Contents lists available at ScienceDirect

Asian Pacific Journal of Tropical Biomedicine

journal homepage: www.elsevier.com/locate/apjtb

Original article http://dx.doi.org/10.1016/j.apjtb.2017.06.005

Antihyperglycemic effect of methanol extract of *Tamarix aphylla* L. Karst (Saltcedar) in streptozocin–nicotinamide induced diabetic rats

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ARTICLE INFO

Article history:
Received 8 May 2016
Received in revised form 22 Sep 2016
Accepted 19 Jun 2017
Available online 28 Jun 2017

Keywords:
Tamarix aphylla leaves
Diabetes
Streptozotocin
Antidiabetic potential
Insulin secretion

ABSTRACT

Objective: To evaluate the antihyperglycemic potential of *Tamarix aphylla* (*T. aphylla*) leaves in STZ–NIC induced diabetes in Wister Albino rats.
Methods: Acute toxicity study was done to check the toxicity of *T. aphylla* (L. Karst) methanol extract. *T. aphylla* leaves extract was administered intraperitoneally (100 mg, 250 mg and 400 mg/kg body weight per day) to diabetic Wister rats for 21 days. The various parameters were studied including fasting blood glucose levels, haemoglobin and glycosylated haemoglobin.
Results: The treatment groups with the extract at three dose levels expressively abridged the intensities of blood glucose and Glycosylated Haemoglobin. The earlier detected reduced level of plasma haemoglobin of the diabetic rats was raised to near normalcy with treatment of extract.
Conclusions: The results of the current study confirm that the leaves extract of *T. aphylla* are nontoxic and have antidiabetic nature.

1. Introduction

Diabetes mellitus is the world major endocrine abnormality of various aetiologies, relating to metabolic disorder of protein, lipids and carbohydrates. Almost all types of diabetes are due to reduction in the secreting levels of insulin *i.e.* Insulin deficiency and a decrease in the response of peripheral tissues to insulin *i.e.* Insulin resistance [1]. The protracted hyperglycaemia of diabetes is related with durable injury, dysfunction, and loss of function of different organs, specifically the blood vessels, nerves, kidneys, heart, and eyes. Numerous pathogenic processes are associated with the development of diabetes mellitus. These range from autoimmune damage of the β-cells of the pancreas with resulting insulin deficiency [2]. World Health Organization (WHO) assesses that today more than 220 million individuals globally have diabetes and this figure is likely to be doubled till 2030 [3].

The basic goal of all diabetes management is to sustain a sufficient blood glucose levels. The treatment of diabetes relies on oral hypoglycaemic drugs and recombinant insulin. Four key classes of oral hypoglycaemic drugs have been used widely: insulin secretagogues, biguanides, thiazolidinediones and alpha-glycosidase inhibitors. Individually every drug class works on diverse mechanism of actions, comprising stimulation of insulin secretion, inhibition of hepatic gluconeogenesis, rise in insulin receptor sensitivity and postponement of digestion and absorption of carbohydrate, respectively. The practice of oral drug is restricted by the side effects together with haematological, cutaneous and gastrointestinal reactions, hypoglycaemic coma and disruption of liver and kidney functions. In count they are not appropriate for use during gestation (Pregnancy) [4]. So, it is indispensable that we shall continue the search for new and, if conceivable, more effective drugs, and the huge reservoirs of phytotherapy could be an ideal alternate [2].

Nature remained a foundation of therapeutic cures for thousands of decades and plant-based therapies are still playing a vital part in the primary health care system of 80% of the worlds underdeveloped and developing nations. Most of the current advertised medicines are dilutions, combinations, reproductions

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Peer review under responsibility of Hainan Medical University. The journal implements double-blind peer review practiced by specially invited international editorial board members.

or distinctions of materials that exist in nature [1]. Right now, there is rising interest in herbal medications due to the associated side effects with oral hypoglycaemic agents (therapeutic agent) for the management of DM. A number of investigators have revealed that cumarins, flavonoids, terpenoids, and a mass of other secondary plant metabolites, together with arginine and glutamic acid display antidiabetic properties [4]. The World Health Organization (WHO) expert committee suggested that plants having hypoglycaemic action may deliver a practical source of novel oral antidiabetic drug for the expansion of pharmaceutical units, or may perform as simple dietetic adjuncts to the current therapies [5].

Tamarix aphylla (*T. aphylla*) L. Karst (Tamaricaceae) is the leading known species of tamarix having height: up to 18 m (*i.e.* 60 feet). This species has a range of common name *i.e.* Athel pine, Athel tree, Athel tamarisk, and Saltcedar. It is a perennial tree, innate across East, North and Central Africa, over the Middle East, and found in parts of southern and Western Asia. The phytochemical screening displayed a conspicuous lack of alkaloids in almost all extracts, then a notable presence of tannins. Others metabolites and bioactive mixtures were identified such as flavonoids, cardiac glycosides, steroids and terpenoids [6]. *T. aphylla* is used as herbal remedies such as diuretic, carminative, anti-inflammatory and for handling internal haematomas [7] tuberculosis, leprosy, smallpox aphrodisiac and tonic, hepatitis, eczema and other skin ailments [8]. While it is systematically certified as an analgesic and antipyretic [9] antimicrobial [10] antifungal [11] and has cardio-protective effect in doxorubicin induced cardiotoxicity [12].

Though *T. aphylla* L. Karst has been examined for its several medicinal properties. Nevertheless, there are no evidences on the antidiabetic action of the plant. Therefore this study was started to assess the antidiabetic activity of methanol extract of *T. aphylla* L. Karst in Streptozocin–Nicotinamide induced diabetic rats.

2. Materials and methods

2.1. Kits and chemicals

Streptozocin was purchased from M.S. Traders local Distributor of Sigma Aldrich Lahore Pakistan. Haematological Parameters like Haemoglobin and HbA1c were performed on Sysmex KX-21N™ Automated Haematology Analyser. Abbott FreeStyle Optium Glucometer was used for the measurement of Fasting Blood Sugar of rats. Glibenclamide and other chemicals were of analytical grade and were obtained from local firms (Pakistan).

2.2. Plant material and extraction

Fresh leaves were collected from the tree of *T. aphylla* L. Karst in the month of April 2015 from Bajaur Agency, Federally Administered Tribal Areas (FATA), Pakistan. The plant was botanically identified and authenticated by Dr. Barkat in the Department of Botany, University of Peshawar, Khyber Pukhtoon Khwa and a voucher specimen (No. 20089 PUP) was deposited at the herbarium of Botany Department. The leaves of the plant were shade dried at (30–35) °C and the dried leaves were ground into coarse powder with auto-mix blender. The powder obtained (3.24 kg) was macerated in 5 L/kg of the extract in 80% methanol for 14 days at room temperature with daily stirring and mixing. The filtrate was concentrated under reduced pressure at 40 °C until extraction solvent was removed. A dark green soluble crude residue was obtained (about 214.27 g, 6.61% w/w). Normal saline was used to dissolve the extract for experimentation.

2.3. Experimental animals

A total of 50 male Wistar rats aged (8–10) weeks weighing (220–320) g were used in this study. All rats were obtained from National Institute of Health, Islamabad, Pakistan. All rats were housed in a well ventilated room having temperature (25 ± 2) °C and humidity (40%–50%) in 12-h light–dark periods. They were fed on normal laboratory food and allowed free access to water for two weeks before the commencement and during the period of the experiment. The experiment was performed according to the guidelines set by the Animal Ethics Committee, Institute of Basic Medical Sciences, Khyber Medical University, Pakistan (DIR/KMU-EB/AA/000159).

2.4. Induction of diabetes in experimental rats

Rats were starved overnight for 12-h before injection. Diabetes was induced in by a single intraperitoneal (*i.p*) injection of STZ [65 mg/kg body weight (b.w.)] dissolved freshly in ice-cold saline for this injection [13]. Control animals received an injection of an equivalent volume of normal saline. Nicotinamide was dissolved in normal saline and administered (180 mg/kg, *i.p*) 15 min before STZ administration [14]. Since, STZ is capable of inducing fatal hypoglycaemia due to massive pancreatic insulin release, the rats were provided with 10% glucose solution after 6 h of STZ administration for the next 24 h to overcome drug induced hypoglycaemia [2]. After 48 h, for the development and aggravation of diabetes, rats with moderate diabetes (*i.e.* fasting blood glucose concentration, > 200 mg/dL) that exhibited hyperglycaemia were selected for further experimentation.

2.5. Acute toxicity studies

Typical healthy male mice were grouped into drug-treated 'test' and vehicle-treated 'control' groups, total making up of seven sets of six mice each. *T. aphylla* (500, 1 000, 1 500, 1 600, 1 650, 1 800 and 2 100 g/kg) was alone injected intraperitoneally to the mice in each of the test groups. Every mice in the control group was treated with Normal Saline only. The animals were studied continuously for 2 h under the following profiles [13] (a) Behavioural profile: Alertness, agitation, irritability, and dread; (b) Neurological profile: impulsive action, reaction, touch reaction, pain response, and gait; (c) Autonomic profile: excretion and urination.

After 24 h period they were observed for any death or lethality.

2.6. Experimental design

In the testing a total of 42 animals were used (6 normal control rats; 30 STZ diabetic living rats and 6 normal extract group rats) were used. The rats were separated into seven sets of six rats in individual group as follow:

Group I: Control rats.
Group II: Streptozotocin induced diabetic rats.

Group III: Diabetic rats treated with *T. aphylla* leaf extract (100 mg/kg body weight/rat/day) in saline solution intraperitoneally for 21 d.
Group IV: Diabetic rats treated with *T. aphylla* leaf extract (250 mg/kg body weight/rat/day) in salt solution intraperitoneally for 21 d.
Group V: Diabetic rats treated through *T. aphylla* leaf extract (400 mg/kg body weight/rat/day) in salt solution intraperitoneally for 21 d.
Group VI: Diabetic rats treated through glibenclamide (5 mg/kg body weight/day) in saline solution intraperitoneally for 21 d.
Group VII: Normal Non-diabetic rats treated with *T. aphylla* leaf extract (250 mg/kg body weight/rat/day) in saline solution intraperitoneally for 21 d.

Throughout the experimental period, body mass was measured at the start and ends of the experiment however blood glucose levels of all the rats were noted at regular breaks. At the end of the trial period, the rats were stopped to eat overnight, sedated, and sacrificed by cervical dislocation. The blood was congregated with and without anticoagulant for plasma and serum portion respectively.

2.7. Phytochemical screening

The phytochemical screening indicated a visible lack of alkaloids in all extracts, and a notable presence of tannins. Others metabolites and biologically active components were recognized such as flavonoids, cardiac glycosides, steroids and terpenoids. Also, no cyanogen glycosides and saponins were noticed [7] (Table 1).

Table 1
Phytochemical compounds identified in different extracts [7].

Metabolite	W. Extract	W–E. Extract	W–M. Extract	W–A. Extract
Steroids	+	+	+	+
Terpenoids	+	+	+	+
Alkaloids	–	–	–	–
Flavonoids	+	+	+	+
Tannins	+	+	+	+
Cardiac glycosides	tr	+	+	+
Cyanogens glycosides	–	–	–	–
Free quinines	–	–	–	–
Anthraquinones	+	–	–	–
Saponins	–	–	–	–
Reducing sugar	+	–	–	–
Gum	tr	–	–	–

Note: tr: trace; W: Water (100%); W–E: Water–Ethanol (30–70; v–v); W–M: Water–Methanol (30–70; v–v); W–A: Water–Acetone (30–70; v–v).

2.8. Biochemical parameters

Blood glucose was measured with e glucometer (Abbott FreeStyle Optium, England) at different intervals *i.e.* 1, 3, 7, 14 and 21 d after daily administration of extract intraperitoneally. After blood glucose estimation on day 21, whole blood was collected by cardiac puncture under mild chloroform anaesthesia from rats. Haemoglobin, glycated haemoglobin levels were evaluated in normal and streptozocin–nicotinamide induced diabetic rats on KX-21N™ Automated Haematology Analyser (Sysmex, America).

2.9. Statistical analysis

Results of the experiment were measure as mean ± SD for six rats in individual experimental group. Statistical evaluation of data was done using SPSS 20.0 version. Hypothesis was then tested by means of one way ANOVA followed by LSD test and a P value of <0.05 was considered statistically significant.

3. Results

3.1. Acute toxicity tests

In acute toxicity study, methanol extract of *T. aphylla* treated animals did not show any alteration in their behavioural pattern. Experiment was carried out on male Wister mice. No mortality was observed in the extract treated mice and beside that performance of the treated mice also appeared normal. There was no lethality or toxic reaction found till 1 500 mg/kg dose until the end of the day.

3.2. Effect of T. aphylla on fasting blood glucose

Blood glucose level was measured in normal control and experimental animals on days 1, 3, 7, 14 and 21 of the treatment. STZ–NIC induced diabetic rats presented a significant rise in the levels of blood glucose when correlated to normal rats. I. P administration of *T. aphylla* (400 mg/kg body weight) causes a significant reduction in FBS (Fasting blood sugar) level compared to diabetic control Table 2.

3.3. Effect of T. aphylla on HbA1c and haemoglobin

Table 2 presents the effect of *T. aphylla* on haemoglobin and glycosylated haemoglobin levels in normal and STZ-induced diabetic rats in next 21 d of treatment. In diabetic rats, there was a significant decrease in haemoglobin (−34.31%) and an upsurge in HbA1c (+80.76%) when was compare to normal

Table 2
Effect of *T. aphylla* on Fasting Blood Glucose in STZ–NIC-induced diabetic rats.

Groups	Fasting blood glucose (mg/dL)					Haemoglobin and HbA1c	
	1st Day	3rd Day	7th Day	14th Day	21th Day	Hb (g/dL)	HbA1c (%)
Normal control	96.3 ± 6.1	98.3 ± 6.9	95.9 ± 6.8	97.8 ± 6.9	97.8 ± 6.9	13.7 ± 1.2	5.2 ± 0.3
Normal + *T. aphylla* 250 mg/kg bw.	94.3 ± 6.3	94.6 ± 7.1	91.8 ± 7.4	89.3 ± 6.6	85.6 ± 7.5	13.5 ± 1.2	5.0 ± 0.3
Diabetic control	285.9 ± 9.9	309.0 ± 13.8	351.7 ± 19.2	380.7 ± 17.9	413.3 ± 24.4	10.2 ± 1.0	9.4 ± 0.8
Diabetic + *T. aphylla* 100 mg/kg bw.	285.3 ± 9.1	274.2 ± 6.8	251.5 ± 8.8	228.2 ± 6.1	213.0 ± 6.5	11.4 ± 1.0	7.7 ± 0.7
Diabetic + *T. aphylla* 250 mg/kg bw.	291.9 ± 8.6	266.3 ± 6.5	236.9 ± 4.9	179.5 ± 8.4	156.6 ± 5.7	12.6 ± 1.1	6.6 ± 0.5
Diabetic + *T. aphylla* 400 mg/kg bw.	288.2 ± 8.1	261.2 ± 4.2	220.5 ± 6.8	149.3 ± 11.2	122.0 ± 9.4	13.1 ± 1.1	5.9 ± 0.4
Diabetic + glibenclamide 5 mg/kg bw.	289.6 ± 9.7	254.2 ± 11.3	204.5 ± 7.4	139.7 ± 9.8	112.58 ± 9.5	13.3 ± 1.2	5.6 ± 0.4

Each value is mean ± SD for six rats in each group.

control rats. I.P administration of *T. aphylla* significantly improve haemoglobin (+22.13%), and decreased HbA1c (−59.32%) when compared to diabetic control rats.

4. Discussion

Diabetes arises from destruction of B-cell due to the toxic effect of STZ, which facilitates preferential uptake into pancreatic B-cells through GLUT2. There is clear evidence that free radicals play an essential role in the mechanism of DNA damage and cytotoxicity by STZ. It has been reported that STZ causes radicals generation by the xanthine oxidase system of pancreatic cells, and stimulates H_2O_2 generation. As result, it leads to DNA fragmentation and necrosis in the pancreatic B-cell islets. Therefore, the rate of insulin synthesis is reduced. Injection of NA, a poly-ADP-ribose synthetase inhibitor, protects the B-cells function by preventing the reduction in the level of NAD (nicotinamide adenine dinucleotide); thereby the inhibition of insulin secretion partially reverses and it prevents the aggravation of experimental diabetes following by administration of STZ. This circumstance contributes a number of characteristics similar to type 2 diabetes mellitus [15]. In our study, we observe high blood glucose level in STZ–NA induced diabetic rats and it might be due to beta cell destructive nature of streptozocin. While by after intraperitoneal administration of *T. aphylla* leaf extract 250 mg/kg, 400 mg/kg and glibenclamide to the diabetic rats markedly decrease blood glucose level from first to fourth week compared to diabetic control rats. Hence it might be assumed that antihyperglycemic nature of *T. aphylla* is due to its protective nature against STZ induced beta cell destruction and possible regeneration of damaged beta cell or increase in insulin secretion or its actions.

Glycated haemoglobin form from non-enzymatic reaction between free amino acid of haemoglobin and glucose by a process call glycosylation [16]. It is used as biomarker for confirmation of long term glycaemic control in patients with diabetes and it also predicts the chances of development and exaggeration of diabetic associated complications [17]. In earlier studies it was revealed that a 10% reduction in HBA1c results in a 35% reduction in risk of retinopathy, 25%–44% reduction in risk of nephropathy and a 30% reduction in risk of neuropathy [18]. In our study we observe increase in level of HbA1c and decrease in level of Hb in diabetic rats compared to normal control rats which further assures the process of glycosylation in diabetic rats due to elevated glucose level. But after administration of *T. aphylla* and glibenclamide to the diabetic rats markedly reduced HbA1c enhance Hb levels compared to diabetic control rats. This result reveals that *T. aphylla* has the ability to prevent hyperglycaemia and its associated complications in diabetes.

Hyperglycaemia is an important risk factor for the cardiovascular diseases (CVD) risk. Animal studies reveal raised blood glucose level result in glycation and peroxidation of protein in body which damages arterial walls [19]. In diabetic patients the prevalence of CVD increases 2–8 folds compare to non-diabetic patients. The increase in coronary heart diseases has emerged as a main cause of morbidity and mortality in diabetic patients all over the globe [20]. Both type 1 and type 2 diabetes are considered independent contributor for coronary heart diseases [21].

In our current study, administration of STZ results in alteration of normal blood glucose, Haemoglobin and HbA1c when compared to normal control rats. The abnormal blood profiles were brought to normalcy when treated with both doses of *T. aphylla* and glibenclamide in STZ–NA induced diabetic rats. The antihyperglycemic action of the extract may be due to proper stabilization of blood glucose level and increase in insulin level after the administration of *T. aphylla* which may normalize the hyperglycaemia in diabetic rats.

The presence of tannins, flavonoids and phenolic compounds in *T. aphylla* may be responsible for the hypoglycaemic activities in diabetic rats.

Data from our study confirm that *T. aphylla* leaves extract possesses blood glucose lowering action in diabetic condition. Moreover it has an ability to prevent diabetic complication associated with raise blood glucose. Hence our finding gives scientific validation to the traditional use of *T. aphylla* leaf extract in the treatment of diabetes.

Conflict of interest statement

We declare that we have no conflict of interest.

Acknowledgements

This research is supported by Institute of Basic Medical Sciences, Khyber Medical University with grant no. of 06472414.

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