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Evaluation of antidiabetic potential of steroidal alkaloid of *Sarcococca saligna*

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ABSTRACT

The demand for natural medicines has increased because of their limited adverse effects. The aim of study is to explore the antidiabetic potential of isolated steroidal alkaloid from *Sarcococca saligna* in streptozotocin induced diabetic rats. To determine the antidiabetic activity of steroidal alkaloids, diabetes was induced in rats by injecting streptozotocin intraperitoneally at a dose of 40 mg/Kg. After a week of STZ injection the treatment were started and the 8th day was considered as the 1st day of treatment and up to four weeks the rats were treated with steroidal alkaloids. Animals were divided into five groups, group 1 considered as a control group by receiving normal saline (1 ml/Kg) twice daily and group 2, 3, 4 were treated with active compound sarcovagine-D, saracodine and holaphylline at the dose of 5 mg/Kg subcutaneously twice a day while group 5 was treated with a standard drug glibenclamide at a dose of 1 mg/Kg/day. The result showed that treated group 2 and 4 reduced the glucose level in blood significantly while group 3 showed moderate glucose reduction. The fructosamine level reduced significantly in treating group 4 from the 2nd week of treatment while group 2 and 3 decreased the level significantly in week 4 in diabetic rats. The treated groups showed gradual decreases the glucose level in 1st and 2nd week of oral glucose tolerance test compared to control group. The group receiving holaphylline **(4)** and sarcovagine-D **(2)** showed good improvements in blood lipids while the effect of compound on body weight showed less significant improvement. The present study concluded that steroid alkaloids from isolated *Sarcococca saligna* possess hypoglycemic effect and improve others diabetes associated complications. Together these finding further research is needed using a range of doses to explore the other possible beneficial effects in diabetes mellitus and its molecular mechanism.

1. Introduction

Diabetes mellitus is characterized by chronic metabolic alteration of glucose that worldwide affected more than 347 million population [1]. Management of diabetes is one of the world health problem due to lack of proper and successful treatment until now has been discovered [2]. Previous study reported high level of blood glucose as a result of inadequate insulin production as well as their activity. Currently, oral

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antidiabetic agents and injectable insulin used for the treatment of diabetes mellitus produces severe adverse effects to human body such as lactic acidosis, liver and renal complications [3] and that's why alternative treatment such as herbal medicines are getting popular for treating diabetes. There are about 800 plant species which possess antihyperglycemic property are being in practice in Asia, Native and South America for treatment [4]. In recent years, herbal medicines have been investigated for their utility in the treatment of diabetes. The use of herbal medicine increases in the management of diabetes in both developing and developed world because of its cost effective [5–7]. The secondary metabolites such as alkaloids, phenols, flavonoids, terpenoids and glycosides isolated from plants have shown positive biological activity. Drugs commonly used for treating diabetes such as metformin and insulin secretagogues are also the isolated from plant origin [8]. The active compounds isolated from plants decrease enzyme actions and other parameters of blood which causes hyperglycemic condition [9].

Sarcococca saligna (D.Don) Muel belong to Buxaceae family is an evergreen dicotyledon shrub with scaly buds, found at altitudes of 4000–9000 feet above the sea level in northern regions of Pakistan. Traditionally *S. saligna* shoots and leaves were used for stomach disorder, blood disorder and also for muscles pain [10]. Steroidal alkaloids isolated from these species were pharmacologically active and has shown different biological activities. The extract fractions of *S. saligna* has been used widely against pain, malaria, rheumatism and skin infections diseases [11]. Steroids alkaloid isolated from *S. saligna* have been used to treat diarrhoea and hypersecretion in mice [12]. Steroidal alkaloids isolated from *S. saligna* have been found to possess antibacterial, antileishmanial and potent acetylcholinestrase inhibitors which can be used in several nervous disorders [11] Some steroidal alkaloids from *S. saligna* have showed hepatoprotective potential [13]. Keeping in view the reported biological significance of *Sarcococa saligna* it has been further investigated in diabetes. Therefore the aim of this study is to evaluate antidiabetic potential of isolated steroidal alkaloids from *Sarcococa saligna*.

2. Material and method

2.1. Plant material

Sarcococca saligna (D.Don) Muel (40 Kg) was collected in June 2014 from Miandam, District Swat, Khyber Pukhtoonkhwa, Pakistan. The plant was identified by Dr. Jilani, a botanist at Department of Botany, University of Peshawar, Pakistan and specimen voucher (But.20098 (pup) was submitted in herbarium section of Botany Department.

2.2. General procedures

Analytical grade reagents were used in chromatography techniques. Different column sizes with alumina (Al_2O_3) and silica gel (SiO_2) were used in chromatography. TLC plates (Merck GF-254) with pre-coated SiO_2 and Dragendroff,s reagent spray was used for visualization. Hitachi UV-3200 spectrophotometer, IR Jasco A-302 model spectrophotometer, Mass spectrometer model Jeol HX-110, Bruker Avance AM-400 and AC-300 NMR instrument were used for structure elucidation of compounds.

2.3. Extraction and isolation

Plant were collected, shade dried and crushed in to powder then was soaked in to MeOH/H_2O mixture ratio 8:2 of 35 liters for 20 days. The methanolic extract was filtered under vacuum and become concentrated (2 Kg). The concentrated methanolic extract was then soluble in distilled water (2 L). The mixture was then defatted with *n*-Haxane (254 gm) to remove the fatty material followed by the extraction of water layer with chloroform at a pH.6 to obtain chloroform fraction (200 gm). The rest of aqueous fractions extracted with ethyl acetate (150 gm) and butanol (100 gm) respectively.

The chloroform fraction was then subjected for further elution through over neutral Al_2O_3 column chromatography. The elution took place by increasing polarities of *n*-Hexane/ ethyl acetate/ diethlyamine to get three different fractions F1 to F3. The fractions F2 (3.8g) were further subjected individually on neutral Al_2O_3 column chromatography. The elution took place by increasing the polarities of solvents *n*-hexane/ethyl acetate/ with a few drops of diethylamine to afford a compound NA-8 (180 mg). Similarly the purified compound NS-55 (220 mg) and NF73-31(135 mg) isolated through elution over neutral Al_2O_3 column chromatography by increasing solvent system polarities of *n*-hexane/ethyl acetate with a few drops of diethyl amine.

NA- 8 compound was isolated which was physically appeared as a white crystalline solid with melting point 173 °C. The spectral data showed that the compound NA-8 (Sarcovagine-D) is known and isolated previous from the *Sarcococa vagans* [14]. Similarly, compound NS-55 apparently seems like white amorphous powder with melting point 242–244 °C. From the analysis of spectral data showed that the compound NS-55 (Saracodine) was known and isolated previous from *Sarcococa saligna* [15]. Compound NF73-31 occurs as a sticky light yellowish powder with melting point 125 °C. The spectral data analysis showed that the compound NF-73-31 (holaphylline) is known but first time isolated from this species and previously reported from *Holarrhena floribunda* plant [16].

2.4. Experimental animals

Male albino rats, 5–7 weeks old, were divided into 5 groups with 10 animals per group. Rats were fed a standard rodent diet. The animals were maintained in a 12 h light/dark cycle at a constant temperature (25 °C), allowed adaptation for one week before carrying out the experiment. The experimental work was approved by the animal ethical committee of the Centre of Biotechnology and Microbiology, University of Peshawar.

2.5. Induction of diabetes

The albino rats were kept in a fasting condition for a whole night and then made diabetic by injecting streptozotocin intraperitoneally, prepared freshly (3 mM) in citrate buffer with a pH 4.5 at dose of (40 mg/Kg) [17]. After a week, rats having stabilized diabetes with a fasting plasma glucose (FPG) level of > 220 mg/dl were considered for the experiment. After a week of STZ injection the treatment was started and the 8th day was considered as the 1st day of treatment with steroidal alkaloids.

2.6. Study design

Group 1 received normal saline twice daily (1 ml/Kg s.c) and served as diabetic control group. Group 2 was treated with active compound sarcovagine-D with a 5 mg/ Kg dose subcutaneously twice a day. Group 3 was treated with compound saracodine subcutaneously at a dose of 5 mg/Kg, twice a day. Similarly Group 4 was treated with holaphylline twice a day subcutaneously at a dose of 5 mg/Kg twice a day. Group 5 was treated with a standard drug glibenclamide at a dose of 1 mg/Kg/day. All the compounds and standard drug were dissolved in 10 ml normal saline

2.7. Sample collection

To determine glucose level, blood was collected in a heparinized glass tube by pricking the capillary vessels in the tail tip. The blood samples were centrifuged at 4 °C for 10 min. Plasma was separated and kept at −20 °C for further determination of fasting plasma glucose (FPG). To determine fructosamine and lipid level in serum, half of blood