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Antihyperglycemic effects of *Amrtottara Kvatha*, an Ayurvedic polyherbal formulation in streptozotocin-induced diabetic rats by suppressing oxidative stress

Research Article

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Abstract

Amrtottara Kvatha [KNM3] (AK) is a widely used Ayurvedic preparation, primarily utilized for hyperpyrexia ('*Jwara*'). In the present study we evaluated the antidiabetic effect of AK against streptozotocin (STZ) induced diabetic murine model. The effects of AK on normoglycemic rats and glucose tolerance were also studied. The antidiabetic effect of AK was evaluated in streptozotocin (STZ, 55 mg/kg, i.p.) induced diabetic rats. AK was administered orally at four doses AK (1/4), AK (1/2), AK (T) and AK (DD) for fourteen days to examine the antidiabetic activity with glibenclamide (5 mg/kg) as reference standard. The effect of AK on blood glucose and insulin levels, biochemical parameters, oxidative stress biomarkers and histopathological examinations were studied. Oral administration of AK at doses AK (1/4), AK (1/2), AK (T) and AK (DD) to rats with streptozotocin induced diabetes showed significant ($P<0.01$) decrease in blood glucose levels with improved insulin levels, liver glycogen and pancreatic protein content. The oxidative stress biomarkers (malondialdehyde and advanced oxidation protein products) were reduced in all AK treated groups with a significant ($P<0.01$) increase in antioxidant enzyme status. Histopathological studies also supported the findings. The present study reports for the first time the antidiabetic effects of *Amrtottara Kvatha* by suppressing oxidative stress induced by streptozotocin in Wistar rats.

Key Words: Anti-diabetic, Glucose tolerance, Glibenclamide, Malondialdehyde, Normoglycemic.

Introduction

Diabetes mellitus is a chronic metabolic syndrome caused by insulin deficiency, insulin inaction or both and it affects carbohydrate, fat and protein metabolism. It is characterized by the complications such as hyperglycemia, polyurea, ketonuria and ketonemia (1). The hyperglycemic condition caused by the impaired secretion of insulin by pancreatic beta cells is Type-1 diabetes and hyperglycemia due to insulin resistance is type-2 diabetes (2). Diabetes is associated with oxidative stress leading to the elevated release of reactive oxygen species (ROS) such as hydrogen peroxide (H_2O_2), hydroxyl radical ($OH^{\bullet}$) and superoxide radical ($O_2^{\bullet}$) and improper functioning of the antioxidant defence mechanism of the body (3). It is one of the major causes of cardiac diseases, stroke, retinopathy, kidney failure and lower limb amputation. Morbidity and mortality directly or indirectly due to diabetes is a major health challenge across the globe. The number of diabetic patients increased from 108 million in 1980 to 422 million in 2014 and the prevalence has been rising more rapidly in low and middle-income countries. It has been estimated that 1.5 million deaths were directly caused by diabetes in the year 2019 (4).

Management of diabetes mellitus employs modifications of diet, change of lifestyle, intake of oral hypoglycemic drugs, administration of exogenous insulin and herbal remedies. The present conventional diabetic treatments reduce insulin demand, stimulate endogenous secretion of insulin from pancreatic beta cells, inhibition of oligo and disaccharides degradation and effective and enhanced action of insulin at the target tissues. Many of the synthetic hypoglycemic drugs have adverse side effects related to their use including gastrointestinal disturbances, weight gain, fluid retention, abdominal discomfort, diarrhoea and lactic acidosis (5). As the treatment protocols currently

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available for diabetes are to be taken life-long and are not free from complications, safer alternatives are always in search of (6). Holistic approach and plant derivatives are gaining much focus in the treatment of diabetes as they are free from side effects. The herbal drugs can improve insulin sensitivity, increase the production of insulin and/or decrease blood glucose levels (7). The pharmacologically active constituents of many Ayurvedic drugs have been identified and their useful effects in drug therapy were determined (8). Some of the herbal capsules containing polyherbal formulation in specific combinations were reported to be antidiabetic (9) and they were characterized as per the standard methods (10).

Amrtottara Kvatha (AK), a unique Ayurvedic polyherbal formulation indicated for *Jwara* (hyperpyrexia) is mentioned in the traditional Kerala Ayurvedic literature, '*Chikitsamanjari*' (11) and '*Sahasrayogam*' (as '*Nagaradi'*) (12). The drugs used in the preparation of AK are fresh stem of *Tinospora cordifolia* (Willd.) Miers (*Amrtha*) (Family: Menispermaceae) dried fruit rind of *Terminalia chebula* Retz. (*Haritaki*) (Family: Combretaceae) and dried rhizome of *Zingiber officinale* Roscoe (*Nagara*) (Family: Zingiberaceae) in the ratio 6:4:2 respectively. It is indicated with adjuvant sugar only or jaggery and salt. But in practice, this combination is given by Ayurvedic physicians in a wide range of conditions including jaundice, rheumatic ailments, oedema, etc. The combination is widely accepted and used one, but not commonly used in diabetes. The individual drugs of this formulation have undergone antidiabetic studies, but no scientific evaluation has been carried out for the antidiabetic effect of the polyherbal formulation. In the present study, the antidiabetic effect of AK (without adjuvant) against streptozotocin induced diabetes in Wistar rats is reported.

Materials and Methods

Collection of plant materials

The plant materials used for the study, the mature fruits of *Terminalia chebula* and dried rhizomes of *Zingiber officinale* were collected from Marayur, Idukki district, Kerala, India in the months of January-March. Fresh specimens of mature stem of *Tinospora cordifolia* were collected from the herbal garden of Jawaharlal Nehru Tropical Botanic Garden and Research Institute (JNTBGRI), Palode, Trivandrum, Kerala, India. The specimens were authenticated by the plant taxonomist of JNTBGRI and voucher specimens were deposited at the Institute's Herbarium (*Tinospora cordifolia*- JNTBGRI 83964, *Terminalia chebula*- JNTBGRI 83965, *Zingiber officinale*- JNTBGRI 83966) for future reference. *T. chebula* fruit pericarp was separated from the fruit by discarding the seeds and endocarp. *T. chebula* and *Z. officinale* were stored separately in air tight zip locked plastic covers. They were coarsely powdered in small quantity and stored.

Preparation of AK

AK was prepared daily during the course of the study in order to administer as freshly prepared. Fresh mature stem of *T. cordifolia* was washed after peeling off the skin, cut into small pieces and mashed with mortar and pestle. The crushed fresh stem of *T. cordifolia* (24 g), 16 g of coarsely powdered (passed through sieve no. 40) *T. chebula* fruit pericarp and 8 g of coarsely powdered (passed through sieve no. 40) rhizome of *Z. officinale* were mixed together to get 48 g crude drug. It was mixed with 384 mL water, boiled and reduced to 96 mL and filtered through clean white cloth (4 layered) according to the *Kvatha* preparation procedure mentioned in '*Sahasrayogam*', The Ayurvedic Formulary of India (AFI) and '*Sarngadhara Samhita*[KNM4] ' (13).

Rat dose of AK comparable to adult human therapeutic dose was calculated using Paget & Barnes conversion factor (14). The different doses of AK used for the study were AK (1/4): Rat dose comparable to 1/4th of the adult human therapeutic dose of AK (0.5 mL/200 g b.wt., p.o.), AK (1/2): Rat dose comparable to half of the adult human therapeutic dose of AK (0.9 mL/ 200 g b.wt., p.o.), AK (T): Rat dose comparable to the adult human therapeutic dose of AK (1.7 mL/200 g b.wt., p.o.), AK (DD): Rat dose comparable to double of the adult human therapeutic dose of AK (3.4 mL/200 g b.wt., p.o.).

Chemicals and apparatus

Streptozotocin (STZ), Glibenclamide, 5, 5-dithio-bis-(2-nitrobenzoic acid) (DTNB) and Nitroblue tetrazolium (NBT) were purchased from Sigma Aldrich, USA. All other chemicals and reagents used for the experiments were of analytical reagent grade. Apparatus such as rotary evaporator (Buchi, Switzerland), Bio-analyser (Fully, Tulip Group, Italy) and CARY 100 UV-Vis spectrophotometer (Agilent Technologies, Malaysia) were used in the study.

Experimental animals

Wistar albino male rats (150 g - 220 g) and Swiss albino male mice (25-30 g) were obtained from the Animal House of Jawaharlal Nehru Tropical Botanical Garden and Research Institute, Palode. They were grouped and housed in poly-acrylic cages (two animals per cage) and maintained under standard laboratory conditions (temperature 24-28̊ C, relative humidity 60-70 % and 12 h dark light cycles. They were fed commercial rat feed (Lipton India Ltd, Mumbai, India) and boiled water, *ad libitum*. Animals were acclimatized for one week before starting the experiments. All experiments involving animals were carried out according to the guidelines of Committee for the Purpose of Control and Supervision of Experiments on animals (CPCSEA), New Delhi, India after getting the approval of Institute's Animal Ethics Committee (IAEC No.18/IAEC/AVC/2013 dated 24.04.2013).

Qualitative phytochemical analysis

Qualitative phytochemical analysis was carried out according to the standard method (15).

Acute toxicity Study

Acute oral toxicity study was performed as per Organization of Economic Cooperation and Development (OECD, 423) guidelines (16). Six groups of 6 mice, males were administered 100, 200, 400, 800, 1600, 3200 mg/kg of AK (p.o.). They were observed continuously for 1 h for gross behavioral changes, symptoms of toxicity and mortality if any and intermittently for the next 6 h and then again 24 h after dosing with AK.

Effect of AK on normoglycemic rats

Wistar albino male rats were divided in to four groups of 6 animals (n=6) each. Group I served as control and received vehicle only. Groups II, III and IV were administered orally with AK (1/2), AK (T) and AK (DD). Blood glucose levels were determined at 0, 1, 2, 3 and 4 h following treatment by taking the blood samples from the tail vein.

Glucose tolerance test

Wistar albino male rats were divided in to five groups of six animals in each group and were fasted for 15 hours. Distilled water (normal control), standard drug (glibenclamide, 5 mg/kg), AK at different doses viz., AK (1/2), AK (T) and AK (DD) were administered orally to the groups. Thirty minutes later, glucose (1.25 g/kg) was orally administered to each rat. Blood samples were taken from tail vein before administering drug and at 0 min (just before glucose loading), 30, 90 and 150 minutes for the assay of glucose (17).

Streptozotocin induced diabetic study

Wistar albino male rats were divided in to seven groups, each group containing six rats. Group I (normal control group without diabetes) received distilled water orally. Diabetes was induced in the remaining 6 groups of rats by intraperitoneal injection of streptozotocin, (STZ, 55 mg/kg. b.w, i.p.). After 24 h, blood sugar levels of the streptozotocin treated animals were determined. The animals showing blood sugar level more than 250 mg/dL were considered diabetic (18). The diabetic animals (groups II-VII), the diabetic control (Group II), standard drug control, glibenclamide 5 mg/kg (Group III), AK ¼ (Group IV), AK ½ (Group V), AK (T) (Group VI) AK (DD) (Group VII) were stabilized for five days. The treatments were started from the sixth day (day 0) and the administration was done once a day for 14 days. Blood samples were taken from tail vein and blood glucose levels were measured on 0th, 7th and 14th day. On 14th day insulin levels were also determined by enzyme linked immunosorbent assay (ELISA) using rat insulin ELISA kit.

On the 15th day, all the animals were sacrificed by carbon dioxide inhalation. The blood was collected by cardiac puncture for biochemical estimation of serum parameters. The liver, pancreas and kidney specimens obtained from the control and treated groups were preserved in 10% buffered formalin and processed for histopathology. Sections of 3-7 µm thickness were stained with haematoxylin-eosin, and examined for histopathological changes. Liver and pancreatic tissues were sliced out and kept in normal saline (0.9%) to estimate liver glycogen, total protein, malondialdehyde (MDA), advanced oxidation protein products (AOPP) and antioxidant enzymes.

The biochemical parameters such as total cholesterol, triglycerides and creatinine were assayed according to the standard methods using commercial kits (Coral Clinical System, Goa, India). Liver glycogen levels were measured by the standard method (19). Total protein was estimated according to Biuret method (20). The concentration of malondialdehyde was determined by the standard method (21). Advanced oxidation protein products levels were determined by the standard method (22). The antioxidant enzymes in liver homogenate were estimated by standard methods. Catalase was estimated by the standard method (23). Reduced glutathione was measured (24) and assay for superoxide dismutase was carried out (25).

Statistical analysis

All the analyses were carried out in triplicate. The statistical significance between the samples was determined by the Analysis of Variance (ANOVA). The results were expressed as mean ± standard deviation (SD), $P \leq 0.01$ was considered to be statistically significant. Significant differences between means were determined by Dunnet's multiple comparison test (26) using the software Graphpad Prism 5.04.

Results

Qualitative phytochemical analysis

The qualitative phytochemical analysis of AK revealed the presence of flavonoids, alkaloids, steroids, phenols and tannin. AK gave positive results for Liebermann Burchard's test indicating the presence of steroids. It formed a white precipitate when treated with Mayer's reagent indicating the presence of alkaloids. Further, it gave positive results for flavonoids, phenolic compounds and tannins.

Acute toxicity Study

In the acute toxicity studies conducted in Swiss albino mice, AK was found to be non-toxic up to 3200 mg/kg. The animals did not exhibit any behavioural changes such as tremor, convulsion, salivation, diarrhea or lethargy during the first four hours of the administration of AK and no mortality was observed up to 14 days. The LD_{50} of AK was therefore greater than 3200 mg/kg, (p.o.) in mice.

Effect of AK in normoglycemic rats

The result showed that there was no significant decrease in blood glucose levels in normoglycemic rats, when treated with the single dose of AK at AK ½ (0.9 mL/200 g b.wt.), AK (T) (1.7 mL/200 g b.wt.) and AK (DD) (3.4 mL/200 g b.wt.) after 0-4 h (Table 1).

Table 1: Effect of *Amrtottara Kvatha* on blood glucose levels of normoglycemic rats

Groups	Blood glucose level (mg/dL) at (hrs.)				
	0	1	2	3	4
Normal control	91.21 ± 1.37	94.80 ± 0.71	92.38 ± 0.64	92.10 ± 0.41	91.55 ± 0.37
AK (1/2)	92.55 ± 0.81	92.11 ± 1.13	91.21 ± 0.94	90.33 ± 0.92	90.50 ± 0.71
AK (T)	92.13 ± 0.61	92.20 ± 0.63	92.33 ± 0.77	92.45 ± 0.84	92.53 ± 0.84
AK (DD)	93.52 ± 1.12	92.94 ± 0.65	91.79 ± 0.20	90.21 ± 0.40	89.59 ± 0.54

Values are the mean ± SD, n=6; One-way ANOVA followed by Dunnet's multiple comparison test, *P<0.01 when compared to normal control.

Effect of AK on glucose tolerance test

The effect of AK on blood glucose levels of hyperglycemic rats due to glucose loading (1.25 g/kg, p.o.) is given in Table 2. There was a significant (P<0.01) glucose lowering effect seen in AK (1/2) treated animals compared to the normal control group. The standard drug, glibenclamide (5 mg/kg, p.o.) exhibited significant (P<0.01) anti-hyperglycemic effect from 0th min to 150th min of glucose administration and prevented elevation in blood glucose levels after glucose loading. At the 30th min (30 min after glucose loading), the groups showed hike in blood glucose levels. In AK treated groups, AK (1/2) and AK (DD), the increase in blood glucose was significantly (P<0.01) reduced when compared to normal control. At the 150th min after glucose loading, AK (1/2) group showed significant (P<0.01) glucose lowering effect (35.54%) when compared to other treated groups and normal control.

Table 2: Effect of *Amrtottara Kvatha* on glucose tolerance in fasted and glucose loaded normal Wistar rats

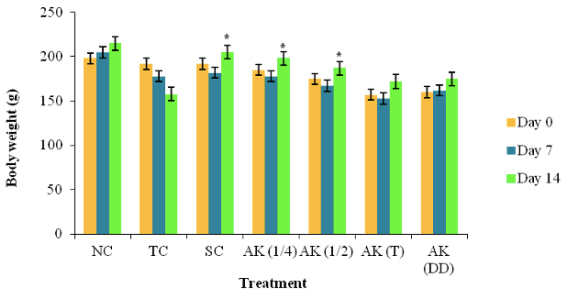
Groups	Blood glucose (mg/dL)				
	Initial (Before drug administration)	0th min (Before glucose loading)	30th min	90th min	150th min
Normal control	66.33 ± 2.80	58.30 ± 1.52	93.31 ± 3.10	69.34 ± 1.51	51.35 ± 1.52
Glibenclamide (5 mg/kg)	67.61 ± 2.52	41.10 ± 2.44	39.32 ± 2.10*	25.11 ± 2.62*	21.13 ± 1.11*
AK (1/2)	65.64 ± 3.52	51.71 ± 3.63	64.11 ± 3.42*	56.13 ± 2.61*	42.31 ± 1.53*
AK (T)	65.13 ± 5.21	59.10 ± 2.22	82.61 ± 4.13*	59.60 ± 1.34*	46.74 ± 1.58*
AK (DD)	66.66 ± 4.70	59.37 ± 2.54	68.17 ± 1.82*	64.36 ± 2.23	49.37 ± 1.34

Values are the mean ± SD, n=6; One-way ANOVA followed by Dunnet's multiple comparison test, *P<0.01 when compared to normal control.

Effect of AK on streptozotocin-induced diabetic rats

In streptozotocin-induced diabetic animals, the toxin control group showed a progressive decline in the body weight of animals after 14 days. All the AK treated groups except AK (DD) showed a decrease in body weight on 7th day of the treatment, but the AK treated groups and glibenclamide standard group showed increase in body weight after 14 days compared to the toxin control group (Figure 1A). The blood glucose level was significantly (P<0.01) increased in diabetic rats compared to normal rats. Oral administration of AK (1/4), AK (1/2) and glibenclamide (5 mg/kg) to diabetic rats significantly (P<0.01) decreased the blood glucose level. On day 7, the standard drug glibenclamide (5 mg/kg) showed 60.37% decrease in glucose levels while AK (1/4), AK (1/2), AK (T) and AK (DD) showed 39.32%, 29.28%, 26.05% and 20.62% decrease respectively. After 14 days' treatment, glibenclamide showed 66.33% decrease in glucose level while AK (1/4), AK (1/2), AK (T) and AK (DD) showed 61.20%, 63.50%, 58.87% and 56.69% decrease respectively (Table 3). The serum insulin level was also improved in AK treated groups and glibenclamide on day 14 which was significantly less in toxin control group (Table 3).

Figure 1 A: Effect of *Amrtottara Kvatha* on body weight of streptozotocin induced diabetic rats after 14 days of treatment



Values are the mean ± SD, n=6; One-way ANOVA with Dunnet's multiple comparison test, *P<0.01 when compared to toxin control.

Table 3: Effect of *Amrtottara Kvatha* on blood glucose and insulin levels of streptozotocin induced Diabetic rats after 14 days treatment

Groups	Blood glucose (mg/dL)			Insulin (µIU/mL)
	Day 0	Day 7	Day 14	Day 14
Normal control	73.32 ± 2.50	73.68 ± 2.71	72.64 ± 2.35	5.10 ± 0.21
Diabetic control	330.31 ± 2.44	482.35 ± 1.50	566.32 ± 0.71	1.73 ± 0.51
Glibenclamide (5 mg/kg)	318.71 ± 1.83	126.31 ± 1.34*	107.82 ± 1.23*	4.67 ± 0.21*
AK (1/4)	323.10 ± 1.11	196.12 ± 1.54*	125.30 ± 1.33*	4.33 ± 0.30*
AK (1/2)	346.11 ± 2.82	244.71 ± 1.23*	126.65 ± 1.91*	4.62 ± 0.44*
AK (T)	380.12 ± 1.20	281.10 ± 2.12*	156.23 ± 1.62*	4.24 ± 0.23*
AK (DD)	381.73 ± 1.65	303.11 ± 1.82*	165.30 ± 1.61*	4.35 ± 0.45*

Values are the mean ± SD, n=6; One-way ANOVA followed by Dunnet's multiple comparison test, *P<0.01 when compared to toxin control.

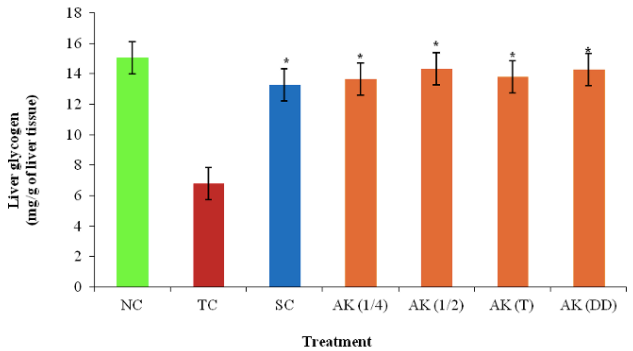
The biochemical parameters such as total cholesterol, triglycerides and creatinine were elevated in diabetic rats compared to the normal control while it was found to be significantly (P<0.01) lowered in AK (1/2) treated group compared to the standard control (Table 4). The liver glycogen level was increased in AK treated animals compared to the toxin control. The liver glycogen in AK (1/2) treated rats were estimated as 14.32 mg/g and it was comparable to the glycogen level in normal rats (15.03 mg/g) (Figure. 1B).

Table 4: Effect of *Amrtottara Kvatha* on biochemical parameters of streptozotocin induced Diabetic rats after 14 days treatment

Groups	Total cholesterol (mg/dL)	Triglycerides (mg/dL)	Creatinine (mg/dL)
Normal control	61.72 ± 1.50	44.18 ± 1.35	0.32 ± 0.13
Diabetic control	92.41 ± 1.74	142.52 ± 1.24	1.24 ± 0.96
Glibenclamide (5 mg/kg)	67.40 ± 1.11*	68.77 ± 1.33*	0.32 ± 0.14*
AK (1/4)	74.12 ± 0.93	89.35 ± 1.31*	0.52 ± 0.31
AK (1/2)	69.20± 1.21*	73.50 ± 1.84*	0.34 ± 0.23*
AK (T)	76.57 ± 1.44	79.92 ± 1.27*	0.40 ± 0.38*
AK (DD)	78.32 ± 1.60	83.55 ± 1.23*	0.56 ± 0.33

Values are the mean ± SD, n=6; One-way ANOVA followed by Dunnet's multiple comparison test, *P<0.01 when compared to toxin control.

Figure 1 B: Effect of *Amrtottara Kvatha* on liver glycogen levels of streptozotocin induced diabetic rats after 14 days of treatment



Values are the mean ± SD, n=6; One-way ANOVA with Dunnet's multiple comparison test, *P<0.01 when compared to toxin control.

The total protein in the pancreatic tissue was significantly (P<0.01) increased in AK administered groups compared to the diabetic control. MDA, an index of lipid peroxidation and advanced oxidation protein products, a marker of protein oxidation were also get elevated in the diabetic control group. MDA and AOPP levels were reduced in all AK treated groups compared to the toxin control (Table 5) indicating the ameliorative effect AK on streptozotocin induced oxidative stress.

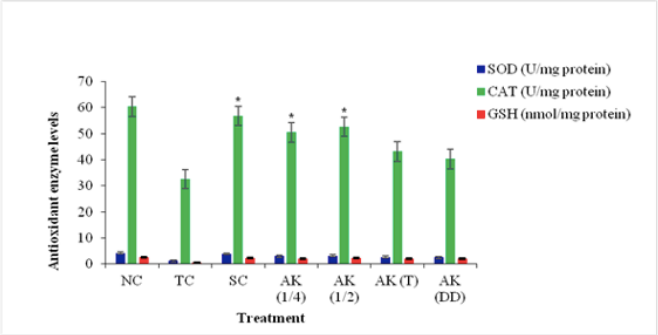
The antioxidant enzymes such as catalase, reduced glutathione and superoxide dismutase that prevent oxidative damage to the tissues were decreased in streptozotocin-induced diabetic rats. The AK treated groups exhibited a significant (P<0.01) increase in the levels of these antioxidant enzymes compared to the toxin control (Figure 1C).

Table 5: Effect of *Amrtottara Kvatha* on oxidative stress of streptozotocin induced diabetic rats after 14 days treatment

Groups	Total protein (mg/g tissue)	MDA (nmol/mg protein)	AOPP (nmol/mg protein)
Normal control	365.23 ± 1.40	0.21 ± 0.10	0.20 ± 1.30
Diabetic control	161.51 ± 1.57	0.72 ± 0.10	0.80 ± 0.20
Glibenclamide (5mg/kg)	355.85 ± 0.74	0.31 ± 0.13	0.31 ± 0.12*
AK (¼)	354.20 ± 1.63	0.33 ± 0.11	0.42 ± 1.20
AK (½)	330.23 ± 0.71	0.31 ± 0.11	0.41 ± 0.30*
AK (T)	355.50 ± 1.54	0.30 ± 0.12	0.50 ± 0.71
AK (DD)	354.11 ± 1.43	0.32 ± 0.11	0.50 ± 0.42

Values are the mean ± SD, n=6; One-way ANOVA followed by Dunnet's multiple comparison test, *P<0.01 when compared to toxin control.

Figure 1 C: Effect of *Amrtottara Kvatha* on antioxidant enzymes status of streptozotocin induced diabetic rats after 14 days of treatment

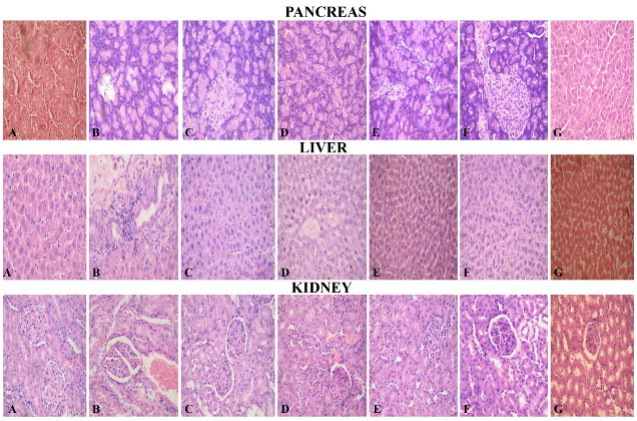


Values are the mean ± SD, n=6; One-way ANOVA with Dunnet's multiple comparison test, *P<0.01 when compared to toxin control.

The histopathological examination of pancreas, liver and kidney confirmed the protective effect of AK against streptozotocin induced diabetes in rats (Figure 2). The pancreas of diabetic rats showed degeneration islet patches. The pancreas of diabetic rats after the treatments with all the doses of AK and glibenclamide (5 mg/kg) showed pancreatic islets almost similar to normal control. The liver of streptozotocin induced diabetic control rats showed degenerated parenchymatous cells with severe necrosis and increased number of Kupffer cells. The histopathological observation of the liver of diabetic

rats treated with AK [AK (1/4), AK (1/2), AK (T), AK (DD)] showed hepatocytes with near normal appearance and minimal necrosis comparable to normal control and glibenclamide groups. The histopathological examination of kidney of toxin control showed broken glomeruli and Bowman's capsule with increased space. The kidney of diabetic rats after the treatment with AK at all the doses and glibenclamide (5 mg/kg) showed glomeruli and Bowman's capsule almost similar to normal control.

Figure 2: Histopathological evidence of the protective effect of *Amrtottara Kvatha* (AK)/ glibenclamide on the pancreas, liver and kidney of streptozotocin induced diabetes in Wistar rats



(A) Normal control, (B) Toxin control shows pancreas degeneration of islets patches, liver showing degenerated parenchymatous cells with severe necrosis and increased number of Kupffer cells and kidney with expanded glomerulus and thickened walls (C) glibenclamide control (5 mg/kg), (D), (E), (F) and (G) diabetic rat after treatment with AK (1/4), AK (1/2), AK (T) and AK (DD) respectively showing moderate histological changes. (Microscopic Magnification: x 400.

Discussion

The present study reports for the first time the effect of Ayurvedic polyherbal drug, AK, as an antidiabetic agent. Glucose tolerance test of AK was carried out in overnight fasted rats. The blood glucose lowering potential of AK can be attributed to the stimulatory effect of AK on β-cells of islets of Langerhans to stimulate insulin secretion (27). The treatment with AK did not show any significant effect in normoglycemic rats. Streptozotocin induced animals exhibited reduced response to insulin in hepatic and peripheral tissue. The results showed that streptozotocin effectively induced diabetes in normal fasted rats as reflected by the levels of blood glucose, body weight and other serum biochemical parameters. Streptozotocin induced diabetes is associated with the characteristic body weight loss resulted by the increased wasting of muscles and loss of tissue proteins. The body weight of diabetic control animals was found to be drastically decreased up on the induction of diabetes. Diabetic rats treated with AK showed a significant increase in body weight compared to untreated toxin control and it may be due to the protective effect of AK against muscle wasting and loss of tissue proteins (28) Streptozotocin produced significant increase in blood glucose level by 250-350 mg/dL by the selective destruction of insulin secreting pancreatic β-cells causing diabetes close to type-2 diabetes of humans (29). After 14 days' treatment, AK (1/4) and AK (1/2) reduced the blood glucose level in streptozotocin induced diabetic rats. This result can be correlated with inhibition of glycogenolysis as suggested by the increased liver glycogen level (30). The insulin level in AK (1/2) treated group had increased significantly after 14 days' treatment and it was almost equal to the standard drug glibenclamide in its efficiency. The increase in insulin level suggested the possible action of improved tissue glucose uptake. Thus the results suggested that the efficacy of AK may be due to the increase in insulin secretion. The histopathological results also supported the findings.

The most common lipid abnormalities associated with diabetes are hypercholesterolemia and hypertriglyceridemia, which are attributed to the excess mobilization of fat from adipose tissue due to underutilization of glucose (31). In the present study, the elevated levels of cholesterol and triglycerides were observed in streptozotocin induced diabetic rats. After treatment with AK, the levels of cholesterol and triglyceride were significantly reduced and comparable to the standard control. This may be achieved by the antihyperlipidemic effect of AK. The serum creatinine level was also elevated in diabetic rats because of the diminished ability of diabetic rats to filter creatinine from blood. AK administered group exhibited significant decrease in serum creatinine level. In the present study, the recovery towards the normalization of biochemical parameters and histopathological architecture caused by AK (1/2) treated group was almost similar to that caused by glibenclamide. Glibenclamide is a sulfonylurea drug used for treating diabetes and it could be considered as a standard drug for comparing the efficacy of anti-hyperglycemic compounds (32).

Streptozotocin induced diabetes results in the increased carbonyl stress and lipid peroxidation. Increased lipid peroxidation is due to the oxidative damage by increased peroxy radicals and hydroxyl radicals. Diabetogenic action of STZ also been attributed to the production of nitric oxide (NO) and reactive oxygen species (ROS) efficiency of this defence mechanism is altered in diabetes, and therefore free radicals will accumulate in the system, finally producing destructive effects in many vital tissues (33). MDA has been widely used as an index of lipid peroxidation and as a marker of oxidative stress. The significant decrease in MDA levels of AK treated groups may be attributed to the protective effect of AK on DNA from oxidative damage by enhancing natural antioxidant mechanism to lower MDA levels in the tissue (34). This revealed the protective effect of AK against oxidative stress induced by streptozotocin. The occurrence of protein oxidative damage in diabetes has been assessed by advanced oxidation protein products

and AOPP is significantly elevated in diabetes by the elevated carbonyl oxidation and oxidant-antioxidant imbalance. AOPP are the derivatives of oxidatively modified albumin, fibrinogen and lipoprotein formed as a result of intense oxidative stress (35). The hyperglycemic condition can promote accumulation of reactive oxygen species and reactive nitrogen species through various metabolic pathways, which results in the increased levels of advanced glycation end products (36). There was an elevation in AOPP in diabetic rats, but AK (1/2) administered group significantly decreased AOPP and showed 51.90% of protection and the standard drug glibenclamide showed 65.82% of protection against AOPP compared to toxin control. The result depicted the capability of AK (1/2) to restore the oxidant-antioxidant imbalance. The antioxidant enzymes such as SOD, catalase and reduced glutathione are responsible for the detoxification of deleterious free radicals (37). The levels of these antioxidant enzymes were found to be reduced in streptozotocin induced diabetic rats. However, the treatment with AK (1/2) and glibenclamide (5 mg/kg) resulted in a significant increase in these antioxidant enzyme levels by which they can effectively prevent the formation of free radicals or scavenge the reactive oxygen species.

The results of the present study also suggested the non-toxic nature of AK up to 3200 mg/kg in mice and this was not surprising as it is extensively used as a traditional medicament to treat various ailments. AK revealed the presence of flavonoids, alkaloids and phenolic compounds in the qualitative phytochemical analysis. The presence of nine compounds such as gallic acid, chebulic acid, tartaric acid, quinic acid, protocatechuic acid-4-glucoside, quercetin, caffeoyl glucose, quercetin-3-glucuronide and quercetin rhamnoside have been reported in AK. Flavonoids and their derivatives can act as potent antidiabetic agents (38). Perhaps the antioxidant compounds such as gallic acid, quercetin, chebulic acid and protocatechuic acid-4-glucoside present in AK might be responsible for the quenching of free radicals formed by the diabetogenic reactions of streptozotocin and they may also strengthen the activity of antioxidant enzymes. The current study showed that AK had reduced oxidative stress induced by streptozotocin. Ellagic acid in AK has been estimated by HPTLC method a 0.05 ± 0.016% w/w (39). It has been reported that dietary ellagic acid can act as a potent antidiabetic agent by improving insulin sensitivity, secretion and decreased postprandial hyperglycemia via inhibition of intestinal α-glucosidase activity (40). The increased insulin levels in diabetic rats treated with AK (1/2) may be due to the presence of ellagic acid.

Conclusion

In conclusion, our study reports for the first time the antidiabetic effects of AK, an Ayurvedic polyherbal drug in streptozotocin induced diabetic rats. Administration of AK at AK (1/2) (Rat dose comparable to half of the adult human therapeutic dose of AK (0.9 mL/200 g b.wt., p.o.) improved the diabetic symptoms with reduced body weight, elevated blood glucose level, hyperlipidemia and oxidant–antioxidant imbalance. These findings indicate that AK is very effective and useful as a traditional medicament for the management of diabetes.

The mechanism behind the effect may be due to the antioxidant and antihyperglycemic properties as well as quenching of free radicals, formed as a result of the diabetogenic reactions of streptozotocin. The polyherbal formulation also strengthens the activities of antioxidant enzymes. However, further research is warranted to determine the precise mechanism of action.

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