EVALUATION OF HYPOLIPIDEMIC ACTIVITY OF *ACHYRANTHES ASPERA* LINN. ON ALLOXAN INDUCED DIABETIC RATS

R.Vidhya¹*, G.Jothi ²

¹Department of Biochemistry, Dharmapuram Ganambigai Govt Arts College(w), Mayiladuthurai-609001. Tamil Nadu.

²Head, Department of Biochemistry, Srimad Andavan Arts and Science College, Trichy 620005. Tamil Nadu.

ABSTRACT

The present study, aims to evaluate the hypolipidemic activity of aqueous extract of *Achyranthes aspera* (L.) against alloxan induced diabetic rats. Wistar strain of albino rats of either sex were divided into five groups comprising of six rats each. Group I served as normal control, group II served as disease control (alloxan induced), group III & IV animals, received aqueous extract of *A. aspera* Linn at a dosage of 250mg/kg body weight and 500mg/kg body weight for 45 days, group V served as standard drug control (glibenclamide 1mg/Kg body weight). After the experimental period the blood and tissue samples were collected and subjected to various biochemical parameters and histopathological studies. There were profound alterations in fasting blood glucose, serum insulin, lipid profile in serum and tissue, urea, creatinine levels in alloxan induced rats. Aqueous extract of *A. aspera* showed an excellent reduction in serum and marked raised tissue cholesterol, triglyceride, and freefattyacid and phospholipid levels. plant extract help in resuming the lipid profile, urea and creatinine to normal and results were comparable to that of standard drug glibenclamide (1mg/kg bw). The alterations were observed to resume (P<0.05) back to normal on treatment with plant drug. The effect of plant extract was found to be dose dependent. The present investigation reveals the antidiabetic potential of aqueous extract of whole plant of *A. aspera* L.

KEY WORDS: Diabetes mellitus, *Achyranthes aspera* Linn., Alloxan, Lipid profile, urea, Creatinine
INTRODUCTION
Diabetes is a syndrome characterized by disordered metabolism and abnormally high blood sugar (hyperglycemia) resulting from low levels of the hormone insulin with or without abnormal resistance to insulin’s effect \(^1\). Diabetes mellitus is a major public health problem worldwide. In 2000, the prevalence of diabetes mellitus was estimated to be 0.19% in people < 20 years old and 8.6% in people > 20 years old. In individuals > 65 years the prevalence of diabetes mellitus was 20.1%. The prevalence of type 2 diabetes mellitus is expected to rise more rapidly in the future because of increasing obesity and reduced activity levels \(^2\).

Recently, some medicinal plants have been reported to be useful in diabetes worldwide and have been used empirically as anti-diabetic and antihyperlipidemic remedies. Antihyperglycemic effect of these attributed to their ability to restore the function of pancreatic tissues by causing an increase in insulin output or inhibit the intestinal absorption of glucose or to the facilitation of metabolites in insulin dependent processes. More than 400 plant species having hypoglycemic activity is available in literature.

*Achyranthes aspera* Linn. belonging to family Amaranthaceae, is commonly found as a weed on way side throughout India. It is known as Apamarg in Sanskrit, Chirchitta in Hindi and Prickly chaff flower in English, Naayuruvi in Tamil. The plant is used for treating asthma, cough, snake bite, hydrophobia, urinary calculi, rabies, influenza, piles, bronchitis, diarrhea, renal dropsy, gonorrhea and abdominal pain etc \(^4,5,6,7,8\). It also stimulates the immunity system and enhances the antigen clearance \(^9\), Anti-inflammatory effect \(^10\), Anticancer activity \(^11\). Elevate thyroid hormones levels and decrease lipid peroxidation \(^12\) and hypoglycemic activity \(^13\).

MATERIAL AND METHODS
Preparation of plant extract
*Achyranthes aspera* Linn. was obtained from places in and around Trichy identified and authenticated by the Botanist Dr. S. John Britto,Director, Rabinet Herbarium, Centre for molecular Systematics, St. Joseph’s College (Campus), Tiruchirappalli-620 002, Tamil Nadu, India. 200gm of whole plant powder (*Achyranthes aspera* Linn.) was taken and extracted with water. To one part of the plant material six parts of water was added, boiled and reduced to one third and the filtrate was evaporated to dryness. Paste form of the extract was obtained then subjected to pre-clinical screening.
Animals
Male and female Wistar strains of albino rats weighing 150-200gm were used as the experimental models. The animals were kept in well ventilated cages and were fed with commercial pellet rat chew and water ad libitum and maintained under standard laboratory conditions (Temperature 24-28°C, relative humidity 60-70%). All the studies were conducted according to the ethical guidelines of CPCSEA after obtaining necessary clearance from the committee (approval No: 790/03/ac/CPCSEA).

Alloxan induction
Diabetes mellitus was induced in a batch of normoglycemic albino rats, starved for 16 hours, 150mg/kg body weight of alloxan monohydrate was dissolved in physiological saline and injected intraperitoneally. This dose of alloxan produced persistent hyperglycemia after 4 days as revealed by determination of urine sugar levels by the analysis if blood and urine sample [14]

Experimental design
The rats were divided into four groups each comprising of rats.
Group I - Normal control
Group II - Animals treated with alloxan in normal saline at a dosage of 150mg/kg body weight IP.
Group III - Animals were treated as in Group II. After 4 days of alloxan induction, treated with *Achyranthes aspera* L. aqueous extract 250-mg/kg body weights orally for 45 days.
Group IV - Animals were treated as in Group II. After 4 days of alloxan induction, treated with *Achyranthes aspera* L. aqueous extract 500-mg/kg body weights orally for 45 days.
Group V - Animals were treated as in Group II. After 4 days of alloxan induction, treated with standard drug glibenclamide 1-mg/kg body weight orally for 45 days.

Collection of blood, liver from the rat
The experiment was determined at end of 45 days and the animals were fasting overnight. After the experimental period animals were sacrificed by cervical decapitation under mild chloroform anesthesia. Blood was collected and serum was separated by centrifugation (for 15min at 2000rpm). The liver were dissected out and washed in ice cold saline. Tissues were cut into small pieces and homogenized in 0.1M phosphate buffer (pH7.4). The homogenate was centrifuged and the supernatant was used for various analysis.
Histopathological studies
The pancreatic tissues from all the groups were subjected to histopathological studies [14]. The tissues were fixed using 10% formalin, routinely processed and embedded in paraffin wax. Paraffin sections (5µm thick) were cut on glass slides and stained with hematoxylin and eosin (H&E) after dewaxing, and examined under a light microscope by a pathologist blinded to the groups studied.

The parameters are studied
Estimation of blood glucose
Blood glucose content was estimated by Folin – Wu’s method [15]. To 0.1 ml of the plasma added to 3.4 ml of water, 0.2 ml of 10% sodium tungstate and 0.2 ml of 2/3 N Sulphuric acid in order to precipitate the protein. Mixed well and centrifuged. To 1 ml of the filtrate added 2 ml of alkaline copper sulphate solution and placed in a boiling water bath for 8 minutes, cooled and added 2 ml of phosphomolybdic acid. Various concentrations of standard solution was taken and it is made up to 2 ml of alkaline copper sulphate solution and placed in a boiling water bath for 8 minutes, cooled and added 2 ml of phosphomolybdic acid. A Blank was also maintained. The blue colour, developed was read at 620 nm.

Extraction of Lipids
Tissue lipids were extracted by Folch method [16]. A known volume of suspension was mixed with 10ml of chloroform methanol mixture and homogenized. The homogenate was filtered through Whatmann filter paper (no.42) into a separating funnel. The filtrate was mixed with 0.2 ml of physiological saline and the mixture was kept overnight undisturbed. The lower phase containing the lipid extract was drained off into reweighed beakers. The upper phase was re- extracted with more chloroform- methane mixture and extracts were dissolved in 1.0 ml of chloroform- methanol mixture and aliquots were for estimation of various lipid components.

Estimation of cholesterol
Cholesterol content was estimated by the Zak's method [17]. 0.1 ml of sample (tissue and serum) was added to 4.9 ml of ferric chloride precipitating reagent and mixed well and centrifuged. From this 2.5 ml of filtrate was taken. A cold ice bath was set and added 2.5 ml of diluting reagent and 4ml of concentrated sulphuric acid with thorough mixing various concentrations of working standard solution were taken and made up to 5 ml with diluting reagent. Added 4 ml of sulphuric acid to all the tubes. A blank was
also maintained. The colour developed was read at 560nm.

**Estimation of triacylglycerol**

Estimation of TG by Foster method \(^{[18]}\). 4.0 ml of isopropanol was added to 0.1 ml of sample and mixed well, followed by 0.4 g of alumina and shaken well for 15 min. Centrifuged at 2000 rpm for 10 min and then 2.0 ml of the supernatant was transferred to appropriately labeled tubes. The tubes were placed in a water bath at 65° C for 15 min for saponification after adding 0.6 ml of the saponification reagent. After cooling 1.0 ml of Sodium meta periodate was added followed by 0.5 ml of acetyl acetone reagent. After mixing, the tubes were kept in a water bath at 65° C for half an hour. The contents were cooled and read at 430 nm.

**Assay of HDL cholesterol**

HDL cholesterol by Friedewald method \(^{[19]}\). To 1.0 ml of sample (tissue and plasma), 0.18 ml of heparin manganese chloride reagent was added and mixed. This was allowed to stand in an ice bath for 30 minutes and then centrifuged in a refrigerated centrifuge 2500 g for 30 minutes. The supernatant contained HDL fraction. Aliquots of the HDL supernatant were estimated for cholesterol, phospholipids and triacylglycerol.

**Aggregation of VLDL**

To 1 ml of plasma was added to 0.15 ml of SDS solution. The contents were mixed well and incubated at 37° C for 2 hours. The contents were centrifuged in a refrigerated centrifuge at 10,000 g for 30 minutes. VLDL aggregated as a pellicle at the top. The supernatant was a mixture containing HDL and LDL fractions. The fractions of lipoproteins were assayed after heparin manganese chloride and SDS precipitation. The values are expressed in mg/dl plasma. After precipitation the cholesterol levels in supernatant was measured to get HDL cholesterol. SDS precipitated VLDL and the cholesterol content in the supernatant was measured for HDL cholesterol, LDL cholesterol and VLDL cholesterol.

\[
\text{LDL cholesterol} = \text{Total serum cholesterol} - \frac{\text{Total serum TGL} - \text{HDL Cholesterol}}{5}
\]

\[
\text{VLDL} = \frac{\text{Total Serum TGL}}{5}
\]

**Estimation of free fatty acid**

Determination of free fatty acid in plasma by Colorimetric method. Non-esterified fatty acids were estimated by the method of Falholt \(^{[20]}\).
0.1ml of lipid extract was evaporated to dryness. 0.1 ml of phosphate buffer 6.0ml of extraction solvent and 2.5 ml of copper reagent were added. All the tubes were shaken vigorously 200mg of activated silicic acid was added and left aside for 30 minutes. The tubes were centrifuged and 3ml of the copper layer was transferred to another tube containing 0.5 ml of diphenyl carbazide and mixed carefully. The absorbance was read at 550nm.

**Estimation of phospholipid**
Phospholipids were estimated by the method of Bartlette [21] by digestion with perchloric acid and the phosphorus liberated was estimated by the method of Fisk and Subbarow [22]. 0.1ml of sample (tissue lipid) was digested with 0.2 ml of perchloric acid over a sand bath. Digestion was continued till it was colorless. The liberated phosphorus was estimated. 4.3 ml of deionised water was added to the digested sample followed by 0.5 ml of ammonium molybdate. After 10min 0.2 ml of ANSA was added. Tubes were well shaken and kept aside for 20 mins. Blue colour read at 620nm. The total phospholipids were estimated by multiplying the value of Pi by 25 and expressed as mg/g wet tissue.

**Assay of plasma insulin**
Plasma Insulin was determined by radioimmunoassay method. It is based on antigen and antibody reaction [23].

**Estimation of urea**
Estimation of blood urea was carried out by Diacetyl monoxime method of the Barker [24]. To 0.1 ml of blood, 3.3 ml of distilled water 0.3 ml of 10% sodium tungstate, 3.3 ml of 2/3 N H₂SO₄ were added and centrifuged for few minutes at 3000 rpm. After centrifugation 2ml of supernatant was taken. To this 2 ml of distilled water, 0.4 ml of DAM reagent and 1.6 ml of H₂SO₄-H₃PO₄ reagent were added. The test tube was incubated in a boiling water bath for 30 minutes. After incubation, the test tube was cooled and the color was read at 480nm using blank.

**Estimation of creatinine**
The colorimetric determination of creatinine by the Jaffes reaction of the Bonsness [25]. In to a series of test tubes various concentration of standard solution was taken and a volume was made up to 3 ml with distilled water. For the test 2 ml of serum was added to 2 ml of sulphuric acid and 2 ml of sodium tungstate and centrifuged. From that 3 ml was taken. Then...
added 1 ml of picric acid and 1 ml of sodium hydroxide to all tubes are heated in a boiling water bath for 15 minutes. The colour developed was read at 500nm.

Statistical analysis:
Hypothesis testing methods included one way analysis of variance (ANOVA) followed by least significant differences test. P values <0.05 were considered as significant. All the results were expressed as means ± standard error mean for six animals in each group.

RESULTS
A significant increase in the level of blood glucose and a decrease in body weight were observed in diabetic rats. After animals were treated with A.aspera aqueous extract gain in body weight and decrease blood glucose level compared to diabetic control. The diabetic rats showed a signify decrease in the levels of plasma insulin. Administration of A.aspera and glibenglamide showed notable increase in serum insulin level (Table-1).

Estimation of tissue and serum lipid profile
The serum and tissue lipid profiles are shown in Table2&3. Aqueous extract of A.aspera showed an excellent reduction of TG, cholesterol and FFA, PL in serum and tissue, when compared diabetic rats were raised in cholesterol, triglyceride, freefattyacid and phospholipid levels. Alloxan induced diabetic animals reduced the HDL cholesterol and cause of significant elevation in LDL and VLDL levels, plant extract help in resuming the lipid profile to normal in liver and serum and results were comparable to that of standard drug glibenclamide (1mg/kg bw)

Estimation of serum urea and creatinine
The kidney functions can be assessed by the level of serum urea and creatinine. Significant increase in serum urea and creatinine was observed in diabetic rats. After administration of aqueous extract is having good potential reduced the creatinine and urea level to normal (Table-4).

Table:1 Effect of treatment A.aspera extract for 45 days on blood glucose, body weight and serum insulin of experimental animals:-

<table>
<thead>
<tr>
<th>S.NO</th>
<th>Groups</th>
<th>Blood glucose mg/dl</th>
<th>Initial body weight(g)</th>
<th>Final body weight(g)</th>
<th>Serum Insulin µu/ml</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Normal</td>
<td>84.3±1.2</td>
<td>150±1.2</td>
<td>160±1.2</td>
<td>23.4±0.7</td>
</tr>
<tr>
<td>2.</td>
<td>Diabetic control</td>
<td>292.3±0.9*</td>
<td>128±1.8*</td>
<td>110±2.0*</td>
<td>9.8±0.5*</td>
</tr>
</tbody>
</table>
Values are Mean ± SEM (n=6)

*P < 0.05.statistically significant when compared Group II with Group I,

**P < 0.05.statistically significant when compared Group III, & IV with Group II.

Table:2 Effect of treatment *A. aspera* extract for 45 days on serum and tissue protein, Cholesterol, Triglyceride of experimental animals:-

<table>
<thead>
<tr>
<th>S.No</th>
<th>Groups</th>
<th>Cholesterol (mg/dl)</th>
<th>Triglyceride (mg/100g)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Serum</td>
<td>Tissue</td>
</tr>
<tr>
<td>1</td>
<td>Normal</td>
<td>70 ±0.5</td>
<td>90.8±0.4</td>
</tr>
<tr>
<td>2</td>
<td>Diabetic control</td>
<td>240±0.3*</td>
<td>220.3±0.6*</td>
</tr>
<tr>
<td>3</td>
<td>Diabetic+<em>A. aspera</em> (250mg/kg)</td>
<td>165±0.2**</td>
<td>170.4±0.5**</td>
</tr>
<tr>
<td>4</td>
<td>Diabetic+<em>A. aspera</em> (500mg/kg)</td>
<td>84±0.6**</td>
<td>100.3±0.2**</td>
</tr>
<tr>
<td>5</td>
<td>Diabetic+glibenclamide (1mg/kg)</td>
<td>89±0.4</td>
<td>112.5±0.7</td>
</tr>
</tbody>
</table>

Values are Mean ± SEM (n=6)

*P < 0.05.statistically significant when compared Group II with Group I,

**P < 0.05.statistically significant when compared Group III, & IV with Group II.
Table 3: Effect of treatment *A. aspera* extract for 45 days on serum and tissue HDL, LDL, VLDL & Tissue PL, FFA of experimental animals:

<table>
<thead>
<tr>
<th>S.NO</th>
<th>Group</th>
<th>HDL</th>
<th>LDL</th>
<th>VLDL</th>
<th>Phospholipid mg/100g</th>
<th>FFA meq/100g</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Serum mg/dl</td>
<td>Tissue mg/100g</td>
<td>Serum mg/dl</td>
<td>Tissue mg/100g</td>
<td>Serum mg/dl</td>
</tr>
<tr>
<td>1.</td>
<td>Normal</td>
<td>42.3±0.3</td>
<td>78±1.2</td>
<td>63.5±0.3</td>
<td>87.9±0.2</td>
<td>15±0.8</td>
</tr>
<tr>
<td>2.</td>
<td>Diabetic control</td>
<td>17.4±0.4*</td>
<td>59.2±1.3*</td>
<td>214.3±0.9*</td>
<td>192.4±0.7*</td>
<td>29.2±0.5*</td>
</tr>
<tr>
<td>3.</td>
<td>Diabetic+A. aspera (250mg/kg)</td>
<td>24.3±0.5**</td>
<td>118±0.9**</td>
<td>148.1±0.4**</td>
<td>153.7±0.3**</td>
<td>21.8±0.7**</td>
</tr>
<tr>
<td>4.</td>
<td>Diabetic+A. aspera (500mg/kg)</td>
<td>41.8±0.7**</td>
<td>76±1.0**</td>
<td>78.0±0.7**</td>
<td>98.3±0.5**</td>
<td>14.4±0.4**</td>
</tr>
<tr>
<td>5.</td>
<td>Diabetic+glibenclamide (1mg/kg)</td>
<td>51.5 ±0.2</td>
<td>70±1.5</td>
<td>82.3±0.5</td>
<td>112.2±0.4</td>
<td>1.7±0.2</td>
</tr>
</tbody>
</table>

Values are Mean ± SEM (n=6)

*P < 0.05 statistically significant when compared Group II with Group I,

**P < 0.05 statistically significant when compared Group III, & IV with Group II.
Table: 4:- Effect of treatment A.aspera extract for 45 days on blood urea, creatinine level of experimental animals:-

<table>
<thead>
<tr>
<th>S.no</th>
<th>Groups</th>
<th>Urea (mg/dl)</th>
<th>Creatinine (mg/dl)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Normal</td>
<td>32±0.5</td>
<td>0.4±0.01</td>
</tr>
<tr>
<td>2.</td>
<td>Diabetic control</td>
<td>91±0.7*</td>
<td>4.5±0.02*</td>
</tr>
<tr>
<td>3.</td>
<td>Diabetic+A. aspera (250mg/kg)</td>
<td>64±0.8**</td>
<td>3.0±0.01**</td>
</tr>
<tr>
<td>4.</td>
<td>Diabetic+A. aspera (500mg/kg)</td>
<td>34±0.5**</td>
<td>0.9±0.04**</td>
</tr>
<tr>
<td>5.</td>
<td>Diabetic+glibenclamide (1mg/kg)</td>
<td>27.0±1.0</td>
<td>1.2±0.08</td>
</tr>
</tbody>
</table>

Values are Mean ± SEM (n=6)

*P < 0.05.statistically significant when compared Group II with Group I,

**P < 0.05.statistically significant when compared Group III, & IV with Group II,

Histopathological studies

Islets of untreated rats appear to have distortion and vascular degeneration. In contrast A.aspera treated animals showed improvement in histoarchitecture considerably high dose (500mg/kg bw) pancreatic tissue was regenerated to be normal. No variation has been noted in reference drug treated tissue (figure- I, II, III, IV and V).

Figure- I Islets of langerhans of normal rats (IS-Islets)

Figure- II Islets of Langerhans of group II rats (Disease control) shows degenerative changes of beta cells (VD-Vascular degeneration)
Figure- III Islets of Langerhans of group III animals are treated with plant extract (250mg/kg bw) shows regeneration in beta cells (GI- Granulated Islets)

Figure-IV Islets of Langerhans of group IV rats are treated with plant extract (500mg/kg bw) shows profound regeneration in beta cells (WGI-Well Granulated Islets)

Figure-V Islets of Langerhans of group V rats are with standard drug (glibenclamide 1mg/kg bw) shows mild degeneration in beta cells (MD- Mild degeneration)
DISCUSSION
Currently-available drug regimens for management of diabetes mellitus have certain drawbacks and therefore, there is a need for safer and more effective antidiabetic drugs [26-28]. Induction of diabetes with alloxan is associated with the characteristic loss of body weight which is due to increased muscle wasting in diabetes. Diabetic rats treated with the aqueous extract showed an increase in body weight. This may be due to its protective effect in controlling muscle wasting [29].

In our study, there was significant elevation in blood glucose level in diabetic control group as compared with normal animals. Over production of glucose by means of excessive hepatic glycogenolysis and gluconeogenesis and decreased utilization of glucose by the tissues is one of the fundamental bases of hyperglycemic in diabetes mellitus [30]. Liver functions as a “glucosat” and play a vital role in the maintenance of blood glucose level and hence it is of interest to examine the possible role of A.aspera on key enzymes of carbohydrate metabolism in liver. Liver is the main site for glycolysis, a process where glucose is degraded and gluconeogenesis [31].

The insulin activity was found to be decreased in diabetic rat. Treatment with A.aspera elevated the insulin level in the β cell of pancreas. A.aspera may stimulate insulin secretion, which may activate islet of pancreas to regeneration Langerhans cell. This may be due to increased insulin secretion, which is responsible for the activation of the glucogenic key enzyme.

Diabetic rats were observed to have increased plasma lipids and decreased tissue lipids, which are responsible for several cardiovascular disorders [32]. Consequently, the excess of fatty acids in the plasma may promote the hepatic conversion of fatty acids into phospholipids and cholesterol, the main product of lipid metabolism. The increase level of TG and cholesterol in the blood of diabetic rats may lead to cardiovascular disease. The accelerated coronary heart disease has emerged as a leading cause of morbidity and mortality in diabetic patients in the world wide [33].

The higher lipid levels seen in diabetic rats were due to increased mobilization of free fatty acids from peripheral depot and also due to lipolysis caused by hormones [34-35]. Andalla et al [36] LDL plays an important role in arteriosclerosis. LDL and VLDL carry cholesterol to the peripheral tissues where it is deposited. Hence high levels of LDL and VLDL are
atherogenic. HDL transports cholesterol from peripheral tissues to the liver and thus aids in its excretion. HDL therefore has a protective effect. After treatment of plant extract cholesterol, VLDL, LDL levels were significantly reduced respectively.

Elevated levels of urea and creatinine seen during increased protein breakdown and may also see in renal disorder one the secondary complication in diabetes mellitus. On treatment with aqueous extract of *A.aspera* the levels of urea and creatinine were maintained to normal.

According to these results, *A.aspera* could be a supplement, as an antioxidant therapy, and may be beneficial for correcting the hyperglycemia and preventing diabetic complications due to lipid peroxidation and free radicals. The *A.aspera* plant is having a hypoglycemic effect and it also controls the antioxidant level. Hence it could be used to improve the lipid metabolism\[^{37}\]. Normalization histopathology of β cells in the islets of Langerhans in pancreas of diabetic rats fed with aqueous extract *A.aspera* (*figure-III and IV*) and also showed mild regenengration of β cells pancreas in standard drug (*figure V*). Hence, the hypoglycemic activity of aqueous extract of *A.aspera* may be due to its protective action against alloxan- mediated damage to the pancreatic beta cells and also possibly because of regeneration of damaged beta cells or increased insulin secretion\[^{38}\].

**CONCLUSION**

Many indigenous Indian medicinal plants are used as remedies against various diseases. In the present scenario herbal medicines and herbal research is coming to lime light. The present investigation evaluates the antidiabetic efficacy of *A.aspera*, further investigation have to be extended by analyzing the mechanism of action of *A.aspera* is combating altered glycemic and lipid profile in diabetic condition.

**REFERENCES**