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Research Article

A Comparative Study on the Anti-Diabetic Potential of Aloe vera Gel and Fenugreek Seeds on Experimentally induced Diabetic Rats

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ABSTRACT: In the present study, the anti-diabetic property of an ethanolic extract of Aloe vera leaf gel and fine crude powder of Fenugreek seeds were compared. Oral administration of Aloe vera gel extract at a concentration of 300 mg/kg body weight and Fenugreek seeds powder extract at a concentration of 2g/kg body weight to alloxan-induced diabetes in rats significantly decreased the levels of blood glucose and glycosylated hemoglobin. The increased levels of lipid peroxidation in the pancreas of diabetic rats were reverted back to near normal levels after the treatment with Aloe vera gel and Fenugreek seeds powder extract. A significant increase was observed in SOD, vitamin C, vitamin E levels in Aloe vera gel and Fenugreek seeds powder extract treated rats. These extracts were also more effective than glibenclamide in restoring the levels to near normal.

KEY WORDS: *Aloe vera*, Fenugreek seeds, Alloxan-induced diabetes, anti-diabetic.

INTRODUCTION

Diabetes mellitus, often referred to simply as diabetes, is a syndrome of disordered metabolism, usually due to defects in either insulin secretion or resistance to its effects, resulting in abnormally high blood sugar levels (hyperglycemia).[1] The signs of diabetes are excessive urine production, thirst, increased fluid intake, blurred vision, weight loss. The secondary complications include cardiovascular, renal, neurological and ocular diseases. [2] Alloxan is widely used to induce diabetes in a wide variety of species by damaging the insulin secreting pancreatic β-cell, resulting in decrease in endogenous insulin release.[3]

Oral hypoglycemic drugs are used for the treatment of diabetes. Glibenclamide is a commonly used hypoglycemic drug which is classified in the category of sulfonylurea drugs. It stimulates insulin secretion from the existing pancreatic cells. Glibenclamide mainly acts by inhibiting ATP-sensitive K^+ (K_{ATP}) channels in the plasma membrane.[4] The inhibition of ATP sensitive channels leads to membrane depolarization, activation of voltage-gated Ca^{2+} channels, increased Ca^{2+} influx, a rise in cytosolic (Ca^{2+}) and thereby insulin release. Glibenclamide is often used as a standard drug in moderate diabetic model to compare the antidiabetic properties of variety of compounds.[5]

The global scenario of medical research is towards herbal treatment. The uses of medicinal plants in the treatment of diabetes mellitus are common practices in wide areas, especially in Eastern States of India such as Assam, Manipur, Mizoram, where people depend solely on herbal treatments. [6] Aloe vera grows in tropical climates and is widely distributed in Africa, Asia and other tropical areas.[7] It is a perennial plant belonging to the family of Liliaceae, which includes about 360 species.[8] Taxonomists often refer to *Aloe barbadensis* as Aloe vera.[9] Aloe vera is a stemless or very short-stemmed succulent plant growing to 60–100 cm (24–39 in) tall. The stems are thick and fleshy, green to grey-green in color, with some varieties showing white flecks on the upper and lower stem surfaces.[10] *Trigonella foenum-graecum* (Fenugreek) is native to the Eastern Mediterranean, Central Asia and Ethiopia, and is much cultivated in India, Pakistan and China.[11] It has a long history of medical uses in Ayurvedic and Chinese medicine, and has been used for numerous indications, including labour induction, indigestion

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and as a general tonic to improve metabolism and health.[12] The present study was carried out to compare the antidiabetic potential of Fenugreek seeds and Aloe vera gel in alloxan induced diabetic rats.

MATERIALS AND METHODS

Experimental animals and chemicals

Healthy male albino rats (140gm- 160gm) were purchased from the animal house, Manaparai. The groups of rats were kept separately in individual stainless steel hoppers. The test animals were characterized by strain, source, sex, weight and age. The animals were kept individually for feeding with conventional laboratory diets and an unlimited supply of drinking water. The experiments were designed as per guidelines of institutional ethical committee. Alloxan monohydrate was procured from the National chemicals Pvt. Ltd. Glibenclamide was used to treat diabetes and was procured from Aventis Pharma Limited, Goa. It was administered with the dosage of 600 μg/kg b.w.[13] . Other chemicals used were of analytical grade and were procured from SISCOM, Thillai nagar, Tiruchirappalli.

Herbal preparations

Fresh leaves of *Aloe barbadensis* were collected from Tiruchirappalli district, Tamil nadu, India in October 2010. The material was identified by Dr. John Britto, Rapinat Herbarium at St. Joseph's College, Tiruchirappalli, Tamil nadu, India. A voucher specimen (KA001 2011/01) was deposited in the St Joseph's College, Tiruchirappalli, Tamil nadu, India. The fresh leaves were washed with water and were cut transversely into pieces. The thick epidermis was selectively removed and fleshy solid gel was cut into small pieces. The gel was refluxed with absolute ethanol and dried in a heating mantle. An aqueous preparation of dried powder was administered with the dosage of 300 mg/kg b.w.[13] Fenugreek seeds were procured from the local market, Tiruchirappalli, India and powdered using an electric grinder and stored in a air tight container at room temperature. Aqueous preparation of this fine crude powder was administered with the dosage of 2 g/kg b.w.[14]

Experimental Design

The rats were divided into four groups (n = 6). Group I served as a control and received 0.9% saline. Alloxan monohydrate was administered at the dosage of 150 mg/kg b.w, i.m.[16] as a diabetes inducer except to the control group. Group II was alloxan induced diabetic rats without further treatment. Group III received *Aloe barbadensis (Aloe vera)* gel (300 mg/kg b.w., oral).[13] Group IV received aqueous extract of *Trigonella foenumgraecum* (fenugreek) (2g/kg b.w., oral).[14] Group V received the standard drug Glibenclamide (600 μg/kg b.w)[15] for 21 days. *Aloe barbadensis, Trigonella foenumgraecum* and Glibenclamide were given as co-treatment.

Study protocol

At the end of experiment, rats were sacrificed by cervical decapitation. Blood was collected and used for further study. The pancreas was dissected, washed using ice cold saline solution (0.9%), homogenized, and centrifuged. The resulting supernatant was used for various biochemical assays.

Blood glucose levels were determined by the method of GOD-PAP.[17] Glycosylated Hemoglobin was determined by ion exchange resin method.[18] TBARS in tissues were estimated by a previously described method.[19] Superoxide dismutase (SOD) activity was determined by a previously described method.[20] Ascorbic acid (Vitamin C)[21] and α-tocopherol[22] were estimated by previously described method.

Statistical analysis

The data obtained in the present investigation was subjected to statistical analysis. All results are expressed as Mean ± S.D.

RESULTS

Table I shows the levels of glucose and glycosylated hemoglobin in normal and alloxan induced diabetes in rats. The glucose level and glycosylated haemoglobin was significantly elevated in the alloxan -induced groups (Group II) when compared to the control group (Group I) of rats. On administration of herbal drugs and standard drug viz Aloe vera, Fenugreek and Glibenclamide to the Group III, IV and V rats, the level of glucose and Glycosylated haemoglobin was significantly reduced when compared to alloxan induced rats (Group II).

Table 2 shows the levels of TBARS in the pancreas of normal and alloxan induced rats. There was a significant elevation in the levels of TBARS in alloxan-induced group (Group II) compared to normal (Group I). On treatment with herbal drugs and standard drug viz, Aloe vera, Fenugreek and Glibenclamide to Group III, IV and V rats, there was significant decrease in the levels of TBARS when compared to alloxan-induced group (Group II).

Table 3 shows the changes in the levels of SOD, vitamin C and Vitamin E in pancreatic tissue in normal and alloxan induced rats. The activities of SOD, vitamin C and Vitamin E were decreased significantly in alloxan-induced group (Group II) when compared to normal (Group I). Herbal drugs and standard drug treatment viz, Aloe vera, Fenugreek and Glibenclamide to Group III, IV and V rats, significantly increased the activities of SOD , vitamin C and Vitamin E in pancreatic tissue compared to alloxan- induced group (Group II).

Table 1: Changes in the level of glucose and glycosylated haemoglobin.

Parameters	Control group	Alloxan induced group	Aloe vera treated group	Fenugreek seed treated group	Glibenclamide treated group
Glucose (mg/dl)	137.75 ± 2.62	314.25 ± 30.24^{*a} 128.1%	152 ± 1.63^{**a} 51.63%	159 ± 5.22^{**a} 49.40%	163 ± 8.52^{**a} 48.13%
Glycosylated Haemoglobin %	6.52 ± 0.38	12.4 ± 0.25^{*a} 90.2%	7.5 ± 0.42^{**a} 63.7%	8 ± 0.31^{**a} 35.4%	8.6 ± 0.16^{**a} 30.6%

Values represent means ± S.D; n = 6;
* - denotes comparison between group I Vs group II; $^{a-}P < 0.001$;
** - denotes comparison between group II Vs group III, IV, V; $^{a-}P < 0.001$.

Table 2: Changes in the activities of TBARS in pancreatic tissue and plasma.

Parameters	Control group	Alloxan induced group	Aloe vera treated group	Fenugreek seed treated group	Glibenclamide treated group
TBARS (mM/mg in tissue)	1.025 ± 0.15	1.5 ± 0.21^{*a} 46.3%	1.1 ± 0.40^{**a} 26.7%	1.15 ± 0.1^{**a} 23.3%	1.17 ± 0.25^{**a} 22%

Values represent means ± S.D; n = 6;
* - denotes comparison between group I Vs group II; $^{a-}P < 0.001$;
** - denotes comparison between group II Vs group III, IV, V; $^{a-}P < 0.001$.

Table 3: Changes in the activities of Antioxidants in pancreatic tissue and plasma.

Para meters	Control group	Alloxan induced group	Aloe vera treated group	Fenugreek seed treated group	Glibenclamide treated group
SOD (units/mg protein)	48 ± 2.16	14 ± 0.81 ^{*a} 70.8%	31.25 ± 2.62^{**a} 123.2%	28.28 ± 4.5^{**a} 102%	21 ± 4.32^{**a} 50%
Vitamin C(mg/dl)	3.9 ± 0.16	2.4 ± 0.21^{*a} 38.5%	2.7 ± 0.29^{**a} 25%	3.1 ± 0.16^{**a} 29.2%	2.9 ± 0.21^{**a} 20.8%
Vitamin E(mg/dl)	1.22 ± 0.18	0.85 ± 0.05^{*a} 30.61%	1.2 ± 0.08^{**a} 41.17%	1.12 ± 0.15^{**a} 31.76%	1.02 ± 0.12^{**a} 20.58%

Values represent means ± S.D; n = 6;
* - denotes comparison between group I Vs group II; $^{a-}P < 0.001$;
** - denotes comparison between group II Vs group III, IV, V; $^{a-}P < 0.001$.

DISCUSSION

Alloxan, a beta cytotoxin, induces diabetes in animals by damaging the pancreas, which decreases the utilization of glucose by the tissues.[23] Excess glucose present in the blood combines with hemoglobin to form glycosylated hemoglobin and is accumulated in glomerulus as advanced glycation end products (AGEs). This accumulation results in kidney hypertrophy and damage. Insulin deficiency leads to various metabolic aberrations in the animals resulting in increased blood glucose,[24] decreased protein content[25] and increased levels of cholesterol and triglycerides.[26] The rate of glycation of hemoglobin is propotional to the concentration of blood glucose.[27]

Administration of alloxan significantly increased the level of glucose and glycosylated haemoglobin when compared to control rats. Alloxan is relatively toxic to insulin producing pancreatic beta cells because it preferentially accumulates in beta cells through uptake via the GLUT2 glucose transporter. Hence the production of insulin is reduced.[3] There was also a significant reduction in the blood glucose and glycosylated hemoglobin levels in *Aloe barbadensis* and *Trigonella foenum graecum* treated diabetic rats thus showing improvement in glycemic status which may be due to its protective effect on pancreatic cells.

Sauvaire *et al*[28] in his *in vitro* studies demonstrated that the amino acid 4-hydroxyisoleucine in *Trigonella foenum graecum* seeds increased glucose-induced insulin release in human and rat pancreatic islet cells. This amino acid appeared to act only on pancreatic beta cells, since the levels of somatostatin and glucagon were not altered. *Trigonella foenum graecum* seeds also exert hypoglycemic effects by stimulating glucose- dependent insulin secretion from pancreatic beta cells, as well as by inhibiting the activities of alpha-amylase and sucrase, two intestinal enzymes involved in carbohydrate metabolism.

In addition to the amino acid in the *Trigonella foenum graecum*, a high-fibre content is also associated in controlling blood glucose level. High fibre diet increases the number of insulin receptor sites. Certain dietary fibres reduce the rate of food passage through the intestine and into the bloodstream,

thereby helping to control the increase in postprandial blood sugar levels. Water-retaining fibres, especially the mucilaginous compounds, such as the gel fibre present in *Trigonella foenum graecum* seeds, reduce the rate of glucose absorption and may also delay gastric emptying thereby preventing the rise in blood sugar levels following a meal.

There are two possible explanations for the antidiabetic property of *Aloe barbadensis*. It may have exerted its effect by preventing the death of β-cells and it may permit recovery of partially destroyed β-cells. Aloe vera may also have initiated cell proliferation.[29]

Oxidative stress that leads to an increased production of reactive oxygen species (ROS) and finally cellular lipid peroxidation has been found to play an important role in the development of diabetes mellitus.[30-32] The mechanism by which production of lipid peroxide (LPO) is enhanced in diabetes is by hypoinsulinemia. It is thought that hypoinsulinemia increases the activity of the enzyme fatty acyl coenzyme A oxidase, which initiates beta-oxidation of fatty acids, resulting in LPO.[33] Increased LPO impairs membrane function by decreasing membrane fluidity and changing the activity of membrane-bound enzymes and receptors. [34, 35]

The increase in liver tissue Thio barbituric acid reactive species (TBARS) levels in the diabetic group animals is in agreement with the well known fact that tissues of diabetic animals exhibit increased oxidative stress and disturbances in antioxidant defense compared to normal controls. *Aloe barbadensis* and *Trigonella foenum graecum* extract treated rats showed reduced TBARS levels.

Oxidative stress in cells results from the increased production of reactive oxygen species. Antioxidant defense potential also decreased due to the increased generation of ROS.[36] The harmful effects of free radical production to the body are protected by the antioxidants.[37] In diabetes, this defense mechanism is altered and leads to damaged tissues.[38] Super oxide dismutase (SOD) scavenges the superoxide radical by converting it to H_2O_2 and molecular oxygen.[39] The activity of SOD was found to be lower in alloxan induced rats. *Aloe barbadensis* and *Trigonella foenum graecum* extract treated rats showed increase in the levels of SOD.

Gupta and Flora[40] reported antioxidant components in *Aloe barbadensis*. They examined lipid peroxidation using rat liver microsomal and mitochondrial enzymes. Among the aloesin derivatives examined, isorabaichromone showed a potent antioxidant activity. Khosla *et al* [14] in their studies reported that flavonoids and polyphenols from fenugreek showed significant antioxidant activity in fenugreek seeds which may be due partly to the presence of flavonoids and polyphenols. Fenugreek was found to contain saponin compounds, namely diosgenin and yamogenin. Trigonelline is the important alkaloid isolated from the seeds. The other important constituents are coumarins, fenugreekine, nicotinic acid, phytic acid, and scopoletin.

Vitamin C is an excellent hydrophilic antioxidant in plasma, because it disappears faster than other antioxidants when plasma is exposed to reactive oxygen species.[41] As an antioxidant, vitamin C's primary role is to neutralize free radicals. Since ascorbic acid is water soluble, it can work both inside and outside the cells to combat free radical damage. Vitamin C is an excellent source of electrons; therefore, it "can donate electrons to free radicals such as hydroxyl and superoxide radicals and quench their reactivity".[42]

Vitamin E is a fat-soluble vitamin that helps to prevent damage to lipids by scavenging free radicals. [43] When highly reactive species attack lipids within membranes or lipoproteins, they set off the chain reaction of lipid. Vitamin E halts this chain reaction by breaking the chain of lipid peroxidation.[44] In diabetic rats treated with herbal extracts, a significant increase in activity of these antioxidants was observed.

Other antioxidants like catalase, reduced glutathione, glutathione peroxidase and glutathione S transferase also play an important role antioxidant status of the rats. Further studies are needed to rule out the role of these enzymes in antioxidant nature of *Aloe barbadensis* and *Trigonella foenum graecum*.

In conclusion, the effect of *Aloe barbadensis* extract, *Trigonella foenum graecum* seed powder extract and glibenclamide showed reduction in blood glucose levels in diabetic rats that might be due to an enhancement in glycemic status and antioxidants levels. It is evident from these results that the levels of all the parameters treated with *Aloe barbadensis* and *Trigonella foenum graecum* was quite comparable with glibenclamide and that *Aloe barbadensis* is better remedy when compared to *Trigonella foenum graecum* and the standard drug.

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