IN VIVO ANTI-DIABETIC ACTIVITY OF THE ENDEMIC
MEDICINAL PLANT CARALLUMA SARKARIAE R.BR. USING STZ
INDUCED MALE WISTAR ALBINO RATS

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ABSTRACT
Caralluma sarkariae is a succulent, endangered and endemic plant from southern Western Ghats. This species has used by ethnomedical practices by traditional healers of Southern Tamilnadu. Traditionally the plant has been used as the treatment of Rheumatism, ulcer, asthma, fever, inflammation, diabetes, stress, cancer and obesity. The methanolic extract of C. sarkariae was investigated for Anti-diabetic activity by STZ induced male wistar albino rats. The result indicated that the methanolic extract at the dose of 150mg/kg b.wt., showed a Significant anti-diabetic activity as compared to the standard drug. The findings of the study suggest that the methanolic extract of C. sarkariae showed significant activities which confirm the traditional usage of this medicinal plant to treat the metabolic disorders.

KEYWORDS: Caralluma sarkariae, methanol extract, Streptozotocin.

INTRODUCTION
Herbal medicine is the oldest form of health care known to humanity and has been used in all cultures throughout history. Even in ancient cultures, tribal people methodically collected information on herbs and developed well-defined herbal pharmacopoeias. Diabetes is one of the most common chronic diseases in the world and affects 5% of the global population. The number of people with diabetes is increasing dramatically due to the population growth, aging, urbanization, obesity and physical inactivity that lead to the major health and socio-economic problems. The Current Scenario demonstrates that the world prevalence of diabetes
will increase up to 7.7% adults by 2030. So, with regard to the issue of socio-economic burden of diabetes, leads to discover of more effective and less side effect therapies.

*Caralluma sarkariae* (Apocynaceae) is an endemic and endangered medicinal plant in Southern Western Ghats. It is a succulent herb found in South and East India. *C. sarkariae* is an indigenous medicinal plant, has a folk (Siddha and Ayurvedha) reputation in rural southern India. Traditionally, the plant has been in use as an anti-inflammatory, diabetes, expectorant, carminative, digestive, stomachache, asthma, anti-stress, cancer, tumor, diabetes, rheumatism, paralysis, joints pains, fever, ulcer and obesity (Zakaria *et al.*, 2008). Thus, the present investigation was carried out to evaluate the anti-diabetic activity of *C. sarkariae* in experimental animal model.

**MATERIALS AND METHODS**

**Collection of plant material**
The whole plant part of *Caralluma sarkariae* R.Br. was collected during the month of January 2016, from Virudhunagar district, Southern Western Ghats of Tamilnadu. The plant was identified and authenticated by a plant taxonomist.

**Anti-diabetic activity**

**Experimental animals**
Male Wistar albino rats were obtained from the Animal house, Agricultural University, Trissur, Kerala. Feed (standard pellet diet) and water were supplied *ad libitum*. All the animals were housed and maintained at a temperature of 20-24ºC, relative humidity of 50-70% and a 12 hrs light/dark cycle. Rats had been housed in five groups of ten in the same cage for 1 week before treatment. The experimental protocol has been approved by the Institution Animal Ethics committee and by the Regulatory body of the government (659/02/a/CPCSEA).

**Induction of experimental diabetes mellitus**
Diabetes was induced in overnight fasted rats by intra-peritoneally injection of 55mg/kg b.wt. of Strepstozotosin (STZ) freshly prepared in ice-cold 0.1M sodium citrate buffer, pH 4.5 (Hamilton *et al.*, 1998). Normal control rats received an equivalent amount of buffer intravenously. STZ is capable of producing fatal hypoglycemia as a result of massive pancreatic insulin release; therefore the animals were treated with 20% glucose solution (15-20 ml) orally after 6 hours. After one hour of STZ administration the animals were fed on
standard pellets and water. The blood glucose level (BGL) was monitored after blood samples collected by tail tipping method using glucose check strips in glucometer (Johnson & Johnson medical (China) Ltd.). After 48 h when the condition of diabetes was stabilized, animals with blood glucose levels above 300mg/dl were selected for the study (Lisa et al., 1998).

**Oral Glucose Tolerance Test (OGTT)**
OGTT of plant extracts was carried out in overnight fasted normal rats, which were equally divided into four groups of six rats each. Group I (normal control) and group II (Diabetic control) were given the normal feed and ordinary tap water, group III diabetic rats given methanolic extract of *C. sarkariae* at the dose of 100mg/kg body weight, daily orally for 21 days consequently and group IV diabetic rats given methanolic extract of *C. sarkariae* at the dose of 150mg/kg body weight daily orally for 21 days consequently. Thereafter, following 30min post extract administration all the animals were fed with glucose (2g/kg). Blood samples were obtained by nicking the tails with a sharp razor (Aydin et al., 1995) and glucose concentrations were determined using a one-touch glucometer (Johnson & Johnson medical Ltd., Mumbai). The glucose concentration was read and documented from the glucometer readings. This method was chosen because it used a very small quantity of whole blood (<5μl) and gave immediate results. Thus an indication, whether the glucose load had been administered successfully via gavage. After the initial measurement of blood glucose was taken using the glucometer (at time 0), glucose was administered orally to each rat. Blood glucose concentrations were then measured at 30, 60, 90 and 120 minutes after the loading dose of 2g/kg glucose.

**Estimation of various parameters**
The hemoglobin (Drabkin and Austin, 1932), glycosylated hemoglobin (Kynoch and Lehmann, 1977), Insulin (Anderson et al., 1993), Creatinine (Owen et al., 1954), Biliurubin (Malloy and Evelyn, 1937), Urea (Varley,1976) and Total cholesterol (Parekh and Jung, 1970) levels were estimated.

**RESULT AND DISCUSSION**
Blood glucose levels of methanolic extract of *C. sarkariae* treated groups at different time intervals after oral glucose tolerance test (OGTT) are shown in Table-1. The maximum rise in blood glucose occurred 30 min after the oral glucose administration. Although the glucose levels started to decline, they remained high after 120 min. The methanolic extract of *C.
sarkariae at the dose of 100mg/kg body wt., & 150mg/kg body wt. and glibenclamide suppressed the rise in glucose levels, at different degrees. Glibenclamide and methanolic extract of C. sarkariae (150mg/kg) significantly suppressed the rise in blood glucose at 90min to 120min after oral glucose administration when compared with diabetic control animals. At the end of 120 min the blood glucose reached near the normal level, in diabetic rat treated with methanolic extract of C. sarkariae. The effect of methanolic plant extract at the dose of 150mg/kg b.wt was more pronounced when compared with the dose of 100mg/kg b.wt given orally.

Hemoglobin and Glycosylated Hemoglobin
The level of total hemoglobin and glycosylated hemoglobin profiles of different experimental groups of control, diabetic induced and treated animals are represented in Table-2. The diabetic rats showed a significant decrease in the level of total hemoglobin and significant increase in the level of glycosylated hemoglobin. The administration of methanolic extract of C. sarkariae and glibenclamide to diabetic induced rats restored the changes in the level of total hemoglobin and glycosylated hemoglobin to near normal levels.

Insulin, Urea, Creatinine, Total bilirubin and Total cholesterol levels
All the diabetic groups II, III and IV had significantly higher levels of plasma urea and creatinine than in control groups, indicating a poor control of diabetes. There was a significant reduction in serum insulin levels in STZ diabetic rats, compared with normal rats. Administration of methanolic extract of C. sarkariae at 150mg/kg body wt. and glibenclamide tended to bring serum insulin towards normal level. Treatment of rats with C. sarkariae extracts produced significant decrease in the urea and serum creatinine levels in comparison with the control group. The diabetic rats showed a significant elevation in the total bilirubin, when compared to the control group. However the extract of C. sarkariae at 150mg/kg body wt., treated groups showed a significant decrease in the levels of total bilirubin and total cholesterol. The diabetic rats administrated with C. sarkariae extract at 150mg/kg body wt., daily for 21 days consequently orally by IGC altered the values of insulin, Urea, Creatinine, total bilirubin and total cholesterol levels were compared to control. All the results were compared with the standard drug, glibenclamide (Table-3).

India has a rich history of using various potent herbs and herbal components for treating diabetes. The use of medicinal plants has a long folk history for the treatment of diabetes mellitus (Shoaib, 1992 and Ernst, 1997). Prior to the development of insulin injection therapy
in 1921, diabetes was managed entirely with indigenous medicinal plants. The increased total bilirubin levels indicate that excess haemoglobin is being destroyed or that the liver is not actively treating the haemoglobin it is receiving (Andullu and Vardycheryalu, 2001). The decrease in protein content during diabetes is reported which may be due to the increase in gluconeogenesis or due to decrease in soluble protein during diabetes mellitus (Belfiore et al., 1972). Insulin deficiency causes excessive catabolism of protein and the aminoacids released are used for gluconeogenesis. The protein damage might be due to the lipid peroxidation reaction. Due to insulin deficiency the protein content is decreased in muscular tissue by proteolysis (Subash babu et al., 2007). The apocyanaceae members also have potential anti-diabetic activity. It would be seen in various plant species for example the methanolic leaf extract (150 mg/kg) and whole plant extract (300mg/kg) of Picralima nitida exhibited significant anti-diabetic activities with 39.40% and 38.48% glycaemia reduction respectively(Teugwa 2013). observed a significant drop in blood glucose with the glycosides extract with reduction in fasting blood glucose levels of 64.4% (250 mg/kg) in hyperglycemic rats. Telosma procumbens extract at the dose of 100mg/kg b.wt. produced significant reduction in blood glucose level in diabetic rats. In diabetic mice, the maximum decrease in glycemia using the high dose of the plant extract was obtained 1 hr (59%) after treatment which is quite comparable to the effect of insulin (65%). These evidences clearly indicate that the ethanolic extract of T. procumbens exhibited antidiabetic potential (Cajuday and Amparado, 2014).

Nerium indicum chloroform extract (NICE) and ethanolic extract (NIEE) showed significant (P<0.001) anti-diabetic activity (Sikarwar et al., 2009). The hydroethanol extract of whole plants (150 mg/Kg) and methanol leave extract of Picralima nitida (300 mg/Kg) exhibited significant anti-diabetic activities with 39.40% and 38.48% glycaemia reduction, respectively. The measurement of stress markers in plasma, liver and kidney after administration of both extracts showed significant reduction in MDA and hydrogen peroxide levels, coupled with a substantial increase in catalase activity (Teugwal et al., 2013).
Table 1: Effect of Caralluma sarkariae methanolic extracts on oral glucose tolerance in normal, diabetic induced and drug treated rats

<table>
<thead>
<tr>
<th>Treatment</th>
<th>0 min(mg/dl)</th>
<th>30 min(mg/dl)</th>
<th>90 min(mg/dl)</th>
<th>120 min(mg/dl)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group I</td>
<td>74.22 ± 8.20</td>
<td>146.5 ± 2.51</td>
<td>138.20 ± 5.33</td>
<td>124.50 ± 1.25</td>
</tr>
<tr>
<td>Group II</td>
<td>76.30 ± 8.91</td>
<td>132.3 ± 1.13</td>
<td>118.70 ± 4.30</td>
<td>96.79 ± 3.38</td>
</tr>
<tr>
<td>Group III</td>
<td>78.54 ± 4.36</td>
<td>128.55 ± 0.05</td>
<td>98.04 ± 2.07</td>
<td>85.19 ± 1.88</td>
</tr>
<tr>
<td>Group IV</td>
<td>77.72 ± 7.10</td>
<td>126.62 ± 4.54</td>
<td>94.14 ± 2.40</td>
<td>83.11 ± 2.09</td>
</tr>
</tbody>
</table>

Each Value is SEM ± 5 individual.

**Group I:** Control rats given normal saline daily for 21 days.

**Group II:** STZ induced diabetic rats given normal saline daily for 21 days.

**Group III:** STZ induced diabetic rats given methanolic extract of *C. sarkariae* drug at the dose of 100 mg/kg body weight, daily orally for 21 days.

**Group IV:** STZ induced diabetic rats given methanolic extract of *C. sarkariae* drug at the dose of 150 mg/kg body weight daily orally for 21 days.

**Group V:** STZ induced diabetic rats given glibenclamide at the dose of 600 µg/kg body weight, daily orally for 21 days.

Table 2: Effect of Caralluma sarkariae methanolic extracts on hemoglobin and glycosylated hemoglobin levels in normal, diabetic induced and drug treated rats

<table>
<thead>
<tr>
<th>Treatment groups</th>
<th>Hemoglobin (mg/dl)</th>
<th>Glycosylated hemoglobin (mg/dl)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group I</td>
<td>11.25±0.15</td>
<td>4.74±0.12</td>
</tr>
<tr>
<td>Group II</td>
<td>6.86±0.25</td>
<td>9.98±0.11</td>
</tr>
<tr>
<td>Group III</td>
<td>7.98±1.94</td>
<td>8.79±0.05</td>
</tr>
<tr>
<td>Group IV</td>
<td>8.91±2.04</td>
<td>7.62±1.51</td>
</tr>
<tr>
<td>Group V</td>
<td>10.62±0.06</td>
<td>5.03±0.08</td>
</tr>
</tbody>
</table>

Each Value is SEM of 5 animals.

**Group I:** Control rats given normal saline daily for 21 days.

**Group II:** STZ induced diabetic rats given normal saline daily for 21 days.

**Group III:** STZ induced diabetic rats given methanolic extract of *C. sarkariae* drug at the dose of 100 mg/kg body weight, daily orally for 21 days.

**Group IV:** STZ induced diabetic rats given methanolic extract of *C. sarkariae* drug at the dose of 150 mg/kg body weight daily orally for 21 days.

**Group V:** STZ induced diabetic rats given glibenclamide at the dose of 600 µg/kg body weight, daily orally for 21 days.
Table 3: Effect of *Caralluma sarkariae* methanolic extracts on the serum insulin, urea, creatinine and Total bilirubin levels of normal, diabetic induced and drug treated rats

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Group I</th>
<th>Group II</th>
<th>Group III</th>
<th>Group IV</th>
<th>Group V</th>
</tr>
</thead>
<tbody>
<tr>
<td>Insulin (MIu/ml)</td>
<td>16.90±0.25</td>
<td>07.24±0.05</td>
<td>11.05±0.33</td>
<td>14.72±0.50</td>
<td>15.97±0.91</td>
</tr>
<tr>
<td>Urea (mg/dl)</td>
<td>18.05±0.11</td>
<td>27.81±2.34</td>
<td>22.49±0.40</td>
<td>19.12±0.23</td>
<td>16.55±1.30</td>
</tr>
<tr>
<td>Creatinine (mg/dl)</td>
<td>0.49±0.05</td>
<td>0.92±0.11</td>
<td>0.79±0.15</td>
<td>0.63±0.18</td>
<td>0.52±0.02</td>
</tr>
<tr>
<td>Total Bilirubin (µmol/L)</td>
<td>0.91±0.25</td>
<td>2.92±0.51</td>
<td>1.67±0.34</td>
<td>1.23±0.18</td>
<td>0.87±0.11</td>
</tr>
<tr>
<td>Total Cholesterol (mg/dl)</td>
<td>133.50±0.82</td>
<td>193.21±1.27</td>
<td>152.44±2.14</td>
<td>139.31±0.25</td>
<td>128.22±0.50</td>
</tr>
</tbody>
</table>

Each Value is SEM of 5 animals.

**Group I**: Control rats given normal saline daily for 21 days.

**Group II**: STZ induced diabetic rats given normal saline daily for 21 days.

**Group III**: STZ induced diabetic rats given methanolic extract of *C. sarkariae* drug at the dose of 100 mg/kg body weight, daily orally for 21 days.

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**Group V**: STZ induced diabetic rats given glibenclamide at the dose of 600 µg/kg body weight, daily orally for 21 days.

**CONCLUSION**

In modern era, the trend towards the use of alternative and complementary green medicine is increasing tremendously and it offers extraordinary chance to the development of herbal medicine. Although, the methanolic extract of *C. sarkariae* exhibited significant anti-diabetic activity. The exact mechanisms underlying the observed pharmacological effect can be elucidated only after the isolation of active components, by using a wide range of experimental models.

**REFERENCES**

