

Comparative Study of Antidiabetic and Antioxidant Activities of the Ethanolic Extracts of Leaves and Bark of *Acacia Farnesiana* (L.) Willd. in Streptozocin Induced Type 1 Diabetic Experimental Animal Models

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Abstract: Objective: The present study was carried out to evaluate the antidiabetic and antioxidant effect of the ethanolic extract of the leaves and bark of *Acacia farnesiana* (L.) Willd. in streptozotocin induced type 1 diabetic animal model and also to explore the probable mechanism of their activities.

Materials and methods.: A single intraperitoneal injection of streptozocin 150mg/kg was injected in mice to induce diabetes. Mice with blood glucose level >200 mg was considered diabetic and taken in the experiment. Oral administration of the ethanolic extract of leaves (EELAF) and bark (EEBAF) of *Acacia farnesiana* were administered p.o for 14 days. Blood glucose was measured at repeated intervals and serum insulin, tissue glycogen and antioxidant parameters were evaluated at the 15th day of experiment. The effect of the drugs on intestinal glucose absorption was evaluated on Wistar rats.

Results: Streptozocin induced diabetic mice showed significant hyperglycaemia and loss of body weight with alteration of serum insulin, tissue glycogen and antioxidant levels. On treatment with EELAF, EEBAF and insulin there was significant fall in blood glucose level and increase in weight with normalization of the impaired glycogen and antioxidant variables. Both extracts also showed significant inhibitory activity on intestinal glucose absorption.

Conclusion: In conclusion, our data suggests that both the plant extracts had potential anti-diabetic and antioxidant activity in STZ induced type 1 diabetic experimental model.

Keywords: Streptozocin, insulin, ethanolic extract.

Introduction

Diabetes mellitus is a group of metabolic diseases characterized by hyperglycemia resulting from defects in insulin secretion, insulin action, or both. ^[1]

Free radicals are formed disproportionately in diabetes by various mechanisms and increasing evidence in both experimental and clinical studies suggests that oxidative stress plays a major role in the pathogenesis of both types of diabetes mellitus. ^[2]

The International Diabetes Federation (IDF) estimates the total number of diabetic subjects to be around 382 million and this is further to be set to rise to 592 million by the year 2035. ^[3]

The approach to the prevention and treatment of diabetes has been transformed since the discovery of insulin ^[4]. Research has been directed to develop newer compounds to complement or supplement the existing treatment for type 1 diabetes. ^[5]

During the last two decades the traditional system of medicine and medicinal plants research has become topics of global interest and importance.^[6] The World Health Organization (WHO) has also substantiated the utilization of herbal remedies for the management of diabetes.^[7] So keeping this in mind the present study has been designed to evaluate and compare the antidiabetic, and antioxidant activities of leaves and bark of *Acacia farnesiana*(L.) Willd.. on Streptozotocin-induced type 1 diabetic mice and also to explore the probable mechanism of action

Acacia farnesiana belongs to the family Fabaceae is a woody shrub with spiny branches and fragrant yellow flowers.^[8] The different parts of the plant are used for its antispasmodic, aphrodisiac, astringent, demulcent, diarrhoea, rheumatism, stimulant^[9] and antidiabetic^[10] properties.

Materials and methods

Chemicals

Analytical grade chemicals were used for all the experiments. The streptozocin was purchased from Hi-Media India limited

Plant material

The leaves along with the bark of *Acacia farnesiana*(L.)Willd was collected in the months of April 2013. The leaves and the bark of the plant material were identified by Dr L. R Saikia in the Department of Life Sciences, Dibrugarh University. Avoucher specimen (V.No. DUL.Sc.2541) was deposited at the Department of Life Sciences, Dibrugarh University

The leaves and bark were washed thoroughly with tap water, shade dried, cut into small pieces and were crushed to moderately coarse powder. It was extracted using 95% ethanol in a percolator. The leaves and bark yield were 35.5% and 27% respectively.

Experimental animal

Both healthy adult male albino mice of 25-40 g and albino male Wistar rats, weighing 150-200 g, were used in the study. They were kept in standard laboratory conditions .Animals had access to standard pellet diet and water given *ad libitum*. All the animals were taken care of under ethical consideration. Permission from the Institutional Animal Ethics Committee for the study of animals was duly obtained.

Phytochemical screening

EELAF and EEBAF were subjected to qualitative phytochemical analysis for alkaloids, flavonoids, tannins, saponins, diterpenes, triterpenes and phenols as per the standard methods^[11]

Acute (oral) Toxicity Study

Acute oral toxicity test for the ethanolic extracts of leaves (EELAF) and bark of *Acacia farnesiana* (EEBAF) were carried out as per OECD Guidelines 425 and was found safe up to 2000 mg/kg p.o for each extract. Two doses of the extracts 400mg/kg and 800mg kg were selected for the study.

Induction of experimental diabetes

Swiss albino mice were selected for the study. A single dose of the streptozotocin (STZ) to be injected in overnight fasted animals was extemporaneously prepared in ice cold citrate buffer (pH 4.5). STZ (150 mg/kg) was injected intrapeitoneally to the animals. Control mice received an equivalent amount of citrate buffer^[12].The mice had free access to 5% of glucose water and basal diet *ad libitum* during the next 24 hours. Hyperglycemia was confirmed by the elevated glucose levels in plasma, determined at 72 h. The animals with blood glucose concentration more than 250 mg/ml were used for the study.^[13]

Method of collection of blood

Under all aseptic and antiseptic measure and under ether inhalation anaesthesia, blood samples were collected via cardiac puncture. Serum was obtained from the collected blood for estimation of serum insulin. ^[14]

Experimental Design for Study of antidiabetic, and Antioxidant Activities

35 albino mice out (of which 30 were diabetic) weighing 25-35 grams were divided into 7 groups of 5 animals each and treated as below.

Normal Control:	Received Normal Saline at a dose of 5 ml/Kg/day p.o.
Diabetic Control:	Received Normal Saline at a dose of 5ml/Kg/day p.o.
EELAF(400) :	Received EELAF at a dose of 400mg/Kg/day p.o.
EELAF (800):	Received EELAF at a dose of 800mg/Kg/day p.o..
EEBAF (400):	Received EEBAF at a dose of 400mg/Kg/day p.o.
EEBAF (800):	Received EEBAF at a dose of 800mg/Kg/day p.o..
Insulin:	Received NPH insulin at a dose of 2U/kg/day s.c. ^[15]

All the animals used for the experiment were kept under observation daily for food and water intake. The drugs were administered orally, once daily, to the animals in the doses given above for continuous two weeks by means of a feeding tube. Fasting blood samples were collected from the tail of each mouse and measured by glucometer.

At the very first day i.e., after 72 hours of induction of diabetes and then on the 8th and 15th day of the experiment blood will be collected from the tail of the mice under aseptic and antiseptic conditions and fasting blood glucose level will be estimated by glucometer.. On the 15th day blood will be collected for the estimation insulin and then animals were euthanized and liver, muscle and heart were taken out with care for estimation of biochemical parameters. The body weight of the animals in each group was recorded on Day 1 and Day 15 of the experiment.

Estimation of other biochemical parameters:

Serum insulin levels were assessed on day 15 of the experiment by radioimmunoassay ^[16].The liver was excised and were homogenized by tissue homogenizer and the supernatant was used for used the determination of lipid peroxidation^[17] enzymatic antioxidant like catalase,^[18] superoxide dismutase(SOD)^[19] Glutathione S-Transferase(GST)^[20] and Reduced Glutathione(GSH)^[21] The total proteins were estimated by Lowery et al. (1951) method.^[22]

Experimental Design for estimation of tissue glycogen

35 mice (out of which 30 were diabetic) were divided into 7 groups of 5 animals each. After 72 hours of induction of diabetes, the mice were kept fasting for 18 hours and then the animals were euthanized for estimation of glycogen level in the liver and muscle tissues. ^[23]

Study groups

Normal Control:	Received Normal Saline at a dose of 5ml/Kg/day p.o.
Diabetic Control:	Received Normal Saline at a dose of 5ml/Kg/day p.o.
EELAF(400) :	Received EELAF at a dose of 400mg/Kg/day p.o.
EELAF (800):	Received EELAF at a dose of 800mg/Kg/day p.o..
EEBAF (400):	Received EEBAF at a dose of 400mg/Kg/day p.o.
EEBAF (800):	Received EEBAF at a dose of 800mg/Kg/day p.o..
Insulin:	Received NPH insulin at a dose of 2U/kg/day s.c. ^[15]

Experimental Design for estimation of intestinal glucose absorption

The experiment for effect of the plant extracts on intestinal glucose absorption was done on male Wistar rats by the method described by Das S et al (2001) with some modifications. ^[24]

The rats were divided into six groups with five animals for each group.

Normal Control:	Received Normal Saline at a dose of 10 ml/Kg/day orally.
EELAF 400 :	Received EELAF at a dose of 400mg/Kg/day orally.
EELAF 800 :	Received EELAF at a dose of 800mg/Kg/day orally.
EEBAF 400 :	Received EEBAF at a dose of 400mg/Kg/day orally.
EEBAF 800 :	Received EEBAF at a dose of 800mg/Kg/day orally.
Standard Drug:	Received Metformin at a dose of 135mg/Kg/day

Statistical Analysis

Mean values were obtained by one-way analysis of variance (ANOVA) followed by Tukey's test, using the computer software, Graph pad Prism 6. The significance of difference between and within various groups was determined. The results are expressed as mean \pm S.E.M. Values of $p < 0.05$ were taken to imply as statistically significant.

Results

Acute toxicity study

Acute toxicity studies revealed the non toxic nature of both the extract. All the animals were alive, healthy, and active during the observation period

Phytochemical constituents

The preliminary phytochemical studies indicated the presence of alkaloids, saponins, steroids, flavonoids, tannins, and glycosides.

Effect of EELAF and EEBAF on fasting blood glucose level in type 1 diabetic mice (Table 1)

In normal control group there is no significant change of fasting blood glucose level. However there was a persistent increase in blood sugar level of streptozotocin induced diabetic control group and both the extract treated groups with insulin with their respective doses showed gradual and moderate antihyperglycemic effect. (Table 1)

Table 1: Effects of EELAF and EEBAF on Fasting Blood Glucose (FBG) levels of Streptozocin induced type 1 diabetic mice

Groups	Mean fasting blood glucose level (mg/100 ml)			
	Day '0' (baseline)	Day '1'(after 72 hours)	Day 8 th	Day 15 th
Normal control	107.8 \pm 3.74	109.2 \pm 2.92	111.2 \pm 2.43	113 \pm 3.262
Diabetic control	107.8 \pm 3.86	265.2 \pm 5.14 ^a	275.8 \pm 3.56 ^a	294.4 \pm 4.93 ^a
EELAF 400	104.6 \pm 2.87	270 \pm 2.966 ^a	238.6 \pm 2.61 ^b	230.60 \pm 3.58 ^b (21.67%)
EELAF 800	101.8 \pm 1.98	266.8 \pm 8.54 ^a	225 \pm 4.51 ^b	215.60 \pm 7.89 ^b (26.76%)
EEBAF 400	107.4 \pm 2.29	280.2 \pm 3.61 ^a	227.4 \pm 1.86 ^b	216.60 \pm 4.12 ^b (26.42%)
EEBAF 800	108.6 \pm 2.83	281.4 \pm 5.26 ^a	205.8 \pm 5.87 ^b	194.40 \pm 5.47 ^b (34%)
Insulin	99 \pm 2.40	277.2 \pm 6.64 ^a	173.6 \pm 2.82 ^b	162.20 \pm 4.31 ^b (44.90%)
ANOVA <i>p</i>	>0.05	>0.05	<0.05	<0.05

Evaluation of antioxidant status of EELAF and EEBAF (Table 2)

There was a significant ($p < 0.05$) elevation in TBARS levels and reduction CAT, SOD, GST and GSH levels in liver of diabetic mice compared to control mice. The administration of plant extracts

and insulin significantly ($p < 0.05$) reversed these changes. In all investigational parameters there was increase activities of the both the extracts with increase of doses. (Table 2)

Table 2: Effects of EELAF and EEBAF on antioxidant levels in liver of Streptozocin induced type 1 diabetic mice.

Liver	Catalase	SOD	GST	GSH	TBARS
	$\mu\text{M H}_2\text{O}_2/\text{min}/\text{mgprotein}$	U/mg protein	$\mu\text{mol}/\text{min}/\text{mg protein}$	mg/100gm wet tissue	nM of MDA /mg protein
Normal control	101.80 ± 6.351	7.08 ± 0.496	5.76 ± 0.625	41.05 ± 1.871	0.88 \pm 0.159
Diabetic control	51.00 $\pm 4.219^a$	2.38 $\pm 0.511^a$	1.80 $\pm 0.626^a$	19.01 \pm 1.461 ^a	2.84 $\pm 0.186^a$
EELAF 400	74.60 $\pm 2.694^b$	4.06 $\pm 0.172^b$	3.73 $\pm 0.343^b$	24.98 \pm 1.479	1.96 $\pm 0.193^b$
EELAF 800	84.80 $\pm 4.352^b$	5.59 $\pm 0.243^b$	4.10 $\pm 0.252^b$	29.98 $\pm 1.771^b$	1.57 $\pm 0.179^b$
EEBAF 400	81.00 \pm 4.827 ^b	4.12 $\pm 0.156^b$	3.61 $\pm 0.130^b$	29.60 $\pm 3.013^b$	1.72 $\pm 0.180^b$
EEBAF 800	85.20 $\pm 4.800^b$	5.16 $\pm 0.271^b$	4.56 $\pm 0.282^b$	34.59 $\pm 1.724^b$	1.57 $\pm 0.110^b$
Insulin	83.20 $\pm 2.657^b$	5.74 $\pm 0.281^b$	4.75 \pm 0.218 ^b	35.42 $\pm 1.434^b$	1.28 $\pm 0.163^b$
Anova P	<0.05	<0.05	<0.05	<0.05	<0.05

Estimation of serum insulin. (Table 3)

On 15th day serum insulin was estimated and there is significant fall ($p < 0.05$) in diabetic group. On administration of EELAF, EEBAF and insulin there is progressive increase in the serum insulin levels. All doses were statistically significant ($p < 0.05$) except EELAF 400mg/kg. (Table 3)

Table 3: Effects of EELAF and EEBAF on serum insulin of Streptozocin induced type 1 diabetic mice

Groups	Serum insulin ($\mu\text{IU}/\text{ml}$)
	Day 15 th
Normal control	23.40 \pm 0.678
Diabetic control	8.40 \pm 1.162 ^a
EELAF 400	11.20 \pm 0.663
EELAF 800	13.00 \pm 0.548 ^b
EEBAF 400	13.40 \pm 0.483 ^b
EEBAF 800	15.00 \pm 1.414 ^b
INSULIN	18.00 \pm 1.225 ^b
ANOVAP	<0.05

Estimation of tissue glycogen levels (Table 4)

There was significant ($p < 0.05$) decrease in glycogen reserves in liver and muscles in diabetic group. On administration of EELAF, EEBAF and insulin there is increase in the glycogen stores in liver and muscle. All doses were statistically significant ($p < 0.05$) except EELAF 400mg/kg in its activity to increase glycogen levels. On the contrary there is significant increase in glycogen store in cardiac muscles in diabetic group. However on treatment with plant extracts and insulin there was continuous fall in the glycogen store in the test groups. All doses being statistically significant ($p < 0.05$) (Table 4).

Table 4: Effects of EELAF and EEBAF on tissue glycogen levels of Streptozocin induced type 1 diabetic mice.

Groups	Liver	Skeletal muscle	Cardiac muscle
	mg/100gm	mg/100gm	mg/100gm
Normal control	53.60 ± 2.56	12.02 ± 0.58	07.93 ± 0.242
Diabetic control	25.20 ± 2.69 ^a	6.82 ± 2.68 ^a	21.12 ± 0.403 ^a
EELAF 400	34.20 ± 2.05	7.55 ± 0.38	14.93 ± 0.415 ^b
EELAF 800	44.80 ± 1.43 ^b	9.53 ± 0.49 ^b	11.33 ± 0.262 ^b
EEBAF 400	43.20 ± 2.97 ^b	8.92 ± 0.38 ^b	12.27 ± 0.628 ^b
EEBAF 800	38.20 ± 1.60 ^b	9.87 ± 0.30 ^b	11.15 ± 0.404 ^b
Insulin	40.40 ± 2.76 ^b	10.69 ± 0.56 ^b	10.17 ± 0.297 ^b
ANOVA P	<0.05	<0.05	<0.05

Effect on intestinal glucose absorption (Table 5)

Both EELAF and EEBAF and metformin in their respective doses showed significantly reduced glucose absorption ($P < 0.05$) when compared with the control. There was also significant difference in the absorption ($p < 0.05$) between EEBAF and EELAF, the effect of EEBAF (800mg/kg) dose being significantly more the EELAF(800mg/kg). (Table 5)

Table 5: Effects of EELAF and EEBAF on intestinal glucose absorption of Streptozocin induced type 1 diabetic mice.

Groups	Glucose absorption (mg/g dry wt/hr)
Normal control	121.60 ± 3.15
EELAF 400	103.80 ± 3.33 ^a
EELAF 800	99.00 ± 3.42 ^a
EEBAF 400	92.60 ± 3.07 ^a
EEBAF 800	79.40 ± 1.94 ^{a,c}
INSULIN	65.00 ± 2.61 ^a
ANOVA P	<0.05

Effect on body weight (Table 6)

At both the doses of 400 mg/kg and 800 mg/kg of EELAF and EEBAF there were significant ($p < 0.01$) weight gains relative to untreated diabetic mice. It also showed a significant ($p < 0.01$) increase in the body weight as compared to their weight at day1 except in diabetic control group. (Table 6)

Table 6: Effects of EELAF and EEBAF on body weight of Streptozocin induced type 1 diabetic mice

Groups	Mean body weight (grams)		
	Day '0' (baseline)	Day '1' (after 72 hours)	Day 15 th
Normal control	26.20 ± 0.58	27.20 ± 0.58	31.00 ± 0.32
Diabetic control	27.20 ± 0.58	25.40 ± 0.40	22.20 ± 0.37 ^a
EELAF 400	27.80 ± 0.80	25.40 ± 0.24	27.80 ± 0.37 ^b
EELAF 800	26.80 ± 0.58	26.40 ± 0.68	29.00 ± 0.84 ^b
EEBAF 400	28.00 ± 0.59	26.60 ± 0.25	29.20 ± 0.63 ^b
EEBAF 800	28.20 ± 0.58	27.00 ± 0.71	29.80 ± 0.58 ^b
INSULIN	27.60 ± 0.60	26.40 ± 0.40	29.80 ± 0.58 ^b
ANOVA P	>0.05	>0.05	<0.05

Values expressed as mean ± SEM (n=5). Statistical analysis: ANOVA followed by Tukey's tests. ^a $p < 0.05$, when compared to Normal Control group, ^b $p < 0.05$, when compared to Diabetic Control group. ^c $p < 0.05$, when compared EEBAF800 to EELAF 800 group.

Discussion

Diabetogenic properties of STZ are mediated through diverse mechanisms including targeted uptake of STZ in beta cells by Glut2 receptors and increased oxidative stress due to nitric oxide release and reactive oxygen species production. As a result, the β - cells undergo destruction by necrosis. [25]

Streptozotocin action in Bcells is accompanied by characteristic alterations in blood insulin and glucose concentrations.[18] There is significant increase in the level of blood glucose and a decrease in plasma insulin were observed in diabetic mice when compared to control mice.

In our study the possible mechanism of *Acacia farnesiana* in antidiabetic action may be through potentiation of pancreatic secretion of insulin from β -cell of islets by progressive regeneration of damage β -cells, or due to enhanced transport of blood glucose to the peripheral tissue thereby enhancing the utilization of glucose.[26-27]. This was clearly evidenced by the increased level of insulin in diabetic mice treated with *Acacia farnesiana* plant extract. However literature search shows that very few beta cells survive in the pancreas in the process of inducing type 1 diabetes by streptozotocin.[28] Therefore, though both the extracts showed increased insulin levels, they could not bring the serum insulin values comparable to normal control group. But the extracts succeeded significantly in bringing control of hyperglycemia in diabetic mice when compared with the diabetic control group which is suggestive of the presence in it of some constituents with insulin-like action. In this context a number of other plants have also been reported to have antidiabetic and insulin release stimulatory effect on account of their various phytochemical constituents.[24]

Decreased intestinal glucose absorption shown by the extracts is another probable mechanism of antidiabetic action which can be attributed to the flavonoids, saponins, polyphenols and tannins in both the extracts.[29] The flavonoids including quercetin are known to inhibit membrane Na⁺, K⁺ ATPase which has a key role in intestinal absorption. The presence of these phytochemicals in the extract (EBC) suggests that it may inhibit membrane Na⁺, K⁺ ATPase and thereby the desired action of reducing glucose absorption by the Sodium/Glucose cotransporter (SGLT1).[30] Tannins and saponins too showed marked loss in glucose transport across the intestine and potent S-GLUT-1 mediated inhibition of glucose absorption from the intestine respectively. [31,32]

Excessive hepatic glycogenolysis and gluconeogenesis associated with decreased utilization of glucose by tissues is the fundamental mechanism underlying hyperglycemia in the diabetic state. This may be due to the lack of or resistance to insulin, which is essential to trigger the activation of glycogen synthase systems.[33] The significant increase of liver glycogen levels in the extract treated diabetic animals may be because of the reactivation of the glycogen synthase system and inhibition of glycogen phosphorylase. Diminished phosphatidylinositol 3-kinase (PI-3K) activation in diabetes has been reported to be associated with impaired skeletal muscle glycogen synthase enzyme.[34] Moreover it could be predicted that glycogen levels in tissues decrease as the influx of glucose is inhibited in the absence of insulin and recovers on insulin treatment.[35]

The glycogen status in the heart has been found to be increased which corresponds to other study done on similar area.[36,37] Increased storage of glycogen in the myocardium results when there is a shift in energy substrate utilization, typically from a carbohydrate metabolism to a lipid metabolism. This switch in energy source produces an excessive accumulation of glycogen within the myocardium, which may accelerate glycogen synthesis or an overall impairment in glycogenolysis, or a combination of the two. [38] EELAF and EEBAF due to its insulin-like action of its phytochemical probably decreased the cardiac glycogen in this study by improving the glucose metabolism.

In diabetes, oxidative stress coexists along with decrease in the antioxidant status; the free radicals so produced results in tissue damage which can lead to detrimental effects. Antioxidant enzymes, including SOD, CAT, GST and GSH are considered to be major antioxidant defense system against free radicals.[39,40] In the present study there is significant increase in the antioxidants levels and decrease in lipid peroxidation as indicated by MDA. The antidiabetic activity of the *Acacia farnesiana* might be attributed to the antioxidant activity, which do protects the existing β -cells from dying by free radical scavenging action which has been initiated after administration of streptozocin.

The antioxidant activity of the EELAF and EEBAF can be linked to high polyphenolic contents as shown by Jayamurthy P *et al*. [41] Gallic acid a component of the plant has direct action on

superoxide, hydroxyl and alkoxy radical with its ability to attenuate oxidative stress. Studies have suggested that Gallic acid have membrane binding property thereby prevents the tissue damage. ^[42]

It has been also identified that saponins and quercetin plays an important role in mitigating oxidative stress. The other phytochemicals alkaloids and terpenoids found in the plant may also have some contribution as the antioxidant. ^[43]

Considering weight of animals, the results from this study revealed a significant loss of body weight of untreated diabetic mice compared to normal control group. This may be due to the increase muscle wasting and loss of adipose tissue resulting from excessive breakdown of tissue protein and fatty acids or may be on account of glycosuria. ^[44]

However, the animals treated with plant extracts and insulin tended to regain the weight of the animals. So it can be also suggested that there might be a protective role of both the plant extract (EEBAF and EELAF) on muscle wasting or due to better glycaemic control

Conclusions

Thus it can be concluded that the EELAF and EEBAF holds enormous potential as an antidiabetic with additional antioxidant activities which also validate their traditional use as an antidiabetic. But further research is necessary to gain a better understanding of its potential therapeutic action.

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