Effect of aqueous pericarp extract of *Litchi chinensis* on hypoglycemic and antihyperglycemic activities in normal and in streptozotocin induced diabetic rats.

Eswar Kumar Kilari*, Rohini Koratana, Swathi Putta

Pharmacology Division, A.U. College of Pharmaceutical Sciences, Andhra University, Visakhapatnam, India

*For correspondence

Dr. K. Eswar Kumar
Assistant Professor-II, A.U. College of Pharmaceutical Sciences, Andhra University, Visakhapatnam, India.
Tel: +91-9440632728
E-mail: ekilari@gmail.com

Received: 12 Jan 2015
Accepted: 1 Mar 2015

**ABSTRACT**

**Aim:** To evaluate the hypoglycemic and antihyperglycemic activities of *Litchi chinensis* in normal and streptozotocin induced diabetic rats.

**Methods:** Diabetes was induced to Wistar albino rats by using streptozotocin - Nicotinamide. The rats were divided into 5 groups each having 6 rats including control group receiving water, the diabetic rats treated with gliclazide (1 mg/kg) and aqueous pericarp extract of *Litchi chinensis* (100, 200 mg/kg). They were fed orally for 12 weeks once daily. Blood was collected by retro orbital plexus method during the study period. At the end of the study period all serum parameters were evaluated.

**Results:** At the end of 12 weeks study period the serum parameters like glucose, triglycerides, total cholesterol, LDL, VLDL, bilirubin, creatinine, total protein, albumin, uric acid and urea were found to be increased in diabetic rats and treatment with APLC there was a significant reduction in all the parameters. The HDL & total protein were found to be decreased in diabetic rats and there was a significant increase in APLC treatment. The APLC was found to have significant antihyperglycemic, antihyperlipidemia activity and offered significant protection against the damage to kidney, which might be due to the antioxidant potential present in the APLC.

**Keywords:** Diabetes mellitus, antihyperglycemic activity, *Litchi chinensis*.

**Introduction**

Diabetes mellitus is the most common endocrine disorder of carbohydrate metabolism is affecting approximately 8.3% of the population worldwide[1]. In 1675 Thomas Willis added the word ‘mellitus’ to the disease, a word from Latin meaning ‘Honey’ a reference to the sweet taste of the urine[2]. Diabetes mellitus is not a single disease entity, but rather a group of metabolic disorders sharing a common underlying feature of hyperglycemia. Hyperglycemia in diabetes, results from defects in insulin secretion, insulin action or most commonly both[3]. Chronic elevation of blood glucose level leads to damage of blood vessels (angiopathy). The endothelial cells lining the blood vessels take in more glucose than normal, since they don’t depend on insulin. They then form more surface glycoproteins than normal, and cause the basement membrane to grow thicker and weaker. In diabetes, the resulting problems are grouped under “microvascular disease” (due to damage to small blood vessels) like retinopathy, nephropathy & neuropathy and “macrovascular disease” (due to damage to the arteries) like cardiomyopathy.

Different groups of oral hypoglycemic agents are currently available with characteristic profiles of side effects[4-7]. The search for antidiabetic agents with little or no side effects is continuous processes. The plant kingdom is a wide field to look for effective oral hypoglycemic agents. More than 300 species have been reported to display hypoglycemic activity[8] but only few of them have been investigated despite the World Health Organization (WHO) recommendation that traditional plant remedy for diabetics warrant further evaluation[9].

Effective control of blood glucose level is a key step in preventing or reverses diabetic complications and improving the quality of life in both type I and type II diabetic patients[10]. In the history of Unani, Ayurveda, Siddha or Homeopathic has been well documented that illness can be managed purely by herbal preparations, thus the diabetic individual could lead a healthy life as non-diabetics. Experiments and clinical trials conducted worldwide have provided dependable evidences on the effects of various herbal formulations in the maintenance of normal blood sugar level[11]. Previous studies conducted on some fruit pericarps indicating that, pericarp are rich...
source of antioxidant capability[12-14].

The present study was attempted to evaluate the fruit pericarp extract of *Litchi chinensis* consists of anthocyanins like Cyanidin-3-glucoside, Cyanidin-3-diglucoside, cyanidin-3-rutinoside[15], malvidin-3-glucoside and quercetin-3-rutinoside[15], pelargonidin 3, 7-diglucoside, rutin and epicatechin[16].

**Materials and Methods**

**Chemicals**

Streptozotocin was purchased from SIGMA Aldrich, St. LOUIS, MO, USA. All other chemicals used for this study were analytical grade.

**Plant Materials**

The ripened litchees (*Litchi chinensis*) were obtained from local market of Visakhapatnam. The peels were manually separated and shade dried. The pericarps were powdered in a grinder to get 40-mesh size powder. The moisture content of pericarp powder was found to be 13.5%. The powder was suspended in 2% gum acacia and used in the experimental studies.

**Animals**

Animals were obtained from the Tina laboratories, Hyderabad. Albino Wistar rats (180-200 g) of male were used in the present study. The animals were housed under standard environmental conditions (23±1°C) with relative humidity of 50±10% and maintain 12:12 dark and light cycle, maintained with free access to water ad libitum standard laboratory diet (70% carbohydrates, 25% proteins, 5% lipids (Hindustan liver Bangalore). After randomization before the experiment, the rats were acclimatized for a period of two weeks. The animal housing and handling were in accordance with CPSCEA guidelines. Our Institute was approved by CPCSEA for conducting animal experiments with the registration No. 516/01/A/CPCSEA. The prior permission for the study was obtained from our Institutional Animal Ethics Committee (IAEC).

**Induction of Diabetes**

The rats were fasted for 18 h prior to the experiment with water ad libitum. The rats were injected intraperitoneally with nicotinamide 100 mg/kg. After 15 minutes Streptozotocin (STZ) was administered by dissolving in citrate buffer at a dose of 55 mg/kg body weight. Animals were treated with 10% glucose to combat the early phase of hypoglycemia. Blood samples were collected after 72 hours of STZ treatment and the induction of diabetes mellitus was confirmed by estimation of fasting blood glucose levels (FBG). Only those rats with blood glucose levels ≥250 mg/dl were included in the study (Day 0).

**Experimental Procedures**

Control group was administered with distilled water, aqueous pericarp extract of *Litchi chinensis* (*APLC*) at 100 mg/kg and 200 mg/kg of rat body weight to group-I, and group-II respectively. Blood samples were withdrawn at 0, 1, 2, 3, 4, 6, 8, 10 & 12 h intervals by retro-orbital puncture method and were analyzed for blood glucose by GOD/POD method using SCREEN MASTER 3000 auto-analysur.

After the induction of diabetes, the rats were grouped into five different groups of each containing six animals. Group I contains control rats received distilled water and fed on normal diet, group II served as diabetic control received vehicle only, group III contains diabetic rats treated with gliclazide at a dose of 1 mg/kg body weight orally, group IV contains diabetic rats received *APLC* at a dose of 100 mg/kg body weight, group V contains diabetic rats received *APLC* at a dose of 200 mg/kg body weight for 12 weeks. Treatment with drugs was started after 72 hours of STZ treatment (i.e. Day 1) and was continued for 12 weeks. All drugs were given orally as a single oral dose. Blood glucose was measured before starting the treatment (day 0) and at 4 week interval thereafter up to the end of the treatment period of 12 weeks and estimated fasting blood glucose by glucose-oxidase-peroxidase (GOD-POD) method[17]. All the treatment groups were compared with diabetic control group.

**Biochemical assays**

At the end of the 12 week study period, rats were fasted overnight and blood samples were withdrawn through the retroorbital plexus using glass capillary. Blood was allowed to clot and serum was separated by centrifugation at 4000 rpm for 10 min. Serum glucose levels were estimated at 0, 1, 2, 3, 4, 6 and 8 h intervals. Serum glycosylated haemoglobin, triglycerides, total cholesterol, HDL, LDL, VLDL, bilirubin, creatinine, albumin, total protein, ura, uric acid and BUN levels were estimated. Serum glucose levels were estimated by GOD/POD method[17]. Triglyceride, total cholesterol, HDL[18,19], Bilirubin[20], creatinine[21], total protein[22], albumin[23], uric acid[24], uric acid[25] was estimated by commercially available kits At the end of the study all the rats were sacrificed and pancreas was studied for histopathological changes.

**Statistical Analysis**

All the data were expressed as mean±SEM. Statistical analysis was carried out using one way ANOVA followed by Dunnet’s multiple comparison test and two way ANOVA followed by Bonferron’s post test.

**Results**

**Hypoglycemic activity**

The treatment with gliclazide at dose of 1 mg/kg body weight was shown to produce a significant reduction in blood glucose levels in normal rats. The percent reduction was found to be 33% (1 h) and 26% (8 h) respectively. The treatment with *APLC* at dose levels of 100 mg/kg and 200 mg/kg body weight shown to produce a dose dependent reduction in blood glucose levels in normal rats and the percent reduction was found to be 35% (2 h) & 27% (6 h); 42% (3 h) and 27% (6 h) respectively. The results were presented in table 1.
Table 1: Effect of APLC on hypoglycemic activity in normal rats during 12 hours.

<table>
<thead>
<tr>
<th>Time (Hrs)</th>
<th>Blood glucose (mg/dL)</th>
<th>(percent blood glucose reduction)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Control</td>
<td>Gliclazide</td>
</tr>
<tr>
<td>0</td>
<td>150.0±2.9 (0.00±0.00)</td>
<td>111.9±6.82 (0.00±0.00)</td>
</tr>
<tr>
<td>1</td>
<td>104.8±2.90 (0.15±0.38)</td>
<td>75.5±7.21 (33.04±2.80)</td>
</tr>
<tr>
<td>2</td>
<td>103.6±2.74 (1.24±0.30)</td>
<td>82.7±3.00 (24.9±4.40)</td>
</tr>
<tr>
<td>3</td>
<td>103.3±2.51 (1.53±0.57)</td>
<td>92.3±4.12 (15.9±6.00)</td>
</tr>
<tr>
<td>4</td>
<td>103.3±2.84 (1.57±0.18)</td>
<td>95.1±2.28 (13.6±4.84)</td>
</tr>
<tr>
<td>6</td>
<td>103.0±3.04 (1.91±0.52)</td>
<td>95.7±4.96 (14.7±5.15)</td>
</tr>
<tr>
<td>8</td>
<td>101.6±2.97 (3.18±0.23)</td>
<td>80.9±3.81 (26.6±4.84)</td>
</tr>
<tr>
<td>10</td>
<td>101.8±2.76 (2.99±0.26)</td>
<td>96.85±3.02 (12.4±3.72)</td>
</tr>
<tr>
<td>12</td>
<td>100.6±2.97 (3.97±0.16)</td>
<td>96.9±3.57 (12.3±3.70)</td>
</tr>
</tbody>
</table>

Table 2: Effect of APLC on antihyperglycemic activity in STZ induced diabetic rats.

<table>
<thead>
<tr>
<th>Group/Time(hrs)</th>
<th>0hr</th>
<th>1hr</th>
<th>2hr</th>
<th>4hr</th>
<th>6hr</th>
<th>8hr</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>102.50±3.05&lt;sup&gt;ns&lt;/sup&gt;</td>
<td>101.50±2.98&lt;sup&gt;ns&lt;/sup&gt;</td>
<td>100.33±2.98&lt;sup&gt;ns&lt;/sup&gt;</td>
<td>99.50±3.19&lt;sup&gt;ns&lt;/sup&gt;</td>
<td>98.50±3.08&lt;sup&gt;ns&lt;/sup&gt;</td>
<td>97.16±2.94&lt;sup&gt;ns&lt;/sup&gt;</td>
</tr>
<tr>
<td>D-Control</td>
<td>453.66±5.71&lt;sup&gt;ns&lt;/sup&gt;</td>
<td>452.33±5.58&lt;sup&gt;ns&lt;/sup&gt;</td>
<td>452.00±5.79&lt;sup&gt;ns&lt;/sup&gt;</td>
<td>450.50±5.76&lt;sup&gt;ns&lt;/sup&gt;</td>
<td>450.00±5.72&lt;sup&gt;ns&lt;/sup&gt;</td>
<td>448.00±5.61&lt;sup&gt;ns&lt;/sup&gt;</td>
</tr>
<tr>
<td>Gliclazide</td>
<td>107±4.5&lt;sup&gt;ns&lt;/sup&gt;</td>
<td>104.3±3.30&lt;sup&gt;ns&lt;/sup&gt;</td>
<td>92.5±4.04&lt;sup&gt;ns&lt;/sup&gt;</td>
<td>72.8±3.7&lt;sup&gt;ns&lt;/sup&gt;</td>
<td>80.3±4.00&lt;sup&gt;ns&lt;/sup&gt;</td>
<td>85.0±3.9&lt;sup&gt;ns&lt;/sup&gt;</td>
</tr>
<tr>
<td>APLC (100mg/kg)</td>
<td>119.50±3.22&lt;sup&gt;ns&lt;/sup&gt;</td>
<td>114.16±3.34&lt;sup&gt;ns&lt;/sup&gt;</td>
<td>106.66±2.99&lt;sup&gt;s&lt;/sup&gt;</td>
<td>100.16±3.09&lt;sup&gt;s&lt;/sup&gt;</td>
<td>82.83±2.08&lt;sup&gt;s&lt;/sup&gt;</td>
<td>95.33±2.33&lt;sup&gt;s&lt;/sup&gt;</td>
</tr>
<tr>
<td>APLC (200mg/kg)</td>
<td>106.00±3.04&lt;sup&gt;ns&lt;/sup&gt;</td>
<td>102.83±3.02&lt;sup&gt;ns&lt;/sup&gt;</td>
<td>95.66±2.92&lt;sup&gt;s&lt;/sup&gt;</td>
<td>87.00±1.86&lt;sup&gt;s&lt;/sup&gt;</td>
<td>67.33±2.87&lt;sup&gt;s&lt;/sup&gt;</td>
<td>83.33±2.15&lt;sup&gt;s&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

p>0.05<sup>ns</sup>, p<0.05*, p<0.01**, p<0.001*** Significance followed by one way ANOVA followed by Dunnet’s multiple comparison test when compared with disease control group.
**Antihyperglycemic activity**

The treatment with gliclazide (1 mg/kg) shown to produce a significant reduction in blood glucose levels in STZ induced diabetic rats. The percent reduction was found to be 32% (4 h). The treatment with APLC (100, 200 mg/kg) to be found were 31%, 36% during 6hr respectively. The results were presented in Table 2.

**Lipid profile**

The serum triglycerides, total cholesterol, LDL and VLDL were found increased in STZ induced diabetic rats. The increased levels were brought back to normal with the treatment of APLC and gliclazide at p<0.05. The serum HDL levels were found to be decreased in STZ induced diabetic rats. The decreased levels were brought back to normal with the treatment with APLC and gliclazide. The results were presented in Graph 1.

**Serum parameters**

The serum HbA1c, total bilirubin, direct bilirubin, creatinine, albumin, uric acid and BUN levels were found to be increased in STZ induced diabetic rats. The increased levels were brought back to normal with APLC and gliclazide treatment. The serum total protein levels were found to be decreased in STZ induced diabetic rats. The decreased levels were brought back to normal with APLC and gliclazide treatment. The results were presented in Table 3.

**Histopathological studies**

The light microscopic examination of endocrine part of pancreas of disease control group revealed the altered structure of both the exocrine and endocrine portions with significant decrease in the number of secretory cells compared to control group. The treatment with the APLC extract and Gliclazide for 12 weeks found to prevent the degenerative changes in STZ induced diabetic rats (figure 1).

**Discussion**

The WHO Expert Committee recommended the importance to investigate and explore hypoglycemic agents from plant origin because plants used in the traditional medicine have fewer side effects than synthetic drugs[26]. The present study discuss about the hypoglycemic and antihyperglycemic effects of APLC. The doses of selected fruit pericarp extract of *Litchi chinensis* were fixed basing on their acute toxicity study in mice and preliminary hypoglycemic studies in Wistar albino rats. The doses that produced optimal and dose dependent reduction in blood glucose levels were selected for hypoglycemic and antihyperglycemic studies.

Some medicinal plants with hypoglycemic properties are known to increase circulating insulin level (pancreatic mechanism) in normoglycemic rats[27]. Another possible mechanism of action is that the extracts might stimulate residual pancreatic mechanism (extra pancreatic), probably increasing peripheral utilization of glucose as postulated by Erah *et al.*, 1996[28]. The APLC was shown significant hypoglycaemic activity with biphasic effect. The biphasic effect might be due to its biphasic absorption or enterohepatic recirculation. We hypothesized that APLC could have a sulfonylurea-like mechanism since they significantly decreased the blood glucose levels in normoglycemic rats. Sulfonylurea compounds lower blood glucose in normal and in diabetic animals by stimulating insulin release from pancreatic β cells and by peripheral utilization of glucose. The APLC was shown to have better hypoglycemic activity compared to standard Gliclazide in normal rats. Streptozotocin (STZ) is used to induce diabetes mellitus in albino Wistar rats along with a poly ADP ribose inhibitor, nicotinamide was administered before 15 minutes of STZ administration to offer partial protection against the action of STZ in rats. The treatment with the APLC significantly reduced the elevated plasma glucose levels in STZ induced diabetic rats. The APLC was shown delayed absorption in diabetic animals compared to normoglycemic animals, might be due to delayed gastric absorption and motility in diabetic condition as diabetes affecting the digestive processes, the motility and nervous control of the entire system of gastrointestinal tract. The effect of diabetes on digestive system can also cause malabsorption[29,30]. The APLC was shown to have better antihyperglycemic activity compared to standard glacial in STZ induced diabetic rats.

Streptozotocin (STZ) induced diabetic rats enhanced the level of glycated hemoglobin (HbA1c) due to raise in levels of glucose in blood which further react with hemoglobin to produce the glycated hemoglobin[31]. The treatment with the APLC significantly lowered the blood glucose levels, which lead to the decrease in the levels of glycated hemoglobin.

The levels of serum lipids are usually elevated in diabetes mellitus[32]. This abnormal high level of serum lipids is mainly due to the uninhibited actions of lipolytic hormones on the fat depots. It is reported that hypercholesterolemia (increased levels of total cholesterol) and hypertriglyceridermia (increased levels of triglycerides) occurs in STZ-induced diabetic rats[32,33]. Under normal circumstances, insulin activates the enzyme lipoprotein lipase, which hydrolyses triglycerides 34. However, in diabetic state lipoprotein lipase is not activated due to insulin deficiency resulting in hypertriglyceridermia. The treatment with APLC significantly reduced the levels of total cholesterol, triglycerides, LDL and VLDL and increased the levels of HDL. The APLC was shown to have better antihyperlipidemic activity compared to standard gliclazide in STZ induced diabetic rats.

The serum bilirubin levels were found to be increased in STZ induced diabetic rats. Rana *et al.*, 1996[35] reported that the increase in serum bilirubin (hyper-bilirubenimia) in STZ induced diabetic rats, may be resulted...
from the decrease of liver uptake, conjugation or increase in total bilirubin, direct bilirubin production from hemolysis. The elevation in serum bilirubin indicates liver damage. The APLC shown to have better hepatoprotective activity than standard gliclazide. The estimation of total protein is useful for measuring gross changes in protein levels caused by various disease states. In diabetic condition the circulating protein binds with free reducing sugars leads to formation amadori products. The APLC was able to increase the protein levels may be by breaking the link between the reducing sugars and amino acids of proteins. Urea is the major nitrogen containing metabolic product of protein metabolism, uric acid is the major product of purine nucleotides, adenosine and guanosine; creatinine is endogenously produced and released into body fluids and its clearance measured as an indicator of glomerular filtration rate[36]. The metabolism of protein is found to be increased in the diabetic rats as indicated by increase in the levels of serum urea, uric acid and decreased levels of proteins as explained above. The APLC shown to decrease the levels of urea and uric acid probably by decreasing the metabolism of proteins.

The treatment with the APLC found to be useful in reducing the damage to the kidney caused due to hyperglycaemia induced by STZ. The APLC was shown to have better and comparable antihyperglycaemic activity with standard gliclazide. Serum creatinine and serum BUN levels measurement is taken as an index of altered GFR in diabetic nephropathy[37]. Our results showed that the level of serum creatinine and BUN levels were significantly elevated in diabetic animals. The treatment with APLC for 12 weeks shown significant reduction in the creatinine and BUN levels.

The light microscopic examination of pancreatic section of control group revealed that the normal structure of the exocrine and endocrine parts of the pancreas. Previous studies reported similar findings and added that the pancreas had a rich capillary network essential for the secretary process[38]. The light microscopic examination of endocrine part of pancreas of disease control group revealed the altered structure of both the exocrine and endocrine portions with significant decrease in the number of secretary cells. The treatment with the APLC extract and Gliclazide for 12 weeks found to prevent the degenerative changes in the pancreas of STZ induced diabetic rats.

It is concluded that, The Litchi chinensis showed better hypoglycemic and antihyperglycemic activity against STZ induced diabetic rats. All the activities might be due to high levels of anthocyanins and polyphenols present in aqueous pericarp extract of Litchi chinensis.

Conflict of intrest statement
Authors have no conflict of interest.

Acknowledgment
The author acknowledges the financial support for completion of research work from Council for Scientific Industrial research, New Delhi (REF: 111177/2K11/1).

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Table 3: Effect of different concentrations of APLC on kidney parameters on normal and Streptozotocin induced diabetic and normal rats.

<table>
<thead>
<tr>
<th>Groups</th>
<th>HbA1c</th>
<th>T.bilirubin</th>
<th>D.bilirubin</th>
<th>Creatinine</th>
<th>Total protein</th>
<th>Albumin</th>
<th>Uric acid</th>
<th>BUN</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>4.37±0.64*</td>
<td>0.75±0.018*</td>
<td>0.69±0.019*</td>
<td>0.69±0.02*</td>
<td>6.15±0.17*</td>
<td>3.72±0.10*</td>
<td>3.63±0.17*</td>
<td>21.95±0.45*</td>
</tr>
<tr>
<td>D.Control</td>
<td>11.68±0.41</td>
<td>1.75±0.055</td>
<td>1.22±0.013</td>
<td>1.90±0.03</td>
<td>3.95±0.23</td>
<td>7.20±0.10</td>
<td>8.14±0.21</td>
<td>36.20±0.20</td>
</tr>
<tr>
<td>Standard</td>
<td>3.88±0.34*</td>
<td>0.72±0.025*</td>
<td>0.690±0.021*</td>
<td>0.78±0.02*</td>
<td>5.87±0.67*</td>
<td>4.10±0.06*</td>
<td>4.13±0.11*</td>
<td>22.44±0.33*</td>
</tr>
<tr>
<td>APLC (100mg/kg)</td>
<td>5.77±0.42*</td>
<td>0.87±0.023*</td>
<td>0.81±0.022</td>
<td>1.01±0.04*</td>
<td>7.96±0.15*</td>
<td>4.68±0.08*</td>
<td>5.14±0.20*</td>
<td>32.58±0.29*</td>
</tr>
<tr>
<td>APLC (200mg/kg)</td>
<td>4.37±0.40*</td>
<td>0.81±0.019*</td>
<td>0.75±0.034</td>
<td>0.83±0.04*</td>
<td>8.37±0.23*</td>
<td>4.07±0.12*</td>
<td>4.19±0.07*</td>
<td>24.67±0.25*</td>
</tr>
</tbody>
</table>

P>0.05**, P<0.001*, P<0.01#, P<0.05 $ two way ANNOVA followed by bonferroni post test when compared with toxicant group.

Graph 1: Effect of APLC on Lipid profile of STZ induced diabetic rats after 12 week study period.