

Antidiabetic effect of *Centella asiatica* extract (whole plant) in streptozotocin nicotinamide-induced diabetic rats

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ABSTRAK

Latar belakang: Pegagan telah digunakan untuk pengobatan diabetes mellitus. Namun dosis efek anti-diabetes ini belum diteliti.

Tujuan: Untuk menguji pengaruh variasi dosis ekstrak pegagan (seluruh bagian tanaman) terhadap kadar gula darah, asupan makanan, dan berat badan pada tikus model diabetes mellitus yang diinduksi Streptozotocin Nicotinamide.

Metode: Tiga puluh enam tikus Wistar jantan dibagi menjadi enam kelompok perlakuan berbeda: kontrol negatif, kontrol positif, kontrol obat (metformin 45 mg/KgBB/hari), ekstrak pegagan 300 mg/KgBB/hari, 600 mg/KgBB/hari, 1200 mg / KgBB / hari. Pemberian ekstrak pegagan (seluruh bagian tanaman) dan metformin dilakukan selama 28 hari. Kadar gula darah dianalisis menggunakan Glucose Oxidase Phenol Aminoantipyrina Peroxidase (GOD-PAP) sebelum dan sesudah perlakuan. Berat badan dan asupan makanan diukur setiap satu minggu. Data dianalisis menggunakan One Way ANOVA dengan tingkat kepercayaan 95%.

Hasil: Pemberian variasi dosis ekstrak pegagan dan metformin secara signifikan menurunkan kadar gula darah, meningkatkan berat badan dan memperbaiki asupan makanan ($p = 0,00$). Kadar gula darah dan asupan makanan pada kelompok ekstrak pegagan (seluruh bagian tanaman) dosis tinggi (1200 mg / KgBB / hari) lebih baik dibanding kelompok dosis rendah (300 mg / KgBB / hari) dan kelompok dosis sedang (600 mg / hari), dan memiliki efek yang sama dengan tikus yang diobati dengan metformin 45 mg / KgBB / hari.

Kesimpulan: Dosis tinggi ekstrak pegagan (1200 mg/KgBB/hari) memiliki efek anti-diabetes yang lebih baik dibanding dosis lain, dan memiliki efek yang sama dengan kontrol obat.

KATA KUNCI: gula darah, diabetes, pegagan, Streptozotocin nicotinamide

ABSTRACT

Background: *Centella asiatica* has been used for healing diabetes mellitus. The dosage of this anti-diabetic effect was yet to be explored.

Objectives: To examine the effect of the variation in the dosage of *C. asiatica* extract (whole plant) on blood glucose levels, food intake, and body weight in Streptozotocin Nicotinamide induced diabetic rats.

Methods: Thirty-six male Wistar rat were divided into six different groups of treatments: negative control, positive control, medication control (metformin 45 mg/KgBW/day), extract of *C. asiatica* 300 mg/KgBW/day, 600 mg/KgBW/day, 1200 mg/KgBW/day. Treatments of *C. asiatica* extract (whole plant) and metformin was done for 28 days. Blood glucose was analyzed using the Glucose Oxidase Phenol Aminoantipyrina Peroxidase (GOD-PAP) before and after treatment. Body weight and food intake were measured every one week. The data were analyzed using One Way ANOVA with 95% confidence level.

Results: The administration of the variation in the dosage of *C. asiatica* extract and metformin significantly decrease blood glucose levels, increase body weight and improve food intake ($p = 0.00$). Blood glucose level and food intake among high dosage of *C. asiatica* extract (whole plant) group (1200 mg/KgBW/day) is better than the low dosage group (300 mg/KgBW/day) and moderate dosage group (600 mg/KgBW/day).

day), and have the same effect with the rats treated with metformin 45 mg/KgBW/day.

Conclusion: High dosage of *C. asiatica* extract (1200 mg/KgBW/day) had a better anti-diabetic effect than other dosages, and had the same effect with the medication control.

KEYWORDS: blood glucose, *Centella asiatica*, diabetes, nicotinamide, Streptozotocin

INTRODUCTION

Diabetes Mellitus (DM) is a metabolic disorder that becomes the main problem of the health in all around the world, the prevalence in the world reaches the number of 382 million people and it is estimated to increase into 592 million people by 2035, while in Indonesia the patients of DM increases from 1.1 percent (1.1 %) in 2007, to 2.1% in 2013 on the basic health research (IDF, 2014; Ministry of Health, 2013).

Type 2 Diabetes Mellitus (T2DM) is a complex metabolic disorder that is marked with the case of hyperglycemia as a result of the failure of cell β pancreas so that there is a resistance for the insulin (3). T2DM happens because of the disturbance of the progressive insulin secretion that becomes the main problem of a resistance to the insulin (4). Severe hyperglycemia and the disturbance of the insulin secretion cause several indications such as polyuria, polydipsia, polyphagia, and the loss of weight (5, 6). Severe hyperglycemia that happens to T2DM patients may poison the cell β pancreas with the glucose and the patients may have the apoptosis (7,8).

The anti-diabetes oral therapy plays an important role in controlling the level of the blood glucose. However, some researchers show that the use of anti-diabetes therapy can cause several side effects such as nauseous, digestion disorder, and diarrhea. Furthermore, if the therapy is used in a long-term, it can cause the other side effects such as the deficiency of vitamin B12, and the raising of the risk of cancer (9-11).

There is a raising of the demand for the herb availability from the plants that have the characteristic of antioxidant and anti-diabetes. *Centella asiatica* has been used as a traditional medicine (herbal medicine) in Indonesia and Asian countries since hundreds of years ago (12). *C. asiatica* is one of the herbs that have the characteristic of antioxidant and

anti-diabetes (13-15). *C. asiatica* contains a high level of antioxidant from the group of pentacyclic triterpenoids (asiatic acid, made classic acid, asiaticoside, and madecassoside) (16-19). The content of triterpenoid contained in *C. asiatica* is known to improve glucose response through increased protein GLUT4 muscle, IR, IRS 1, and IRS 2 (20-24).

The use of *C. asiatica* extract as the herb availability has more positive sides such as effective, relatively cheaper, low level of side effects, and low level of toxicity (20). The use of triterpene marker on the extract of *C. asiatica* is more effective to the T2D patients in increasing the secretion of the insulin compared to the anti-diabetes oral medicine (21. 25-27). The use of Ethanol extract of the *C. asiatica* leaves, a dosage of 300 mg/KgBW/day for 28 days is proven in normalizing the level of blood glucose in Type 2 Diabetic Model Rats – the induction of high-fat diet and the *Streptozotocin*, but the use of the variation in the dosage (300, 600, 1200 mg/KgBW/day) of ethanol extract from all parts of *C. asiatica* (roots, stems and leaves) to blood glucose levels, body weight and feed intake in the type 2 diabetic model rats–streptozotocin-nicotinamide (STZ-NA) induced diabetic rats is unknown. Therefore, the aims of this study are to evaluate the effect of *C. asiatica* extract as anti-diabetic to blood glucose levels, body weight and feed intake in Streptozotocin-Nicotinamide (STZ-NA) induced diabetic rats.

MATERIALS AND METHODS

This study was experimental research with the randomized post-test only with controlled group design. The study was conducted from March to September 2017. The whole methods that were used had been approved by the Health Research Ethics Committee Dr. Moewardi General Hospital

and School of Medicine, Universitas Sebelas Maret (128/II/HREC/2017).

The sample of *C. asiatica* was obtained from commercial products. *C. asiatica* was extracted with the ethanol of 70% (ChemPur, Karlsruhe, Germany) (28). The *C. asiatica* extract was made done at LPPT-Unit 1 (Lembaga Penelitian dan Pengujian Terpadu) Universitas Gadjah Mada Yogyakarta – Unit 1.

Thirty six male albino Wistar rats (*Rattus norvegicus*) in the age of 8–10 weeks in the body weight of 200 ± 50 gram were divided into 6 different groups of treatments:

1. Negative control (standard feed- comfeed, aquades)
2. Positive control (standard feed- comfeed, aquades, STZ-NA)
3. Medication control (standard feed- comfeed, aquades, STZ-NA, metformin 45 mg/KgBW/day)
4. CAE treatment dosage 1 (standard feed- comfeed, aquades, STZ-NA, CAE 300 mg/KgBW/day)
5. CAE treatment dosage 2 (standard feed- comfeed, aquades, STZ-NA, CAE 600 mg/KgBW/day)
6. CAE treatment dosage 3 (standard feed- comfeed, aquades, STZ-NA, CAE 1200 mg/KgBW/day)

Animal breeding was attempted based on the modification procedures by Nain et al. (2012) (29). Thirty six male Wistar rats were placed in a large hygienic polypropylene enclosure containing 6 insulated rats, filled with one rat per bulkhead. The rats were maintained in a room with a temperature control (22 ± 20 °C) 12 hours of bright light and 12 hours dark cycle (lights turned on at 07.00 am), humidity 70 - 90%. The STZ-NA induction was performed on the modification of the Ortiz-Andrade et al. (2008) with a dosage of streptozotocin 65 mg/KgBW and nicotinamide 230 mg/KgBW, rats expressed hyperglycemia if the blood glucose level was ≥ 150 mg / dL (30).

The measurement of glucose blood levels using the Glucose Oxidase Phenol Aminoantipyrina Peroxidase (GOD-PAP) method using Glucose GOD FS kit from Diagnostic System International

(Diasys, Holzheim, Germany), was done before treatment and 28 days after the administration of *C. asiatica* extract. The measurements were made in the morning before feeding, blood samples were obtained from the orbital vein end. A total of ± 0.5 ml of blood samples which were taken had been collected into Eppendorf, centrifuged for 10 minutes at 2500 rpm, the serum was taken and the enzymatic blood glucose levels were determined by GOD-PAP method (31).

Each rat was weighed by using digital scales and measured every seven days during the research. While the intake of feed in each week was calculated from the average total feed intake in one week (the feed gave minus the remaining feed) as measured using a digital scales. The maintenance and the manufacture of laboratory rats, the measurement of blood glucose levels, the body weight and the feed intake were done in Laboratorium Pusat Pangan dan Gizi PAU Universitas Gadjah Mada. The statistical analysis was performed using SPSS for Windows release 20.0. The data obtained were tested for normality using Shapiro-Wilk test and homogeneity using Levene's test. The data variant is normally distributed and homogeneous such as blood glucose levels and the body weight, it is followed by the One-Way ANOVA (Analysis of Variance) test to determine the average difference between the six groups, followed by the Tukey high significant difference (HSD) test to identify the different groups of names significantly. The normality and homogeneity requirements of the data are not met such as feed intake, then a comparable nonparametric test is a Kruskal-Wallis test. If there are significant differences, it will be continued with the Mann-Whitney test. The differences in blood glucose levels, weight, and feed intake before and after the treatment in all groups were tested for paired t-test. There is a significant difference if p-value < 0.05 (32).

RESULTS

***Centella asiatica* extract reduced blood glucose levels in STZ-NA induced diabetic rats**

The analysis of blood glucose level before STZ-NA induction had a normal and homogeneous variation ($p > 0.05$). Measurement of rat blood

glucose level was done 3 times: before induced STZ-NA, three days after induction of STZ-NA, and 28 days after treatment of *C. asiatica* extract dose 300, 600 and 1200 mg/kgBW/day and metformin 45 mg/KgBW/day which can be seen in **Table 1**.

The mean of the blood glucose levels before and after the treatment differed significantly ($p=0.00$). Giving ethanol extract of *C. asiatica* in the dosage of 300 mg/KgBW/day (P1), 600 mg/KgBW/day (P2), 1200 mg/KgBW/day (P3) and metformin 45 mg/KgBW/day (MC) was proven to be able to decrease the rat's blood glucose levels significantly ($p=0.00$). The result of the measurement of blood glucose level in the group treated with extract of *C. asiatica* in a dosage of 300 mg/KgBW/day (P1) showed that this treatment could decrease the blood glucose level significantly ($p=0.00$) as much as 73.92 ± 4.16 mg / dL, but some rats still had the hyperglycemia (> 150 mg / dL) (**Table 1**).

Blood glucose levels of rats which were treated with the extracts of *C. asiatica* in the dosage of 600 mg/KgBW/day decreased and did not have hyperglycemia, but this condition was not in the normal category. The normal blood glucose level of

rats is 50-135 mg / dL (40). The results of Tukey HSD test showed a decrease in blood glucose level of rats treated with extract of *C. asiatica* 1200 mg/KgBW/day (P3), this was similar to the group that was given metformin 45 mg/KgBW/day (MC), and has reached the normal condition (**Table 1**). However, the effect of the decrease in the blood glucose level in the group with extract of *C. asiatica* 1200 mg/KgBW/day (P3) was 126.36 ± 6.88 mg/dL, better than the medication control group (metformin 45 mg / Kg of body weight) 120.70 ± 6.65 mg/dL (**Table 1**).

***Centella asiatica* extract improved food intake in STZ-NA induced diabetic rats**

The effect of the variation in the dosage of *C. asiatica* extract to food intake of rats can be seen in **Table 2**.

There was a significant change in the rats' feed intake before and after the treatment. However, based on Mann-Whitney's further tests, it was known that the intake of feed before the administration of *C. asiatica* extract in the negative control group, the positive control group, treatment dosage 1, dosage 2 and dosage 3 were the same. While the medication

Table 1. Blood Glucose Level (mg/dl) before and after treatment

Group	Pre test (mg/dL)	Post test (mg/dL)	Difference (Δ) (mg/dL)
Negative control	77.04 ± 3.33^a	79.63 ± 2.65^a	-2.59 ± 1.29
Positive control	256.83 ± 7.27^{bc}	261.66 ± 8.69^b	-4.83 ± 2.42
Medication control	253.28 ± 6.28^c	132.58 ± 4.10^e	120.70 ± 6.65
CAE 300mg/KgBW (Dosage 1)	261.78 ± 4.58^b	187.86 ± 1.89^c	73.92 ± 4.16
CAE 600 mg/KgBW (Dosage 2)	257.10 ± 7.10^{bc}	143.35 ± 2.23^d	113.75 ± 7.44
CAE 1200 mg/KgBW (Dosage 3)	257.56 ± 5.69^{bc}	131.21 ± 2.84^e	126.36 ± 6.88

n=6; CAE= *Centella asiatica* extract; Delta (Δ)= the difference of average blood glucose levels before and after the treatment

a,b,c,d,e) Different notations indicated a significant difference based on the *One-Way Anova* test ($P < 0.05$).

Table 2. Food intake (gram) before and after treatment

Group	Pre test (gram)	Post test (gram)
Negative control	12.67 ± 1.03^a	13.17 ± 0.75^a
Positive control	13.50 ± 0.055^a	14.00 ± 0.63^b
Medication control	12.33 ± 0.52^b	12.33 ± 0.78^e
CAE 300mg/KgBW (Dosage 1)	12.83 ± 1.17^a	12.17 ± 0.75^c
CAE 600 mg/KgBW (Dosage 2)	12.83 ± 0.41^{ab}	12.43 ± 0.84^d
CAE 1200 mg/KgBW (Dosage 3)	13.17 ± 0.75^a	13.00 ± 0.89^e

n=6; CAE= *Centella asiatica* extract;

a,b,c,d,e) Different notations indicated a significant difference based on the *Kruskal Wallis* test ($P < 0.05$).

control group was different from the other groups, but the same as the treatment group dosage 2. It can be seen that the polyphagia conditions that occur in rats have not been maximized. However, after 28 days there was an increase in feed intake in the positive control group and differed significantly with the other groups, as well as proving the presence of polyphagia conditions (Table 2).

The mean feed intake before and after the treatment differed significantly ($p < 0.05$). There was a significant decrease in intake of drug control rats, rats treated with extracts of *C. asiatica* dose 300 and 1200 mg/KgBW/day, but in rats given *C. asiatica* extract dose 600 mg/KgBW/day increased feed intake. There was a significant decrease in the intake of the same feed in the group of rats treated with *C. asiatica* extract in a dosage of 1200 mg/KgBW/day and given metformin of 45 mg/BW/ day (Table 2).

Centella asiatica extract increased body weight in STZ-NA induced diabetic rats

The development of body weight was monitored periodically; before the induction of STZ-NA, 3 days after the induction and declared as DM type 2 which was the beginning of the treatment day-0, day-7, day-14, day-21, and day-28 after treatment (Figure 1).

The results showed that in the negative control group, healthy rats had significant weight gain in each week ($p < 0.05$). After the induction of STZ-NA, there was significant weight loss in the positive group, medication control group, and treatment group. This indicates that the induction of STZ-NA causes weight loss in rats. After the treatment,

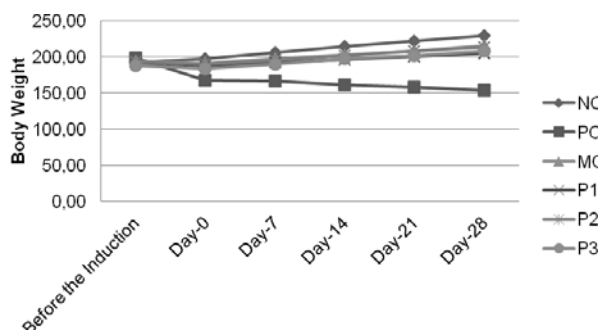


Figure 1. The mean of body weight during 28 days

the experimental group of *C. asiatica* extract and medication control group had significant weight gain until the end of the study ($p < 0.05$). While in the positive control group, the group of untreated diabetic type 2 model rats experienced significant weight loss each week ($p = 0.00$).

The mean weight of rats after the treatment for 28 days of *C. asiatica* extract with 300 mg/KgBW/day with a dosage of 1200 mg/KgBW/day, a dosage of 600 mg/KgBW/day with metformin 45 mg/KgBW/day was the same as presented in Table 3.

DISCUSSION

The laboratory rats experienced the hyperglycemia 3 days after the induction of Streptozotocin-Nicotinamide (STZ-NA) with the blood glucose level > 150 mg / dL. There were no dead rats. This was due to the effect of Nicotinamide (NA) protection against excess cytotoxic β effects of STZ so that until the research was complete there were no dead rats even on the rats with the positive control (33).

Table 3. Body weight (gram) before and after treatment

Group	Pre test (gram)	Post test (gram)	Difference (Δ) (gram)
Negative control	197.83 \pm 7.36 ^a	229.83 \pm 6.24 ^a	-32.00 \pm 1.79
Positive control	195.17 \pm 6.82 ^{ab}	179.17 \pm 6.01 ^b	16.00 \pm 1.41
Medication control	188.17 \pm 5.15 ^b	204.83 \pm 5.27 ^c	-16.67 \pm 0.52
CAE 300mg/KgBW (Dosage 1)	189.67 \pm 2.88 ^b	213.83 \pm 2.48 ^d	-24.17 \pm 1.17
CAE 600 mg/KgBW (Dosage 2)	187.17 \pm 2.32 ^b	207.83 \pm 2.40 ^c	-20.66 \pm 0.52
CAE 1200 mg/KgBW (Dosage 3)	191.17 \pm 3.06 ^b	215.17 \pm 3.71 ^d	-24.00 \pm 0.89

n=6; CAE= *Centella asiatica* extract; Delta (Δ)= the difference of average blood glucose levels before and after the treatment

^{a,b,c,d,e} Different notations indicated a significant difference based on the One-Way ANOVA test ($p < 0.05$).

The T2DM model rats were induced by using Streptozotocin-Nicotinamide because it has several advantages: (1) stable moderate hyperglycemia that does not require exogenous insulin to survive; (2) decreased β -cell by 40% and decreased insulin production by 60% (3) glucose intolerance due to the impaired insulin secretion; (4) the presence of glucose-stimulated insulin secretion (though it was disturbed); and (6) weight loss, polyphagia and polydipsia (34). The giving of *C. asiatica* extract till the dosage of 1200 mg/KgBW for 28 days did not cause death in rats. This is in line with previous study in Rahman et al. (2012), the provision of *C. asiatica* extract up to a dosage of 3000 mg/KgBW/day in laboratory rats showed no signs of acute toxicity and death (35). Before the intake of *C. asiatica* extract was done (pretest), the mean of the blood glucose level of the treatment group that was given with *C. asiatica* extract was the same as the medication control group, and the positive control group (T2D control), but different from the negative control group ($p=0.00$) (**Table 1**). It was proved that before the treatment was carried out in the group of *C. asiatica* extract treatment and medication control group experienced the same hyperglycemia condition, same as the condition in the positive control group, and the making of type 2 diabetic model rats was successfully performed. The increasing of blood glucose levels (hyperglycemia) in type 2 diabetes occurred due to the energy metabolic disturbances (glucose absorption, glucose utilization, and TCA cycles), the increasing of gluconeogenesis (the increasing of endogenous glucose production) and glycogenolysis (36).

The giving of *C. asiatica* extract dosage 300 mg/KgBW/day could decrease the blood glucose level but couldn't reach the normal limit (>150 mg/dL) (**Table 1**). This was not in line with the research of Maulidani *et al.* (2016) which states that the use of *C. asiatica* extract in a dosage of 300 mg/KgBW/day for 28 days can normalize the blood glucose levels (37). The difference of the results of this study was possible because of the differences in the inductions that were given in the making of the T2D model rats, part of *C. asiatica* that was extracted and the origin of *C. asiatica* used. Part of *C. asiatica* that

was extracted and the origin of *C. asiatica* (location and environmental condition of *C. asiatica* planted) is one of the factors that influence the quality and quantity of phenolic bioactive compounds and the *C. asiatica*'s triterpenoid (17,38,39).

The giving of *C. asiatica* extract dosage 1200 mg/KgBW/day could decrease the blood glucose level equivalent to metformin. *Centella asiatica* is a herb that is used as a traditional medicine (herbal medicine) in Indonesia and Asian countries since hundreds of years ago (12). *C. asiatica* contains a variety of chemical components, but the most is the antioxidant component of the triterpenoid group. *C. asiatica* has higher antioxidant content (89%) when it is compared to grape seed (83%) and vitamin C (88%) (11,41). The results of the phytochemical test showed that *C. asiatica* contains the antioxidant of β -carotene, β -sitosterol, cholesterol, camphor, kaempferol, stigmasterol, flavonoid saponin, and the largest are pentacyclic triterpenoid groups (asiatic acid, made classic acid, asiaticoside, and madecassoside) (16-19).

Triterpenoid contained in *Centella asiatica* is a compound that plays a role in lowering blood glucose levels by improving glucose response through increased protein GLUT4 muscle, IR, IRS 1, and IRS 2 (20-24). Giving the *C. asiatica* extracts can restore changes in carbohydrate metabolism enzyme levels, prevent lipid peroxidation, increase antioxidant status, and increase fat metabolism enzymes, thus lowering blood glucose levels and increasing plasma insulin levels. (21,25,27).

Based on **Table 2**, it was known that there was a significant change in the rats' feed intake before and after the treatment. Polyphagia in T2D occurs due to the insulin in the blood is not enough to enter the blood glucose into the cell as a source of energy, thus causing the body always feel hungry (4). The giving of *C. asiatica* extract dosage 1200 mg/KgBW/day could decrease equivalent to metformin (**Table 2**). A decrease in feed intake may be due to *C. asiatica* extract improves the polyphagia condition and increases rat's leptin hormone. Leptin is a hormone involved in regulating weight, feed intake, and energy expenditure. Giving an ethanol extract of *C. asiatica* can decrease feed intake and

increase weight in laboratory animals that have leptin deficiency (42).

Type 2 Diabetes Mellitus is a complex metabolic disorder that is marked with the case of hyperglycemia as a result of the failure of cell β pancreas so that there is a resistance for the insulin (3). T2D happens because of the disturbance of the progressive insulin secretion that becomes the main problem of a resistance to the insulin (4). Insulin secretion disorders lead to weight loss (5,6). The disturbance of insulin secretion causes the blood glucose disruption to enter the cells in the muscle and fat tissue so that the energy source is obtained from the splitting of stagnant energy that is reserved and from the fat tissue through glycogenolysis and lipolysis. The process of glycogenolysis and lipolysis that occur continuously causes the decrease of the mass of the muscle and fat tissue and leads to weight loss. (4).

The given of *C. asiatica* extract resulted in the same weight gain of rat's as metformin (**Table 3**). Weight gain occurs because *C. asiatica* extract has been shown to increase the levels of pyruvate, alanine, lactate, and crevice intermediate substances, leading to the increase of glycolysis, the decrease of gluconeogenesis and the increase of Kreb cycle' (34). The administration of *C. asiatica* extract in T2D model rats was significantly shown to restore the major enzyme activity in the increase of glycoses, such as hexokinase, glucose-6-phosphate dehydrogenase (G6PDH), and pyruvate kinase (PK) (11,21).

CONCLUSIONS AND RECOMMENDATIONS

The extracts of 1200 mg/KgBW/day of *Centella asiatica* had a better antidiabetic effect than dosage 300 and 600 mg/KgBW/day, and had the same effect with metformin 45 mg/KgBW/day. A similar study needs to be done but with longer hyperglycemia conditions before the treatment, so that the signs and symptoms of type 2 diabetes in rats are more visible.

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