



ANTIDIABETIC ACTIVITY, ALPHA-AMYLASE AND ALPHA-GLUCOSIDASE INHIBITORY EFFECT OF *PASTINACA SATIVA* EXTRACT

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Pastinaca sativa belonging to the family Apiaceae, is a tropical tree used in many countries as a herbal drug for the care of diabetic patients. However, the methodical rationale for this medical use is very limited. The aim of this analysis was to examine the antidiabetic activity of the *Pastinaca sativa* methanolic extract and the possible mechanisms underlying that activity. The extract hypoglycemic behavior was investigated in typical diabetic rats induced with alloxan. Finally, an effect of the *Pastinaca sativa* extract (Crude extract of *Pastinaca sativa*-CEPS) on the activity of α -amylase and α -glucosidase was examined in vitro. CEPS showed IC₅₀ equal to $91.69 \pm 1.5 \mu\text{g/mL}$ for α -amylase enzyme inhibition activity. Normal acarbose (control) demonstrated IC₅₀ equal to $83.25 \pm 1.28 \mu\text{g/mL}$. CEPS displayed IC₅₀ values of $88.05 \pm 1.25 \mu\text{g/mL}$ for α -glucosidase enzyme. Under similar laboratory conditions, acarbose displayed an IC₅₀ value of $51.00 \pm 1.23 \mu\text{g/mL}$. CEPS exhibited IC₅₀ less than $100 \mu\text{g/mL}$ would be considered as healthy. The extract at 400 mg/kg greatly decreased the region under the blood glucose level curve in a typical rats test for oral glucose tolerance. A single dose of the extract decreased significantly in the alloxan-induced diabetic model, similar to glibenclamide (standard), from 208.33 mg/dl to 106.38 mg/dl at 200 mg/kg CEPS and from 209.82 mg/dl to 111.65 mg/dl at 400 mg/kg CEPS. These findings were comparable with 0.5 mg/kg of glibenclamide indicating a substantial decrease from 205.55 mg/dl on 7th day to 84.88 mg/dl. The *Pastinaca sativa* methanolic extract possesses strong antidiabetic activity in vivo. Besides, the extract has also been shown to have a significant inhibitory activity of α -amylase and α -glucosidase, which might lead to its anti-hyperglycemic function when used in diabetic patients.

INTRODUCTION

Diabetes mellitus, one of the most common metabolic disorders, was estimated to affect 415 million people globally in 2015, with that number expected to rise to 642 million by 2040¹. Patients of chronic hyperglycemia are at an increased risk of both macrovascular (heart attacks, peripheral arterial disease, and/or stroke) and microvascular

complications such as retinopathy, nephropathy, and neuropathy². Free radicals and reactive oxygen species can be generated by cells when blood glucose levels are elevated. As a result of the accumulation of free radicals, disruption to cellular macromolecules such as lipids, proteins, and nucleic acids occurs, resulting in diabetes progression and complications³. Plants derived natural products were classified as naturally

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occurring antioxidant compounds^{4&5}. It also appears that plant extracts and their derivatives have many other pharmacological functions including antimicrobial, anticancer, anti-inflammatory and anti-diabetic function.

As a result, traditional medicines have been used as a vital source of new medications to treat a number of disorders, including diabetes mellitus⁶. *Pastinaca sativa* belongs to the family Apiaceae⁷. Experimental documents reports that *Pastinaca sativa* have polyacetylene, furanocoumarin chemical constituents this are having medicinal properties^{8&9}. The *Pastinaca sativa* is sparsely distributed in India, W. Peninsula, Ceylon, and China. It is present in the tropical Himalayas in India, Burma, Andhra Pradesh, and the state of Telangana. This plant is historically used as an antibacterial^{10&11}. While some of the plants are reputed for their activities in the indigenous medicine systems¹², further scientific assessment is still needed. Additionally, the antidiabetic ability effects on α -amylase, α -glucosidase activities were also explored as potential pathways causing the extract.

MATERIALS AND METHODS

Plant extraction

The *Pastinaca sativa* (PS) plant was collected from the Balaji Institute of Pharmaceutical Sciences, Laknepally, Warangal, Telangana State, India's medicinal gardens in July, 2018. The plant authenticated by Professor Rana Kausar, Department of botany, Osmania University, Hyderabad, State of Telangana. The entire collected dried plant was powdered in an electronic grinder. The coarse powder was subjected to methanol extraction (CEPS) in Soxhlet apparatus for about five cycles. After that, the liquid is cleaned, dried in a desiccator, and processed for further use.

Chemicals and reagents

Alloxan and glibenclamide had been acquired by Sisco Research Laboratories Pvt Ltd. The blood glucose levels were measured using M/s Boehringer Mannheim, India Ltd's Haemo-Glukotest (20-800 R) glucose strips. Many of the chemicals and reagents used in the analysis were of high quality.

Preliminary phytochemical screening

Diverse phytoconstituents such as alkaloids, flavonoids, steroids, tannins, glycosides, triterpenoids, and saponins were screened for *Pastinaca sativa*¹³.

Animals

Male Wistar rats weighing 150-200 g were collected. The Institutional Animal Ethics Committee of Balaji Institute of Pharmaceutical Sciences, Laknepally, Narsampet, Warangal, TS, has approved the animal protocol (CPCSEA/IAEC/BIPS/2019/1/1). For acclimatization, animals were given a pelleted diet and free access to water at a temperature of 25°C and a relative humidity of 45-55%, with 12 hrs. for each of the dark and light phases.

Examination of the effect on α -amylase activity

The α -amylase process was carried out using the starch-iodine system¹⁴⁻¹⁶. A 390 μ L phosphate buffer (0.02 M comprising 0.006 M NaCl, pH 7.0) containing different amounts of the extract was combined with 10 μ L of α -amylase solution (0.025 mg/mL). After 10-minute incubation at 37°C, 100 μ L of 1% starch solution was applied and the mixture was re-incubated for 1 hr. The absorbance at A₅₆₂ nm was measured after 5mL of purified water was applied, followed by 0.1liters of one percent iodine solution. The same reaction conditions were used for α -amylase testing, substratum determinations, and blank determinations. The percentage of enzyme activity inhibition was determined using the formula (percent) = (A-C) X100/(B-C), where A represents sample absorption, B represents blank absorption (without α -amylase), and C represents absorption regulation (without starch).

Examination of the effect on α -glucosidase activity

As already reported¹⁷ the inhibitory function of α -glucosidase has been measured. In brief, a mixture of 75 μ L of α -glucosidase (Sigma-Aldrich, USA), 225 μ L of 80 mM buffer of phosphate pH 7.0, and 10-100 μ L of various extract concentrations or acarbose inhibitor α -glucosidase (Fluka, USA) were incubate at 37°C for 10min. The absorbance was measured

optically by using a spectrophotometer at A_{510} nm. Effects are defined as the concentration at which α -glucosidase development is inhibited by 50 percent (IC_{50}).

Acute toxicity studies

Health adult female rats were divided into 3 groups (n= 5) and were starving overnight. The rats were administered orally with a reducing dosage level of the *Pastinaca sativa* extract (5000, 1750 mg/kg) (OECD Guidelines No. TG425), and one group was retained as a control. The animals were monitored regularly for 4 hrs. at a 30 min. interval under a behavioural, physiological, and autonomic profile that included toxicity and mortality, as well as occasionally for any symptoms of acute toxicity up to 14 days after 6 hrs. and then 24 hrs. thereafter.

Oral glucose tolerance test in normal rats

The effect of CEPS on blood glucose levels was initially tested using an oral glucose scale. Standard rats fasted for 6 hrs. were randomly divided into four separate classes (n= 6), as follows. Group-1: animals receiving oral glucose 2 gm/kg; Group-2: animals receiving oral glibenclamide 0.5 mg/kg and glucose solution 2 gm/kg; Group-3: animals receiving oral CEPS 20 0mg/kg and oral glucose solution 2 gm/kg; Group-4: animals receiving oral CEPS 400 mg/kg and oral glucose solution 2 gm/kg^{18& 19}.

Anti-diabetic activity in alloxan-induced diabetic rats

Fasted rats were given a 150 mg/kg intraperitoneal injection of alloxan monohydrate dissolved in a cold 0.85 percent saline solution to induce type II diabetes²⁰. Diabetic induction was assessed after three days of alloxan injection. Class II diabetic rats were used in the trial and had glucose levels above 200 mg/dl (survived without insulin)²¹. The rats were divided into five classes at random, as follows. Group-1: normal control animals receiving 2 ml/kg 1 percent oral NaCMC; group-2: alloxan (150 mg/kg) diabetic animals receiving 2 ml/kg 1 percent oral

NaCMC; group-3: alloxan (150 mg/kg) diabetic animals receiving glibenclamide 0.5 mg/kg orally; group-4: alloxan (150 mg/kg) diabetic animals receiving CEPS 200 mg /kg orally in 1 percent NaCMC; group-5: alloxan (150 mg/kg) diabetic animals receiving CEPS 200 mg/kg orally in 1 percent. Blood glucose levels were measured at 0, 1, 2, 4, and 6 hrs. after a single dose of drug administration to ascertain the extract's acute anti-diabetic effect, as seen in previous research²² dividing the rats into seven groups of six rats each.

Statistical analysis

Values have been expressed as mean \pm SEM (n= 6). One-way variance analysis (ANOVA) and the Dunnett test were included in the statistical analysis. Origin pro Software was found to be statistically significant at $p < 0.001$.

RESULT AND DISCUSSION

Acute toxicity studies

Acute oral toxicity tests have shown the non-toxic nature of neither CEPS nor any deep toxic reactions detected at a dosage of 5000 mg/kg b.wt, p.o. was observed. That implicitly pronounces a plant extract safety profile. The high oral lethal dose values of PS (LD_{50} value > 5000 mg kg^{-1} b.wt., p.o.) indicate its low acute toxicity.

Preliminary phytochemical analysis

The existence of alkaloids, flavonoids, steroids, glycosides, triterpenoids, and saponins in CEPS was shown by a preliminary phytochemical study.

Examination of the effect on α -amylase activity

In the present study, CEPS displayed significant concentration-dependent inhibition of the α -amylase enzyme. CEPS exhibited IC_{50} less than 100 μ g/mL will be considered as healthy. The CEPS showed IC_{50} 91.69 ± 1.52 μ g/mL enzyme inhibition activity (slope $y = 0.265X + 25.7$). Under identical laboratory conditions (Figure-1), regular acarbose showed an IC_{50} of 83.25 ± 1.28 μ g/mL (Slope; $y = 0.451x + 12.45$)

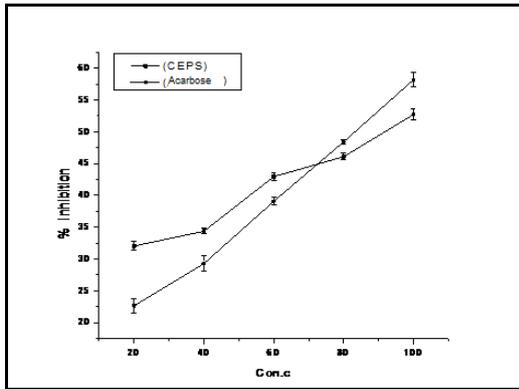


Fig.1: α -amylase inhibition activity of CEPS and acarbose All values were reported as Mean \pm S.E.M (n= 6)

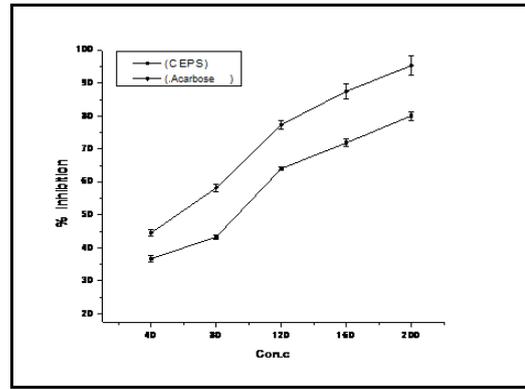


Fig. 2: α -glucosidase inhibition activity of CEPS and acarbose All values were reported as Mean \pm S.E.M (n= 6)

Examination of the effect on α -glucosidase activity

The α -glucosidase enzyme is one of the products of diabetes control drugs. The enzyme requires polysaccharide conversion into monosaccharide, which can be consumed by the intestine. Of the plant extracts analyzed, IC₅₀ was less than 100 μ g/mL. CEPS demonstrated IC₅₀ levels of 88.05 \pm 1.25 μ g/mL ($y = 0.288x + 24.64$) inhibition of the enzymes. At related laboratory conditions, acarbose displayed an IC₅₀ of 51.00 \pm 1.23 μ g/mL ($y = 0.327x + 33.32$) (Figure-2).

Oral glucose tolerance test in normal rats

Blood sugar levels in glucose-fed rats were measured at different periods at dose 200 mg/kg and 400 mg/kg of CEPS (Figure-3). The mean blood glucose level in rats treated with CEPS decreased from 86.89 mg/dl to 85.45mg dl at 200 mg/kg body weight and from 92.87 mg/dl to 85.41 mg/dl at 400 mg/kg dosage. CEPS or glibenclamide has been shown to have greatly increased resistance to glucose in normal rats. Rats administrated CEPS (400mg/kg) and glibenclamide (2 mg/kg) significantly showed reduced in glucose elevation level after 30 min relative to the control group ($P < 0.05$).

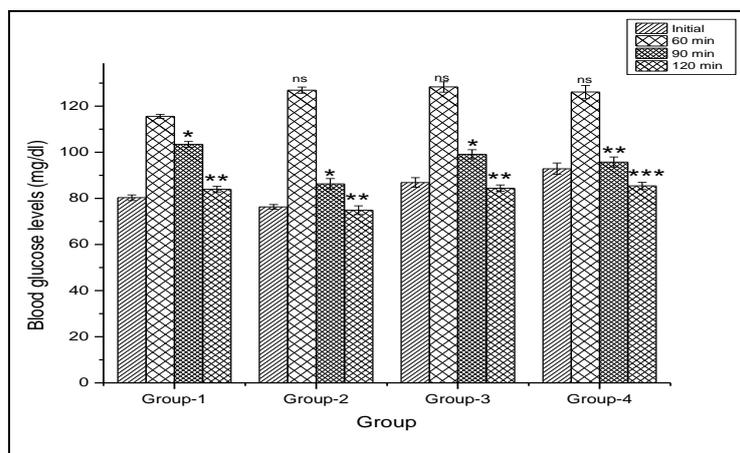


Fig. 3: Effect of CEPS on oral glucose tolerance test

Each value represents the mean \pm SEM. n= 6 number of animals in each group. ^a $P < 0.001$ vs vehicle control, * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$, Compared to respective CEPS treated control groups.

Group-1: animals receiving oral glucose 2 gm/kg; **Group-2:** animals receiving oral glibenclamide 0.5 mg/kg and glucose solution 2gm/kg; **Group-3:** animals receiving oral CEPS 200mg/kg and oral glucose solution 2 gm/kg; **Group-4:** animals receiving oral CEPS 400mg/kg and oral glucose solution 2 gm/kg

Antidiabetic effect of CEPS extract in alloxan diabetic rats

The Antidiabetic effect of the different concentration of the CEPS on diabetic rat's blood sugar levels was seen in Figure-4. Blood glucose levels lowered from 208.33 mg/dl to 106.38 mg/dl at CEPS 200mg/kg and from 209.82mg/dl to 111.65 mg/dl at CEPS 400 mg/kg. These findings were comparable with 0.5 mg/kg of glibenclamide indicating a substantial decrease from 205.55 mg/dl on 7th day to 84.88 mg/dl.

The α -amylase inhibitors are essential for preventing glucose release from the dietary supply of carbohydrates and increasing glucose absorption, resulting in lower postprandial plasma glucose levels²³. The care goal for diabetic patients is to ensure adherence to normal glycemic regulation thresholds in both fasting and post-prandial circumstances. Several natural sources were used to examine

the inhibition of glucose development from carbohydrates in the gut or the removal of glucose from the intestine. Alpha-1, 4-glycosidic hydrolysis interactions with starch, glycogen, and various oligosaccharides are catalyzed by α -amylase. In addition, alpha-glucosidase breaks down disaccharides into basic sugars that are quickly consumed in the intestines. Rao et al. 2017²⁴ found that minimizing their involvement in the human digestive tract is an important method for treating diabetes. CEP inhibits alpha-amylase and alpha-glucosidase effectively, according to our findings. The results of this research backed up the traditional case for herbal anti-diabetic action. As a result, the author expanded the study to include acute toxicity tests and *in-vivo* models to promote antidiabetic activity. The findings of this study backed up the conventional hypothesis of herbal antidiabetic action.

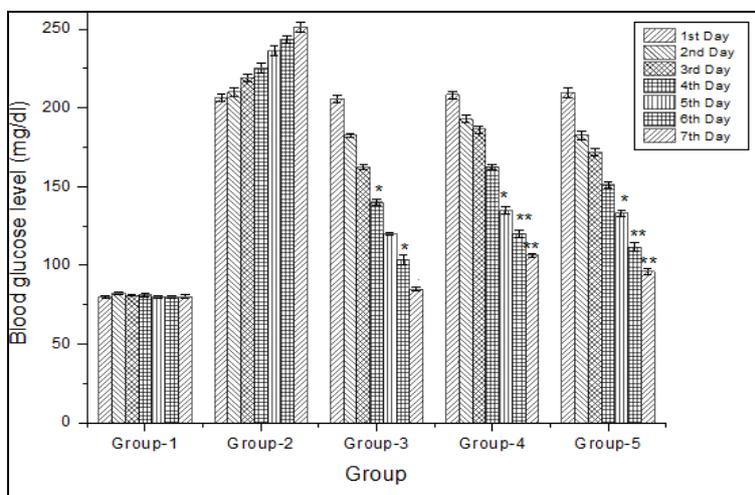


Fig.4: Effect CEPS on blood glucose levels in diabetic rats

Each value represents the mean \pm SEM. $n = 6$ number of animals in each group. ^a $P < 0.001$ vs vehicle control, ^{*} $P < 0.05$, ^{**} $P < 0.01$, ^{***} $P < 0.001$, Compared to respective CEPS treated control groups.

Group-1: normal control animals receiving 2 ml/kg 1 percent oral NaCMC; **Group-2:** alloxan (150 mg/kg) diabetic animals receiving 2 ml/kg 1 percent oral NaCMC; **group-3:** alloxan (150 mg/kg) diabetic animals receiving glibenclamide 0.5 mg /kg orally; **group-4:** alloxan (150 mg/kg) diabetic animals receiving CEPS 200 mg / kg orally in 1 percent NaCMC; **group-5:** alloxan (150 mg/kg) diabetic animals receiving CEPS 200 mg/kg orally in 1 percent.

Diabetes is a prevalent metabolic condition that can cause multiple organ damage and symptoms²⁵. Increased postprandial glucose levels raise the risk of cardiovascular disease, which is the leading cause of mortality among diabetics. Micro-vascular injury can also be caused by postprandial surges due to oxidation of low density lipoprotein (LDL) and a pro-atherogenic mechanism²⁶. Controlling blood glucose levels is an important technique for managing diabetes and associated consequences. Diet rich in carbohydrate in food is rapidly absorbed in the gut with the help of the α -amylase and α -glucosidase enzymes, which break carbohydrate down to simple absorbable sugars (monosaccharides), a high-carbohydrate diet produces a fast rise in blood glucose levels²⁶. Oral hypoglycemic medications that inhibit saccharide hydrolyzing enzymes (α -amylase and α -glucosidase) have been found to be effective in the treatment of hyperglycemia, particularly in individuals with Type II diabetes²⁷. These inhibitors slow carbohydrate digestion and lengthen total carbohydrate digestion time, resulting in a slower rate of glucose absorption and a lower postprandial plasma glucose increase²⁸. Synthetic hypoglycemic medicines like acarbose, miglitol, and voglibose are used in combination with other anti-diabetic treatments, however these inhibitors have been linked to gastrointestinal side effects such as stomach pain, flatulence, and diarrhea. As a result, there is a rising interest in finding novel and efficient α -amylase and α -glucosidase inhibitors from plants those have little or no adverse effects²⁹.

The anti-diabetic activity of CEPS was investigated in this study on stable and alloxan-diabetic rats. An oral glucose tolerance test was used to assess the extract's hypoglycemic effect in standard rats. A 6-hrs. quick is considered to be the perfect time for rats to develop an oral glucose tolerance test (OGTT)^{30, 31}. The rise in blood glucose in each group reported the successful loading of oral glucose after 30 min. The antidiabetic medication glibenclamide used as a supportive aid in this research decreases postprandial hyperglycemia by increasing insulin release from the β cell. Based on our preliminary review the dosage for oral administration was selected at 400 mg / kg.

As compared to a vehicle control, the extract and glibenclamide increased glucose tolerance. The antidiabetic activity of CEPS may be attributed to the flavonoid effect. The successful biological concepts of most hypoglycemic and antidiabetic medicinal plants are considered to be flavonoids³². If the antidiabetic activity of CEPS, near that seen in glibenclamide, may be attributed to increased insulin secretion remains to be seen. However, the extract should be further subject to bioactivity-guided drug development to isolate the lead compound responsible for the antidiabetic activity and alternative modes of action.

Conclusion

In conclusion, the *Pastinaca Sativa* methanolic extract possesses strong antidiabetic activity in vivo. In addition, the extract displayed a significant inhibitory activity of α -amylase and α -glucosidase which may lead to its anti-hyperglycemic function when used in diabetic patients. The findings obtained in this analysis provide empirical evidence for corroborating the use of *Pastinaca sativa* for the conventional treatment of diabetics. Besides, molecular experiments and isolation of the active ingredient in the extract are strongly required to elucidate the plant's mechanism of action.

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نشرة العلوم الصيدلانية جامعة أسيوط



النشاط المضاد لمرض السكري لمستخلص نبات البستيناكا ساتيفا و تأثيره المثبط على ألفا أميلاز وألفا جلوكوزيداز

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براسانس دس ن ب ك^١ - شاكرا فارسي ج^١

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^٢ قسم الفارماكولوجي ، معهد بالاجي للعلوم الصيدلانية ، لكانبالي ، نارسامبت ، وارانجال ، تيلانجانا ، الهند

٥٠٦٣٣١

ينتمي نبات البستيناكا ساتيفا إلى العائلة الخيمية، وهي شجرة استوائية تستخدم في العديد من البلدان كدواء عشبي لمرضى السكري. ومع ذلك، فإن الأساس العلمي المنهجي لهذا الاستخدام الطبي محدود للغاية. كان الهدف من هذا التحليل هو فحص النشاط المضاد لمرض السكري لمستخلص الميثانول من باستيناكا ساتيفا والآليات المحتملة وراء هذا النشاط. تم فحص نشاط الخلاصة كخافضة لسكر الدم في الفئران المصابة بداء السكري والتي تم اصابتها باستخدام الألوكسان. أخيراً، تم فحص تأثير مستخلص البستيناكا ساتيفا (المستخلص الخام من البستيناكا ساتيفا - س ي ب س) على نشاط ألفا أميلاز وألفا جلوكوزيداز في المختبر. س ي ب س أظهر قيم التثبيط النصفية (IC50) يساوي ٩١.٦٩ ± ١.٥ ميكروجرام/مل لنشاط تثبيط إنزيم ألفا أميلاز. أظهر أكاربوز (كعنصر تحكم) IC50 يساوي ٨٣.٢٥ ± ١.٢٨ ميكروجرام/مل. س ي ب س أظهر قيم IC50 تبلغ ٨٨.٠٥ ± ١.٢٥ ميكروجرام/مل لإنزيم ألفا جلوكوزيداز. في ظل ظروف معملية مماثلة، عرض أكاربوز قيمة IC50 تبلغ ٥١.٠٠ ± ١.٢٣ ميكروجرام/مل. أظهر س ي ب س أن IC50 أقل من ١٠٠ ميكروجرام/مل يعتبر صحيحاً. يقلل المستخلص عند ٤٠٠ مجم / كجم بشكل كبير من المنطقة الواقعة تحت منحنى مستوى السكر في الدم في اختبار الفئران لتحمل الجلوكوز عن طريق الفم. خفضت جرعة واحدة من المستخلص بشكل ملحوظ مستوى السكر في نموذج السكري والتي تم اصابة الفئران باستخدام الألوكسان، بالمقارنة بالجليبينكلاميد (مرجع)، من ٢٠٨.٣٣ مجم / ديسيلتر إلى ١٠٦.٣٨ مجم / ديسيلتر عند ٢٠٠ مجم / كجم من س ي ب س ومن ٢٠٩.٨٢ مجم / ديسيلتر إلى ١١١.٦٥ مجم / ديسيلتر عند ٤٠٠ مجم / كجم من س ي ب س. كانت هذه النتائج مقابلة لنتائج ٠.٥ مجم / كجم من الجلبيبيكلاميد مما يشير إلى انخفاض كبير من ٢٠٥.٥٥ مجم / ديسيلتر في اليوم السابع إلى ٨٤.٨٨ مجم / ديسيلتر. أظهرت النتائج لمستخلص الميثانول باستيناكا ساتيفا نشاطاً قوياً مضاداً لمرض السكر في الجسم الحي. إلى جانب ذلك، تبين أيضاً أن المستخلص له نشاط مثبط كبير ألفا أميلاز وألفا جلوكوزيداز، مما قد يؤدي إلى تخفيضه لمستوى السكر في مرضى السكري.