STUDIES ON THE BETA CELLS OF ISLETS OF LANGERHANS OF ALLOXAN INDUCED DIABETIC ALBINO RATS TREATED WITH ALCOHOLIC SEED EXTRACT OF CAESALPINIA BONDUCELLA

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KEYWORDS
Diabetes
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Beta cells
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ABSTRACT
Present investigation was undertaken to evaluate the potential anti-diabetic activity of alcoholic seed extract of Caesalpinia bonducella (ASECB) on islets of albino rats. The rats were divided into four groups, Control, Diabetic, Diabetic + 200 mg ASECB and Diabetic + 400 mg ASECB. Oral administration of different doses of ASECB to diabetic rats was conducted for a period of 14 days. Blood glucose level was estimated on 0, 3rd, 10th and 17th day. Histopathology of the pancreas and morphometry of beta cells was studied. The body weight of the diabetic control rats was significantly reduced. The body weight was regained and there was significant increase in the final body weight in treated groups. Treated animals showed a significant decrease in blood glucose level. The number of beta cells in diabetic rats compared to controls was significantly reduced. Treated rats showed significant increase in number of beta cells. The islet size in diabetic group significantly decreased when compared to control. The animals treated with ASECB showed a significant increase in islet size. Thus, it is suggested ASECB could serve as a good oral hypoglycemic agent.

INTRODUCTION
Diabetes Mellitus (DM) is a most common, metabolic, hereditary and endocrine disorder that disturbs the metabolism of carbohydrates, fats and proteins. It is characterized by hyperglycemia and glycosuria produced by deficiency in production of insulin by the endocrine pancreas leading to excess glucose in blood. Chronic hyperglycemia during diabetes causes glycation of body proteins that in turn lead to secondary complications affecting eyes, kidney, heart, nerves, blood vessels, liver and many other vital organs causing nephropathy, neuropathy, retinopathy, atherosclerosis, weight loss, sexual dysfunction and may lead to drowsiness to coma. The symptoms of diabetes are increased blood sugar, body weight loss, weakness, frequent urination, severe thirst, polyuria, blurred vision etc.

A world wide survey reported that DM is affecting nearly 10% of the population every year (Vetrichelvan et al., 2002). The number of people suffering from the disease worldwide is increasing at an alarming rate with projected 366 million peoples likely to be diabetic by the year 2030 as against 191 million estimated in 2000 (Wild et al., 2004). This disease is the seventh leading cause of death in the world. The number of people with diabetes multiplies worldwide. It is projected to become one of the world main disablers and killers within the next 25 years (Li WL et al., 2004). The incidence of DM is high all over the world especially in Asia. The countries with largest number of DM are India, China and the United states (Hruban and Wilentz, 2004). Recent studies on geographical and ethnic influences have shown that people of Indian origin are highly prone to diabetes. It is estimated that between 10-12% of the urban population and 4-6% of the rural population of India have diabetes now. Statistical projections about India suggest that the number of diabetics will rise from 15 million in 1995 to 79.4 million by 2025, making it the country with the highest number of diabetic in the world (King et al., 1998, Boyle et al., 2001). World Health Organization (WHO) has issued a warning that India will be the diabetic capital of the world. Changes in human behavior and life styles over the last century have resulted in a dramatic increase in the incidence of diabetes worldwide (Grover et al., 2005). Based on etiology, DM is classified into 1. Type-I (Insulin dependent DM), 2. Type-II (Non-insulin dependent DM) and 3. Gestational Diabetes (GD).

Different types of oral hypoglycemic agents such as biguanides, sulphonylurea are available along with insulin for the treatment of DM (Holman and Turner, 1991). But side effects are associated with their uses (Rao et al., 1997, Valiathan, 1998). Synthetic hypoglycemic drugs can produce side effects including coma, disturbances of liver and kidney function. In addition they are not suitable for use during pregnancy (Larner, 1985). Further insulin therapy has short comings such as ineffectiveness following oral administration, need for constant refrigeration and fatal hypoglycemia in the event of excess dosage (Anuradha et al., 2001) in which blood sugar level falls. A fall below 55mg/dL produces severe symptoms leading to “insulin shock” and death. At present antidiabetic drugs like glibpiride, glipizide, pioglitazone,
rosiglitazone and metformin do not cure diabetes and produce side effects leading to other complications. Hence now-a-days herbal drugs are gaining popularity in the treatment of DM (Chandan Kumar et al., 2013). With this background present investigation has been designed.

Plants are the important source of drugs; in fact many of the currently available drugs were derived either directly or indirectly from the plants. Since ancient times, plant remedies have been used to relieve diabetes. In the 6th century BC Sushruta, an Indian physician recommended plant remedies for the treatment of DM. Herbal remedies from medicinal plants have been used traditionally. Prior to development of insulin and other hypoglycemic synthetic drugs, DM was managed with medicinal plants. Several such plants show hypoglycemic activity when taken orally. Certain plants of medicinal value are recommended for better management of DM and have shown better results and are used in many parts of the world. In many places throughout the world, DM is kept under control by the use of medicinal plant treatment, although this type of treatment has not been taken seriously by the medical field. Medicinal plants and their products have been widely used for treatments of DM all around the world with less known scientific basis of their functions (Patwardhan et al., 2004; Said et al., 2007). The WHO says that the use of medicinal plants to treat DM is a topic that needs further research (Gray et al., 1999). The WHO approves the use of plant drugs for different diseases including DM (Devaki et al., 2011). There are more than 1200 plants worldwide that are used in the treatment of DM and a number of plants have shown effective hypoglycemic activity after laboratory testing (Eddouks et al., 2005). The search for more effective and safer antidiabetic agents has become an area of active research.

Although herbal medicines have long been used effectively in treating DM in Asian communities and throughout the world, yet the therapeutic efficacy, mechanism of action and safety of most of the herbals used has not been worked out. Only a small number of plants have received scientific and medical evaluation to assess their efficacy. Caesalpinia bonducella is a well known traditional plant used in folklore medicine around the world and especially greater parts of India. The powdered seed kernel of C. bonducella is used by the local people of Assam in the treatment of diabetes (Sharma and Basu, 2009). All parts of this plant has medicinal properties (Kirtikar and Basu, 1988).

Objective

The objective of the present study is to evaluate the antidiabetic effect of ASECB and to find out whether ASECB has any role in the recovery and regeneration of damaged beta cells of islets in alloxan induced diabetic male wistar albino rats or otherwise. In addition, to study its effect on body weight.

Though hypoglycaemic property of C. bonducella is reported, yet its therapeutic efficacy, its effect on pancreatic endocrine cells and its safety has not been worked out. Thus there is a need to investigate on this plant. Therefore the present work was under taken and the main idea behind the present investigation is to show that the C. bonducella seed extract has antidiabetic effect and it effectively helps to regenerate islet and beta cells in the pancreas.

MATERIALS AND METHODS

Experimental animals

Adult male albino rats of Wistar strain developed from Norwegian rat (Ratus norvegicus, Family: Muridae, Order: Rodentia) aged about 80 - 90 days, weighing between 200-240 gms bred and maintained under standard laboratory conditions with proper day-light darkness schedule in animal house of Department of post graduate studies and research in Zoology, Karnatak University, Dharwad, were selected as an experimental model in the present investigation. They were housed in individual polypropylene rat cages under controlled temperature conditions. They were fed with rat pellet feed supplied by M/s Krish Scientist’s Shoppe, agents for scientist’s choice laboratory animal feed, Bangalore, India, and water ad libitum throughout the experimental period. These are quite moderately prolific strain, rather resistant to infectious. The experiments were designed as per guidelines of Institutional Animal Ethics Committee (IAEC), vide Registration No. 639/02/a/CPCSEA.

Induction of diabetes

Alloxan monohydrate (C$_4$H$_2$N$_2$O$_5$H$_2$O) was used as diabetes inducer in rats. The purpose of choosing alloxan monohydrate as the diabetes –inducing agent was that it is known to produce DM irreversibly with a single dose administration by selective necrotic action on the beta cells of pancreas (Anonymous, 1996). Alloxan is well known for its selective pancreatic islet cell toxicity and has been extensively used in inducing DM in animals. DM was induced in normal healthy male albino rats by single intraperitoneal freshly prepared injection of alloxan (150 mg/kg body weight) (Desai and Bhide, 1985) dissolved in normal saline (2 ml/kg BW). The higher dose of alloxan usually caused death before severe diabetes could develop. The animals were allowed to fast for 12 hours prior to alloxan injection. After 3 days of alloxan injection, the glucose level was measured. Rats showing fasting glucose levels > 250 mg/dL were considered as diabetic and selected for the investigation.

Selection of plant

Caesalpinia bonducella (Linn.) Roxb.

The plant belongs to family Caesalpiniiaceae is a thorny shrub found throughout India. The branches are armed with hooks and prickles. The leaves 3cm long and the flowers pale yellow in color. The fruits are inflated pods with prickles having 1 or 2 seeds. The seeds are hard, globular, grey colored, shiny smooth surface with yellowish white kernel.

Preparation of plant extract

C. bonducella plant seeds were collected from the local market in Dharwad. The seeds were shade dried, coarsely powdered using a mixer and sieved to get uniform powder. The powdered material (500gm) was extracted with 95% ethanol in Soxhelt apparatus. The extract was then evaporated under reduced pressure to obtain a greenish black jelly residue. The extracts were then stored in airtight glass containers and refrigerated till further use.

Acute toxicity studies

Acute oral toxicity test for ASECB was carried out. When
administered orally, ASECB was found to be relatively non-toxic (Ali et al., 2008). As such the limit test at 2000 mg/kg was performed. There was no mortality recorded among the rats up to the maximum dose of 500 mg/kg BW.

Experimental design
A total of 20 rats (5 normal, 15 diabetic) were used. The rats (n = 5) were randomly divided into 4 groups. All the experimental rats were placed on normal diet throughout the experiment. Group-I served as control and did not receive any treatment. Alloxan was administered and induced diabetes in Group-II, III and IV.

Group I (Control): Were administered 1ml of distilled water/rat/day orally.

Group II (Diabetic control): Were administered 1ml of distilled water/rat/day orally.

Group III (Diabetic-treated): Were administered 200 mg ASECB/kg BW orally/rat/1ml for 14 days.

Group IV (Diabetic-treated): Were administered 400 mg ASECB/kg BW orally/rat/1ml for 14 days.

The body weight of the rats in all the groups was recorded regularly throughout the duration of the experiment. All animals were observed daily for general health and behavior in the cages.

Blood sample collection and biochemical estimation
Blood glucose level was estimated using ONE TOUCH HORIZON Blood Glucose Monitoring Systems (2004 Life scan Johnson and Johnson Ltd. Inc. Milpitas, CA 95035 USA) using haemoglucostrips. The blood glucose level was estimated before the treatment (0 day), 3rd, 10th and 17th day. For glucose level estimation, blood sample was taken from the tip of the tail of each rat of different groups under mild ether anesthesia. Tail was thoroughly washed with warm water and dried before the sample was taken. At the end of the experiment animals were kept overnight fast but the animals had free access of water and sacrificed after a short exposure to sodium pentobarbital. Blood (1ml) was collected by direct cardiac puncture using a syringe from each rat into sample tubes for blood glucose level estimation, blood sample was taken from the tip of the tail of each rat of different groups under mild ether anesthesia. Tail was thoroughly washed with warm water and dried before the sample was taken. At the end of the experiment animals were kept overnight fast but the animals had free access of water and sacrificed after a short exposure to sodium pentobarbital. Blood (1ml) was collected by direct cardiac puncture using a syringe from each rat into sample tubes for analysis. Pancreas were surgically removed immediately, blotted to remove blood traces and stored in 10% formalin for histopathological studies.

Histopathological studies
The fixed pancreatic tissue were dehydrated in ascending grades of ethanol, cleared in xylene and embedded in paraffin wax. Sections of 5 microns thickness were prepared and first stained with basic dyes Haematoxilin and Eosin (H and E) according to Conn procedure (Conn, 1946) and later sections were specifically stained with Gomeri aldehyde fuchsin (GAF) for beta cells as described by Gomeri (Gomeri, 1950). The histological sections were examined for intracellular changes and results were recorded on microphotographs.

Statistical evaluation
All values were expressed as mean ± S.E. Statistical evaluation was done using one-way analysis of variance (ANOVA) (Winer, 1971). P values of < 0.05 were taken as significant.

RESULTS
Body weight
The results of the present study are summarized in Table 1-3. The body weight of all the animals of different groups at the commencement of the experiment (0 day) is almost same. In the controls the body weight gradually increased. During the experimental period the body weight of the experimental animals were weighed on 0, 3rd, 10th and 17th day. Table 1 shows the mean body weight of the experimental animals of different groups recorded on different days. In Group-II, animals which are diabetes induced, the mean body weight at the day of commencement (0 day) was 207.0±6.24, which gradually reduced in the course of the experiment. The mean body weight on the 17th day was 176.20±8.67 gms. There was a significant decrease (P<0.05) in body weight at the end of the experiment compared to controls. The experimental animals of Group-III and IV which are also diabetic and treated with 200 mg and 400 mg of ASECB, respectively, initially (day 3) showed decrease in body weight after the induction of diabetes, but subsequently animals gained weight and there was significant increase in their final body weight when compared with diabetic group. However, the body weight gained in Group-III and IV was not significant when compared to controls.

Glucose level estimation
Table 2 shows the blood glucose level of the experimental animals of different groups. In controls, the blood glucose levels are almost similar throughout the experimental period. Further, there was no much difference in the glucose level of the experimental animals of different groups on the day of commencement (0 day) of the experiment. In Group-II due to inducing of diabetes by alloxan on the commencement of experiment (0 day), as expected there was abrupt rise in the glucose level on 3rd day of the experiment and it remained elevated throughout till the last day of the experiment. High elevation of glucose level is because of alloxan injection, which causes destruction of beta cells, which in turn brings an increase in blood glucose level. The mean blood glucose level recorded on the 3rd day (after induction) was 255.0±3.22, 220.8±7.70, 223.8±6.12, 212.0±4.66, 208.0±5.57, 195.6±6.70, 213.6±6.70, 186.80±6.27, 223.8±6.12, 219.8±5.30, 212.0±4.66, 208.0±5.57, 195.6±6.70, 213.6±6.70, 186.80±6.27.

Table 1: Effect of treatment with alcoholic seed extract of Caesalpinia bonducella (ASECB) on body weight (gm) of alloxan induced diabetic albino rats

<table>
<thead>
<tr>
<th>Groups</th>
<th>Day 0</th>
<th>Day 3</th>
<th>Day 10</th>
<th>Day 17</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group-I (control)</td>
<td>203.4±5.78</td>
<td>203.8±5.57</td>
<td>212.0±4.66</td>
<td>219.8±5.30</td>
</tr>
<tr>
<td>Group-II (Diabetic)</td>
<td>207.0±6.24</td>
<td>195.6±6.70</td>
<td>186.80±6.27</td>
<td>176.20±8.67</td>
</tr>
<tr>
<td>Group-III (Diabetic + 200mg ASECB)</td>
<td>218.0±7.51</td>
<td>213.6±6.70</td>
<td>223.8±6.12</td>
<td>233.8±5.30</td>
</tr>
<tr>
<td>Group-IV (Diabetic + 400mg ASECB)</td>
<td>208.0±8.60</td>
<td>206.8±7.25</td>
<td>220.8±7.70</td>
<td>239.8±7.85</td>
</tr>
</tbody>
</table>

NOTE: Groups with similar superscript letters (a, b, c) in the given column and similar superscript letters (x, y, z) in the given rows indicates not significant. While groups with dissimilar letters indicates significant difference (p<0.05) from each other.
Histopathology of pancreas

In normal control rats (Group-I), the pancreatic parenchyma is normal. Islets were scattered and quite widely distributed in the exocrine portion (acini) of the pancreatic tissue. The islets are of varying size and mostly spherical or oval in shape and were embedded in the acini. (Fig. 1 and 2) Islets contained uniformly distributed alpha and beta cells. Alpha cells are quite smaller distributed at periphery of the islets. Beta cells are spherical comparatively larger and distributed uniformly in the centre and all over the islets. The cytoplasm of the beta cells was granular with centrally located round nucleus. In diabetic animals of Group-II, the islets are damaged and scattered irregularly with no definite shape and atrophic. (Fig. 3 and 4) The cells of the islets were overlapped and crowded; as a result large empty spaces are left out in the islet. Some of the cells have lost nuclei. Septal spaces between the lobules are not uniform and were widened. (Fig. 5) Islets are shrunken and some were ruptured. (Fig. 4 and 5) There was hyperplasia of islets cells and congestion in pancreatic parenchyma. Many of the beta cells destroyed due to alloxan injection. Microscopic observation reveals that the other cells of islet namely alpha cells and delta cells are not affected. Pancreatic tissue of Group-III and IV which are treated with ASECB revealed that the islets appear to have return to original shape and size. Septal spaces between the lobules were reduced to some extent. Beta cells were restored to their normal size and shape. The restorations of beta cells in treated groups suggests a recovery and normal function of the islet cells, which in turn might have decreased the blood glucose level.

Table 3: Effect of treatment of alcoholic seed extract of Caesalpinia bonducella (ASECB) on Beta cell number per Islet and Islet size (μm) in alloxan induced diabetic albino rats

<table>
<thead>
<tr>
<th>Groups</th>
<th>Day 0</th>
<th>Day 3</th>
<th>Day10</th>
<th>Day 17</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group-I (control)</td>
<td>86.23±5.64</td>
<td>85.80±4.91</td>
<td>86.00±3.35</td>
<td>86.00±3.35</td>
</tr>
<tr>
<td>Group-II (Diabetic)</td>
<td>87.00±7.23</td>
<td>253.00±3.22</td>
<td>279.00±3.49</td>
<td>302.40±3.88</td>
</tr>
<tr>
<td>Group-III (Diabetic + 200 mg ASECB)</td>
<td>85.80±3.81</td>
<td>252.20±4.15</td>
<td>177.20±8.38</td>
<td>102.80±7.37</td>
</tr>
<tr>
<td>Group-IV (Diabetic + 400 mg ASECB)</td>
<td>89.80±4.42</td>
<td>250.40±9.47</td>
<td>173.80±8.75</td>
<td>89.60±1.72</td>
</tr>
</tbody>
</table>

Table 2: Effect of treatment of alcoholic seed extract of Caesalpinia bonducella (ASECB) on glucose level (mg/dL) of alloxan induced diabetic albino rats

<table>
<thead>
<tr>
<th>Groups</th>
<th>Day 0</th>
<th>Day 3</th>
<th>Day10</th>
<th>Day 17</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group-I (control)</td>
<td>88.60±3.35</td>
<td>88.60±3.35</td>
<td>88.60±3.35</td>
<td>88.60±3.35</td>
</tr>
<tr>
<td>Group-II (Diabetic)</td>
<td>24.72±1.43</td>
<td>263.72±3.38</td>
<td>465.32±6.62</td>
<td>631.16±6.98</td>
</tr>
<tr>
<td>Group-II (Diabetic + 200 mg ASECB)</td>
<td>85.80±3.81</td>
<td>252.20±4.15</td>
<td>177.20±8.38</td>
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Note: Group with similar superscript letter (a, b, c) in the given column and similar superscript letter (x, y, z) in the given rows indicates not significant. While groups with dissimilar letter indicates significant difference (p<0.05) from each other.

which rose to 279.0±3.49 & 302.40±3.88 on 10th and 17th day of the experiment respectively. Diabetic rats of Group III and IV which are fed with 200 mg and 400 mg ASECB, respectively, and from 4th day showed a decrease in blood glucose level. The mean blood glucose level of Group III recorded on day 0, 3rd, 10th and 17th day was 85.80±3.81, 252.20±4.15, 177.20±8.38 and 102.80±7.37 respectively. Similarly the mean blood glucose level of Group-IV estimated on day 0, 3, 10 and day 17 was 89.80±4.42, 250.40±9.47, 173.80±8.75 and 89.60±1.72 respectively. The blood glucose level of Group-IV which was high when checked on day 3, was significantly reduced (P<0.05) and almost restored normalcy on 17th day. When comparison was made between diabetic (Group-II) and treated (Group-IV), mean blood glucose levels were found to decline sharply from 255.0±3.22 to 89.60±1.72 mg/dL on day 17 after oral treatment of ASECB. The reduction value of glucose level in Group-IV was close to 89.60±1.72 mg/dL on day 17 after oral treatment of ASECB. Diabetic rats of Group III and IV which are fed with 200 mg and 400 mg ASECB, respectively, and from 4th day showed a decrease in blood glucose level. The mean blood glucose level of Group III recorded on day 0, 3rd, 10th and 17th day was 85.80±3.81, 252.20±4.15, 177.20±8.38 and 102.80±7.37 respectively. Similarly the mean blood glucose level of Group-IV estimated on day 0, 3, 10 and day 17 was 89.80±4.42, 250.40±9.47, 173.80±8.75 and 89.60±1.72 respectively. The blood glucose level of Group-IV which was high when checked on day 3, was significantly reduced (P<0.05) and almost restored normalcy on 17th day. When comparison was made between diabetic (Group-II) and treated (Group-IV), mean blood glucose levels were found to decline sharply from 255.0±3.22 to 89.60±1.72 mg/dL on day 17 after oral treatment of ASECB. The reduction value of glucose level in Group-IV was close to 89.60±1.72 mg/dL on day 17 after oral treatment of ASECB.

Betal cell count and islet diameter

Table 3 shows the mean values and ANOVA results of beta cell count and islet size. The mean beta cell number per islet in Group I, II, III and IV was 62.12±1.84, 24.72±1.43, 48.28±4.20 and 54.46±0.84 respectively. A microscopic observation of pancreas section of Group II reveals that the number of beta cell in an islet has significantly reduced when compared to controls. The number of beta cell in Group III and IV which were treated with ASECB from day 4 onwards, revealed an increase in beta cell count. The increase of beta cell in Group-III and IV compared to Group-I and II was statistically significant (P<0.05). The mean number of beta cell in Group-IV has reached to that of Group-I (control). The mean size of the islets treated animals Group-II and IV was significantly higher than diabetic control group (Group-I) (P<0.05). The significant increase of islet size in treated animals is due to ASECB.
drugs have side effects, costly and other draw backs. Therefore, investigation is on for the discovery of new drugs which have no side effects and cheap. Several medicinal plants have been suggested as a rich source of antidiabetic property. *C. bonducella* is one among them, which has been used traditionally by the tribal people of Andaman and Nicobar islands for the relief of DM.

Blood sugar lowering activity of *C. bonducella* has been primarily evaluated with significant results in rabbit (Rao et al., 1994). Sharma et al. (1997) have also reported earlier the

**Abbreviations:** IL- Islet of Langerhans, AC- Acini, IS- Interlobular septa, SIL- Shrunken islet of Langerhans, ID- Interlobular duct, H&E = Haematoxilin and Eosin, GAF = Gomori Aldehyde Fuchsin
hypoglycemic action of the C. bonduc cella in alloxan induced diabetic rats. The increase in blood glucose level and decrease in islet size and beta cell population in Group-II (Diabetic) when compared to normal control is due to alloxan injection. Islet necrosis is a direct effect of alloxan, which in turn decreases the insulin level. The reduction in beta cell count can be as low as 50% during diabetics. Treatment with ASECB to the diabetic animals (Group-III and IV) results in the weight gain, restoration of glucose level, beta cell number and islet size to almost normalcy. Observations suggest that the ASECB has glucose lowering activity.

At this investigative stage, it is not possible to explain the exact mechanism of action of ASECB on the endocrine pancreas, but however some of the following probable mechanism of action (Hypothesis) can be proposed.

Phytochemical analysis of C. bonduc cella has revealed the presence of alkaloid, flavonoids, glycosides, saponins, tannins and triterpenoids having antioxidant properties (Gaur et al., 2008). These biologically active constituents might have helped to gain in body weight and also restoration of blood glucose level. Table-1 reveals that the mean body weight of experimental animals of Group-II (Diabetic-untreated) was significantly reduced. Weight loss is one of the clinical symptoms of DM. This weight loss may be due to the degeneration of the adipocytes and muscle tissues to make up for the energy lost from the body due to frequent urination and over conversion of glycogen to glucose (Ene et al., 2007).

This loss of body weight in diabetes is due to increased lipolysis and increased muscle wasting and loss of tissue proteins caused by insulin deficiency (Tripathi, 2004). The seed extract of C. bonduc cella might have prevented this lipolysis and proteolysis by ameliorating the extent of insulin deficiency and thereby, caused an increase in body weight (Sharma and Das, 2009). DM causes failure to use of glucose for energy that leads to increased utilization and decreased storage of protein responsible for reduction of body weight essentially by depletion of the body proteins (Guyton and Hall, 2000).

There are reports that some plants contains mucilages and minerals like calcium, zinc, magnesium, manganese and copper had remarkable hypoglycemic activity decreasing blood glucose level in diabetic rats within 15 days (Akbar and Iqbal, 1991; Ibrahim et al., 1997).

Burcalain et al., (1995) reported that the hypoglycemic action of the extract of herbal plants in diabetic rats may be possible through the insulinomimetic action or by other mechanism such as stimulation of glucose uptake by peripheral tissue, inhibition of endogenous glucose production or activation of gluconeogenesis in liver and muscles.

In diabetes the excess glucose present in the blood reacts with hemoglobin to form glycosylated hemoglobin. The rate of glycosylation is directly proportional to concentration of blood glucose and with improvement of glycemic control glycosylated hemoglobin also decreases (Monnier and Cerami, 1982). Hence, the estimation of glycosylation of hemoglobin is a well established parameter useful in the management and prognosis of the disease (Chang and Nobel, 1979). It might be suggested that the hypoglycemic action of the ASECB appeared as a result of direct metabolic effect on tissue and/or increase in insulin secretion (Shanmugasundaram et al., 1990, Farva et al., 1986). The antidiabetic activity of Caesalpinia bonduc (L.) Roxb might be attributed to the presence of flavonoids, known to be natural antioxidants, which protect the existing beta cells from dying by their free radical scavenging action (Keneto et al., 1999). Kathanna et al. (2008) reported the presence of many phyto constituents including saponines in the leaf extract of Caesalpinia bonduc (L.) Roxb. Thus the saponines present in the Caesalpinia bonduc (L.) Roxb. might contribute to its antihyperglycaemiac action. It is suggested that another possible mechanism may be that the alkaloids may cause inhibition of mitochondrial function that increases the AMP/ATP ratio, which could explain the activation pathway in the treatment of diabetes (Sharma et al., 2010).

All of the above said actions may be responsible for the abolition of diabetic complications.

It is concluded from the present study, that ASECB has anti-diabetic effect. Further studies are in progress to isolate and identify the active hypoglycemic phyto constituents of the extract and to elucidate their actual mechanism of action by estimating the insulin titers.

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