"Evaluation of *Anogeissus acuminata* (Roxb. ex DC.) in Diabetes mellitus and its complications."

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A. Title of the thesis and abstract

Title: Evaluation of *Anogeissus acuminata* (Roxb. ex DC.) in Diabetes mellitus and its complications.

Abstract

Objective

To evaluate the effect of methanolic extracts of *Anogeissus acuminata* (AA) in Diabetes mellitus and its complications like diabetic neuropathy, nephropathy and cardiovascular complications.

Methods

Type 1 DM was induced by injecting Streptozotocin (STZ), 50 mg/kg, i.p. in 6 hour fasted rats. Rats with DM were treated with methanolic extracts of AA for 8 weeks at doses 100 and 300 mg/kg, orally. Human NPH Insulin (4 IU/kg, s.c.) was used as standard treatment. Plasma glucose levels (at1, 2, 4, 8 weeks) and oxidative stress parameters (at 2 and 4 weeks) were assessed. Effect on diabetic nephropathy was evaluated by recording urinary protein excretion, kidney weights, serum creatinine, blood urea nitrogen (BUN) levels (at 8 weeks). Effect on neuropathy was evaluated by hot plate test (at 4 weeks), formalin test (at 6 weeks), intestinal charcoal meal test and sciatic nerve conduction velocity (at 8 weeks).

Type 2 diabetes mellitus was induced in fructose fed rats by injection of STZ 40 mg/kg, i.p. in 6 hr fasted rats. Glibenclamide 5 mg/kg, p.o. was used as standard drug. Plasma glucose levels (at 2,8 and 11 weeks), glycated Hb (at 11 weeks), lipid levels (at 2 and 12 weeks) and oxidative stress parameters (at 2 and 12 weeks) were evaluated at specified time points. At the end of 12 weeks of treatment, mean blood pressure was determined by carotid artery cannulation, heart rate and force of contraction of isolated heart was determined using Langendorff heart technique. Heart weight, left ventricular weight and Serum CK-MB and LDH levels were measured. Preliminary phytochemical screening of both extracts was done as per standard protocol and was followed by HPTLC fingerprinting. *In vitro* anti-oxidant and PTP 1B inhibitory activity of the extracts was carried out.

Results

Methanolic extracts of AA produced statistically significant ($p< 0.05$) hypoglycemic and antioxidant effect in animals with T1DM. Urinary protein excretion, serum creatinine and blood urea nitrogen levels were also reduced significantly in all AA treated groups ($p< 0.05$). Kidney hypertrophy could be attenuated remarkably as reflected by significantly lower kidney weight/100 gm body weight ratio ($p< 0.05$). AA treated rats exhibited less thermal and chemical hyperalgesia as well as significantly better charcoal meal transit and nerve conduction velocity as compared to diabetic rats ($p< 0.05$). AA extracts also resulted in significant reduction in plasma glucose, HbA_{1C}, lipid and oxidative stress parameters in Fructose fed STZ injected rats. It also resulted in improvement in cardiac hypertrophy, haemodynamic and oxidative stress parameters in these animals.

Conclusion

The results suggest that methanolic extracts of AA exerted hypoglycemic and antioxidant effect on diabetic rats, which was comparable to that of standard. The treatment was also able to attenuate development of diabetic complications like nephropathy, neuropathy and cardiovascular complications.

Keywords

Anogeissus acuminata, Diabetes mellitus, Diabetic nephropathy, Diabetic neuropathy, Cardiovascular complications.

B. Brief definition of state of the art of the topic

There has been drastic change in lifestyle and food habits in last decades, especially in the developing countries like India. This has also resulted in changes in status of health and disease. Prevalence of metabolic disorders like diabetes mellitus and hypertension has risen to epidemic levels. India is about to become diabetes hub of the world [1]. Against the voluminous increase in prevalence of diabetes there has been little progress in drugs used in management of diabetes mellitus (DM). Most drugs used in treatment of DM address the issue of hyperglycemia. However, the recent findings have shown that, many factors other

than carbohydrate metabolism play important role in pathogenesis of DM. Factors like chronic inflammation, activation of immune system, oxidative stress, derangement of protein and lipid metabolism all play important roles in disease progression and development of diabetic complications [2, 3]. In this setting of the disease, addressing only one aspect of the pathogenesis cannot sufficiently prevent the disease progression and development of diabetic complications.

Many plants have been used in medicine since ancient time. Although undermined by development of modern medicine, herbal medicine has continued to be used in many parts of world due to many reasons, some of them being their effectiveness and better safety profiles. Many scientists propose the multiple mechanisms possessed by a herbal drug to be beneficial in multifactorial diseases. Further investigation on traditional medicinal herbs has been recommended by WHO Expert Committee on diabetes [4].

Anogeissus acuminata (AA) belongs to the family Combretaceae. This plant is used in different parts of world by locales as medicine in different ailments including DM [5,6]. Moreover, the plant is rich in phenolics, which are potential candidates for varied pharmacological actions. AA extracts have demonstrated anti-inflammatory, anti-oxidant, analgesic, neuroprotective, HIV reverse transcriptase inhibitory activity in different studies [7-11]. It has also demonstrated hypoglycemic action in alloxan induced DM in mice [12]. Thus, it was hypothesised that AA may be a good antidiabetic agent on virtue of its varied actions and constituents and may be able to prevent the development of diabetic complications on long term. Therefore, it was evaluated in the present study for its antidiabetic action in two types of DM models and in models of diabetic complications like diabetic nephropathy, neuropathy and cardiac complications.

C. Definition of problem

The major morbidity caused by DM is due to its associated complications. Although drugs are available for lowering blood glucose levels, a useful approach in the treatment of DM can be to encompass other beneficial pharmacological properties in addition to antihyperglycemic activity, which can be beneficial in prevention of its complications. However, there are no such drugs

available in market, which can prevent development of diabetic complications while controlling the blood glucose levels.

D. Objective and scope of work

Following are the objectives of present research work

1. Evaluation of extracts of AA for antihyperglycemic effect on animal models of DM representing type 1 and type 2 DM.

2. Evaluation of effect on other factors involved in progression of DM like oxidative stress, lipid levels and insulin resistance.

3. Evaluation of effects of AA treatment on development of diabetic nephropathy, neuropathy and cardiovascular complications.

4. Phytochemical evaluation of extracts of AA and their *in vitro* assessment for various pharmacological effects.

Scope of research: The research involves evaluation of ability of the drug to reduce blood glucose levels in two types of DM and its effect on various parameters of diabetic nephropathy, neuropathy and cardiac complications. The plant extract was evaluated for presence of various chemical constituents and quantification of major constituents.

E. Original contribution by thesis

The effect of AA extracts have not yet been evaluated for effect on diabetic complications, thus, this thesis will provide a scientific basis for its further development as an effective treatment for DM.

F. Methodology of research, results, comparisons

Plant Material and Extraction

Aerial parts and bark of the plant were collected from Khedbrahma, Gujarat in the month of March. Herbarium of the collected sample was submitted for authentication at NISCAIR, Delhi with provided reference no. NISCAIR/RHMD/consult/2013/2290/70. Leaves and bark of plant

were dried in shade and made into a coarse powder. Methanolic extract was prepared using Soxhlet extractor. The yield for leaves and bark was 16% and 14.9% w/w respectively in terms of dried starting material.

Gas chromatographic evaluation of the extracts was done for residual solvent. The phytochemical investigation of the methanolic extracts of leaf and bark of AA was carried out using standard protocol [13]. Estimation of tannin content of the extract was performed by using AOAC Official Method, 1965 [14]. Flavonoids content was determined by Aluminum chloride method using quercetin as standard [15]**.**

HPTLC fingerprinting analysis of the extracts was performed using following parameters

Stationary phase: Merck Silica gel 60F254 TLC precoated aluminum plates.

Sample Volume: 20µl of freshly prepared

Mobile phase: toluene: ethyl acetate: formic acid (4.5:3.0:0.2, *v/v/v*)

Animals

Adult male wistar rats of 170-200 gm body weight were used for experiment. Animals were maintained as per CPCSEA guidelines. Protocols for the experiment were approved by Institutional Animal Ethics Committee (Protocol No. PIPH 26/13, PIPH 35/13).

Experimental Studies

The effect of AA extracts are evaluated in two animal models of diabetes mellitus. Disease identical to Type I DM was induced by using Streptozotocin as inducing agent [16], while that identical to type II DM was developed by Streptozotocin injection in fructose fed rats [17]. Validity of the models was established by assessing insulin sensitivity in diseased animals [18].

Induction of DM

Type 1 diabetes mellitus was induced in male wistar rats by injection of STZ (Himedia Labs, Mumbai) in citrate buffer (pH: 4.5) at a dose of 50 mg/kg i.p. After 48 hours blood glucose levels (BGL) were determined using a glucometer (One Touch Glucometer, Jhonson and Jhonson Ltd). Animals with BGL> 250 mg/dl were considered diabetic and were divided in six

groups. Animals untreated with STZ were kept in normal control group. Treatment was continued for 8 weeks as per following plan for various groups.

Group I: Normal Control (NC): Blank acacia suspension, 1ml, orally

Group II: Diabetic Control (DC): Blank acacia suspension, 1ml, orally

Group III: Standard treatment (Std): Human NPH Insulin (4 IU/kg/day), Subcutaneously

Group IV: Test 1 (LE100): Leaf extract suspended in water using acacia as suspending agent, 100 mg/kg bw, orally

Group V: Test 2 (LE300): Leaf extract suspended in water using acacia as suspending agent, 300 mg/kg bw, orally

Group VI: Test 3 (BE100): Bark extract suspended in water using acacia as suspending agent, 100 mg/kg bw, orally

Group VII: Test 4 (BE300): Bark extract suspended in water using acacia as suspending agent, 300 mg/kg bw, orally

Type 2 DM was induced by Fructose + STZ model [17]. The animals were given 10% fructose solution in place of drinking water for 3 weeks, after 3 weeks 40 mg/kg, i.p. STZ was injected in 6 hr fasted rats.48 hours after the STZ injection, blood glucose levels were measured. Animals with blood glucose levels above 250 mg/dl were considered diabetic. Animals were divided in 6 groups with 6 animals in each. Animals untreated with STZ were kept in normal control group. Treatment was given for 12 weeks in following manner.

Group I: Normal Control (NC): Blank acacia suspension, 1ml, orally

Group II: Diabetic Control (DC): Blank acacia suspension, 1ml, orally

Group III: Standard treatment (Std): Glibenclamide (5 mg/kg b.w.), orally

Group IV: Test 1 (LE100): Leaf extract suspended in water using acacia as suspending agent, 100 mg/kg bw, orally

Group V: Test 2 (LE300): Leaf extract suspended in water using acacia as suspending agent, 300 mg/kg bw, orally

Group VI: Test 3 (BE100): Bark extract suspended in water using acacia as suspending agent, 100 mg/kg bw, orally

Group VII: Test 4 (BE300): Bark extract suspended in water using acacia as suspending agent, 300 mg/kg bw, orally

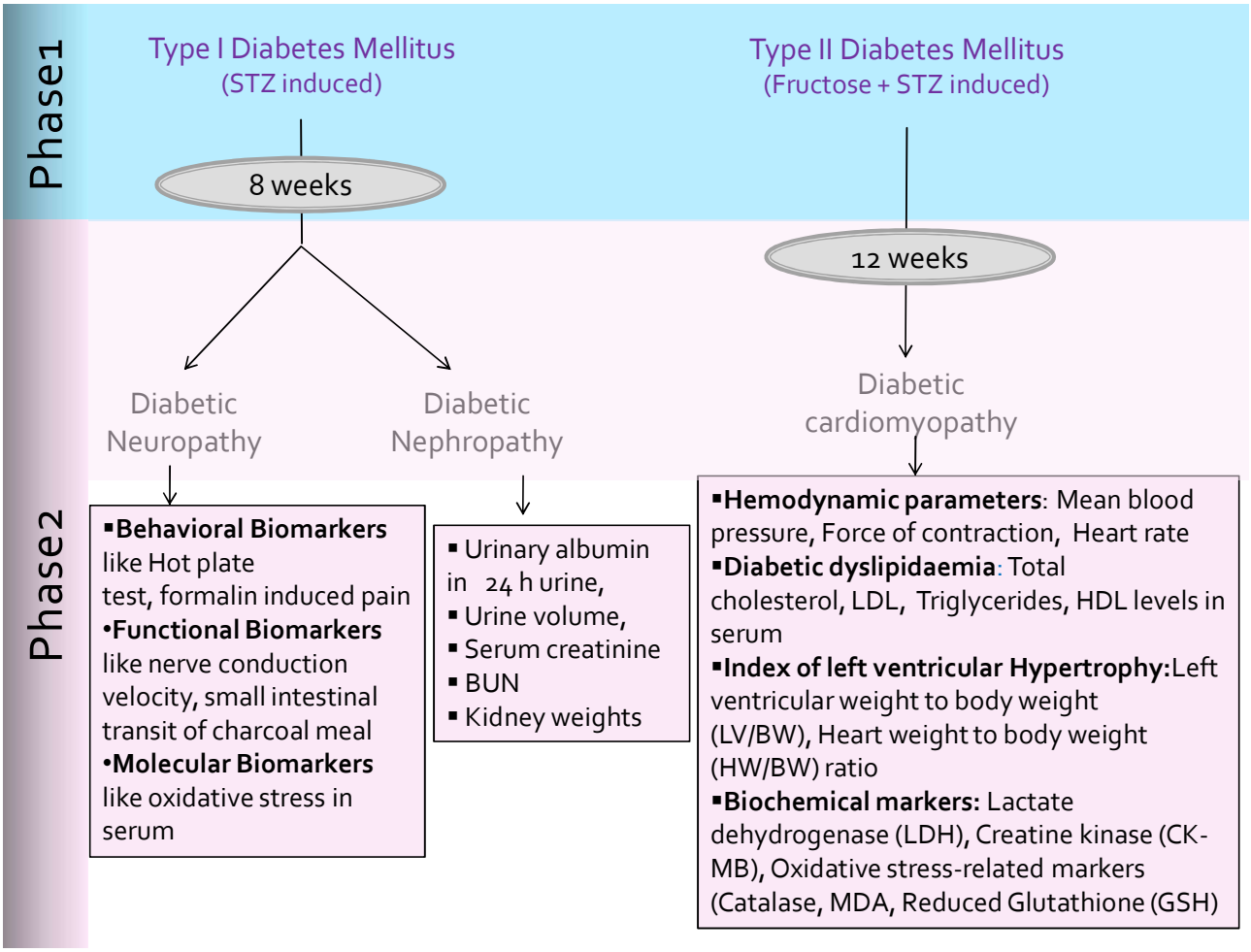

Figure 1: Schematic presentation of Experimental design and parameters evaluated

The study was divided in two phases. Phase 1 consisted of evaluation of antihyperglycaemic action of extracts in two models of DM. Phase 2 consisted of evaluation of extracts in complications of DM. Various parameters for evaluation of diabetic neuropathy [19, 20], nephropathy [21-23] and cardiovascular complications [24, 25] are presented above in Figure 1.

In addition to this, *in vitro* antioxidant activity, *in vitro* PTP1B inhibitory activity of the extracts was evaluated. Phytochemical evaluation of the extracts was performed.

Statistical Analysis

Data were expressed as mean ± SEM. Statistical analysis was done using one-way or two way analysis of variance (ANOVA) followed by Tukey's Multiple comparison test or Bonferroni posttest using GraphPad Prism version 5.30 for Windows, GraphPad Software, San Diego California USA. P values <0.05 were considered as significant.

Results:

1. **Analysis of the plant extracts**

1.1. Gas chromatographic evaluation of the extracts

Evaluation showed that the methanol residues were under USP permeable limit (3000 ppm) in both the extracts.

1.2. Phytochemical investigation

Tannins and flavonoids were found to be the major constituents in the extract. 24.57% and 13.63% w/w tannins were found to be present in leaf and bark extracts respectively. Whereas 14.5% and 57% w/w flavonoids were present in leaf and bark extracts respectively**.**

1.3. HPTLC fingerprinting analysis

Several peaks were observed however, one peak in leaf extract corresponded with the Rf of Gallic acid and one peak in bark extract corresponded with quercetin.

2. **Characterization of induced DM for detecting insulin sensitivity using Intravenous insulin Tolerance Test**

On 15th day of induction of diabetes, Insulin (0.1 IU/kg) was administered by i.v injection and blood samples were collected at 3, 10, 20, 30 minutes for the measurement of plasma glucose. The value is presented as a percentage reduction in plasma glucose level. A graph was plotted for percentage reduction in plasma glucose level Vs time and slope (K_{ITT}) of the lines were determined. K_{ITT} > 2.0 %/min represented normal insulin sensitivity while that less than 1.5 %/min indicated reduced insulin sensitivity. Animals with DM induced by 50 mg/kg, STZ i.p. had K_{ITT} 2.189 ±0.35 %/min while those with fructose + STZ induced DM had K_{ITT} of 0.68 ± 0.22 %/min. This indicated that the STZ induced DM had normal insulin sensitivity resembling T1DM, while fructose + STZ animals had reduced insulin sensitivity resembling clinical type 2 DM.

3. Studies in STZ induced DM model

3.1. Effect of AA Treatment on Body Weight, Food Intake and Water Intake

In the present study, induction of diabetes resulted in loss of body weight and increased food and water intake. Treatment with AA extract was found to prevent weight loss, hyperphagia and polydipsia when compared with animals of DC group.

3.2. Effect on Plasma Glucose Levels and Oxidative Stress Parameters

Rats of DC group showed significantly elevated levels of plasma glucose as compared to normal rats. Plasma glucose levels were significantly lower in AA extract and insulin treated groups (150.8±10.8, 127.7±7.7, 166.8±9.1, 146.0±12.9 and 161.5±7.0 mg/dl in LE100, LE300, BE100, BE300 and standard group respectively) as compared to DC group (357.5±8.6 mg/dl) at 8 weeks of treatment. DM in rats also resulted in significantly increased lipid peroxidation and reduced glutathione and catalase levels. Treatment with AA extracts for 8 weeks significantly reduced lipid peroxidation and increased glutathione and catalase levels.

3.3. Effect on Diabetic Neuropathy

3.3.1. Thermal Nociceptive Threshold

Induction of diabetes caused a significant hyperalgesia demonstrated by a decreased response time (11±1.2 S) on hot plate as compared to normal rats (22 ±1.2 S) at 4 weeks post induction of DM. Treatment with AA extract showed a significant increase in nociceptive response time reflecting less disturbed nociception in treated rats (18± 0.76 S, 20±1.9 S, 19.0± 1.2 S, 20.0±1.4 S and 19±1.7 S respectively for LE100, LE300, BE100, BE300 and standard).

3.3.2. Formalin Test

Rats in DC group showed significantly increased number of limb flinches (33.3 ± 1.8) as compared to normal rats (12.8 ± 0.83) in formalin test at 6 weeks of treatment. Animals treated with AA extracts exhibited less chemical allodynia as reflected by reduced number of limb flinches (26± 1.9, 23±1.5, 25.1± 1, 24.1±1.6 and 24.5±1.5 respectively for LE100, LE300, BE100, BE300 and standard).

3.3.3. Charcoal Meal Test

Diabetes resulted in reduced gastrointestinal motility due to autonomic neuropathy. This was reflected as less distance travelled by charcoal meal in diabetic animal (52.5±2.3 cm) as compared to that in normal animal (78.7±3.19 cm). AA treatment resulted in significant increase in distance travelled by charcoal as compared to DC rats (63.6± 1.6, 68.2±1.7, 63.5± 2.9, 65.2±3.1 and 65±1.6 respectively for LE100, LE300, BE100, BE300 and standard).

3.3.4. Nerve Conduction Velocity

Nerve conduction velocity was reduced in diabetic animals (35.4±1.1 m/s) as compared to that in normal controls (48.9±0.74 m/s). Treatment with AA extract for 8 weeks resulted in significantly higher nerve conduction velocity as compared to diabetic control animals (40.6± 1.4, 43.8±1.7, 40.6± 1.0, 42.3±0.8 and 41.6±0.9 m/s respectively for LE100, LE300, BE100, BE300 and standard).

3.4. Effect on Diabetic nephropathy

3.4.1. Serum creatinine and BUN levels

Rats in DC group exhibited high levels of serum creatinine (1.09± 0.03 mg/dl) and BUN (189.3± 13.4 mg/dl) levels as compared to normal animals (0.61 ± 0.05 mg/dl and 61.8 ±5.4 mg/dl respectively). AA treated animals showed significantly lower levels of serum creatinine (0.78±0.05, 0.71±0.07, 0.79±0.07, 0.68±0.10 and 0.89±0.10 mg/dl for LE100, LE300, BE100, BE300 and standard group respectively) and BUN (129.8±17.6, 111.1±12.9, 128.5±12.9, 107.6±11.7 and 119.5±11.17 mg/dl for LE100, LE300, BE100, BE300 and standard group respectively).

3.4.2. Urinary protein excretion

Diabetic animals showed marked urinary protein excretion. AA treated animals showed significantly less urinary protein excretion (3.5±0.3, 1.8±0.3, 3.6±0.2, 1.7±0.2 mg/day in LE100, LE300, BE100 and BE300 respectively) as compared to those in untreated diabetic rats (6.5±0.2 mg/day).

3.4.3. Kidney weight to body weight ratio

Uncontrolled DM on long term resulted in kidney hypertrophy and fluid accumulation. Treatment with AA extracts could prevent this as reflected by significantly lower KW/100 gm body wt (0.69±0.05, 0.62±0.04, 0.65±0.06, 0.61±0.07 and 0.69±0.07 respectively for LE100, LE300, BE100, BE300 and standard groups) as compared to diabetic animals (0.94±0.04).

4. Studies in fructose + STZ induced DM model

4.1. Effect on Plasma Glucose, Glycated Hb and lipid levels

Methanolic extract of AA produced a significant hypoglycemic effect as seen by plasma glucose levels of 346.6±16.86 Vs 116.3±14.66, 116.3±9.93 mg/dl for DC, LE300 and BE300 respectively at 11 weeks. A consistent reduction in PGL was evident from the values of

HbA1C measured at 11 weeks (11.6±0.44 Vs 7.83±0.7, 7.5±0.61 % for DC, LE300 and BE300 respectively). LE300 and BE300 treatment also caused a significant reduction in total cholesterol, triglyceride and LDL levels, while increase in HDL levels as compared to DC rats.

4.2. Effect on Insulin resistance and pancreatic beta cell function

DC rats had higher insulin levels (54.9±5.52 µU/ml) as compared to normal rats (47.4±4.7 µU/ml). However, higher levels of insulin in DC rats were quite lower when seen in proportion to the plasma glucose level in these animals. This may be due to exhausted beta cell function resulting from insulin resistance and glucose toxicity. AA treatment reduced the insulin levels (53.6±7.02 and 52.7±5.56 µU/ml respectively for LE300 and BE300). Although, there was found no significant difference in insulin levels between control and treated groups, it reveals a beneficial effect on functioning of beta cells when seen in conjugation with glucose levels. Insulin resistance and beta cell function were determined using Homeostatic Model Assessment method. IR was significantly lower in all AA treated groups except BE100. DC rats also had significantly diminished beta cell function, which was found to be significantly higher in standard, LE300 and BE300 groups.

4.3. Effect on Oxidative Stress Parameters

Serum MDA, GSH and catalase levels were determined at 2 weeks and 12 weeks of treatment. All AA treated groups had significantly reduced oxidative stress markers, supporting the observation that the extracts have strong antioxidant activity.

4.4. Evaluation in cardiovascular complications

These parameters were evaluated at 12 weeks after development of DM, as this was the expected duration to develop cardiovascular complications in diabetic rats.

4.4.1. Effect on Hemodynamic parameters

Carotid artery of anaesthetised rat was cannulated to measure mean blood pressure (MBP) using student physiograph. DC rats had significantly higher MBP as compared to NC rats. Animals treated with standard drug, LE300 and BE300 had significantly lower MBP as compared to DC rats. Hearts from the animals were quickly removed and mounted on Langendorff assembly to measure heart rate and force of contraction using force transducer of student physiograph. It was observed that DC rats had significantly lower heart rate as compared to NC rats, while that in LE300 and BE300 treated rats was significantly higher compared to DC rats. There were no significant differences in force of contraction between the groups. After this heart weight and left ventricular weight were recorded and left ventricular (LV) hypertrophy and cardiac hypertrophy index was measured. DC rats had significantly higher ratios indicating hypertrophy of LV and heart. LE300 and BE300 treatment could prevent both cardiac and LV hypertrophy, while standard treatment, LE100 and BE100 treated animals had significantly lower cardiac hypertrophy index.

4.4.2. Effect on biochemical parameters

Serum LDH and Creatine Kinase MB levels are sensitive markers of cardiac muscle damage. LDH and CK-MB levels in serum were significantly elevated in DC rats (182.16±12.26 and 28.71±3.14 respectively), while those were significantly lower in standard (100.9±6.93 and 17.53±2.46) and AA treated groups (106.95±9.8, 87.21±13.7, 120.66±21.0, 112.83±12.3 U/L LDH and 18.61±3.4, 17.25±1.8, 21.21±3.4, 18.15±1.9 U/L CK-MB for LE100, LE300, BE100 and BE300 respectively), indicating less damage to myocardium in these animals.

5. *In-vitro* studies

5.1. *In vitro* antioxidant activity of extracts

In vitro antioxidant activity was analyzed by 1,1-Diphenyl-2-picrylhydrazyl (DPPH) radical scavenging assay [26], reducing power assay [27] and TBA (thiobarbituric acid) assay [28], using vitamin C as a standard. EC50 value for Vitamin C, leaf and bark of the plant in DPPH

assay were 31.06 ± 1.16, 39.20 ± 1.26 and 35.44 ± 1.22 µg/ml, respectively [29]. The extracts also exhibited significant reducing power and inhibition of lipid peroxidation comparable to that by Vitamin C.

5.2. *In Vitro* PTP1B inhibitory action

Antidiabetic potential was evaluated using *in vitro* protein tyrosine phosphatase 1B (PTP1B) inhibition assay using Suramin as a standard [30]. Both extracts demonstrated significant PTP1B inhibitory activity with IC50 value 65.60 ±1.14 and 43.78 ±1.20 µg/ml for leaf and bark extracts respectively against 6.61 ±1.63 µg/ml for Suramin.

G. Achievement of objectives

1. The methanolic extracts of AA were found to exert antihyperglycemic effect on animal models of DM representing type 1 and type 2 DM.

2. In addition to this, they also had favorable effect on other factors involved in progression of DM like oxidative stress, lipid levels and insulin resistance.

3. The extracts showed beneficial effects on diabetic nephropathy, neuropathy and cardiovascular complications.

4. Tannins and Flavonoids were found to be the major constituents of plant. Plant extract also exhibited potent *in vitro* anti-oxidant and PTP1B inhibitory effect.

H. Conclusion

It was observed that methanolic extracts of AA had antidiabetic action in Type I as well as Type II DM models. It could also prevent development of diabetic neuropathy, nephropathy and cardiovascular complications of DM. It showed significant anti-oxidant and lipid lowering effects in a dose dependent manner. Phytochemical investigation revealed tannins and flavonoids to be the major constituents in the plant.

I. List of papers published and Copies of the same

A) Poster presentation:

Title: Evaluation of hypoglycemic and antioxidant activity of methanolic extracts of Anogeissus acuminata (Roxb. Ex DC.) in Streptozotocin induced diabetes mellitus in rats.
At National Conference on Diabetes and its complications organized by Nirma University, Ahmedabad on 6-7 September, 2013.

B) Papers Published

1) Navale AM, Paranjape AN. Role of inflammation in development of diabetic complications and commonly used inflammatory markers with respect to diabetic complications. International Journal of Pharmacy and Pharmaceutical Sciences, 2013, 2(5), 1-5.

2) Navale AM, Paranjape AN, Pithadia A, Mansuri J. Upcoming Drugs in Treatment of Diabetes Mellitus: Peering into the Crystal ball. PHARMAGENE, 2013, 1(2), 51-56.

3) Navale AM, Paranjape AN. In vitro antioxidant and PTP inhibitory activity of methanolic extract of Anogeissus acuminata leaf and bark. Journal of Pharmacy Research Vol 10 (1), 65-68.

4) Navale AM, Paranjape AN. Glucose transporters: physiological and pathological roles. Biophysical Reviews, 2016, 8(1), 5-9.

5) Navale AM, Paranjape AN. Phytochemical screening and HPTLC fingerprinting of Anogeissus acuminata extracts. International Journal of Research in Ayurveda & Pharmacy, 2016, 7 (Suppl 2), 207-9. DOI: 10.7897/2277-4343.07288.

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