ANTI-DIABETIC EFFECTS OF Withania somnifera ROOT AND LEAF EXTRACTS ON STREPTOZOTOCIN INDUCED DIABETIC RATS

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Abstract: The present investigation explores the possibilities of using the root and leaf extracts of an important plant Withania somnifera targeting DM and to examine their hypoglycaemic and hypolipidaemic effects on Streptozotocin-induced diabetic rats. Withania somnifera root extracts (WSREt) and leaf extracts (WSLEt) were orally administered daily to diabetic rats for eight weeks. After the treatment period, blood glucose and serum enzymes like aspartate transaminase (AST), alanine transaminase (ALT) and alkaline phosphatase (ALP), total cholesterol, triglycerides, HDL-c high density lipoprotein-bound cholesterol, LDL-c low density lipoprotein-bound cholesterol I, LDH and serum proteins levels were determined. The levels of blood glucose, AST, ALT, ALP, LDH, serum lipids except HDL-c (high density lipoprotein-bound cholesterol) were significantly increased, but total protein albumin, albumin : globulin (A : G) ratio, were significantly decreased in streptozotocin-induced diabetic rats. Treatment of the diabetic rats with root and leaf extracts altered the changes of the above parameters and restored them after eight weeks of treatment, indicating that the extracts possess hypoglycaemic and hypolipidaemic properties, hence useful in diabetes mellitus.

Key words: Diabetes mellitus, Withania somnifera, Streptozotocin

INTRODUCTION

Withania somnifera Dunal (Solanaceae), known as Ashwagandha in Sanskrit or Winter cherry in English, is one of the most valuable plants of the traditional Indian systems of medicines. The plant is used in more than 100 formulations of Ayurveda, Unani and Sidha and is therapeutically equivalent to ginseng [1], hence called popularly as ‘Indian ginseng’.

The pharmacological effects of the roots of W. somnifera are attributed to the presence of withanolides, a group of steroidal lactones [4]. Its leaves are used in Ayurvedic and Unani systems for treatment of tumors and tubercular glands [5]. A number of withanolide steroidal lactones have been isolated from the leaves of W. somnifera [6] that exhibit antibacterial, anti-fungal and antitumor properties[7]. There are a number of reports elucidating the chemical and pharmacological properties of W. somnifera [8-10].

W. somnifera is a shrubby plant cultivated in India, parts of East Asia and Africa which offers tremendous potential as an energizing medicinal herb. Ayurvedic practitioners have used the roots of this plant for centuries with success as a tonic to increase vitality and longevity, as well as to treat health conditions as diverse as tumors and arthritis. Recent laboratory studies have begun to confirm what Ayurvedic practitioners have known for years that W. somnifera deserves attention as an herbal...
therapy to ease or even eliminate many of today’s common health problems.

Hypoglycaemic activity of ‘Trasina’ (an ayurvedic formulation) containing extracts of *W. somnifera* as one of the important constituents has been established beyond doubt and this activity may be due to its antioxidant properties [11]. It has been in used for a very long time for all age groups and for both sexes and even during pregnancy without any side effects. The traditional uses and antidiabetic activities of *W. somnifera* have been reviewed [12]. Hypoglycaemic effects [13] and the effects of *W. somnifera* on insulin sensitivity in NIDDM rats [14] have been reported.

The chemistry and nutritional properties of phenolic compounds, including flavonoids, have been extensively reviewed [15]. Flavonoids are commonly found in all plants and also possess hypoglycemic and antidiabetic activities [16]. Therefore, the present study was aimed at determining the antidiabetic effects of *W. somnifera* root (WSREt) and leaf (WSLEt) extracts on streptozotocin induced DM.

**MATERIALS AND METHODS**

Twenty four male healthy adult albino Wistar strain rats (150 – 180 g bw), procured from an authorized firm in Bhubaneswar, were used in the experimental study. They were housed in plastic cages with filter tops under controlled conditions of 12 h light/12 h dark cycle, 50% humidity and 28 ± 2 °C. They all received a standard pellet diet and water *ad libitum*. The standard pellet diet contained 21% crude protein, 5% fat, 4% crude fiber, 8% ash, 1% calcium, 0.6% phosphorus, 2% vitamins and 55% nitrogen free extract (carbohydrates). The animals were maintained in the department of pharmaceutical sciences as per the principles and guidelines of the ethical committee for animal care of the Utkal University (Odisha,) in accordance with the Indian National Law on Animal Care and Use (CPCSEA).

Seeds of *W. somnifera* (L.) were procured from the Central Institute of Medicinal and Aromatic Plants (CIMAP), Lucknow, India and the plants were grown in the experimental garden in Post Graduate Department of Botany, Utkal University. The plants were collected in the month of August 2011 and the plant parts like root and leaf were selected for the antidiabetic activity. The parts (root and leaf) of the plant were collected from field grown plants in six months after planting. The materials were cleaned and dried in shade, and then ground to a fine powder.

About 500 g of dry powder was extracted with ethanol (80%) at 70 °C by continuous hot percolation using a Soxhlet apparatus. The extraction was continued for 24 h. The ethanolic extract was then filtered and kept in oven at 40°C for 24 h to evaporate the ethanol from it. The concentrated extract was then dissolved in as little water as possible and washed three times with chloroform. The residual layer was extracted three times with ethyl acetate. All the extracts were finally pooled and concentrated using the rotary evaporator. A dark brown residue (extract) was obtained. The yield of extracts from root and leaf was about 76 and 72 g, respectively.

The rats were injected with streptozotocin dissolved in sterile normal saline at a dose of 150 mg/kg bw, intraperitoneally. The rats were then kept for the next 24 h on 5% glucose solution bottles in their cages to prevent hypoglycaemia [36]. After a fortnight, rats with moderate diabetes having glycosuria (indicated by Benedict’s test for urine) and hyperglycaemia with blood glucose range of 250 – 300 mg/dl were used for the experiment. Blood was collected from the eyes (venous pool) by sino-ocular puncture for the estimation of the blood glucose. In the experiment a total number of 24 rats (18 diabetic induced rats and six normal rats) were used. Diabetes was induced in rats two weeks before starting the treatment. The rats were divided into four groups as follows after the induction of streptozotocin diabetes and each group comprised of six rats.

**Group 1**: Normal control rats received only distilled water during the experimental period.

**Group 2**: Diabetic control- freshly prepared streptozotocin in normal saline was administered in a single dose of 150 mg/kg bw through intraperitoneally to overnight fasted rats and the animals were allowed to develop diabetes for two weeks.

**Group 3**: Diabetic rats were daily treated with WSREt at a dose of 200 mg/kg bw dissolved in distilled water using an intragastric tube for eight weeks.

**Group 4**: Diabetic rats were daily treated with WSLEt at a dose of 200 mg/kg bw dissolved in distilled water using an intragastric tube for eight weeks.
The rats were carefully monitored every day. The sugar levels of blood of all the rats were determined. After eight weeks of treatment the rats were sacrificed by cervical dislocation. Blood was collected and processed for the biochemical estimations such as blood glucose in serum/plasma by GOD/POD method, total cholestrol by enzymatic method, triglyceride, HDL cholesterol by PEG/CHOD-PAP, LDL cholesterol by direct enzymatic method, alanine aminotransferase (SGPT) by mod. IFCC method, aspartate aminotransferase (SGOT) by mod. IFCC, lactate dehydrogenase by UV kinetic IFCC method, total protein, albumin & globulin by biuret and BCG dye binding method.

RESULTS AND DISCUSSION

Effects of WSREt and WSLEt on blood glucose: The levels of blood glucose were significantly increased in streptozotocin induced diabetic rats when compared with those of normal control rats. Administration of WSREt and WSLEt at doses of 200 mg/kg bw to diabetic rats tends to bring the values to near normal. Among these two doses of WSREt and WSLEt, the dose of WSREt showed better results (Table 1). It is not clear how WSREt and WSLEt cure diabetic rats under hyperglycaemic conditions. The possibility may be that WSREt and WSLEt may increase the pancreatic secretion of insulin from the cells of islets of Langerhan’s or both extracts may act like insulin substitutes. Further studies should be done to elucidate the actual mechanism of the decrease in blood sugar.

Effects of WSREt and WSLEt on serum lipid profile like TC, TG, HDL-c and LDL-c: Levels of serum lipids like TC, TG, serum high density lipoprotein-bound cholesterol (HDL-c), and low-density lipoprotein-bound cholesterol (LDL-c) and its effect on administering WSREt and WSLEt are presented in Table 1. The rise in blood sugar is accompanied by the increase in TC, TG, LDL-c, and fall
of HDL-c in diabetic rats. The levels of serum TC TG and LDL-c were significantly increased in diabetic rats when compared to those of normal control rats, while the level of serum HDL-c (70%) was significantly decreased in diabetic rats when compared to that of normal control rats (Table 1).

The abnormal high concentration of serum lipids is mainly due to increase in the mobilization of free fatty acids from the peripheral fat deposits, because insulin inhibits the hormone sensitive lipase production. Administering WSREt and WSLEt to diabetic rats tends to bring the values to near normal. Thus, WSREt and WSLEt treatments exhibited hypocholesterolaemic, hypotriglyceridaemic and hypophospholipidaemic effects while at the same time increasing the HDL-c. W. somnifera is known to have antioxidant properties [17] and this may reduce the susceptibility of lipids to oxidation and stabilize the membrane lipids thereby reducing oxidative stress.

Effects of WSREt and WSLEt on lactate dehydrogenase (LDH): The LDH levels were significantly increased in diabetic rats when compared to normal control rats. LDH levels showed significant decrease in WSREt and WSLEt treated diabetic rats. Organs rich in LDH are liver, heart and skeletal muscles. In myocardial infarction, serum LDH level begins to rise by 8-12 hours of attack, reaches to a peak within 48-72 hours and returns to normal after 1-2 weeks. Elevated serum LDH levels indicate cardiac damage and a profound risk of coronary heart disease.

Effects of WSREt and WSLEt on serum protein, albumin and A:G ratio: The levels of total protein in serum and albumin and albumin : globulin (A:G) ratio in serum were significantly decreased in diabetic rats when compared to those of normal control rats. On the other hand, no change was observed in the level of serum globulin in diabetic rats when compared to that of normal control rats (Table 3). The total protein levels in serum and albumin and A:G ratio in serum were significantly increased in diabetic rats treated with WSREt when compared to those of diabetic rats. Hypoalbuminemia was observed in DM, which is consisted with other report in that the altered A:G ratio was observed in diabetic rats [18]. Distinct metabolic renal alterations lead to a negative nitrogen balance, enhanced proteolysis and lowered protein synthesis in experimental diabetes [19]. The reversal of the changes by WSREt and WSLEt therapy proves that the insulin deficiency had been sufficiently corrected. Serum albumin and A:G ratio as well as total protein never deviated from the normal range throughout the treatment period in WSREt and WSLEt treated diabetic rats. W. somnifera has been reported to produce anabolic effects, enhancing the synthesis of certain modulator proteins in rat liver and increasing the bw in humans [20].

Effects of WSREt and WSLEt on liver function enzymes like aspartate transaminase (AST), alanine transaminase (ALT) and alkaline phosphatase (ALP): In the present study the activities of AST, ALT, and ALP in serum were altered in diabetic rats. The changes in the levels of AST, ALT, and ALP are directly related to changes in metabolism in which the enzymes are involved. The increased activities of transaminases, which are active in the absence of insulin due to the availability of amino acids in the blood of DM and are also responsible for the increased gluconeogenesis and ketogenesis. AST and ALT levels were restored to their respective normal levels in the WSREt and WSLEt treated groups. AST and ALT levels also act as an indicator of liver function hence restoration of normal level of these enzymes indicates that the normal functioning of liver. Increased activities of serum ACP and ALP have been observed in diabetic rats [22]. Streptozotocin treated diabetes caused lipid peroxide mediated tissue damage in the pancreas, liver, kidney, and heart. The increase in the levels of these enzymes in diabetes may be as a result of the leaking out from the tissues and then migrating into the blood stream [23]. Diabetes and hyperlipidaemia also cause cell damage by altering the cell membrane architecture, which results in enhanced activities of ALP in diabetic rats. In WSREt, WSLEt treated groups, the cell damage might be reverted and which may leads to the decreased activities of ALP.

From the above results, it may be concluded that the W. somnifera root and leaf extracts possess antidiabetic and antihyperlipidaemic activities in streptozotocin-induced diabetic rats. The antidiabetic and antihyperlipidaemic effect was more pronounced in WSREt (Root extracts) compared to WSLEt (Leaf extracts). Further investigations on phytochemical characterization of W. somnifera is required to identify the specific compound(s) involved in the observed hypoglycaemic and hypolipidaemic activities.
REFERENCES