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Antidiabetic, antihyperlipidemic and *in vivo* antioxidant potential of aqueous extract of *Anogeissus latifolia* bark in type 2 diabetic rats

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ABSTRACT

Objective: To evaluate the antidiabetic, antihyperlipidemic and *in vivo* antioxidant potential of aqueous extract of *Anogeissus latifolia* bark (AEALB) in streptozotocin-nicotinamide (STZ-NIN)-induced type 2 diabetic rats. **Methods:** Oral acute toxicity for AEALB was performed in rats at 2 g/kg dose. In rats, type 2 diabetes was induced by administration of STZ-NIN (65 mg/kg-110 mg/kg, i.p). AEALB 100, 200 mg/kg dose was administered orally to the diabetic rats up to four weeks. The body weight and blood glucose levels were determined at the end of every week. The haemoglobin, glycosylated haemoglobin, serum lipid profiles and other biochemical parameters were measured on fourth week. The antioxidants level in liver, kidney and pancreas of diabetic rats treated with AEALB were estimated. **Results:** In the acute toxicity study, AEALB was non-toxic at 2 g/kg in rats. The increased body weight, haemoglobin and decreased blood glucose, glycosylated haemoglobin and other biochemical parameters level was observed in diabetic rats treated with both doses of AEALB compared to diabetic control rats. In diabetic rats, AEALB administration, altered lipid profiles and antioxidants level were reversed to near normal than diabetic control rats. **Conclusion:** Aqueous extract of *Anogeissus latifolia* bark possess significant antidiabetic, antihyperlipidemic and *in vivo* antioxidant activity in type 2 diabetic rats.

1. Introduction

Diabetes is a chronic metabolic disease characterized by hyperglycemia caused by dysfunction in carbohydrate, protein and fat metabolism due to the insulin produced by the body is insufficient, or cells do not respond properly to the insulin that is produced. This hyperglycemic state produces the classical symptoms of polyuria, polydipsia and polyphagia.[1] Globally, the occurrence of diabetes is estimated to increase, from 4 % in 1995 to 5.4 % by the year 2025. India has highest number of diabetic people with current figure of 40.9 million.[2] It has become a serious metabolic disorder and is now one of the leading causes of death in the world. Hyperglycaemia is associated with long term damage and failure of various organs such as eyes, kidneys, liver, nerves, heart, and blood vessels.[3] The abnormal changes in lipid and protein metabolism leads to progression of diabetic complications. Hyperglycaemia modifies the proteins such as elastin, collagen present in various tissues to glycoproteins responsible for the retinopathy, neuropathy, atherosclerosis and nephropathy.[4] During diabetes, lipoprotein oxidation and advanced glycation end products (AGEs) formed by non-enzymatic glycosylation of proteins generates free radicals. Experimental evidence supports the role of free radicals in the pathogenesis of diabetes and its complications. Free radicals damages cellular molecules, DNA, proteins and lipids which leads to dysfunction of normal function of cells.[5]

Although several therapies are currently used in the treatment of diabetes, draw backs such as cost, hypoglycemia, weight gain, gastrointestinal disturbances and liver toxicity are major concerns to search for alternative approach or medicine to treat or control diabetes.[6] Traditionally used medicinal plants on the other hand can provide an alternative approach to treat diabetes. Presently available traditional medicines to control or

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manage diabetes contain number of medicinal plants.[7] These are antioxidant rich therapies and are virtually free of adverse effects. The World Health Organization (WHO) has listed as many as 21,000 plants, which are used for medicinal purposes around the world. 2500 of such species are in India, out of which more than 150 species are used commercially on a large scale.[8]

Anogeissus latifolia Wall. (*A. latifolia*) is a fairly large tree (Family: Combretaceae), commonly found in the dry deciduous forests throughout India. It is an important timber source with the leaves and bark used for tanning. Traditionally, the bark has been used in the treatment of various disorders like skin diseases, snake and scorpion bite, stomach diseases, colic, wounds, diabetes, inflammation, cough and diarrhoea.[9] The bark has been reported for antioxidant[10], wound healing[11], antiulcer and antimicrobial[12] and hepatoprotective[13] activities. Also, leaf and bark have been reported for their anthelmintic activity [14] and antihyperglycemic activity.[15] The exudate, gum ghatti, collected from this plant was reported for hypolipidemic activity.[16] Even though, anti-hyperglycaemic activity of *A. latifolia* bark was reported, the published study was short term and failed to document the efficacy of *A. latifolia* bark extracts in type 2 diabetic rats for long term. Moreover, *in vivo* antioxidant levels in various tissues as well as anti-hyperlipidemic activity in type 2 diabetic rats were not studied. Hence, our objective was to investigate the antidiabetic, antihyperlipidemic and *in vivo* antioxidant activity of aqueous extract of *Anogeissus latifolia* bark (AEALB) in streptozotocin-nicotinamide induced type 2 diabetic rats.

2. Materials and methods

2.1. Plant material and extraction

Anogeissus latifolia bark was collected from Coimbatore, Tamil Nadu and authenticated by G.V.S Murthy, Scientist, Botanical Survey of India (BSI), Coimbatore, Tamil Nadu. The authentication certificate number was BSI/SRC/5/23/Tech.1146. The bark was cleaned, shade dried and powdered coarsely. The aqueous extract of *Anogeissus latifolia* bark was prepared by soaking bark powder (150 g) in one liter of distilled water over night and boiled for 30 minutes and filtered which was concentrated under water bath. Then, it was stored in refrigerator at 2-8℃ up to completion of study and yield of the extract was 10.62 % (w/w).

2.2. Experimental animals

Male Wistar albino rats (160-180 g) were used to evaluate the anti-diabetic activity. In acute toxicity study, female Wistar albino rats (140-160 g) were used and all animals were kept and maintained under standard laboratory conditions. The animals were fed with standard laboratory diet and allowed to drink water *ad libitum*. The experiments were conducted in accordance with protocol duly approved (KMCRET/M.Pharm/05/2010-11) by the institutional animal ethical committee (IAEC) of KMCH College of Pharmacy, Coimbatore-48.

2.3. Chemicals

Streptozotocin, nicotinamide and all other chemicals used in this study are of analytical grade and were procured from Himedia Laboratories, Mumbai, India. The biochemical parameters estimation was performed using commercially available kits (Piramal Healthcare Limited, Lab Diagnostic Division, Mumbai, India).

2.4. Acute toxicity study

Acute oral toxicity study was performed as per Organization for Economic Cooperation and Development (OECD) guideline 423.[17] After the oral administration of AEALB (2 g/kg), rats are observed individually once during the first 30 min, periodically during the first 24 h, with special attention given during the first 4 h, and daily thereafter, for a total of 14 days.

2.5. Induction of experimental diabetes

Type 2 diabetes was induced in overnight fasted rats by administration of nicotinamide (110 mg/kg, i.p), 15 minutes prior to the injection of streptozotocin (65 mg/kg, i.p.) after dissolving in freshly prepared cold citrate buffer (0.1 M, pH 4.5).[18] STZ induces fatal hypoglycemia as a result of massive pancreatic insulin release, the rats were provided with 5% dextrose solution after 6 h of STZ administration for next 24 h to prevent hypoglycemia.[19] Diabetes was confirmed 72 h after induction by measurement of tail vein blood glucose levels with the glucose meter (Glucocard™ 01-mini, Arkray Factory, Inc., Japan) by glucose oxidase-peroxidase method using strips. Diabetic rats were kept 14 days under standard laboratory condition for the stabilization of blood glucose level.[20] After 14 days of induction of diabetes, fasting blood glucose was again determined and only animals with a fasting blood glucose level greater than 200 mg/dl were selected for the study.

2.6. Antidiabetic activity assessment

The rats were divided into five groups each consisting of a minimum of six rats.

Group 1: Control rats received propylene glycol (5 ml/kg).
Group 2: Diabetic rats received propylene glycol (5 ml/kg).
Group 3: Diabetic rats received AEALB (100 mg/kg).
Group 4: Diabetic rats received AEALB (200 mg/kg).
Group 5: Diabetic rats received standard drug glibenclamide (5 mg/kg).

The propylene glycol, AEALB and glibenclamide were administered orally by gavage to the respective group animals for 28 days. The propylene glycol was chosen as a vehicle due to its non-toxic nature and also it is used as one of the common vehicle in many studies. Throughout the study period, AEALB and glibenclamide was freshly suspended in distilled water and propylene glycol respectively before oral administration. The fasting body weight, blood glucose levels were estimated on every week up to four weeks periodically.

2.7. Biochemical parameters estimation

At the end of experimental period, overnight fasted animals received respective treatment and after 1 h, all animals were anaesthetized with ketamine (50 mg/kg, i.p.) and blood samples were collected through retro-orbital plexus puncture and stored in with or without disodium ethylenediamine tetra acetate depending on the respective biochemical parameter estimation. The blood glucose, haemoglobin (Hb), glycosylated haemoglobin (HbA1c) was estimated using whole blood. The total cholesterol (TC), triglycerides (TG), HDL, serum glutamate-pyruvate transaminase (SGPT) and serum glutamate-oxaloacetate transaminase (SGOT), creatinine, albumin, total protein (TP), uric acid and urea in serum were estimated as per respective standard procedure given in the kit using semi-autoanalyzer (Photometer 5010V5+, Germany). The LDL and VLDL levels was calculated by the following equation,[5]

VLDL = Triglycerides/5
LDL = TC - (HDL+VLDL)

2.8. Estimation of antioxidant levels

Liver, kidney and pancreas were dissected out and washed immediately with ice cold saline to remove blood. The antioxidant enzymes such as superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPx), reduced glutathione (GSH) and lipid peroxidation by measuring thiobarbituric acid reactive substances (TBARS), was estimated in liver, kidney and pancreas.[21]

2.9. Statistical analysis

All the data expressed as mean ± SEM were evaluated by one-way analysis of variance (ANOVA), followed by Dunnett's multiple comparisons test using Prism Graphpad version 5.0 and values of $P<0.05$ were considered as statistically significant.

3. Results

3.1. Body weight and blood glucose level changes in diabetic rats

In the present study, STZ-NIN-induced diabetic rats showed significant ($P<0.001$) reduction in body weight and elevation in blood glucose level compared to control rats. Administration of AEALB (100 and 200 mg/kg) and glibenclamide (5 mg/kg) significantly ($P<0.001$, $P<0.01$ and $P<0.05$) increased the body weight from second to fourth weeks compared to diabetic control rats (Table1). Administration of AEALB 100 mg/kg and glibenclamide (5 mg/kg) significantly ($P<0.001$, $P<0.01$) reduced blood glucose levels from first week to fourth week. But, in the case of AEALB 200 mg/kg, significant reduction ($P<0.001$) was observed from second week onwards than diabetic control rats (Table 2). Also, AEALB 100 mg/kg was significantly ($P<0.001$) more effective to reduce blood glucose level than AEALB 200 mg/kg dose.

3.2. Lipid profiles level changes in diabetic rats

In our study, induction of diabetes significantly ($P<0.001$) altered the normal lipid profile levels compared to control rats. Administration of both doses of AEALB and glibenclamide significantly ($P<0.001$) decreased TC, TG, LDL,

Table 1.
Effect of AEALB on body weight in STZ-NIN-induced diabetic rats

Group	Dose (mg/kg)	Body weight (g)					
		Before STZ induction	0 day	1st week	2nd week	3rd week	4th week
Normal control	Vehicle	159.7±2.91	168.3±3.07	179.2±3.16	191.5±2.86	203.0±2.01	216.5±2.93
Diabetic control	Vehicle	170.2±3.14	129.8±3.30[a]	126.3±2.23[a]	121.0±5.43[a]	116.5±2.34[a]	109.2±4.55[a]
AEALB	100	171.3±5.89	132.2±5.70[a]	134.2±6.18[ns]	136.2±3.08[b]	138.2±4.46[b]	141.3±2.60[c]
AEALB	200	179.0±3.98	133.0±4.41[a]	134.0±2.93[ns]	135.7±2.30[b]	136.5±5.83[b]	138.2±3.53[b]
Glibenclamide	5	175.7±4.69	132.7±2.66[a]	134.3±3.80[ns]	137.2±4.90[c]	139.2±4.76[c]	142.5±2.99[d]

Data's are expressed as Mean ± SEM (n = 6). Vehicle - Propylene glycol (5 ml/kg).
[a]$P<0.001$ Diabetic control compared with normal control.
[b]$P<0.05$ AEALB 100, 200 mg/kg and glibenclamide 5 mg/kg compared with diabetic control.
[c]$P<0.01$AEALB 100 and glibenclamide 5 mg/kg compared with diabetic control.
[d]$P<0.001$Glibenclamide 5 mg/kg compared with diabetic control.
ns- No significant compared with diabetic control.

Table 2.
Effect of AEALB on blood glucose level in STZ-NIN- induced diabetic rats.

Group	Dose (mg/kg)	Blood glucose (mg/dL)					
		Before STZ induction	0 day	1st week	2nd week	3rd week	4th week
Normal control	Vehicle	70.0 ± 3.24	79.0 ± 2.43	86.5 ± 2.48	72.0 ± 2.40	82.0 ± 1.88	83.3 ± 3.42
Diabetic control	Vehicle	65.0 ± 2.38	401.0 ± 3.13[a]	376.7 ± 2.62[a]	398.2 ± 2.34[a]	403.3 ± 3.87[a]	380.5 ± 4.34[a]
AEALB	100	73.7 ± 4.67	398.7 ± 4.20[a]	388.5 ± 3.06[ns]	303.0 ± 4.66[b,c]	225.5 ± 4.09[b,c]	149.8 ± 2.50[b,c]
AEALB	200	80.5 ± 4.88	402.8 ± 2.76[a]	394.3 ± 3.30[ns]	353.8 ± 4.15[b]	281.5 ± 3.99[b]	190.3 ± 3.07[b]
Glibenclamide	5	62.8 ± 4.63	395.5 ± 4.63[a]	341.0 ± 5.15[b]	279.5 ± 2.64[b]	196.7 ± 3.78[b]	112.0 ± 4.16[b]

Data's are expressed as Mean ± SEM (n = 6). Vehicle - Propylene glycol (5 ml/kg).
[a]$P<0.001$ Diabetic control compared with normal control.
[b]$P<0.001$AEALB 100, 200 mg/kg and glibenclamide 5 mg/kg compared with diabetic control.
[c]$P<0.001$AEALB 100 compared with AEALB 200 mg/kg.
ns- No significant compared with diabetic control and AEALB 200 mg/kg.

Table 3.
Effect of AEALB on lipid profiles level in STZ-NIN-induced diabetic rats

Group	Dose (mg/kg)	Lipid profiles (mg/dL)				
		CHL	TG	HDL	LDL	VLDL
Normal control	Vehicle	93.67±3.45	96.0±3.02	30.15±1.35	44.63±2.93	18.77±0.93
Diabetic control	Vehicle	183.90±3.55[a]	213.3±2.89[a]	18.37±1.25[a]	119.80±2.17[a]	42.20±0.58[a]
AEALB	100	124.00±2.62[b,d]	130.7±3.05[b,d]	29.17±1.57[b,ns]	55.36±3.10[b,d]	22.01±0.85[b,e]
AEALB	200	146.30±2.34[b]	150.5±2.04[b]	26.88±1.67[c]	79.75±2.50[b]	25.58±0.71[b]
Glibenclamide	5	96.50±2.86[b]	100.3±2.12[b]	31.53±1.72[b]	43.17±2.24[b]	20.83±0.66[b]

Data's are expressed as Mean ± SEM (n = 6). Vehicle - Propylene glycol (5 ml/kg).
[a]$P<0.001$ Diabetic control compared with normal control.
[b]$P<0.001$AEALB 100, 200 mg/kg and glibenclamide 5 mg/kg compared with diabetic control.
[c]$P<0.01$AEALB 200 mg/kg compared with diabetic control.
[d]$P<0.001$AEALB 100 mg/kg compared with AEALB 200 mg/kg.
[e]$P<0.05$AEALB 100 mg/kg compared with AEALB 200 mg/kg.
ns- No significant compared with AEALB 200 mg/kg.

Table 4.
Effect of AEALB on TP, Hb, HbA1c and albumin in STZ-NIN-induced diabetic rats

Group	Dose (mg/kg)	TP (g/dL)	Hb (g%)	HbA1c (%)	Albumin (g/dL)
Normal control	Vehicle	7.48±0.26	13.03±0.24	6.06±0.25	2.10±0.03
Diabetic control	Vehicle	4.73±0.19[a]	6.86±0.32[a]	9.85±0.30[a]	1.08±0.04[a]
AEALB	100	6.05±0.22[b,ns]	11.08±0.26[c,e]	7.61±0.12[c,ns]	1.66±0.07[c,ns]
AEALB	200	6.08±0.34[b]	8.58±0.56[d]	8.37±0.31[d]	1.50±0.05[c]
Glibenclamide	5	6.11±0.30[b]	12.53±0.20[c]	6.69±0.13[c]	1.96±0.08[c]

Data's are expressed as mean ± SEM (n = 6). Vehicle - Propylene glycol (5 ml/kg).
[a]$P<0.001$ Diabetic control compared with normal control.
[b]$P<0.05$ AEALB 100, 200 mg/kg and glibenclamide 5 mg/kg compared with diabetic control.
[c]$P<0.001$ AEALB 100, 200 mg/kg and glibenclamide 5 mg/kg compared with diabetic control.
[d]$P<0.01$ AEALB 200 mg/kg compared with diabetic control group.
[e]$P<0.001$ AEALB 100 mg/kg compared with AEALB 200 mg/kg.
ns- No significant compared with AEALB 200 mg/kg.

Table 5.
Effect of AEALB on SGOT, SGPT, creatinine, urea and uric acid in STZ-NIN-induced diabetic rats

Group	Dose (mg/kg)	SGOT (U/L)	SGPT (U/L)	Creatinine (mg/dL)	Urea (mg/dL)	Uric acid (mg/dL)
Normal control	Vehicle	128.3±5.29	33.5±5.36	0.40±0.08	40.15±5.57	1.93±0.06
Diabetic control	Vehicle	301.2±7.71[a]	217.5±3.05[a]	0.73±0.03[a]	120.10±6.47[a]	3.56±0.07[a]
AEALB	100	182.2±4.67[b,e]	114.5±3.81[b,f]	0.46±0.02[c,ns]	69.77±5.86[b,g]	2.43±0.04[b,ns]
AEALB	200	209.2±4.05[b]	142.5±2.83[b]	0.50±0.05[d]	96.57±6.75[ns]	2.68±0.09[b]
Glibenclamide	5	153.8±3.86[b]	57.83±3.40[b]	0.43±0.04[c]	54.42±4.37[b]	2.06±0.08[b]

Data's are expressed as the mean ± SEM, (n = 6). Vehicle - Propylene glycol (5 mL/kg).
[a]$P<0.001$ Diabetic control compared with normal control.
[b]$P<0.001$AEALB 100, 200 mg/kg and glibenclamide 5 mg/kg compared with diabetic control.
[c]$P<0.01$ AEALB 100 mg/kg and glibenclamide 5 mg/kg compared with diabetic control.
[d]$P<0.05$ AEALB 200 mg/kg compared with diabetic control.
[e]$P<0.01$ AEALB 100 mg/kg compared with AEALB 200 mg/kg.
[f]$P<0.001$ AEALB 100 mg/kg compared with AEALB 200 mg/kg.
[g]$P<0.05$ AEALB 100 mg/kg compared with AEALB 200 mg/kg; ns- No significant compared with diabetic control and AEALB 200 mg/kg.

VLDL levels and also significantly ($P<0.001$, $P<0.01$, $P<0.05$) increased the HDL level compared to diabetic control rats. Oral administration of AEALB 100 mg/kg has significant ($P<0.001$ and $P<0.05$) greater reduction of elevated lipid profile levels in diabetic rats than AEALB 200 mg/kg dose (Table 3).

3.3. Biochemical parameters level changes in diabetic rats

The decreased Hb, TP, albumin and increased HbA1c levels were noticed in diabetic control rats compared to control rats. Administration of AEALB 100 and 200 mg/kg and glibenclamide significantly ($P<0.001$, $P<0.01$, $P<0.05$) increased Hb, TP, albumin levels and reduced HbA1c levels compared to diabetic control rats. AEALB 100 mg/kg has showed significant ($P<0.001$) higher improved in Hb levels than AEALB 200 mg/kg dose (Table 4). Also, the serum creatinine, SGOT, SGPT, urea and uric acid levels were elevated significantly ($P<0.001$) in STZ-NIN-induced diabetic rats compared to control rats. Both the doses of AEALB and glibenclamide treatment significantly ($P<0.001$, $P<0.01$, $P<0.05$) reduced above parameters compared to diabetic control rats. In diabetic rats, AEALB treatment significantly ($P<0.001$ and $P<0.05$) reduced SGOT, SGPT and urea levels compared to AEALB 200 mg/kg (Table 5).

3.4. Antioxidants levels restoration in diabetic rats

A significant ($P<0.001$) reduction of SOD, CAT, GPx and GSH levels was observed in liver, kidney and pancreas of diabetic control rats compared to control rats. Administration of AEALB (100 and 200 mg/kg) and glibenclamide (5 mg/kg)

Table 6.
Effect of AEALB on liver, pancreas and kidney antioxidants in STZ-NIN-induced diabetic rats

Organs	Group	Dose (mg/kg)	SOD(U/mg protein)	CAT(μM of H_2O_2 consumed/ min/mg protein)	GSH(μg of GSH/ mg protein)	GPx(μg of GSH utilized/ min/mg protein)	TBARS(μM MDA/ min/mg protein)
Liver	Normal control	Vehicle	32.85±1.38	758.5±39.56	35.48±2.24	29.85±1.77	47.37±2.92
	Diabetic control	Vehicle	8.213±1.16[a]	509.9±23.05[a]	21.97±1.72[b]	21.47±1.22[b]	89.14±2.29[a]
	AEALB	100	22.12±2.34[c,ns]	743.3±19.98[d,f]	32.44±2.12[e,ns]	31.58±1.16[c,ns]	68.46±2.93[c,ns]
	AEALB	200	17.01±1.40[d]	669.6±41.31[e]	31.26±2.14[ns]	27.80±1.55[e]	69.90±1.80[c]
	Glibenclamide	5	26.43±1.67[c]	755.4±48.50[c]	33.79±2.85[d]	31.87±1.20[c]	68.69±2.99[c]
Pancreas	Normal control	Vehicle	11.54±0.54	1147.00±106.41	80.73±4.05	222.10±9.78	68.59±4.18
	Diabetic control	Vehicle	2.48±0.34[a]	680.00±108.60[b]	43.48±2.64[a]	99.17±8.42[a]	140.71±6.09[a]
	AEALB	100	9.83±0.84[c,ns]	1215.00±110.62[e,f]	68.52±2.60[c,ns]	205.72±8.76[c,f]	94.17±5.65[c,ns]
	AEALB	200	8.81±0.89[c]	1064.00±108.11[d]	60.94±3.37[d]	173.12±7.57[c]	103.20±4.78[c]
	Glibenclamide	5	9.62±1.09[c]	1240.00±88.02[d]	72.74±3.98[c]	204.70±8.67[c]	99.45±5.74[c]
Kidney	Normal control	Vehicle	2.23±0.21	194.3±7.07	35.45±2.85	30.31±2.01	25.05±2.21
	Diabetic control	Vehicle	0.99±0.15[b]	156.2±4.59[b]	20.35±1.28[a]	13.36±2.32[b]	57.07±2.47[a]
	AEALB	100	2.49±0.22[c,ns]	207.8±5.04[c,ns]	33.38±1.88[d,ns]	26.99±2.96[e,ns]	26.56±2.93[c,g]
	AEALB	200	2.19±0.32[d]	203.3±4.68[c]	30.11±1.20[e]	25.94±3.99[ns]	41.65±2.69[d]
	Glibenclamide	5	2.08±0.13[e]	207.2±8.29[c]	36.64±2.46[c]	27.78±4.24[e]	32.38±3.58[c]

Data's are expressed as Mean ± SEM (n = 6). Vehicle - Propylene glycol (5 ml/kg). In each organ, comparison carried out individually between the groups.
[a]*P*<0.001 Diabetic control compared with normal control.
[b]*P*<0.01 Diabetic control compared with normal control.
[c]*P*<0.001 AEALB 100, 200 mg/kg and glibenclamide 5 mg/kg compared with diabetic control.
[d]*P*<0.01 AEALB 100, 200 mg/kg and glibenclamide 5 mg/kg compared with diabetic control.
[e]*P*<0.05 AEALB 100, 200 mg/kg and glibenclamide 5 mg/kg compared with diabetic control.
[f]*P*<0.05 AEALB 100 mg/kg compared with AEALB 200 mg/kg.
[g]*P*<0.01 AEALB 100 mg/kg compared with AEALB 200 mg/kg.
ns- No significant compared with AEALB 200 mg/kg.

significantly (*P*<0.001, *P*<0.01, *P*<0.05) increased the SOD, CAT, GPx and GSH levels compared to diabetic control rats in above tissues. Diabetes induction also showed a significant (*P*<0.001) increase in TBARS in diabetic control rats compared to control rats. Both the doses of AEALB and glibenclamide treatment significantly (*P*<0.001) decreased TBARS levels compared to the diabetic control rats in liver, kidney and pancreas. The pancreas GPx was increased and kidney lipid peroxidation was decreased after the administration of AEALB 100 mg/kg significantly than AEALB 200 mg/kg in diabetic rats (Table 6).

4. Discussion

Non-insulin dependent diabetes mellitus or type 2 diabetes is much more prevalent form of diabetes accounting for more than 90% of all diabetes cases and causes serious socioeconomic problems especially in developing countries.[22] STZ and nicotinamide are commonly used laboratory chemicals to induce experimental type 2 diabetes in animals. STZ mediated alkylation of pancreatic deoxyribonucleic acid and generation of superoxide, hydrogen peroxide, nitric oxide and hydroxyl radicals are responsible for β-cells damage and necrosis.[23] Nicotinamide administration along with STZ reduces pancreatic β-cells damage by prevention of activation of poly ADP-ribosylation which leads to type 2 diabetic condition in animals.[24]

In diabetic condition, elevated blood glucose, reduction in body weight, polyuria, polydipsia and polyphagia are commonly observed. In our study, induction of diabetes by STZ-NIN produced increase in blood glucose level, decrease in body weight and polyuria. In diabetic rats, observed reduction in body weight was possible due to catabolism of fats and protein.[25] The administration of AEALB improves body weight compared to diabetic control rats which indicates preventive effect of AEALB on degradation of structural proteins. The increase in blood glucose level after STZ-NIN administration may be due to insulin deficiency or resistance state in diabetic rats. AEALB treatment significantly reduced blood glucose level in diabetic rats which represents reversal of insulin resistance or increasing insulin secretion possibly by regeneration of damaged pancreatic β-cells in STZ-NIN-induced diabetic rats.[26]

The most common lipid abnormalities in diabetes are hypertriglyceridemia and hypercholesterolemia.[27] The abnormal high concentrations of serum lipids in diabetic animals are mainly due to an increased mobilization of free fatty acids from peripheral fat depots.[28] In the present study, we have noticed significantly increased levels of serum TC, TG, VLDL and LDL as well as marked reduction in serum HDL level in diabetic rats. Administration of both the doses of AEALB decreased levels of TC, LDL, VLDL and TG levels as well as increased the level of HDL in diabetic rats. The above action could be beneficial in preventing diabetic complications like coronary heart diseases and atherosclerosis in diabetic condition.

In diabetic condition, occurrence of reduction in protein and albumin may be due to proteinuria, albuminuria or increased protein catabolism, which are clinical markers in diabetic nephropathy.[29] The protein and albumin level was reduced after the induction of diabetes and treatment of AEALB increased both levels considerably in diabetic rats towards normal level. This action possibly is through increase in the insulin mediated amino acid uptake, enhancement of protein synthesis and/or inhibition of protein degradation.[30]In diabetes, elevated levels of serum urea, uric acid and creatinine are observed which may be

due to renal damage caused by abnormal glucose regulation or elevated glucose and glycosylated protein tissue levels.[31] In present study, significant increase in serum urea, uric acid and creatinine levels were observed in diabetic rats compared to normal control rats which indicates impaired renal function in diabetic rats. The treatment with AEALB lowered the above parameters significantly compared to diabetic control rats and it showed protective effect of AEALB on the kidneys. Also, increased serum SGOT and SGPT levels were reported in diabetes and it may be due to liver dysfunction.[32] In this study, increased level of SGOT and SGPT was observed in STZ-NIN-induced diabetic rats which may have occurred by leakage of enzymes from the liver cytosol into the blood stream; it represents the toxicity of STZ on liver. Diabetic rats treated with AEALB significantly reduced both enzyme levels which represents the protective action of AEALB on liver in diabetic condition. In diabetes, HbA1c is considered as a diagnostic marker and helps to know about degree of protein glycation, long-term blood sugar level and correlation of diabetes associated complications.[33,34] The reduced Hb and elevated HbA1c levels were observed in diabetic rats than normal control rats which may be due to hyperglycemia. AEALB oral administration to the diabetic rats showed significant decreased HbA1c and increased Hb levels. The above action of AEALB may be due to its potential to control hyperglycemia in diabetic rats.

Hyperglycemia is associated with formation of reactive oxygen species (ROS) which causes damage particularly to liver, kidney and also pancreas. The level of lipid peroxidation (TBARS) and reactive oxygen species (superoxide anion, hydrogen peroxide (H_2O_2), and hydroxyl radical) are common markers of oxidative stress in diabetic rats.[35] Lipid peroxidation refers to the oxidative degradation of lipids that impairs cell membrane functions resulting in cell damage and leading to several pathologies and cytotoxicity.[36] Malondialdehyde (MDA) is one of the end product of lipid peroxidation which is usually measured as a marker for oxidative stress.[37] In diabetes, hypoinsulinaemia increases the activity of enzyme fatty acyl-coenzyme A oxidase, which initiates β oxidation of fatty acids, resulting in lipid peroxidation.[38] Our study results showed a significant elevation of MDA level in the liver, kidney and pancreas of diabetic rats which indicates the occurrence of lipid peroxidation in these tissues. Oral administration of both the doses of AEALB significantly lowered MDA level in diabetic rats and it shows the preventive action of AEALB on lipid peroxidation.

Superoxide dismutase, a metalloprotein, is primarily involved in the antioxidant defence by scavenging the superoxide radicals. In hyperglycemia, glucose undergoes auto-oxidation and produces superoxide radicals which lead to lipid peroxidation.[39] Catalase, a hemeprotein, localized in the peroxisomes or microperoxisomes catalyses the decomposition of $H_2O_2$and protects the cell from oxidative damage produced by H_2O_2.[40] In diabetic rats, decreased level of these antioxidant enzymes was reported and it indicates the development of an imbalance between ROS production and antioxidant scavenging systems. In the present study, SOD and CAT levels were significantly decreased in liver, kidney and pancreas of diabetic rats which shows disturbance of antioxidant status. But, after the administration of AEALB, significantly increased levels of SOD and CAT were noticed in diabetic rats compared to diabetic control rats and this action supports the protective effect of AEALB on above organs against free radical mediated damage.

Reduced glutathione is an important biomolecule responsible for the elimination of reactive intermediates by reduction of hydroperoxidase in the presence of glutathione peroxidase. GSH also functions as a free radical scavenger and helps to repair free radical mediated biological damage.[41] Glutathione peroxidiase, catalyses the reaction of hydroperoxides with reduced glutathione to form glutathione disulphide and the reduction products of the hydroperoxide.[42]In this study, GSH and GPx levels were decreased in liver, kidney and pancreas of diabetic rats than normal control rats and it clearly indicates defective function of GSH and GPx in diabetic rats. But, increased level of GSH and GPx were noticed in diabetic rats of above tissues after the administration of AEALB compared to diabetic control rats which shows the free radical scavenging ability of AEALB in diabetic condition.

Our study findings supports that aqueous extract of *Anogeissus latifolia* bark has significant antidiabetic and antihyperlipidemic effect in type 2 diabetic rats. Also, *in vivo* antioxidant results provide more scientific support to the published *in vitro* antioxidant study.

Conflict of interest statement

We declare that we have no conflict of interest.

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