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Evaluation of antidiabetic effect of total calystegines extracted from *Hyoscyamus albus*

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ABSTRACT

Background: Hyoscyamus albus L. (*Solanaceae*) an old medicinal plant is a rich source of tropane and nortropane alkaloids which confers to this plant a number of very interesting and beneficial therapeutic effects.
Purpose: Calystegines that are polyhydroxylated alkaloids and imino-sugars poccess significant glycosidases inhibitory activities and are therefore good candidats for the treatment of diabetes mellitus.
Study design: Calystegines extracted from *Hyoscyamys albus* seeds were tested for teir acute oral toxicity and investigated for their *in-vivo* antidiabetic effect on Streptozotocine induced diabetes in mice.
Methodes: Calystegines were extracted from the seeds plant using an Ion exchange column; the remaining extract was then administrated orally to mice at several single doses for acute toxicity assay. A dose of 130 mg/kg streptozotocine was injected to mice to induce diabetes mellitus, and diabetic mice were treated orally during 20 days with 10 mg/kg and 20 mg/kg calystegines and 20 mg/kg glibenclamide as the reference drug.
Results: Acute oral toxicity showed that calystegines are not toxic up to a dose of 2000 mg/kg with absence of any signs of intoxication and damages in Liver and kidney tissues.
The nortropane alkaloids markedly reduced blood glucose levels and lipid parameters of diabetic mice to normal concentrations after 20 days of treatment at 10 mg/kg and 20 mg/ kg ($p < 0.05$). Histopathological study of diabetic mice pancreas indicated that calystegines of *Hyoscyamus albus* have minimized streptozotocine damages on β-cells of islets of langerhans, stimulated β-cells regeneration and improved with this insulin secretion.
Conclusion: The findings of this study suggest that calystegines are potent antidiabetic agents with antihyperglicemic and hypolipidemic effects, and a protective fonction on pancreas in streptozotocin induced diabetes in mice.

1. Introduction

Diabetes mellitus (DM) is a chronic and complex metabolic/endocrine disorder, resulting from insulin deficiency, characterized by abnormal increase in the blood sugar level, altered metabolism of carbohydrates, protein and lipids, and an increased risk of vascular and renal complications [1].

More than 90% of the cases are type II diabetes, which is characterized by insulin resistance in target tissues and progressive insulin secretory dysfunction. This metabolic disease often leads to microvascular and macrovascular complications, such as retinopathy, neuropathy, nephropathy, atherosclerosis, and heart disease [22].

Postprandial hyperglycemia plays an important role in the development of this pathology and complications associated with the disease; therefore the control of postprandial hyperglycemia is suggested to be important in the treatment of diabetes and prevention of cardiovascular complications [8].

Though different types of oral hypoglycemic agents are available along with insulin for the treatment of diabetes mellitus, there is a growing interest in herbal remedies due to the side

Abbreviations: ALAT, alanine amino transferase; ANOVA, analysis of variance; ASAT, aspartate amino transferase; Caly, calystegines; CCK, *Cholecystokinin*; DM, diabetes mellitus; DNJ, 1-Deoxynojirimycin; EC, enzyme comission; GL, amylo-1,6-glucosidase; Gib, Glibenclamide; GP, glycogen phosphorylase; HDL, high density lipoprotein; IC_{50}, inhibition concentration at 50%; LDL, low density lipoprotein; LSD, least significant difference; OECD, Organization for Economic Cooperation and Development; OGTT, Oral glucose tolerance test; PLA, phosphatase alkaline; STZ, streptozotocin; TRG, triglycerides; VLDL, very low density lipoprotein.

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effects associated with these therapeutic agents. The investigation of anti-diabetic agents of plant origin which are used in traditional medicine is thus of great importance [11].

Hyoscyamus albus L. or white henbane is a Mediteranean plant belongs to *Solanaceae* family which ranks as one of the most important to human beings. All *Hyoscyamus* species are rich sources of tropane alkaloids, mainly hyoscyamine and scopolamine, which are used for their mydriatic, antispasmodic, anticholinergic, analgesic and sedative properties [18].

Recently, a new groupe of polyhydroxylated nortropane alkaloides named calystegines have been isolated from different species of *Solanaceae* like in *Datura*, *Atropa* and *Hyoscyamus* [13,2].

The discovery of polyhydroxy alkaloids, otherwise known as imino-sugars, raised an importante interest of those compounds with structural similarities can pocess glycosidase-inhibitory properties in a reversible and competitive manner by mimicking the pyranosyl or furanosyl moiety of their natural substrates [17,27].

The aim of the present study is accessed on the investigation of the possible antidiabetic effect of calystegines extracted from Algerian *Hyoscyamus albus* seeds using an *in-vivo* experimental model of diabetes induced by Streptozotocine in mice.

2. Materials and methods

2.1. Plant material

Hyoscyamus albus seeds were collected from wild plants growing in Bejaia (Algeria) in August 2014. Seeds were dried in a ventilated room ($30 \pm 3\,^{\circ}C$) and then ground to a fine powder.

2.2. Chemicals

Solvents used for extraction and GC–MS analysis were from HPLC grad, resins (Amrelite IR 120B, H^+, Dowex 1×2, Cl^-), Streptozotocin (≥97%), Glibenclamide (≥99%) and Glucose (≥99.5%) were purshased from Sigma Aldrich (Barcelona, Spain), other reagents used for diagnostic were from analytical grad and purchased from Roche Diagnostics France (Meyla, France).

2.3. Extraction of total calystegines

Total calystegines were isolated according to the procedure described by Bekkouche et al. [6]. Briefly, 50 g of *Hyoscyamus albus* powdered seeds where defated with 250 ml petroleum ether priore to hydroalchoolic extraction.

Crud extract was obtained by macerating three times defated seeds powder in 250 ml aqueous methanol (50/50; v/v) for 24 h. The remaining dried extract was then applied to a cation exchange column (Amberlite IR 120B, H^+ form). The column was washed with water to remove non-binding contaminants. Calystegines and bound compounds were eluted with 2 N NH_4OH. The concentrated residue was then applied to an anion exchange column (Dowex 1×2, Cl^- form) and eluted with water to obtain a purified plant extract that contains total calystegines with an extraction yield of 0.32%.

2.4. Gas chromatography–mass spectrometry analysis

GC–MS was employed to characterize the calystegines of *Hyoscyamus albus* seeds extract using the modified method of Bekkouche et al. [6]. After derivatization with 50 μl of trimethylsilyltrifluoroacetamide (MSTFA), a GCMS-QP2010 plus system (Shimadzu, Kyoto, Japan) equipped with a DB–5 ms column (30 m × 0.25 mm I.D. × 0.25 μm df, Quadrex Corporation, Woodbridge, CT) was used. The separation was performed according to the following oven temperature program: initial temperature was 100 °C and held for 5 min, then it was raised to 300 °C at 10 °C/min, and this value was maintained for 5 min. The injection volume was 0.5 μl in split mode (split ratio 1:10) with the injector temperature at 250 °C.

The carrier gas was He at 36.5 cm/s. The MS detection parameters were: interface temperature, 280 °C; Ion source temperature, 250 °C; mass range, *m/z* 50–600; scan speed, 2500 amu/seg; and event time, 0.20 seg. The data collection and handling were performed using the GCMS solution (ver. 2.50SU3, Shimadzu) software.

2.5. Animals

Male and female Albino mice weighting from 20 to 25 g were purchased from Paster Institute in Algiers (Algeria).

Animals were housed in standard cages at a controlled room (temperature $25 \pm 3\,^{\circ}C$ and humidity $50 \pm 10\%$) and were kept on a 12 h light/dark cycle. They were fed with standard diet and water *ad libitum* and acclimated 10 days before they were used and fasted for 12 h before testing.

All experiments were approved by the Animal Ethics Committee of the University of Bejaia (Algeria) and conducted in strict compliance with internationally accepted principles for laboratory animals (directive N° 2010/63/EU of 22 September 2010 which updates and replaces directive N° 86/609/EEC of 24 November 1986).

2.6. Acute toxicity

Acute oral toxicity of *Hyoscyamus albus* seeds calystegines was tested according to acute-toxic-class method guideline n°423 of the Organization for Economic Cooperation and Development [20].

Thirty six Albino female mice were devised into six groups each containing six animals. Calystegines extract was administered orally at single doses of 5, 20, 50, 300 and 2000 mg/kg. Saline solution was administered to the control group.

The animals were observed individually during 4 h following the administration and at least twice daily to assess the number of deaths and possible toxicological symptoms during 14 days.

At the 15th day of experiment, mice were anesthetized, sacrificed, blood was collected in heparinized tubes to realise hepatic and renal function tests using an *Architect ci4100*® instrument (*Abbott Diagnostics*, Illinois, U.S.A), liver and kidney were removed to perform histopathological studies.

2.7. Hypoglycemic activity: Oral Glucose Tolerance Test

Four groups (n = 6) of healthy Albino male mice were used for oral glucose tolerance test. All animals were fasted 12 h prior to the experiment; calystegines were administrated at 10 mg/kg and 20 mg/kg 30 min before oral administration of 2 g/kg glucose.

Group control received only salin and Glibenclamide at 20 mg/kg was used as reference drug.

Blood glucose level was mesured at 0, 30, 60, 90 and 120 min after glucose administration using a standard glucometer (*OneTouch*® *Ultra*®, LifeScan Canada).

2.8. Antihyperglycemic activity in streptozotocin-induced hyperglycemia model

2.8.1. Induction of experimental diabetes

Hyperglycemia was induced by intraperitoneal injection of Streptozotocin at a dose of 130 mg/kg dissolved in 0.1 M citrate buffer, pH 4.5 on healthy and overnight fasted Albino male mice.