, including an increase in blood glucose, and changes in carbohydrate, lipid, and protein metabolism in different body tissues, such as nail and hair[1,2]. An increase in blood glucose initiates a series of cascade reactions, which finally leads to an increase in free radicals' production (including oxygen free radicals) in various body tissues[3,4]. The high potency of these compounds for chemical reactions damages cells and tissues. Several

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reports have been published concerning the involvement of Reactive Oxygen Species (ROS) in the tissue damages[5] among which the high level of ROS in pancreatic islets and changes in oxidative stress markers in laboratory animals can be noted[6]. Aerobic cells can be protected against free radical particularly ROS by antioxidants compounds, such as glutathione, Vitamins E and C, as well as super Oxide Dismutase (SOD), glutathione Peroxidase (GPx), and catalase enzymes[7,8]. On the other hand, studies has also showed a significant decline both in non-enzymatic antioxidants ( including rehabilitated glutathione (GSH) and Vitamin E) and enzymatic antioxidants (such as SOD, catalase, and GPx in diabetic rat)[9,10]. It is also shown that the free radicals can cause diabetic damages in different organs like pancreas and liver by declining SOD, catalase, and antioxidants activities[11,10]. Free radicals can also damage the unsaturated fatty acid in cell membranes[12]. The combination of fatty acids in cell membranes can also affect cell membrane-related phenomena such as the interaction between insulin and its receptors[13]. In addition, it has been indicated that fatty acid composition of membrane phospholipids in insulin target tissues like liver and skeletal muscles, which is an important factor affecting both insulin secretion and its biological activity[14]. Red blood cells are also susceptible to oxidative damage, due to the presence of fatty acid in their membrane and high concentration of oxygen and hemoglobin[11]. Hence, it is beneficial to use antioxidants compounds (particularly natural antioxidants) and omega3 fatty acid to prevent the oxidative damage.

Vitamin E plus Selenium is one of the important foods compounds which not only have high antioxidant properties but it can also affect different biological processes of the body. Shamsi et al.[15] has also shown that Vitamin E decreases blood glucose in diabetic rats and reduces diabetic disorders. It was also reported that Vitamin E declines Malondialdehyde (MDA) and increases GSH and SOD in diabetic rats[16].

Vitamin E prevents lipid peroxidation and protect cells against peroxide radicals thus it is the most important antioxidant in biological membrane which can neutralize free radicals[17]. Selenium is the only trace element which enters the genetic code as selenocysteine. This element can be extensively found in selenoproteins, namely GPx enzyme, through which Selenium antioxidant effect is activated[18]. Reports available on the efficacy of Selenium in the diabetes have indicate a decline in the effect of streptozotocin (STZ) by Selenium and its positive effect on GPX enzyme activity in laboratory rats[19,20].

*Portulaca oleracea* is a rich source of omega-3 polyunsaturated fatty acids (alpha-linolenic acid), different vitamins (A, C, and E), and minerals which has different pharmacological (such as antioxidant, anticancer, anti-inflammatory, and antimicrobial) properties[21].

Although bioactive compounds of *Portulaca oleracea* can have beneficial effects against the diabetes[22]. There is a few research addressing the anti-diabetic effects of this plant in previous years[23,24]. Thus, the aim of present study was to compare the effect of consumption of *Portulaca oleracea* extracts with natural antioxidants (vitamin E + selenium) and omega3 fatty acids on the serum levels of glucose, blood insulin, and histopathology of the pancreatic tissues.

## 2. Materials and Methods

### *2.1. Animals*

A total of 96 male Wistar rats aged 2-3 months, with average weight of 220 g have been bought from Razi institute, Iran, and kept in laboratory conditions with free access to water and commercial food daily. Experimental animals have been kept in standard cages with minimum 50 percent humidity, 24°C temperature and 12 hours dark/light cycle with appropriate ventilation in a particular cage. Out-and-out functions on animals, coincided on university morality committee. The rats divided into four groups with 4 replicates for each group accidently. The groups contained the control group of diabetic rats, which received the standard ration daily, the second group of diabetic rats were fed standard ration plus *Portulaca oleracea* extract (1.5 mg/kg/day) via gastric feeding tube daily, the third group of diabetic rats were fed standard ration plus omega 3 (500 mg/kg/day) via gastric feeding tube daily, the fourth group of diabetic rats were fed standard plus Vitamin E (400 iu/kg/day) and Selenium (0.5 mg/kg/day) via gastric feeding tube daily.

### *2.2. Extraction of Portulaca oleracea*

Aforementioned atmospheric parts of *Portulaca oleracea* prepared from the farm of Islamic Azad University, Tabriz, Iran and kept in a dark glass battle at 10°C temperature away from direct sunlight. 250 g of the intended powder was extracted by ethanol-water solvent (70% ethanol-30% water) three times at normal laboratory temperature based on the method of Abdullah and Kusumaningtyas[25]. The extracts were mixed and condensed with reduced pressure so that its volume reached to 500 ml, which was equal to 0.5 g of the powder per milliliter was soluble. For further investigation, the extract was divided into equal volumes (25 ml) and stored at -20°C temperature.

### 2.3. *Diabetes infusion*

The rats were diabetic via IP Injection of STZ solution at dosage of 60 mg/kg which dissolved in buffer citrate 0.1 at pH=4.5. At second day, blood samples were collected through tail vein from animals, under anesthesia with chloroform. Rats with fasting blood sugar higher than 250 mg/dl were considered diabetic and were used in present study.

### *2.4. Data analysis method*

#### *2.4.1. Micrometric perusal methods*

The pancreas tissue incisions were done and at least 10

islets were collected from each group. Then the large parts of the islets were measured by using graded eye lens and calibrated slide. The percentage proportion of the alfa and beta cells in each islet was counted.

#### *2.4.2. Blood sample*

The last day of treatment was done on day 28 and the samples of blood and pancreas tissue were collected under euthanasia with chloroform to determine serum glucose and blood insulin levels. The tissue samples were placed in 10% formalin and immediately referred to pathology laboratory, Islamic Azad University, Karaj, Iran for investigating the pathological changes.

#### *2.4.3. Statistical analysis*

The obtained data from histopathological study was analyzed by using SPSS software (version 19). The results were analyzed in one-way variance analysis (ANOVA) and reported as mean +_standard error. The statistical differences between the treatments and the control groups were checked via Tukey test at the significance level of $p < 0.05$.

### *2.5. Ethical approval*

All procedures were approved by the Animal Care Committee of Veterinary Medicine, Karaj Branch, Islamic Azad University. The principles of laboratory animal care were followed, and specific international laws were observed.

## 3. Results

### *3.1. Blood sugar and insulin levels*

The blood glucose level in control rats were significantly higher than treated rats with *Portulaca oleracea* and Vitamin E + Selenium on day 14 ($p < 0.05$, Table 1). The blood glucose level in treated rats were significantly lower than control group on day 28 ($p < 0.05$, Table 1).

Blood insulin in treated rats increased significantly in comparison with the rats of control group on days 14 and 28 ($p < 0.05$, Table 1).

### *3.2. Weight changes*

After treatment of diabetic rats with *Portulaca oleracea* for four weeks, there was a significant difference (180 g) in weight of diabetic rats compared to omega-3 (165 g), Vitamin E + Selenium (172 g) and control groups (142 g, $p < 0.05$, Table 1).

### *3.3. Histopathologic findings*

In present study, *Portulaca oleracea*, omega-3 and Vitamin E + Selenium had crucial role in pancreatic islets regeneration. The minimum islets diameter in omega-3 group on day 14 was significantly more than other groups ($p < 0.05$). The regenerated pancreatic islets had increased significantly in treated diabetic rats with *Portulaca oleracea* in comparison with control group at fourth week ($p < 0.05$, Table 2). The diameter of pancreatic islets in control group decreased significantly in comparison with treated groups on day 28 ($p < 0.05$, Figure 1).

In treated diabetic rats with omega-3 and *Portulaca oleracea*, the percentage of beta cells was significantly more than Vitamin E+ Selenium and control groups on day 14 ($p < 0.05$, Table 2). In control group, significant degenerative changes were seen in number of beta cells on day 28 compared to other groups ($p < 0.05$, figures 1-4).

The percentage of alpha cells in treated rats with *Portulaca oleracea* on day 14 was significantly more than the other groups ($p < 0.05$, Table 2). At end of the study the percentage of alpha cells in the control group showed a significant increase ($p < 0.05$) compared to other groups on day 28 (Table 2).

**Table 1.** Blood sugar, insulin level, and weight after second and fourth weeks of treatment in diabetic rats.

| | control group | | Treated diabetic group with *Portulaca oleracea* | | Treated diabetic group with omega-3 | | Treated diabetic group with (Vitamin E + Selenium) | |
|---|---|---|---|---|---|---|---|---|
| | Second week | Fourth week | Second week | Fourth week | Second week | Fourth week | Second week | Fourth week |
| **Blood glucose (mg/dl)** | $490^{a}$ | $580^{a}$ | $350^{c}$ | $250^{c}$ | $441^{ab}$ | $205^{cd}$ | $308^{cd}$ | $269^{cb}$ |
| **Insulin (IU/ml)** | $7^{c}$ | $6^{cd}$ | $13^{a}$ | $15^{a}$ | $11^{b}$ | $13^{ab}$ | $14^{a}$ | $14^{a}$ |
| **Weight (g)** | $142^{f}$ | | $180^{a}$ | | $165^{c}$ | | $172^{b}$ | |

$^{a,b,c,d,e}$: Different superscripts letters in the same week mean significant differences ($p < 0.05$)

**Table 2.** Stereological results of pancreatic islets after second and fourth weeks of treatment in diabetic rats

| | control group | | *Portulaca oleracea* group | | omega-3 group | | Vitamin E + Selenium group | |
|---|---|---|---|---|---|---|---|---|
| | Second week | Fourth week | Second week | Fourth week | Second week | Fourth week | Second week | Fourth week |
| **Minimum islets diameter (μ)** | $70^{bc}$ | $45^{d}$ | $72.32^{b}$ | $62.33^{b}$ | $84.33^{a}$ | $70^{a}$ | $73^{b}$ | $68.33^{a}$ |
| **Beta cells (%)** | $67.66^{b}$ | $35.66^{f}$ | $70^{a}$ | $64^{b}$ | $72^{a}$ | $68.33^{a}$ | $66^{b}$ | $62.33^{bc}$ |
| **Alpha cells (%)** | $30.33^{c}$ | $62^{a}$ | $41.46^{a}$ | $37.66^{c}$ | $26^{cd}$ | $29.33^{d}$ | $32^{bc}$ | $34^{c}$ |

$^{a,b,c,d,e}$: Different superscripts letters in the same week mean significant differences ($p < 0.05$)

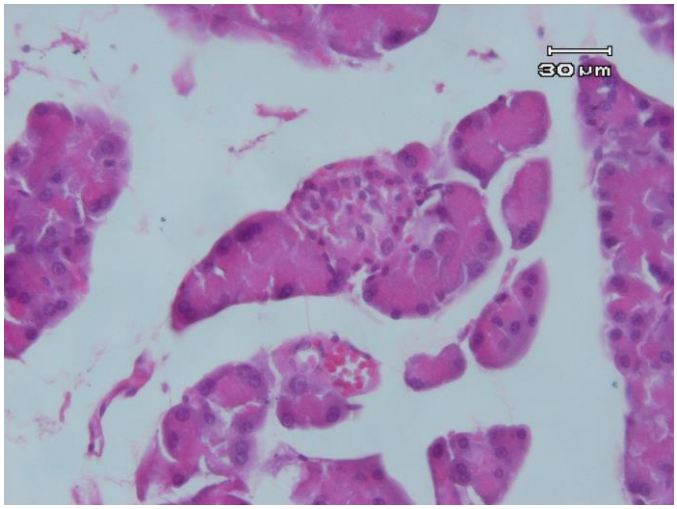


**Figure 1.** Histopathological view of pancreas tissue of diabetic rats in control group on day 28. The number of pancreatic islets and beta cells has been extremely reduced (H&E, X 400)

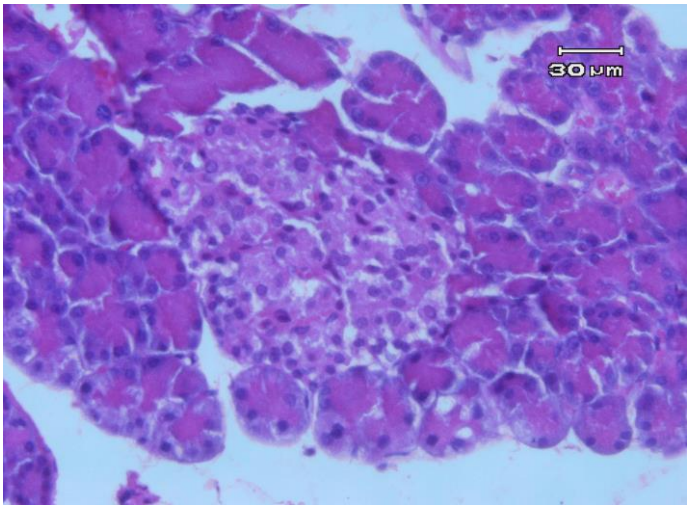


**Figure 2.** Histopathological view of pancreas tissue in diabetic rats treated with *Portulaca oleracea* on day 28. The number of beta cells increased along with decrease in inflammatory cells (H&E, X 400)

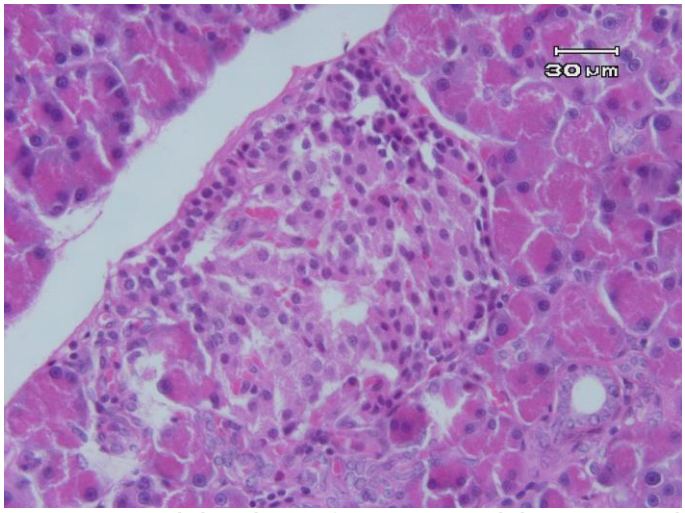


**Figure 3.** Histopathological view of pancreas tissue diabetic rats treated with omega 3 on day 28. The number of beta cells increased along with decrease in inflammatory cells (H&E, X 400)

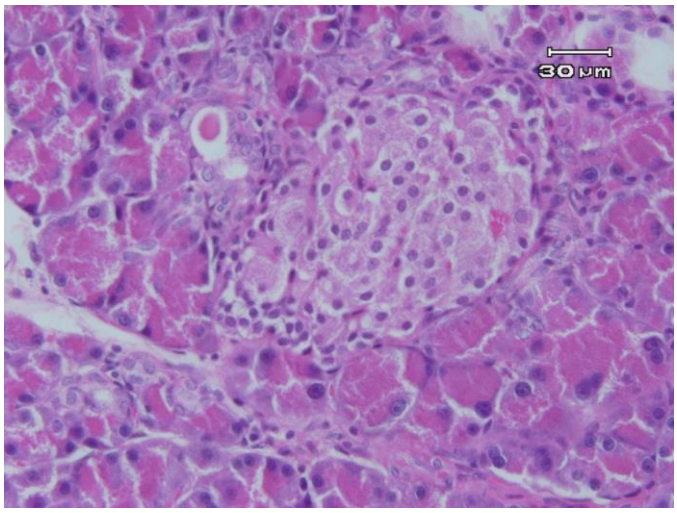


**Figure 4.** Histopathological view of pancreas tissue in diabetic rats treated with Vitamin E + Selenium. The number of beta cells increased along with a decrease in inflammatory cells (H&E, X 400)

## 4. Discussion

Although the pathogenesis of diabetes induced by chemicals and the inflammation of the pancreatic islets has not been fully identified[22], streptozotocin causes damage to DNA molecules and beta cells by alkylating basal and alloxan when producing free radicals[26, 27]. The penetration of mononuclear inflammatory cells, especially lymphocytes, can form an autoimmune reaction leading to the loss of cells in the pancreatic Islets, especially beta cells[28, 29].However, the inflammation process is a complementary component for an autoimmune response that is performed against beta cells in some animal models of diabetes[26].

The results of the present study revealed a decrease in inflammation of pancreatic islets as well as a significant increase in insulin levels and hypoglycemia of the treated rats. Moreover, histopathological findings indicated the regeneration of pancreatic islets that were destroyed by STZ. Furthermore, changes in pancreatic tissue in untreated diabetic rats were similar to those of patients with type 1 (Insulin-dependent) diabetes mellitus.

Since there has been no report of suitable medication for the treatment of some acute and chronic pain conditions, especially in the state of diabetes mellitus with no side effects, researchers have focused on the medicinal plants and their effective extracted substances[30].

*Portulaca oleracea* extract contains pharmacological active agents, such as alkaloids, glycosides, terpenoids, sterols, and flavonoids. It may be stated that some of these compounds can reduce the severity of autoimmune reactions and the inflammation process to some extent that leads to the destruction of beta cells. Consequently, the destruction of the remaining cells is prevented which provides ample opportunity for the proliferation of these cells and the regeneration of the pancreatic islets. It was found that the consumption of *Portulaca oleracea* extract

caused the regeneration of pancreatic islets in diabetic rats with STZ due to the presence of flavonoids, such as quercetin, existing in the aerial parts of the plant which can release insulin by changes in $Ca^{++}$ metabolism[31]. These compounds have insulin-like properties and may cause beta cell regeneration[31]. The results of a study addressing the effect of *Portulaca oleracea* extracts on alloxan-induced diabetic rats have indicated a significant decrease in the Hemoglobin A1C (Hb A1C), serum levels of glucose, Tumor Necrosis Factor-alpha (TNF-α), and Interleukin 6 ( IL-6) of *Portulaca oleracea* pre-treated diabetic rats confirming that *Portulaca oleracea* is a general tissue-protective and regenerative agent[32] and also demonstrated the anti-diabetic effect of hydro-ethanolic extract *Portulaca oleracea* seeds on diabetic animals.

In an investigation by Dehghan et al.[33], it was indicated that 16 weeks of aerobic training or/and *Portulaca oleracea* seed consumption were effective in the regulation of diabetic parameters and biomarkers associated with atherosclerosis in women with Type 2 Diabetes (T2D). Another relevant study revealed that diabetes significantly impaired brain abilities and swimming training and *Portulaca oleracea* synergistically reversed and ameliorated neurobehavioral dysfunction in type 2 diabetic rats[34].

It should be noted that no significant toxic marks have yet been reported about *Portulaca oleracea* [35]. water, minerals, pectin, protein, carbohydrates, fatty acids, especially omega-3 unsaturated fatty acids, antioxidants, and numerous minerals (such as ferritin, copper, manganese, potassium, calcium, and cessation) are found in different parts of this plant[36,37]. *Portulaca oleracea* is the richest plant source with omega-3 fatty acids [36]. Its antioxidant compounds include Olefatoferol, Ascorbic acid, and Glutathione[4]. In addition, the antioxidant properties of the plant extract have been confirmed in laboratory studies [12]. In a study, it was found that aqueous and ethanolic extracts of *Portulaca oleracea* leaves can produce various antioxidants [38]. Moreover, is quinoline as the main alkaloid of *Portulaca oleracea* had a significant stimulating effect on insulin secretion and improving glucose uptake[39].

There are reports on the effectiveness of Selenium in diabetes as well as its significant role in reducing the effect of STZ, and enhancing the activity of GPx enzyme in rats[19,20]. However, Selenium has a narrow therapeutic index, and the increase in consumption can have toxic effects[40].

Vitamin E is known as the most important anti-oxidant of biological membranes that can neutralize free radicals[17]. Jamilian and Ravanbakhsh[41] reported that Vitamin E plus omega-3 fatty acid supplementation in gestational diabetes mellitus (GDM) women had beneficial effects on biomarkers of inflammation and oxidative stress. Baburao Jain and Anand Jain[42] demonstrated that Vitamin E supplementation has an important role in delaying the onset of diabetic complications as well as slowing down the progression of the complications. Another meta-analysis indicated that the supplementation of Vitamin E may be a valuable strategy for controlling diabetes complications and enhancing antioxidant capacity[43].

The essential fatty acids are reported to have a low level in different tissues of diabetic patients. Therefore, the use of omega-3 fatty acids can be effective in reducing the effects of fatty deficiencies[44].The causes of low levels of essential fatty acids in diabetic patients are unclear, but some researchers assert that diabetics have less ability to convert linoleic acid into Eicosa Pentaenoic Acid and Docosa Hexaenoic Acid. In one of the latest studies on the antidiabetic effects of Vitamins C, A, and E as well as omega-3 fatty acids, it was found that lipid peroxidation and malondialdehyde levels were reduced due to the decreased production of free radicals or inhibition of oxidative damage[45].

## 5. Conclusion

In treated diabetic rats with *Portulaca oleracea*, regenerated pancreatic islets levels were more than two groups of omega-3 and Vitamin E + Selenium. Moreover, inflammatory cells decrease in one pancreatic islet were better than omega3 group. Minimum islets diameter in the omega-3 group on day 14 was more than other groups but it significantly increased at the end of the study, compared to the control group. Beta cells percentage in two groups of omega-3 and *Portulaca oleracea* was more than Vitamin E + Selenium group.

At the end of the study, blood glucose levels in the omega-3 group significantly decreased, compared to *Portulaca oleracea* extract and Vitamin E + Selenium groups. At the end of the study, blood insulin levels increased significantly in *Portulaca oleracea* extract group, compared to other groups.

## Declarations

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### *Competing interests*

The authors declare that they have no competing interests.

### *Authors' contribution*

Mahdi Alyari Gavaher designed the study and performed the sampling and practical procedures. Daryoush Babazadeh revise the draft of the manuscript, remove the language errors and check the final version of the article. Farhang Sasani and Alireza Sadeghi revise the draft of the manuscript and reported the histopathological findings. Veghar Hejazi performed the statistical analysis. Arman Moshaveri and Pouria Ahmadi Simab wrote the

draft of the manuscript. All authors check the final proof of the article and the statistical results.

### *Availability of data and materials*

All data and related findings of the thesis are prepared for publishing in the present journal.

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