ABSTRACT

Diabetes mellitus is a metabolic disorder in which the carbohydrates and lipid metabolism is improperly regulated by insulin. Many indigenous Indian medicinal plants have been found to be successfully used to treat Diabetes. Herbal drug are considered to be less toxic and free from side effects than synthetic ones. Alloxan induced diabetes has been observed to cause massive reduction of β-cells of islets of langerhan’s leading to hyperglycemia. Rats treated with Alloxan (150mg/kg), for 72 hours, after showed an increase in the concentration of glucose, triglycerides, cholesterol, LDL cholesterol, SGOT, SGPT and decreases in the level of HDL. In the present investigation and attempt is made to study the beneficial effects of Basella rubra L. in Alloxan induces diabetic rats. The diabetes-induced rats were fed with Basella rubra L. (200 and 400 mg/kg body weight orally through gavage) when tested after ingestion they reduced the concentration of glucose, triglycerides, cholesterol, LDL, SGOT, SGPT and raised the level of HDL in the blood of Alloxan induced rats. The results were compared with glibenclamide treated group. In histopathology of liver and kidney the changes caused after induction of diabetes was global microvascular steatosis liver and vacuoles observed in diabetes rats in kidney section. After fed with plant extract no histopathological changes were noticed. The Anthocyanins in plants can reduce the levels of sugars in diabetic condition and also help in reverses in histopathological changes in liver and kidney.
KEYWORDS: Basella rubra L., Anthocyanins, Glibenclamide, Alloxan, Blood glucose levels, Histopathology.

1. INTRODUCTION
Diabetes mellitus is a metabolic disease characterized by high blood glucose level resulting from defects in insulin secretion, insulin action or both.\(^1\) According to the International Diabetes Federation (IDF), type 2 diabetes currently affects 246 million people worldwide and is expected to increase to 380 million by 2025.\(^2\) The study of natural colorants is an extensive and active area of investigation due to the growing interest of substituting synthetic colorants with toxic effects in humans.\(^3\) Carotenoids and anthocyanins are amongst the most utilised vegetable colorants in the food industry.\(^4\) Carotenoids are liposoluble, stable and able to colour food products from yellow to red.\(^5\) They are obtained mostly from carrots, tomatoes and peppers.\(^6\) On the other hand, the anthocyanins are water-soluble and less stable than carotenoids; they are extracted from grapes, berries, red cabbage, apples, radishes, tulips, roses and orchids, amongst others.\(^7\) The significant property of anthocyanins is their antioxidant activity, which plays a vital role in the prevention of neuronal and cardiovascular illnesses, cancer and diabetes, among others.\(^8\) ANTs, which belong to the flavonoid class, are secondary metabolites of higher plants and are responsible for the blue, red and purple colors of fruits, vegetables and flowers. There is a huge variety of anthocyanins spread in nature. The main differences between them are the number of hydroxylated groups, the nature and the number of bonded sugars to their structure, the aliphatic or aromatic carboxylates bonded to the sugar in the molecule and the position of these bonds.\(^9\)

Anthocyanins (ANTs) are pigments widely found in fruits and vegetables and, therefore, often consumed in a normal diet. Interest in this important class of flavonoids has been growing in the scientific community due to recent evidence of their beneficial effects on health. Numerous publications have reported the antioxidant effects of ANTs\(^10\), such as vasoprotective\(^11\), anti-inflammatory, anticarcinogenic\(^12\), antiobesity\(^13\) and antidiabetic effects.\(^14\)

*Bella rubra* L. known as Malabar spinach is also known as cyclone spinach. It belongs to *Basellaceae* family. It is a climbing perennial plant. They are thick, rugose, succulent and colored from green to purple.\(^15\) Anthocyanins are responsible for the blue, red, violet and purple coloration in most species of plant kingdom.\(^16\)
2. MATERIAL AND METHODS

2.1 Plant Collection and Authentication

*Basella rubra* L. Plant material was collected in the month of November to December from Nalgonda district, Telangana, India. This plant was identified and authenticated by Prof. P. Ramachandra Reddy, Professor of Botany department, University College of science, Osmania University, Hyderabad, Telangana and The Plant Voucher No.0596. The plant specimen was submitted in Department of Pharmacognosy, Nalanda College of Pharmacy.

![Basella rubra L.](image)

Figure 1. Plant of *Basella rubra* L.

2.2 Preparation of methanol extract of *Basella rubra* L.

The plant was collected then leaves were separated from the plant and thoroughly washed and shade dried. After drying the leaves were coarsely powdered. Then it was kept for maceration process by using methanol which is acidified with 0.1MHCL. Anthocyanins were extracted from 5 gm of leaf powder with methanol acidified with 0.1 m HCl. The powders were extracted with 20 ml portions of solvent until the solvent become colorless.[17] Then the menstruum was filtered using muslin cloth. The filtrate was then concentrated by evaporation by keeping at room temperature and extract was stored in refrigerator at 4°C for use in experiments.

2.3 Preliminary phytochemical screening of the extract

The preliminary phytochemical analysis was carried out for the *Basella rubra* L. methanolic extract using standard phytochemical methods.[18]
2.4 Screening for anti-diabetic activity of methanolic extract of *Basella rubra* L. leaves

2.4. a) Animals

Albino wistar rats of male sex weighing about 150-200gms were employed for the anti-diabetic studies. They were housed in standard environment condition and fed with standard rodent diet with water *ad libitum*. The animals were kept in cages under the standard conditions according CPCSEA guidelines (Temperature 25±2ºc, 12 h light and 12 h dark cycle). Ethical clearance for the animal study was obtained from Institutional Animal Ethical Committee (IAEC). Reference number: NCOP/IAEC/approved/75/2016. And as per OECD 423 guidelines.

2.5 Induction of experimental diabetes

The experimental animals were fasted overnight and then made diabetic by a single Intraperitoneal injection of Alloxan (150 mg/kg body weight). Alloxan was prepared just prior to injection in normal saline. The animals were allowed to drink 5% glucose solution overnight to overcome the drug induced hypoglycemia. Diabetes was confirmed by elevated blood glucose level determined at 72 hrs. Animals with fasting blood glucose level more than 250mg/dl were considered as diabetic rats.

2.6 Experimental design

In the experiment, the rats were divided into 5 groups for the evaluation of histopathological and biochemical parameters with six animals in each group.

**GROUP I** - Normal control rats.

**GROUP II** - Diabetic control rats

**GROUP III** – Diabetic rats given Glibenclamide (600 µg/kg b.w./Rat/day) orally for 28 days.

**GROUP IV** - Diabetic rats given *Basella rubra* L. (200 mg/kg b.w./Rats/day) orally for 28 days.

**GROUP V** - Diabetic rats given *Basella rubra* L. (400 mg/kg b.w./Rats/day) orally for 28 days.

Every 7 days the blood was withdrawn from tail vein and glucose levels were measured by using Glucometer (one touch) and body weight were monitored and for 28 days of treatment. At the end of the study Blood was collected by retro orbital puncture, under mild ether anesthesia after overnight fasting (or) Blood samples were obtained from hearts of overnight
fasted rats using micro-capillary technique and allowed to clot for 20 min in laboratory temperature and then allowed for serum separation.

Glycosylated Hemoglobin was estimated\textsuperscript{[19]}, Lipid profile [cholesterol, high density lipoproteins (HDL), low density lipoproteins (LDL), triglycerides] levels in serum were determined according to the instruction of manufacturer (EXCEL DIAGNOSTICS PVT.LTD, Hyderabad, India). Serum glutamic oxaloacetic transminase (SGOT), serum glutamic-pyruvic transminase (SGPT) was determined by the method of Reitman and Frankel, 1957.\textsuperscript{[20]}

2.7 Statistical analyses
Results were expressed as means±sd and the difference between the groups were tested by one-way analysis of variance (ANOVA) followed by the Student-Newman-Keuls test using the software “GraphPad Instat”. The p<0.05 were considered as statistically significant.

2.8 The histopathological study
After blood sampling for the biochemical analyses, at the end of the 28\textsuperscript{th} day the animals were sacrificed, quickly dissected and small slices of liver, kidney were taken and fixed in 10% formalin. The specimen were dehydrated in ascending grades of ethanol, cleared in xylene and embedded in paraffin wax. Sections of 6 mm in thickness were prepared and stained with haematoxylin and eosin\textsuperscript{[21]} and subjected to microscopical examination.

3. RESULTS
The phytochemical analysis of \textit{Basella rubra} L. methanol extract revealed the presence of carbohydrates, proteins, tannins, flavonoids (anthocyanins), sterols, saponins, alkaloids, phenolic glycosides. In flavanoids mostly it consists of anthocyanins. The simple quantitative analysis of the extract was based on the intensity of colour change.

Table 1 showed Body weight was found to be elevated in normal control and a substantial increase in body weight was also observed in diabetic rats. On treatment of diabetic rats with glibenclamide (group-III), a reduction in body weight was observed. \textit{Basella rubra} L. at 200 & 400mg/kg showed a decrease in Body weight which was less compared with the alloxan treated group.

Table 2 showed Single dose intra-peritoneal (i,p) treatment of rats with alloxan monohydrate (150 mg/kg) significantly increases the blood glucose levels after 72 hours of alloxan
administration and a consistent elevation of glucose was observed up to the end of our study. Treatment with *Basella rubra* L. exerted good control over the blood glucose at both dose levels compared with Glibenclamide.

Table 3 showed the alloxan group increased level of cholesterol, triglyceride, LDL and decreased level of HDL-cholesterol in diabetic rats compared to normal control. Oral administration of Basella rubra L. methanolic extract for 28 days significantly reduced the cholesterol, triglycerides and LDL levels and significantly increased the HDL-cholesterol level when compared with diabetic rats.

Table 4 showed the levels of HbA1c observed were significantly low in the groups treated with Glibenclamide, *Basella rubra* L. at both dose levels compared with the negative control.

**Table-1. Effect of oral administration of *Basella rubra* L. methanol extract on body weight in Alloxan induced diabetic rats.**

<table>
<thead>
<tr>
<th>S.NO</th>
<th>Groups</th>
<th>Body Weight (gm)</th>
<th>Initial</th>
<th>1 st week</th>
<th>2 nd week</th>
<th>3 rd week</th>
<th>4 th week</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Group I</td>
<td></td>
<td>170.17±8.61</td>
<td>170.50±8.77</td>
<td>172.83±8.71</td>
<td>172.33±8.80</td>
<td>176.33±8.80</td>
</tr>
<tr>
<td>2</td>
<td>Group II</td>
<td></td>
<td>170.67±4.84</td>
<td>172.67±4.84</td>
<td>175.67±4.83</td>
<td>179.67±4.84</td>
<td>183.67±4.84</td>
</tr>
<tr>
<td>3</td>
<td>Group III</td>
<td></td>
<td>169.83±4.94</td>
<td>165.33±4.54</td>
<td>159.50±4.86</td>
<td>154.33±4.94</td>
<td>148.67±4.96</td>
</tr>
<tr>
<td>4</td>
<td>Group IV</td>
<td></td>
<td>171.17±4.59**</td>
<td>178.17±4.59**</td>
<td>169.17±4.66**</td>
<td>162.17±4.59**</td>
<td>161.17±4.59**</td>
</tr>
<tr>
<td>5</td>
<td>Group V</td>
<td></td>
<td>169.37±5.80*</td>
<td>170.37±5.80*</td>
<td>174.37±5.80*</td>
<td>168.37±5.80*</td>
<td>160.37±4.80*</td>
</tr>
</tbody>
</table>

* Values deviate significantly from diabetic control; ** Values deviate very significantly (P ≤ 0.05).

**Table-2. Effect of oral administration of *Basella rubra* L. methanol extract on blood glucose levels in alloxan induced diabetic rats.**

<table>
<thead>
<tr>
<th>S.NO</th>
<th>Groups</th>
<th>Initial</th>
<th>1 st week</th>
<th>2 nd week</th>
<th>3 rd week</th>
<th>4 th week</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Group-I</td>
<td>98.3±6.52</td>
<td>93.05±3.18</td>
<td>90.85±4.12</td>
<td>85.75±4.98</td>
<td>99.25±2.01</td>
</tr>
<tr>
<td>2</td>
<td>Group-II</td>
<td>87.00±5.01</td>
<td>215.00±8.093</td>
<td>243.5±23.16</td>
<td>250.1±32.22</td>
<td>248.2±20.26</td>
</tr>
<tr>
<td>3</td>
<td>Group-III</td>
<td>92.4±3.63</td>
<td>204.6±10.25</td>
<td>164±13.28</td>
<td>134.3±4.06</td>
<td>102.9±5.51</td>
</tr>
<tr>
<td>4</td>
<td>Group-IV</td>
<td>90.23±2.89**</td>
<td>220.7±12.36**</td>
<td>188.5±7.46**</td>
<td>146.60±10.18**</td>
<td>112.7±3.68**</td>
</tr>
<tr>
<td>5</td>
<td>Group-V</td>
<td>85.95±4.32**</td>
<td>215.00±8.093**</td>
<td>167.8±13.28**</td>
<td>129.66±9.08**</td>
<td>99.35±4.21*</td>
</tr>
</tbody>
</table>

* Values deviate significantly from diabetic control; ** Values deviate very significantly (P ≤0.05) when compared with diabetic control values.
Table 3. Effect of oral administration of *Basella rubra* L. methanol extract on lipid profile in alloxan induced diabetic rats after 28 days.

<table>
<thead>
<tr>
<th>S.NO</th>
<th>Groups</th>
<th>Cholesterol (mg/dl)</th>
<th>Triglycerides (mg/dl)</th>
<th>HDL (mg/dl)</th>
<th>LDL (mg/dl)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Group I</td>
<td>76.54±3.06</td>
<td>26.50±1.08</td>
<td>50.41±3.56</td>
<td>17.46±0.84</td>
</tr>
<tr>
<td>2</td>
<td>Group II</td>
<td>140.42±2.68</td>
<td>130.23±6.51</td>
<td>32.77±0.98</td>
<td>78.20±4.61</td>
</tr>
<tr>
<td>3</td>
<td>Group III</td>
<td>79.93±1.42**</td>
<td>42.46±2.26**</td>
<td>48.73±1.88**</td>
<td>31.87±2.65**</td>
</tr>
<tr>
<td>4</td>
<td>Group IV</td>
<td>80.45±3.64**</td>
<td>30.97±1.61**</td>
<td>49.03±4.23**</td>
<td>29.52±2.47**</td>
</tr>
<tr>
<td>5</td>
<td>Group V</td>
<td>78.32±2.54**</td>
<td>29.84±4.73**</td>
<td>48.52±3.20**</td>
<td>23.64±3.44**</td>
</tr>
</tbody>
</table>

* Values deviate significantly from diabetic control; ** values deviate very significantly (*P*≤0.05) when compared with diabetic control values.

Table 4. Effect of oral administration of *Basella rubra* L. methanolic extract on Glycosylated hemoglobin in alloxan induced after 28 days.

<table>
<thead>
<tr>
<th>S.NO</th>
<th>Groups</th>
<th>% HbA1C</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Group I</td>
<td>4.00±0.17</td>
</tr>
<tr>
<td>2</td>
<td>Group II</td>
<td>10.27±0.35</td>
</tr>
<tr>
<td>3</td>
<td>Group III</td>
<td>5.80±0.29**</td>
</tr>
<tr>
<td>4</td>
<td>Group IV</td>
<td>6.78±0.32*</td>
</tr>
<tr>
<td>5</td>
<td>Group V</td>
<td>5.36±0.41**</td>
</tr>
</tbody>
</table>

* Values deviate significantly from diabetic control; ** Values deviate very significantly (*P*≤0.05) when compared with diabetic control values.

Table 5. Effect of oral administration of LN methanol extract on serum marker enzymes in alloxan induced diabetic rats after 28 days.

<table>
<thead>
<tr>
<th>S.NO</th>
<th>Groups</th>
<th>SGOT</th>
<th>SGPT</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Group I</td>
<td>104.83±4.97</td>
<td>43.42±1.88</td>
</tr>
<tr>
<td>2</td>
<td>Group II</td>
<td>323.45±12.64</td>
<td>120.18±7.86</td>
</tr>
<tr>
<td>3</td>
<td>Group III</td>
<td>198.00±8.04**</td>
<td>77.55±0.90**</td>
</tr>
<tr>
<td>4</td>
<td>Group IV</td>
<td>248.87±27.93*</td>
<td>91.83±2.01**</td>
</tr>
<tr>
<td>5</td>
<td>Group V</td>
<td>187.53±19.31**</td>
<td>60.27±2.58**</td>
</tr>
</tbody>
</table>

* Values deviate significantly from diabetic control; ** Values deviate very significantly (*P*≤0.05) when compared with diabetic control values.
Figure-2. Liver histopathology. A) Normal control B) diabetic control C) Diabetic+ Glibenclamide (600 µg/kg b.w./Rat/day). D) Diabetic + *Basella rubra* L. methanolic extract (200mg/kg b.w./Rat/day ) E) Diabetic + *Basella rubra* L. methanolic extract (400 mg/kg b.w./Rat/day).
Figure-3. Kidney histopathology. A) Normal control B) diabetic control C) Diabetic+ Glibenclamide (600µg/kg,b.w./Rat/day). D) Diabetic + Basella rubra L. methanolic extract (200mg/kg b.w./Rat/day)  E) Diabetic + Basella rubra L. methanolic extract (400 mg/kg, b.w./Rat/day).

Histopathology examination of liver (figure 2) showed Group-I Normal structure, Group –II & III the Hepatocytes showed the global Micro vesicular steatosis. And Groups IV &V
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(Alloxan+ Basella rubra L.) Showed the Hepatocytes, & Portal tracts appeared normal. No steatosis was observed.

Histopathology examination of kidney (figure 3) showed Group-I normal structure of Glomeruli. Group-II In alloxan Treated diabetic Group Kidney showed damages in the cells & various sizes of vacuoles is observed. Group III showed mild damages in the cells of the kidney. Groups –IV & V (Alloxan + Basella rubra L.) showed tubules showed normal histological structure.

DISCUSSION
The Methanolic extract of leaves of Basella rubra L. exhibited significant potential anti-diabetic activity in alloxan-induced Diabetic rats. The number of functionally intact β-cells in the islet organ is of decisive importance in the development course and outcome of DM. The renewal of β-cells in diabetes has been studied in animal models. The total β-cell mass reflects the balance between the renewal and loss of these cells. It was also suggested that regeneration of islet β-cells following destruction by alloxan may be the primary cause of the recovery of alloxan – injected rats by the effects of the drug.

In our study, damage to pancreas in alloxan-treated diabetic control and regeneration of β-cells by Glibenclamide and also the Basella rubra L. extract was observed. In this present study, blood glucose levels return to normal in healthy rats fed with extract of leaves of Basella rubra L. appeared normal No mortality was observed, indicating that there were no acute toxic effects for the leaves of Basella rubra L.

In our study, body weight was found to be elevated in normal control and a substantial increase in body weight was also observed in diabetic rats. Treatment for 4 weeks on diabetic rats with Glibenclamide (600µg/kg) (group-III), a reduction in body weight was observed. Basella rubra L. at 200mg/kg & 400mg/kg showed a decrease in Body weight which was less compared with the alloxan treated group and close the values of normal rats group.

In our study, Single dose intraperitoneal (i.p) treatment of rats with alloxan monohydrate (150 mg/kg) significantly increases the blood glucose levels after 72 hours of alloxan administration and a consistent elevation of glucose was observed up to the end of our study. Treatment with methanolic extract of Basella rubra L., exerted good control over the blood glucose at both dose levels compared with Glibenclamide.
The levels of HbA1c observed were significantly low in the groups treated with Glibenclamide and *Basella rubra* L. at both dose levels compared with the negative control and comparable with Glibenclamide.

In our study, Plasma cholesterol levels were reduced with the *Basella rubra* L. group at 400mg/kg than glibenclamide. Continuous treatment with *Basella rubra* L. at 400mg/kg for 4 weeks was found to significantly lower the cholesterol levels. Extract at 200 mg/kg showed a slight increasing cholesterol level compared to Glibenclamide. The values are statistically significant compared to positive control.

In our study, Plasma triglyceride levels were reduced with the *Basella rubra* L. treated groups at 200 and 400mg/kg than glibenclamide. Continuous treatment with low and high doses of *Basella rubra* L. for 4 weeks it was significantly lowered the triglyceride levels.

In our study, Glibenclamide treated group, Low and high doses of *Basella rubra* L. treated groups exhibited a significant decline in the LDL levels for high dose respectively compared to the alloxan group and significant increase compared to control group.

In our study, Glibenclamide treated group, Low & high doses of *Basella rubra* L. treated groups exhibited a significant increase in the HDL levels respectively compared to the alloxan treated group which significantly decreases compared to control.

Alloxan exposure to animal causes a rise in the level of liver marker enzymes. Glibenclamide treated group, Low & high doses of *Basella rubra* L. treated groups exhibited a significant decrease in SGOT levels and SGPT levels respectively compared to the alloxan treated group which significantly increases compared to control. The extracts at the dose of 400 mg/kg showed the maximum significant decrease in the SGOT levels and SGPT levels that indicates higher liver protective activity of the Plant.

In the histopathology small Changes in the both liver and kidney sections were seen to be significant and a decrease in the cellular damage in the group of *Basella rubra* L. and comparable with the alloxan induced Diabetic groups.

**CONCLUSION**

The finding of study indicate that consumption of *Basella rubra* L. extract exerts significant antidiabetic and hypolipidemic effect in alloxan induced diabetic rats. No Histopathology
changes were observed in both the Plant extracts treated groups. The potential antidiabetic and associated hypolipidemic activities of the Basella rubra L. plant were due to the presence of the phytochemical constituents such as anthocyanins, a type of flavonoid with potential anti-oxidant property.

In future, this study can be extended by the isolation of constituents present in the plant extract and its diabetic activity.

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