ANTIHYPERTHYPERGLYCEMIC AND HEAMATOLOGICAL EFFECT OF COMBINATION OF BOUGAINVILLEA SPECTABILIS AND CATHARANTHUS ROSEUS LEAVES ON ALLOXAN-INDUCED DIABETIC WISTAR RATS

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ABSTRACT

Bougainvillea spectabilis and Catharanthus roseus leaves are known to have medicinal properties. Antihyperglycemic property and other beneficial effects of their plant extract were evaluated in Alloxan-induced diabetic Wistar rats. A total of 24 mature rats weighing 200±10g were used for this study. They were divided into four groups of 6 rats each. Group I was conserved as normal control. Group II, III and IV were made diabetic by intraperitoneal injection of Alloxan. After being confirmed as diabetic, Group II was conserved as diabetic control, Group III was administrated plant extract (Bougainvillea spectabilis and Catharanthus roseus) at 300 mg kg⁻¹ body weight and Group IV was administrated standard drug, Glibenclamide at 5 mg kg⁻¹ body weight for defined time intervals. The blood glucose level and hematological parameters viz. red blood and white blood cells and some of their functional indices were evaluated in diabetic treated groups on 7th, 14th and 21st day after daily oral administration of plant extracts and were compared with the respective controls. The result of study revealed that the plant extract at 300 mg kg⁻¹ body weight exhibited significantly hypoglycemic activity at defined interval and had beneficial effect on all studied hematological parameters viz. RBC, WBC, Hb, PVC, SGOT and SGPT.
KEYWORDS: *Bougainvillea Spectabilis, Catharanthus Roseus*, Diabetes, Hematology, Wistar Rat.

INTRODUCTION

Diabetes mellitus is caused by lack of insulin secretion by the pancreas (type I diabetes) or by insufficient insulin secretion to compensate for decreased sensitivity to the effects of insulin (type II diabetes). (Guyton and Hall, 2006). Patients with diabetes experience significant morbidity and mortality from microvascular (retinopathy, neuropathy, nephropathy etc) and macrovascular complications *viz.* heart attack, stroke and peripheral vascular disease (Halder *et al.*, 2003 and Merlin *et al.*, 2005). The population of humans suffering from this disorder is increasing at epidemic rate and about 366 million people are likely to become diabetic by the year 2030 (Wild *et al.*, 2004). Management of diabetes is still a challenging task. According to recommendations by the WHO expert committee on diabetes mellitus, investigations on hypoglycemic agents from medicinal plants have become important (Viturro *et al.*, 1999). Medicinal plant extracts may have adjuvant effects to existing antidiabetic agents. Many compounds isolated from these plants are used in combinational therapy for diabetes (Srivastava *et al.*, 2012).

*Bougainvillea spectabilis* belonging to the family Nyctaginaceae. The genus *Bougainvillea* of flowering plants native to South America from Brazil west to Peru and south to southern Argentina. The leaves of this genus are reported to have medicinal properties *viz.* antiviral, antibacterial, anti-inflammatory, larvicidal and antifertility potential (Joshi *et al.*, 1984; Andrea *et al.*, 1997 and Umamaheswari *et al.*, 2008). The alcoholic extract of the leaf has been reported to possess hypoglycemic effect and has been used for the management of diabetes mellitus. The hypoglycemic principle of the leaf extract has been isolated and named Pinitol (Bates *et al.*, 2000). Despite the isolation of the hypoglycemic principle, the crude extract is still being used for the management of diabetes mellitus in some local settings today.

*Catharanthus roseus* is known with various names (Madagascar periwinkle; *Vinca rosea; Lochnera rosea*) in India and all over world. *Catharanthus roseus* contains more than 400 known alkaloids, some of which are approved as antineoplastic agents to treat leukemia, Hodgkin’s disease, malignant lymphomas, neuroblastoma, rhabdomyosarcoma, Wilmer’s tumor and other cancers. Its vasodilating and memory enhancing properties have been shown to alleviate vascular dementia and Alzheimler’s disease (Hindmarch *et al.*, 1991 and Fishhof
et al., 1996). Water decoction of the leaves and/or the whole plant is used as household remedy for diabetes in several countries viz. Brazil, Cook Islands, Dominica, England, Jamaica, Pakistan, Taiwan and West Indies (Don, 1999).

The major objective of this investigation was to study the antihyperglycemic activity and hematological alterations due to the methanolic extract of combination of Bougainvillea spectabilis and Catharanthus roseus leaves (1:1) in Alloxan-induced diabetic rats.

MATERIALS AND METHODS
Collection of Plant and Preparation of Extract
The leaves of Bougainvillea spectabilis and Catharanthus roseus were collected during the month of March and April 2013 from Bundelkhand University Campus, Jhansi, U.P. Medicinal were subsequently authenticated and identified in the Ayurveda Research Institute Jhansi, U.P. The freshly collected leaves were air dried under shade at room temperature for 7 days. Drying the plant materials were pounded separately using mortar and pestle into smaller particles and grounded to fine powder using an electric blender. The powdered material of the both plants in the ratio of 1:1 was extracted with methanol in a Soxhlet apparatus and evaporated to dryness yielding a semisolid crude extract. This crude extract was weighted and used in preparation of doses.

Experimental Animals
The study was conducted in sexually mature, male albino rats of Wistar strain (200±10g), purchased from DRDE (Defense Research Development Establishment) Gwalior. Prior to study, the ethical clearance was obtained from the Institutional Animal Ethical Committee (BU/Pharm/IAEC/12/34) New Delhi. All the protocols and the experiments were conducted in strict compliance with the ethical principles and guidelines provided by Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA). The animals were acclimatized to the experimental room at temperature of 25-30°C, controlled humidity conditions (50-55%) and 12 hours light and 12 hours dark cycle. They were fed with rat pelleted diet (Amrut Feeds, Pranov Agro Ltd. Sangli) and water ad-libitum.

Induction of diabetes
Diabetes was induced in rats by single intraperitoneal injection of Alloxan (Alloxan monohydrate, CDH, Bombay Ltd.). Alloxan monohydrate was dissolved in ice-cold physiological saline (0.9% NaCl) to constitute a 10% (w/v) solution and a dose of 100 mgkg⁻¹
b.wt. of rat was selected to induce diabetes. The fasting blood glucose level of rats was measured after 72 hours of Alloxan injection using One Touch® glucometer (Lifescan, Johnson & Johnson, California). The rats with effective and permanent elevated blood glucose levels (above 200 mgdl⁻¹) were selected for the study.

**Experimental Design**
The research work was carried out for 4 weeks. In the first week diabetes was induced in rats and rats were allowed to acclimatize to laboratory environment. The following 3 weeks were investigational period with crude methanolic extract of *Bougainvillea spectabilis* and *Catharanthus roseus* leaves. Twenty-four rats were grouped into four groups of six rats each, in following experimental design.

Group I: Normal control.

Group II: Diabetic control.

Group III: Diabetic; will receive plant extract at 300mgkg⁻¹ b.wt.

Group IV: Diabetic; will receive standard drug, Glibenclamide at 5mgkg⁻¹ b.wt.

After daily administration of doses for the intervals of 7, 14 and 21 days, anesthesia was given to animals by using diethyl ether. The blood samples were collected from each rat by puncturing the optical vein of rat eye *i.e.* Retro-Orbital Plexus with the help of capillaries. The collected blood samples were analyzed for following parameters.

**Clinical Chemistry**
(a) Blood Sugar (Accu-Chek Glucometer).
(b) SGOT and SGPT (Reitman & Frankel Method, 1957),

**Haematocellular Components**
(c) Red Blood Cell Count (Naubaur’s Chamber).
(d) White Blood Cell Count (Naubaur’s Chamber).
(e) Haemoglobin Percentage (Sahli’s Apparatus).
(f) Packed Cell Volume (Wintrobes and Landsberg, 1935).

**Statistical analysis**
The results were expressed as Mean ± S.E. significance of differences compared to the control was determined using student’s t-test.
RESULTS AND DISCUSSION

The blood glucose levels of four groups of experimental albino rats at the end of defined treatment durations are shown in table I. During daily co-administration of methanolic extract of Bougainvillea spectabilis and Catharanthus roseus leaves at a dose of 300 mg kg\(^{-1}\) b.wt. and standard drug, Glibenclamide at a dose of 5 mg kg\(^{-1}\) b.wt. for 7, 14 and 21 days respectively, the blood glucose level decreased significantly as compared to the diabetic control group. The reduction is blood glucose levels of diabetic rats treated with combination of Bougainvillea spectabilis and Catharanthus roseus extract for was 41.54% while reduction in blood glucose levels of diabetic rats treated with standard drug Glibenclamide was 54.09%, compared with their respective diabetic controls at the end of 21 days (p<0.05). Jawla et al., (2011) reported the similar results by the administration of the root bark of Bougainvillea spectabilis at different doses and intervals. Highest hypoglycemic activity was observed with root bark extract at 100 mg kg\(^{-1}\) day\(^{-1}\) after 7 days.

In the present study, RBC count, Hb% and PCV decreased significantly due to administration of Alloxan in albino Wistar rats as compared to normal control group. The occurrence of anemia in diabetes mellitus has been reported due to the increased non-enzymatic glycosylation of RBC membrane proteins (Oyedemi et al., 2011). Oxidation of these proteins and hyperglycemia in diabetes mellitus causes an increase in the production of lipid peroxides that lead to haemolysis of RBC (Arun et al., 2002). During daily co-administration of methanolic extract of Bougainvillea spectabilis and Catharanthus roseus leaves at a dose of 300 mg kg\(^{-1}\) b.wt and standard drug, Glibenclamide at a dose of 5 mg kg\(^{-1}\) b.wt. for 7, 14 and 21 days respectively, the RBC count, Hb% and PCV increased significantly as compared to the diabetic control group and recouped towards the normal group (Table III).

WBC play vital role in body’s immune system and protect the body from invading foreign substances. In the present study, WBC count increased significantly due to administration of Alloxan in albino Wistar rats as compared to normal control group. During daily co-administration of methanolic extract of Bougainvillea spectabilis and Catharanthus roseus leaves at a dose of 300 mg kg\(^{-1}\) b.wt. and standard drug, Glibenclamide at a dose of 5 mg kg\(^{-1}\) b.wt. for 7, 14 and 21 days respectively, the WBC count decreased significantly as compared to the diabetic control group (Table II). The present study revealed the WBCs count in diabetic group showed a significant increase. This increase in WBCs count may be due to the increased hemopoitic activity as a result of the hemolysis of RBCs in diabetic rats and may be
a response to stressful condition after antigen (Alloxan) injection (Sovneyl et al., 1990).

Furthermore, the increase in WBC in the diabetic group is ameliorated by the co-administration of *Bougainvillea spectabilis* and *Catharanthus roseus* extract. Therefore, the plant extract could reduce the destructive effect of Alloxan-induced diabetes. This indicates that the treatment with plant extract affects the defense mechanism and immune response to inhibit the inflammation resulting from Alloxan-induced diabetes (Shakoori et al., 1992).

Alanine amino transferases are ectoplasmic enzymes found in very low concentration in the liver which are released into plasma following hepatocellular damage (James et al., 2007). SGOT and SGPT level increased significantly due to administration of Alloxan in albino Wistar rats as compared to normal control group. During daily co-administration of methanolic extract of *Bougainvillea spectabilis* and *Catharanthus roseus* leaves at a dose of 300 mgkg\(^{-1}\) b.wt and standard drug, Glibenclamide at a dose of 5 mgkg\(^{-1}\) b.wt for 7, 14 and 21 days respectively, the SGOT and SGPT level decreased significantly as compared to the diabetic control group and recouped towards the SGOT and SGPT level of normal control group (Table III).

Table. I: Effect of combination of *Bougainvillea spectabilis* and *Catharanthus roseus* extract on Blood Glucose Levels of diabetic albino rats.

<table>
<thead>
<tr>
<th>Duration of Treatment</th>
<th>Normal Control</th>
<th>Diabetic Control</th>
<th>Diabetic + Plant Extract (300mgkg(^{-1}) b.wt.)</th>
<th>Diabetic + Glibenclamide (5mgkg(^{-1}) b.wt.)</th>
</tr>
</thead>
<tbody>
<tr>
<td>7 DAYS</td>
<td>93.83±1.49</td>
<td>256.33±1.24*</td>
<td>190.00±2.83*</td>
<td>141.83±2.45*</td>
</tr>
<tr>
<td>14 DAYS</td>
<td>94.33±1.12</td>
<td>261.50±0.93*</td>
<td>164.83±3.96*</td>
<td>126.83±0.86*</td>
</tr>
<tr>
<td>21 DAYS</td>
<td>94.16±0.43</td>
<td>267.30±1.53*</td>
<td>138.66±3.07*</td>
<td>106.50±3.12*</td>
</tr>
</tbody>
</table>

Values are given as Mean ± SE of 6 animals in each group.*Values are statistically significant at p<0.05.

Table. II: Effect of combination of *Bougainvillea spectabilis* and *Catharanthus roseus* extract on Heamatological parameters of diabetic albino rats.

<table>
<thead>
<tr>
<th>Groups</th>
<th>Duration of Treatment</th>
<th>RBC(million mm(^{-3}))</th>
<th>Hb (g dl(^{-1}))</th>
<th>PCV (%)</th>
<th>WBC (1000 mm(^{-3}))</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal Control</td>
<td>7 Days</td>
<td>8.8±0.2</td>
<td>14.1±0.04</td>
<td>40.2±0.1</td>
<td>8.6±0.2</td>
</tr>
<tr>
<td></td>
<td>14 Days</td>
<td>8.9±0.4</td>
<td>14.3±0.11</td>
<td>40.5±0.3</td>
<td>8.7±0.3</td>
</tr>
<tr>
<td></td>
<td>21 Days</td>
<td>8.7±0.2</td>
<td>13.9±0.05</td>
<td>40.4±0.1</td>
<td>8.5±0.2</td>
</tr>
<tr>
<td>Diabetic Control</td>
<td>7 Days</td>
<td>7.2±0.5</td>
<td>11.2±0.02*</td>
<td>33.4±0.4*</td>
<td>16.6±1.1*</td>
</tr>
<tr>
<td></td>
<td>14 Days</td>
<td>7.0±0.1*</td>
<td>10.8±0.03*</td>
<td>32.9±0.1*</td>
<td>16.5±0.4*</td>
</tr>
<tr>
<td></td>
<td>21 Days</td>
<td>6.9±0.2*</td>
<td>10.5±0.12*</td>
<td>32.8±0.2*</td>
<td>16.7±0.2*</td>
</tr>
</tbody>
</table>
Values are given as Mean ± SE of 6 animals in each group. *Values are statistically significant at p<0.05.

Table. III: Effect of combination of Bougainvillea spectabilis and Catharanthus roseus extract on SGOT and SGPT of diabetic albino rats.

<table>
<thead>
<tr>
<th>Groups</th>
<th>Duration of Treatment</th>
<th>Liver Enzymes</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>SGOT (IU L⁻¹)</td>
</tr>
<tr>
<td>Normal Control</td>
<td>7 Days</td>
<td>42.52±1.5</td>
</tr>
<tr>
<td></td>
<td>14 Days</td>
<td>44.15±1.8</td>
</tr>
<tr>
<td></td>
<td>21 Days</td>
<td>40.00±0.9</td>
</tr>
<tr>
<td>Diabetic Control</td>
<td>7 Days</td>
<td>69.61±20*</td>
</tr>
<tr>
<td></td>
<td>14 Days</td>
<td>72.50±1.8*</td>
</tr>
<tr>
<td></td>
<td>21 Days</td>
<td>70.12±1.5*</td>
</tr>
<tr>
<td>Diabetic+ Plant Extract</td>
<td>7 Days</td>
<td>51.15±0.5</td>
</tr>
<tr>
<td>(300mg kg⁻¹ b.wt.)</td>
<td>14 Days</td>
<td>45.52±1.2*</td>
</tr>
<tr>
<td></td>
<td>21 Days</td>
<td>40.02±0.6*</td>
</tr>
<tr>
<td>Diabetic+ Glibenclamide (5mg kg⁻¹ b.wt.)</td>
<td>7 Days</td>
<td>50.34±1.0</td>
</tr>
<tr>
<td></td>
<td>14 Days</td>
<td>44.20±1.1*</td>
</tr>
<tr>
<td></td>
<td>21 Days</td>
<td>39.42±1.0*</td>
</tr>
</tbody>
</table>

CONCLUSION

India is a developing country with a large portion of people suffering from diabetes mellitus and its complications. As a large section of the population dwell with poor health infrastructures. The high costs for therapeutic treatment has compelled to look for alternative and cost effective methods to minimize complications associated with diabetes. Therefore, medicinal plants and their products are more convenient for the treatment of diabetes mellitus due to their easy availability, low cost, minimum side effects and greater acceptance amongst the users. This study indicated use of methanolic extracts of combination of Bougainvillea spectabilis and Catharanthus roseus leaves exhibited significant antihyperglycemic effect and also possessed beneficial effects on hematological parameters in diabetic rats.
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REFERENCES


