

Effects of emodin on blood glucose and body weight in type 1 diabetic rats**Abstract**

Objective: To analyze the effect of emodin on blood glucose and body weight in rats with type 1 diabetes.

Methods: A total of 45 healthy SD rats were selected followed by being adaptively fed for one week and then from them 15 rats were randomly selected out as the control group and the remaining 30 healthy SD rats were given intraperitoneal injection of streptozotocin to establish diabetic model with the control group receiving intraperitoneal injection of citrate buffer solution of same doses; At the fifth day, the rats were fasted for 6h and appropriately fed with water. Then the tail blood was collected to measure the fasting blood glucose of rats and this step was repeated 15d after the injection of streptozotocin. The diabetic rat model was established successfully when the blood glucose was detected to be no less than 16.9mmol/L; Thirty diabetic model rats were randomly divided into diabetes group and emodin group with 15 cases in each group (n = 15); 15d later, the rats in diabetes group and control group were treated with intragastric administration of PBS, 5ml/kg, and the rats in emodin group were given intragastric administration of emodin suspension (8g/L), 5ml/kg. The changes of fasting blood glucose and body weight were observed and compared in three groups from the time point of before model establishment to 10 weeks after successful establishment of model.

Results: There was no significant difference among three groups in fasting blood glucose and body weight at the initial stage of establishing model; In diabetes group and emodin group, the rat's body weight in T2 was significantly lower than that in T1 with the body weight decreasing more significantly in diabetes group ($P < 0.05$), while the blood glucose in T2 was significantly higher than that in T1 with the blood glucose increasing more significantly in diabetes group ($P < 0.05$).

Conclusion: Emodin can relieve hyperglycemia in type I diabetic rats but has little influence on body weight.

Keywords: type 1 diabetes ; rats; emodin; body weight; blood sugar

Introduction

Diabetes, more common in endocrine system diseases, is most often diagnosed in middle and elder age group but recent medical survey reveals its incidence has increased year by year in young people. According to a study in 2013, the number of diabetic patients has been close to 400 million in the adults of the world, which seriously threatens people's lives and health [1-3]. Emodin is an orange-yellow needle crystal, orange in acetone and yellow in methanol. Its melting point reaches 256°C~257°C with specific reaction of anthraquinone. It is the main active ingredient of rhubarb almost insoluble in water but soluble in ethanol and alkali solution. Emodin can inhibit the transportation of sodium and potassium ion from intestine to cell, cause water retention in the intestine, stimulate peristalsis and thereby play the function of diarrhea with weak effect. The in vivo distribution and pharmacokinetic study show

that when the rats were given gastric infusion of ^{14}C emodin at the dose of 50mg/kg, the excretion in urine is 18% of the dose given within 24 hours and 22% of the given dose with 72 hours. Most of the rhein in the urine are excreted within 24 hours. There are very few metabolites in urine 72 hours after administration and they appear to exist mainly in the free,unbounded form. The total of emodin and rhein reaches 16% of given doses, the content of glucose aldehyde or sulfate is only about 3% in emodin with another 3% of other radioactive residues. Within 24 hours and 120 hours after administration, the emodin in the stool is mainly in free state, accounting for respectively 48% and 68% of given dose. Six hours after administration,the emodin has high concentration in bile excretion and the excretion reaches 49% of given dose within 15 hours. The emodin appearing to exist mainly in the form of glucuronic acid ester or sulfate accounts for 70% and the radioactivity of most organs decreases obviously 3~5 days after administration, but until the fifth day there remains a very high radiation activity in the kidney. The radioactivity of the mesentery and adipose tissue increases greatly 72~120 hours after administration. Within 0~48 hours after gavage administration of emodin at the dose of 91mg/kg in the rats, the overall yield of anthraquinone derivatives excreted through urinary and feces is up to 53% of given dose in which the total amount of excretion in urine and feces is 2% in 0~24 hours. The total anthraquinone derivatives in the bile reach a peak 4 hours after administration and then decrease gradually. Bile is one of major routes of emodin excretion.

Modern pharmacological studies have shown that emodin has the function of anti-inflammation, improving microvascular disease, improving immunity, promoting recovery of blood lipid metabolism and relieving insulin resistance. In recent years, medical research has found that emodin can reduce the blood sugar concentration of diabetic rats and cause weight loss but there are few studies on its effect of type 1 diabetes mellitus[4-7]. The aim of this study was to investigate whether emodin can cause changes in body weight and blood sugar in type 1 diabetic rats based on the association between emodin and type 1 diabetes mellitus.

Data and methods

General data

A total of 50 healthy SD rats were selected. They all were male and weighted 198-205g, (200.54 ± 1.74) g on the average, 3-month old with the fasting blood glucose of (5.48 ± 0.32) mmol/L.

Research instruments and reagents

The reagents applied this study mainly included streptozotocin, emodin, glucose detector and citric acid buffer. Among them, the citric acid buffer was made as follow: (1) citric acid plus Shuangyan water and sodium citrate plus Shuangyan water were made respectively into A and B liquid, shown as table , equal amount of which then were mixed with pH value adjusted to 4.2-4.5 as the citric acid buffer used in the study. Mixture of STZ and citric acid buffer was made in the proportion of 1:100 as C liquid.

Table 1 Mixture of A and B liquid

mixture	Reagent 1	dosage	Reagent 2	dosage
A liquid	citric acid	2.10g	Shuangyan water	100ml
B liquid	sodium citrate	2.94g	Shuangyan water	100ml

1.3 establishment of type I diabetic rat model

A total of 45 healthy SD rats were adaptively fed for one week and the tail blood was collected to measure the fasting blood glucose, which showed normal value. Then 15 from 45 rats were randomly selected out as the control group and the remaining 30 healthy SD rats were given intraperitoneal injection of C liquid with the dosage calculated in accordance with the dose of 65mg/kg in streptozotocin and the rat's weight to establish diabetic rats model and the control group were injected with citrate buffer of the same dose; At the fifth day, the rats were fasted within previous 12h and appropriately fed with water. Then the tail blood was collected to measure the fasting blood glucose of rats and this step was repeated 15d after the injection of streptozotocin. The diabetic rat model was successfully established when the blood glucose was detected to be no less than 16.9mmol/L shown as in Table 2. In this study, 15d after the beginning of model construction, 30 rats was successfully modeled with the success rate of 100.00% [8-10].

Table 2 Analysis of fasting blood glucose concentration before and after establishment of type 1 diabetic rats

time	fasting glucose (mmol/L)	blood statistic	<i>P</i> value
before establishment	5.68 ± 0.22	—	—
the fifth day after establishment	16.28 ± 1.46	$t_1=50.0937$	$P_1=0.0000$
the fifteenth day after establishment	24.88 ± 2.39	$t_2=56.5661$	$P_2=0.0000$

Note: t_1 and P_1 represented the statistical value of the comparison between before establishment and 5d after establishment and t_2 , P_2 represented the statistical value of the comparison between before establishment and 15d after establishment

Grouping experiment

Thirty diabetic model rats were randomly divided into diabetes group and emodin group with 15 cases in each group (n = 15); 15d later, the rats in diabetes group and control group were treated with intragastric administration of PBS, 5ml/kg, and the rats in emodin group were given intragastric administration of emodin suspension (8g/L), 5ml/kg. The changes of fasting blood glucose and body weight were observed and compared in three groups from the time point of before model establishment to 10 weeks after successful establishment of model with T1 representing the former time point and T2 the latter [11-15].

Statistical analysis

SPSS13.0 statistical software was selected in the study for data processing. The measurement data were described as " $\bar{x} \pm s$ " and checked by t test, $P < 0.05$ suggested there was obvious difference in the data of statistical significance.

Results

General physiological condition of rats before and after establishment of type 1 diabetes model

Before the establishment of type 1 diabetes model, the rats in the diabetic group were able to move freely with glossy fur and sensitive response. After injection of C they were listless with the furs gradually turning yellow and the body weight slowly decreasing. Besides the consumption of edible feed and intake of water increased gradually with rising excretion. With rather damp bedding, the rats were subjected to reduced activity, lags in response and moderate sensitiveness.

The rats in the emodin group had the same performance as those in the diabetes group before intragastric administration of emodin suspension while after the administration they remained dispirited and gradually angular with dim and relatively loose fur. Their reaction was not flexible with slow movement and some rats showed hair loss on the back with especially serious failure occasional seen.

The rats in the control group did not change significantly before and after the model establishment, Their activity changed little with the consumption of food and water as well as urine and stool remaining unchanged. There were not obvious changes in the rats' daily state.

Changes in blood glucose and body weight before and after the establishment of type 1 diabetes model

There was no significant difference in fasting blood glucose and body weight among the three groups in the initial stage of model construction; the rat weight of diabetes group and emodin group was obviously lower compared with the result 10 weeks after successful model construction with the weight in the diabetes group decreasing more significantly ($P < 0.05$): (1) comparison between T2 and T1 in diabetes group: $t = 3.8607$, $P = 0.0006$; (2) comparison of T2 between diabetes group and control group: $t = 32.4768$, $P = 0.0000$; (3) comparison between T2 and T1 in emodin group: $t = 4.2950$, $P = 0.0002$; (4) comparison of T2 between emodin group and control group: $t = 7.2111$, $P = 0.0000$; (5) comparison of T2 between diabetes group and emodin group: $t = 0.9813$, $P = 0.3316$.

The blood glucose of diabetes group and emodin group was significantly higher than that before model construction with higher ascending range seen in the diabetes group ($P < 0.05$): (1) comparison between T2 and T1 in diabetes group: $t = 27.4976$, $P = 0.0000$; (2) comparison of T2 between diabetes group and control group: $t = 27.4700$, $P = 0.0000$; (3) comparison between T2 and T1 in emodin group: $t = 7.3288$, $P = 0.0000$; (4) comparison of T2 between emodin group and control group: $t = 7.2111$, $P = 0.0000$; (5) comparison of T2 between diabetes group and emodin group: $t = 3.7007$, $P = 0.0009$ shown in Table 3.

Table 3 blood glucose and weight changes in rats before and after model construction

group	blood glucose (mmol/L)		weight (g)	
	T1	T2	T1	T2
control group:	5.55 ± 0.36	5.58 ± 0.41	200.35 ± 4.87	421.22 ± 3.96
diabetes group	5.68 ± 0.22	29.87 ± 3.40	200.02 ± 3.03	170.30 ± 29.66
emodin group	5.33 ± 0.35	21.22 ± 8.39	199.39 ± 6.36	179.22 ± 18.51

Discussion

Diabetes mellitus has a moderately high incidence in clinical findings with many triggering factors like heredity, environment and autoimmunity. The study of diabetic medicine involves many different kinds of experimental animals such as dogs, rabbits and pigs in which the rat is most commonly used with fast growth, low cost and easy cultivation as well as obtainment. In this regard the SD rat one of large numbers of species with is highest use rate. STZ has a specific function of destruction on pancreatic beta cells, which can lead to the decrease of insulin secretion and induce type I diabetes mellitus[16-20]. At present,it is the most commonly used model for type I diabetes mellitus in medical research with the advantages of high success rate in model construction and moderately low toxicity in animal tissue. In this course the use of STZ is one of the most critical step and when the injection volume of STZ is less than 40mg/kg,it will give rise to the failure of model establishment. In this study, STZ was injected intraperitoneally into rats at the dosage of 65mg/kg and the success rate of model establishment was 100%.

Emodin, a kind of anthraquinones, is one of effective components of herbal medicine rhubarb after refinement, It can protect the nerve with prevention as well as treatment of Alzheimer's disease and also has the function of antibiosis, anti-inflammatory, antiviral and antitumor. Besides it can effectively induce part of cells to cause their apoptosis with the effect of reducing blood sugar and regulating lipid and inhibiting immune system activity. According to literature reports, emodin has the effect of AMPK activation and enables to increase glycolysis rate as well as accelerate glucose metabolism in vivo. It should be noted that emodin has certain toxicity, especially the high concentration of emodin will damage the liver and kidney, but it has little effect on the results of this study[21-23].

In the study we selected 45 healthy SD rats as the objects and divided them into control group ,diabetes group and emodin group with 15 rats included in each; among them, type 1 diabetic rat model was constructed by intraperitoneal injection of STZ followed by intragastric administration as the establishment of emodin group. The changes of fasting blood glucose and body weight were observed and compared in three groups from the time point of before model establishment to 10 weeks after successful establishment of model. It turned out that there was no significant difference among three groups in fasting blood glucose and body weight at the initial stage of establishing model; In diabetes group and emodin group, the rat's body weight in T2 was significantly lower than that in T1 with the body weight decreasing more significantly in diabetes group ($P < 0.05$), while the blood glucose in T2 was

significantly higher than that in T1 with the blood glucose increasing more significantly in diabetes group ($P < 0.05$), suggesting that emodin can alleviate hyperglycemia in type 1 diabetic rats but has little effect on body weight.

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