Antidiabetic and Antioxidant Activity of *Coccinea grandis* Voigt Stem Extract in Streptozotocin Induced Diabetic Rats

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**ABSTRACT**

**Objective:** In the present study, the antidiabetic and antioxidant study of stem part of *Coccinea grandis* Voigt plant extracts in Streptozotocin induced diabetic rats were investigated. **Materials and methods:** Fifty four Wistar albino rats were used with nine groups and with six rats in each group. 45 mg/kg body weight streptozotocin was administered to group 2 to 9. Group 2 was diabetic control. Group 3 was given with glibipride as standard drug. Group 4 and 5 were given petroleum ether extract 250 and 500 mg/kg respectively. Group 6 and 7 were given 250 and 500 mg/kg chloroform extract respectively. Group 8 and 9 were given 250 and 500 mg/kg hydro alcoholic extract respectively. Antidiabetic activity of the extracts was assessed by serum glucose level on glucose kit. Superoxide dismutase (SOD), Catalase (CAT) and lipid peroxidation studies were assessed with histopathology. **Result:** The chronic study data on diabetic rats cleared the administration of all extracts significantly reduced blood glucose level and lipid peroxidation level with better antioxidant activity. **Conclusion:** From the study, the petroleum ether, chloroform and hydro alcoholic extracts of stem part of *Coccinea grandis* Voigt plant have shown antidiabetic and antioxidant potential.

**Keywords:** Antidiabetic activity, antioxidant activity, Lipid peroxidation, Superoxide dismutase, Catalase.

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1. **INTRODUCTION**

Diabetes mellitus is a metabolic disorder characterized by deficient blood insulin level. This may be cause because of lack of sensitivity of receptors to the insulin or autoimmune destruction of pancreatic ß cells of Langerhans which leads to abnormal glucose homeostasis and elevated blood glucose level.[1] Insulin is a hormone secreted in pancreas which allows body to use glucose as a source of energy. Insulin helps to maintain blood glucose level within the normal limit.[2]

According to WHO survey in 2016, 422 million adults are living with diabetes mellitus globally. In India, more than 62 million people suffered by diabetes mellitus. By 2030, it was predicted that 79.4 million people would be diabetic in India. The prevalence of diabetes is more in India due to changes in lifestyle, trend of urbanization, global nutrition transition, genetic factor, environmental influences, rising living standards. Diabetes mellitus is categorized into mainly two types. Type I Diabetes mellitus (Insulin dependent DM) and type II Diabetes mellitus (Non-insulin dependent DM). [3]

Amongst these types, 90% people are having type II Diabetes mellitus. (National diabetes Fact Sheet 2005). [4] Diabetes mellitus is one of the fatal disorders in the world and it is an 6th leading cause of death. [5] The death rate in diabetic people is double to that of normal people. Diabetes mellitus affects many major organs of body. Many complications are associated with diabetes mellitus. It can cause kidney failure, blindness, impotence, cerebrovascular disorder, cardiovascular disorder.

Medicinal plants have become useful remedies for the treatment of diabetes mellitus and its complication because of polyphenol,[6] saponins glycosides, flavonoids, sterol constituents present in the plants have ability to reduce the blood glucose level and cholesterol level. It has been described in ancient literature about the use of natural medicinal plants or herbal products in diabetes mellitus. Antidiabetic effect of these plants is may be due to their ability to recover the disturbed function of pancreas or
decreases the intestinal glucose absorption. Reported data shown that more than 800 plants possess antidiabetic potential. [7] The plants consist of phytoconstituents like saponins, flavonoids, alkaloids, glycosides, and sterol. These phytoconstituents contribute various medicinal properties. From experimental studies it seems that medicinal plants have potential to reduce blood glucose level or delay the complications associated with diabetes mellitus. The isolated and optimized phytoconstituents from the plants could serve as a better candidate in drug discovery for diabetes. [8]

Many research studies growing in the field of herbal medicine in the past few decades. Research studies in the isolation of phytoconstituents showing therapeutic effect on various diseases have become good approach in herbal area. WHO has suggested efficiency of phytochemicals in reducing various diseases have become good approach in herbal area. The stem part of Coccinea grandis Voigt plant of Ashta, Sangli and adjacent villages of Sangli in the month of October. The authentication of plant was carried out from Botanical Survey of India Pune. The voucher specimen number is BS/WRD/IDEN.CER./2019/H3/138. The stem was cut into small pieces and dried under shade for 10 to 15 days.

2.3. Preparation of extract

The dried pieces of stem of plant were crushed in an electrical grinder and made coarse powder for further extraction. 2 kg coarse powder was subjected to continuous hot soxhlet extraction by using petroleum ether, chloroform and ethyl acetate respectively. The filtrate obtained from continuous hot extraction of each solvent was evaporated to dryness. Further obtained marc was macerated by using methanol and water. Filtrate was evaporated to dryness. The yields of petroleum ether, chloroform, ethyl acetate and water methanol extracts were 16 gm., 13 gm., 8 gm. and 10 gm. respectively. These extracts were stored at 0-4°C in the refrigerator.

2.4. Experimental animals

Wistar albino rats weighing 200–300 g were obtained from the central animal house of H. S. K. College of Pharmacy and Research Centre, Bagalkot. The animals were housed at room temperature (22±2 °C) with 65 ± 10% relative humidity for 12 hr. dark and light cycle and given standard laboratory feed and water ad libitum. The study was approved and conducted as per the norms of the Institutional Animal Ethics Committee (Appasheeb Birnale College of Pharmacy, Sangli) on 22 January 2018. Reference No. IAEC/ABCP/08/2017-18.

2.5. Acute toxicity study (LD50)

The acute toxicity of Coccinea grandis stem extracts were studied according to the OECD guidelines 425 and the female Swiss albino mice (25-30g) were used for acute toxicity. Lethal dose (LD50) was calculated with the help of acute oral toxicity (AOT) software. Based on the result of the study, the screening doses of extracts selected for activity. The extracts found to be safe and found no mortality at 2000mg/kg body weight in female mice. Dose selected for the activity were 250 mg/kg and 500 mg/kg/BW.

2.6. Oral Glucose Tolerance Test (OGTT)

The acclimatized animals were fasted for 24 hours with water ad libitum, fasted animals were divided into seven groups. The dose administered after withdrawing the 0 hours of blood sample and at an interval of 1½, 3, 5 hours after the 250mg/kg and 500mg/kg administration. Blood samples were collected from retro-orbital plexus under anesthesia and were centrifuged at 3000 rpm for 10 minutes to obtain the serum, and used for estimation of glucose by using glucose kit.
2.7. Induction of Experimental Diabetes

After 1 week of acclimatization, the rats were subjected to a 16-h fast. Diabetes was induced with a single injection of streptozotocin (STZ) 45 mg/kg body weight by intraperitoneal route\(^\text{[14]}\). Initial drug induced hypoglycemia was reduced by giving 20% glucose solution overnight. After 5 days the of streptozotocin injection, the fasting blood glucose level above 250 mg/dL was taken as diabetic.\(^{[15]}\)

2.8. Experimental design

In the experiment, a total of 54 rats (6 normal; 48 STZ diabetic rats) were used. The rats were divided into 9 groups of 6 animals each.

**Treatment group:**

1. Group-I: Control rats were administered with 0.5 mL of 0.9% saline orally [Non-diabetic].
2. Group-II: Streptozotocin (45 mg/kg body weight) induced diabetic rats.
3. Group-III: Standard drug Glibenpiride (10 mg/kg body weight) was given to diabetic rats\(^{[16]}\).
4. Group-IV: 250 mg/kg of Petroleum ether extract was given to diabetic rats.
5. Group-V: 500 mg/kg of Petroleum ether extract was given to diabetic rats.
6. Group-VI: 250 mg/kg of Chloroform extract was given to diabetic rats.
7. Group-VII: 500 mg/kg of Chloroform ether extract was given to diabetic rats.
8. Group-VIII: 250 mg/kg of Hydro alcoholic extract was given to diabetic rats.
9. Group-IX: 500 mg/kg of Hydro alcoholic extract was given to diabetic rats.

Above treatment was given for 14 days orally.

2.9. Biochemical Estimation

2.9.1. Measurement of blood glucose level

The blood glucose levels of rats were measured after 5 days, 10 days and 14 days by using Glucose oxidase-peroxidase (GOD-POD) estimation kit method.

2.9.2. Lipid peroxidation (LPO)

Thiobarbituric acid reactive substances (TBARS) in the homogenate were estimated by using Fraga et al. (1981)\(^{[17]}\) method. Briefly, the 0.5 ml of 10% homogenate, 0.5 ml saline and 1.0 ml 10% TCA were mixed well and centrifuged at 3000rpm for 20 minutes. In 1 ml protein free supernatant solution, 0.25 ml of Thiobarbituric acid was added, mixed well and boiled for 1 hour at 95°C. The absorption was measured at 532 nm. The amount of lipid peroxidation was expressed as TBARS nmoles /mg of protein.

2.9.3. Superoxide dismutase (SOD)

Superoxide dismutase activity was determined based on the ability of SOD to inhibit the auto-oxidation of epinephrine to adrenochrome at alkaline pH.

25 μL of the supernatant solution obtained from the centrifuged liver homogenate was first added to 50mM sodium carbonate buffer (10.2) Then 0.1mM epinephrine was added in a total volume of 2 ml of buffer medium. Absorption was measured at 480 nm. The SOD activity was calculated in terms of (U/mg of protein).\(^{[18]}\)

2.9.4. Catalase (CAT)

The assay mixture consisted of 1.95 ml phosphate buffer (0.05 M, pH 7.0), 1.0 ml hydrogen peroxide (0.019 M), and 0.05 ml homogenate (10%, w/v) in a total volume of 3.0 ml. Changes in absorbance were recorded at 240 nm. Catalase activity was calculated in terms of units /mg protein.\(^{[19]}\)

2.10. Histopathology

The pancreas from each group was dissected out and placed in 10 % buffered neutral formal saline solution and then used. These tissues were embedded in paraffin. These fixed tissues were cut and stained with eosin and hematoxylin. The tissue section was observed under microscope.\(^{[20]}\)

2.11. Statistical analysis

All data was expressed as mean ±SEM. The results were analyzed by one way analysis of variance (ANOVA) followed by Dunnet’s test. The significant levels were p<0.05, p<0.01, and p<0.001.

3.0. RESULTS

The acute toxicity study showed the non-toxicity of Coccinia grandis Voigt plant extracts. The lower dose of 250 mg/kg and higher dose of 500mg/kg were selected for anti diabetic study.

Table 1: Effect of Petroleum ether, Chloroform and Hydro alcoholic extracts on normal group (hypoglycemic activity)

<table>
<thead>
<tr>
<th>Sr. no</th>
<th>Treatment</th>
<th>Serum (blood)glucose levels in mg/dl with</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>0 hrs.</td>
</tr>
<tr>
<td>1</td>
<td>Normal</td>
<td>94.17 ± 2.226</td>
</tr>
<tr>
<td>2</td>
<td>Petroleum ether extract-250mg/kg</td>
<td>89.51 ± 3.131</td>
</tr>
<tr>
<td>3</td>
<td>Petroleum ether extract-500mg/kg</td>
<td>99.87 ± 2.429</td>
</tr>
<tr>
<td>4</td>
<td>Chloroform extract-250mg/kg</td>
<td>100.4 ± 2.923</td>
</tr>
<tr>
<td>5</td>
<td>Chloroform extract-500mg/kg</td>
<td>99.2 ± 2.153</td>
</tr>
<tr>
<td>6</td>
<td>Me-water-Extract-250 mg/kg</td>
<td>87.65 ± 2.131</td>
</tr>
<tr>
<td>7</td>
<td>Me-water-Extract-500 mg/kg</td>
<td>91.21 ± 2.572</td>
</tr>
</tbody>
</table>

All value are expressed as mean ± SEM, n=6 in each, * P<0.05, **P<0.01, ***P<0.001 as (paired t test) compared to 0 hour within the group. The values in parentheses represent % change glycemic index.
There was a reduction in blood glucose level appeared in normal rats.

Effect of petroleum ether, chloroform and hydro alcoholic extracts on blood glucose level of streptozotocin induced diabetic rats after 3, 5, 10 and 14 day were shown in table no.2.

Table. No. 2. Effect of Petroleum ether, Chloroform and Hydro alcoholic extracts on streptozotocin induced diabetic rats (Chronic study)

<table>
<thead>
<tr>
<th>Sr. no</th>
<th>Treatment</th>
<th>Serum glucose levels in mg/dl</th>
<th>3rd day</th>
<th>5th day</th>
<th>10th day</th>
<th>14th day</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Normal</td>
<td></td>
<td>97.91 ± 6.296</td>
<td>101.9 ± 6.425</td>
<td>95.80 ± 5.975</td>
<td>99.74 ± 6.223</td>
</tr>
<tr>
<td>2</td>
<td>Control</td>
<td></td>
<td>340.5 ± 9.107*</td>
<td>331.0 ± 9.599*</td>
<td>334.1 ± 9.088*</td>
<td>318.5 ± 10.54*</td>
</tr>
<tr>
<td>3</td>
<td>Petroleum ether extract-250mg/kg</td>
<td></td>
<td>318.0 ± 14.69</td>
<td>225.9 ± 9.819***</td>
<td>142.0 ± 14.44***</td>
<td>97.30 ± 4.271***</td>
</tr>
<tr>
<td>4</td>
<td>Petroleum ether extract-500mg/kg</td>
<td></td>
<td>284.4 ± 28.37</td>
<td>246.5 ± 29.65**</td>
<td>150.2 ± 16.70***</td>
<td>109.5 ± 5.902***</td>
</tr>
<tr>
<td>5</td>
<td>Chloroform extract-250mg/kg</td>
<td></td>
<td>293.5 ± 25.62</td>
<td>232.2 ± 14.71*</td>
<td>175.1 ± 10.92***</td>
<td>128.0 ± 11.62***</td>
</tr>
<tr>
<td>6</td>
<td>Chloroform extract-500mg/kg</td>
<td></td>
<td>282.1 ± 25.62</td>
<td>156.2 ± 14.45***</td>
<td>119.02 ± 11.58***</td>
<td></td>
</tr>
<tr>
<td>7</td>
<td>Me-water-Extract-250mg/kg</td>
<td></td>
<td>261.23 ± 12.41**</td>
<td>221.1 ± 11.21***</td>
<td>187.8 ± 25.98***</td>
<td>119.02 ± 11.58***</td>
</tr>
<tr>
<td>8</td>
<td>Me-water-Extract-500mg/kg</td>
<td></td>
<td>224.17 ± 9.21**</td>
<td>169.1 ± 13.11***</td>
<td>135.1 ± 11.27***</td>
<td>115.12 ± 9.11***</td>
</tr>
<tr>
<td>9</td>
<td>Glimepiride (10mg/kg)</td>
<td></td>
<td>198.5 ± 12.20**</td>
<td>188.0 ± 4.898**</td>
<td>149.4 ± 4.413***</td>
<td>90.26 ± 5.203***</td>
</tr>
</tbody>
</table>

All value are expressed as mean ± SEM, n=6 in each, *P<0.01 as compared to normal group, **P<0.01, ***P<0.001 as compared to control group. One way ANOVA followed by Dunnet’s test. The values in parentheses represent % change glucose level.

Glimepiride 10 mg/kg body weight was used standard. The study showed the reduction in blood glucose level in diabetic rats. Table No.3. Showed the effect of Petroleum ether, Chloroform and Hydro alcoholic extracts on SOD, CAT and lipid peroxidation. The lipid peroxidation was decreased whereas the SOD and CAT values were increased to the normal.

Table. No. 3. Effect of Petroleum ether, Chloroform and Hydro alcoholic extracts on biochemical parameters

<table>
<thead>
<tr>
<th>Sr. no</th>
<th>Treatment</th>
<th>BIOCHEMICAL MARKERS [14th day]</th>
<th>SOD (U/mg of protein)</th>
<th>Catalase (U/mg of protein)</th>
<th>Lipid peroxidation (nmoles/mg of protein)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Normal</td>
<td></td>
<td>542.9 ± 41.1</td>
<td>0.185 ± 0.022</td>
<td>89.00 ± 8.05</td>
</tr>
<tr>
<td>2</td>
<td>Control</td>
<td></td>
<td>311.2 ± 33.42</td>
<td>0.014 ± 0.017*</td>
<td>563.7 ± 9.90*</td>
</tr>
<tr>
<td>3</td>
<td>Petroleum ether extract-250mg/kg</td>
<td></td>
<td>726.4 ± 22.51</td>
<td>0.178 ± 0.019***</td>
<td>163.8 ± 2.73***</td>
</tr>
<tr>
<td>4</td>
<td>Petroleum ether extract-500mg/kg</td>
<td></td>
<td>894.1 ± 221.3</td>
<td>0.209 ± 0.020***</td>
<td>158.2 ± 7.20**</td>
</tr>
<tr>
<td>5</td>
<td>Chloroform extract-250mg/kg</td>
<td></td>
<td>1041 ± 44.1*</td>
<td>0.333 ± 0.041***</td>
<td>173.1 ± 2.20**</td>
</tr>
<tr>
<td>6</td>
<td>Chloroform extract-500mg/kg</td>
<td></td>
<td>1061 ± 36.1*</td>
<td>0.357 ± 0.051***</td>
<td>189.1 ± 4.50***</td>
</tr>
<tr>
<td>7</td>
<td>Me-water-Extract-250mg/kg</td>
<td></td>
<td>689 ± 12.2*</td>
<td>0.145 ± 0.045***</td>
<td>198.2 ± 3.25***</td>
</tr>
<tr>
<td>8</td>
<td>Me-water-Extract-500mg/kg</td>
<td></td>
<td>783 ± 10.2*</td>
<td>0.132 ± 0.015***</td>
<td>210.1 ± 2.11**</td>
</tr>
<tr>
<td>10</td>
<td>Glimepiride 4mg/kg</td>
<td></td>
<td>317 ± 21.3</td>
<td>0.142 ± 0.011**</td>
<td>178.1 ± 2.22**</td>
</tr>
</tbody>
</table>

All value are expressed as mean ± SEM, n=6 in each, *P<0.001 as compared to normal group; **P<0.01, ***P<0.001as compared to control group. One way ANOVA followed by Dunnet’s test.
Histopathological observations of Coccinea grandis Voigt plant extracts (250mg/kg and 500 mg/kg) treated for 14 days in streptozotocin induced diabetic rat pancreas of different groups were shown in fig 1.

Fig. No.1. Histopathological study

Normal (A)
STZ 45mg/kg (B)
Glimepiride 4mg/kg (C)
500 mg/kg Pet ether (D)
500 mg/kg Chl. Extract (E)
500mg/kg of Hydro Alcoholic extract (F)

Fig 1. Normal (A): Showed normal pancreatic architecture, no intracellur gap and density of cells normal. Control (B): STZ given animals showed complete destruction of pancreas, intracellular gap, decreased cell density and altered architecture. Glimepiride (C) and extracts: D, E, F Indicated the decreased intracellular gap, regain of architecture and increased density of cells.

Indicates density of cells: →
Intra cellular gap ←

4.0. DISCUSSION
Streptozotocin (45 mg/kg body weight) was used for inducing diabetes in Wistar albino rats.

Acute toxicity studies showed the non-toxicity and safety of Pet ether, chloroform and hydro alcoholic extracts of stem part of Coccinea grandis plant at 2000 mg/kg body weight dose.

From Streptozotocin induced hyperglycemic (chronic study) data, all extracts showed better tendency to decrease the blood glucose level on 14th day of treatment. It was clear from hypoglycemic activity conducted on normal group of rats, the extracts has ability to lower the blood glucose level in normal group.

Estimation has been conducted at 0 hrs. 1 and half hrs. 3 hrs. and 5 hrs. Histopathological studies revealed the destruction of pancreas in STZ induced diabetic rat with intracellular gap, decreased cell density and altered architecture as shown in fig 1. The diabetic rats treated by glimepiride (STD) and pet ether, chloroform and hydro alcoholic extracts indicated decreased intracellular gap, regain of architecture and increased cell density. Elevated level of lipid peroxidation in diabetic rats was controlled by standard glimepiride drug and all extracts.SOD and catalase activities were increased to normal level in diabetic rats because of antioxidant activity of pet ether, chloroform and hydro alcoholic extracts of stem Coccinea grandis plant. It revealed attenuation of oxidative stress.

Traditional plants contain flavonoids, phenolic compounds, glycosides, alkaloids. It was supported from ancient literature the medicinal uses of plants in different diseases. The plants rich in flavonoids, phenolic compounds, glycosides possess antidiabetic and antioxidant activities.

5.0. CONCLUSION
The present study showed the antidiabetic and antioxidant activity of pet ether, chloroform and hydro alcoholic extracts of stem part of Coccinea grandis plant in streptozotocin induced diabetic rats. These extracts showed promising reduction in blood glucose level in diabetic rats and reduces oxidative stress like standard glimepiride. Further studies are in progress to isolate bioactive constituents from Coccinea grandis stem extracts for antidiabetic activity. Further, exploration of biochemical mechanism involving antidiabetic activity needs to be done.

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CONFLICT OF INTEREST STATEMENT
There is no conflict of interest.
REFERENCES:


