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EFFECT OF AILANTHUS ALTISSIMA (MILL.) SWINGLE AND AILANTHUS EXCELSA (ROXB) STEM BARK EXTRACTS ON STREPTOZOTOCIN INDUCED DIABETES

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ABSTRACT:

The inhibitory effects of methanol 70% extracts of Ailanthus altissima and Ailanthus excelsa stem bark on streptozotocin (ST)–induced diabetic mellitus were studied using ST–treated diabetic model. Ailanthus altissima and Ailanthus excelsa stem bark extracts have a good effect on ATP/ADP ratio and also Ailanthus altissima and Ailanthus excelsa stem bark extracts at concentration 200 µg/ml showed an increase in insulin production in pancreatic islet cells.

Keyword: *Ailanthus altissima, Ailanthus excelsa, stem bark, antidiabetic activity.*

INTRODUCTION

Natural products have been recognized as sources of therapeutic agents for treating a lot of diseases. Ailanthus plants of the family Simaroubaceae are of great interest due to its several medicinal properties. A. altissima plant is used for treatment dysentry, gastric and intestinal upsets.1 The bark of A. altissima is prescribed to treat anemia and also used as antispasmodic, antiasthmatic, cardiac depressant, and for treatment of epilepsy2 and various biological activities of A. altissima were proved as antituberculosis, antimalarial, antitumor and antiherpes.3-11 A. excelsa plant is used to treat skin eruption12 and in treating bronchitis, asthma and in conditions of dysentery,13 and also various biological activities of A. excelsa were proved as antileukemic,14,15 antibacterial16 antifungal17 and antifertility.18 The diabetogenic streptozotocin (ST) is selectively toxic to insulin-secretion β-cells of pancreatic islets. Acute exposure of isolated islets to ST in vitro results in reduction in β-cells number. This study was carried out to determine Ailanthus altissima and Ailanthus excelsa stem bark. antidiabetic activity.

MATERIALS AND METHODS

Stem bark of Ailanthus altissima and Ailanthus excelsa was collected from Zoo garden, Giza, Cairo, Egypt in may 2008. The two plants were identified by Dr. Kamal El-Batanony (Professor of Taxonomy and Botany, Faculty of Science, Cairo Univ.). Pancreatic islets were isolated from male mice by collagenase digestion and density gradient centrifugation.22 ST and sodium nitroprusside (SNP) were obtained from Simga (St. Louis, MO, USA). ST was dissolved as 0.5 mM solution in citrate buffer (10 mM, pH 7.4, 115 mM NaCl, 24 mM $NaCo_3$, 5 mM KCl, 2.5 mM$CaCL_2$, 1 mM $MgCl_2$, 3 mM D-glucose, 0.1 bovine serum albumin) to a final concentration of 1-10 mM.

Plant extraction and phytochemical screening: 1 kg of air dried powered stem bark of Ailanthus altissima and Ailanthus excelsa was extracted with methanol : water mixture (70:30) in a continous extraction apparatus (soxhlet apparatus) till exhaustion, then concentrated under reduced pressure. Phytochemical screening of both methanol (70%) extracts of Ailanthus altissima and Ailanthus excelsa stem bark is compiled in Table 1.

Insulin secretion: To stimulate pancreatic insulin secretion, glucose must be metabolized in pancreatic β-cell. Insulin release was measured by enzyme-linked immunosorbent assay (ELISA) as described19 from islets (9.5 x 10-4) µU insulin/islet cell, corresponding to 150 cells/condition). the two extracts were assayed for their effect on ATP/ADP ratio and insulin excretion and the results of the effects of the extracts are compiled in Tables 2, 3.

Results and Discussion: Phytochemical screening of Ailanthus altissima and Ailanthus excelsa stem bark showed that the presence of the following chemical constituents: carbohydrates and/or glycosides, condensed tannins, alkaloids, coumarins, sterols and/or triterpenes, quassinoids and traces of flavonoids.

Table 1: Results of phytochemical screening of Ailanthus altissima and Ailanthus excelsa stem bark

Chemical Constituents	*Ailanthus altissima* stem bark	*Ailanthus excelsa* stem bark
1. Carbohydrates and/or glycosides	+ ve	+ ve
2. Tannins a. Condensed tannins b. Hydrolysable tannins	 + ve - ve	 + ve - ve
3. Alkaloids and/or nitrogenous bases	++ ve	++ ve
4. Flavonoids	traces	traces
5. Sterols and/or triterpenes	+ ve	+ ve
6. Saponins	- ve	- ve
7. Coumarins	+ ve	+ ve
8. quassinoids	+ ve	+ ve

+ ve denotes the presence of the constituents, - ve denotes the absence of the constituents

EFFECT OF THE EXTRACTS ON ST-INDUCED ATP/ADP RATIO OF ISLETS:

To examine the effects of the extracts on ST-induced ATP/ADP ratio of islets, islets (200/condition) were treated with ST(5 mM) for 1 h and 20 h, then the extracts with the concentration of 200 μg/ml was directly treated to each ST-treated cell for 20 h to see the effects of the extracts on ST-treated cells and each ATP/ADP ratio. There was a significant effect of ST on ATP/ADP ratio (Table 2). But there was no significant effect on the total adenine nucleotide contents of islets following either 1 or 20 h exposure to ST. So the extracts were effective for improvement of ATP/ADP ratio to some extent.

Table 2: ST-induced inhibition of the activity of adenine nucleotides in pancreatic islets and effect of the extracts (200 μg/ml)- treatment to the ST-treated cells

	Control (1h)	Control (20 h)	ST (1 h)	ST (20 h)	Sample **1** (1 h)	Sample **1** (20 h)	Sample **2** (1 h)	Sample **2** (20 h)
ATP/ADP	10.4±1.7	11.1±1.1	7.3±0.6	3.3±0.3*	10.7±2.5	10.4±3.5	11.5±2.6	10.8±1.7

Sample 1: methanol (70%) extract of Ailanthus altissima stem bark, Sample 2: methanol (70%) extract of Ailanthus excelsa stem bark, Adenine nucleotides were determined as described.20 Retention times for ATP and ADP were 6.54± 0.13 and 21.43± 0.14 min, respectively. Intracellular concentrations of ATP and ADP in controls were 2.54± 0.12 and 0.23 ± 0.01 mM, respectively (calculated assuming a islet intracellular water space of 0.71 pl/cell.21 Results are means ± SE for 5 experiments. Statistics: *P < 0.05 for difference from control (Student,s t-test).

EFFECT OF THE EXTRACTS ON ST-INDUCED INHIBITION OF INSULIN SECRETION OF ISLETS:

Table 3 shows the effects of short (1 h) and lnog term exposures (20 h) to the agent on insulin secretion in islets. ST (5 mM) suppressed glucose insulin secretion (6.15 μU/106 cells to 0.94 μU/106 cells) for I h incubation and more strongly inhibited the secretion for 20 h incubation (6.34 μU/106 cells to 0.36 μU/106 cells).

Table 3: ST-induced inhibition of the insulin secretion in pancreatic islets and effect of the extracts (200 μg/ml)-treatment to the ST-treated cells

	Control (1h)	Control (20 h)	ST (1 h)	ST (20 h)	Sample **1** (1 h)	Sample **1** (20 h)	Sample **2** (1 h)	Sample **2** (20 h)
Insulin (μU/10^6 cells)	6.1±0.8	6.5±0.4	0.8±0.2**	0.3±0.04**	4.7±1.2 #	5.4±1.6 # #	4.3±0.4 #	5.6±.05 # #

Sample 1: methanol (70%) extract of Ailanthus altissima stem bark, Sample 2: methanol (70%) extract of Ailanthus excelsa stem bark, cells were cultured in the presence or absence of ST (5 mM) for 1 and 20 hrs. Insulin secretion was determined by ELISA. Results are means ± SE for 5 experiments. Statistics: $P < 0.05$ and $P < 0.01$ for difference from control islet cells (Student,s t-test). #, # #, difference with ST. From the results compiled in Tables 2, 3 it can be concluded that the extracts, methanol (70%) of Ailanthus altissima stem bark and methanol (70%) of Ailanthus excelsa stem bark are effective tropical medicine to treat the diabetes.

Conflict of interest

There is no conflict of interest associated with the authors of this paper.

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