ANTIDIABETIC POTENTIAL OF ETHANOLIC EXTRACTS OF *CITRUS MAXIMA* FRUIT PEEL AND *ANVILLEA GARCINII*

Kharjul Mangesh1*, Gali Vidyasagar2, Kharjul Ashwini3

1Research Scholar, Department of Pharmacy, JJT University, Jhunjhunu- 333001, Rajasthan,  
2Professor & Principal, Veerayatan Institute of Pharmacy, Jakhaniya, Gujarat  
3Dept. of Pharmacognosy, Shivajirao S. Jondhle College of Pharmacy, Asangaon, Maharashtra

Abstract:
This study was designed to assess the effect of ethanolic extract of fruit peels of *Citrus maxima* & ethanolic extract of dried whole plant of *Anvillea garcinii* on streptozotocin-induced diabetic rats and glucose tolerance test. Experiment was performed as described in the standard bibliography, literatures and text books. The reputed journals and publications are obtained from college library and through web search. Streptozotocin (STZ) injected intravenously (65mg/kg) into the tail vein of the rat under nitrous oxideranesthesia to induce diabetes. Animals will be randomly divided into 5 groups of 6 each and assigned as Group 1: Normal control (normal saline), Group 2: Diabetic control (Streptozotocin 65mg/Kg), Group 3: Diabetic + *Citrus maxima* (200mg/Kg), Group 4: Diabetic + *Anvillea garcinii*(300mg/Kg), Group 5: Diabetic + Glibenclamide (500mcg/Kg). After every 24 hours the body weight was measured & blood glucoses were measured. Serum was isolated from the blood collected on 40th day of experimental work and serum total cholesterol (TC), triglyceride (TG) and HDL-cholesterol were estimated by using diagnostic kits. The oral glucose tolerance test was performed in overnight fasted normal animals. Ethanolic extracts of *Citrus maxima* fruit peel & *Anvillea garcinii*, significantly shown their antidiabetic potential by decreasing blood glucose levels and maintaining body weight and serum lipid concentrations to normal. Though the ethanolic extracts demonstrated hypo glycemic and lipid lowering activity, still there is study warranted for correlating the mechanisms of glycemic control (involving receptor level) and phytochemical constituents (focusing on active antidiabetic contents).

Key words: Diabetes, glucose tolerance, serum lipid, ethanolic extract, *Citrus maxima* fruit peel, *Anvillea garcinii*, Glibenclamide

INTRODUCTION
A complex pathophysiology that combines progressive insulin resistance and β-cell failure and has a heritable basis is characteristic of type 2 diabetes mellitus (T2DM). Diabetes also can be related to the gestational hormonal environment, genetic defects, other infections, and certain drugs.1 Symptoms of diabetes include excess urine, hunger, thinning, thirst & blurred vision. The plasma insulin concentration is insufficient to control normal glucose homeostasis due to insulin resistance in peripheral tissue and an insulin secretory defect of the beta cell.

Corresponding Author*
Kharjul Mangesh
which characterises type 2 diabetes mellitus (formerly called NIDDM, type II or adult-onset). \[2\] Insulin resistance related to intra-abdominal (visceral) obesity seen in T2DM along with hypertension and dyslipidemia (high triglyceride and low HDL-cholesterol levels; postprandial hyperlipidemia). T2DM is common form of diabetes mellitus, highly associated with a family history of diabetes, older age, obesity and lack of exercise. It is more common in women, especially women with a history of gestational diabetes, and in Blacks, Hispanics and Native Americans. Impaired glucose tolerance is result of insulin resistance and hyperinsulinemia.

**Glibenclamide:**

It is popular anti-diabetic drug from sulfonylurea class used for treatment of T2DM. It acts by inhibiting ATP-sensitive potassium channels in pancreatic beta cells. This leads to insulin release as a result of cell membrane depolarization, opening of voltage-dependent calcium channels and triggering an increase in intracellular calcium into the beta cell.\[3\] Glibenclamide is 100 folds more potent than Tolbutamide, hence may cause severe hypoglycemia and may lead to even coma state.\[4\] Advanced studies show that it is associated with a marginal decrease in circulating lipids. Also cause rare side effects like weight gain due to fluid retention and edema, photosensitization reaction, cholestatic jaundice.\[5\]

**Anvillea garcinii**

Anvillea garcinii sp. radiata Coss.etDur.is a small woody shrub, densely branched, 20-50 cm. high. The leaves are green-grey, small, roughly triangular, with a large petiole and strongly toothed limb. The big solitary capitules have a diameter of 3-5 cm, with long ligules. The flowers are all yellow orange, the outside ones 25 mm. long. The fruit is a non-fleshy; indehiscent prismatic cypsela (‘achene’ from two carpels). It usually flowers in spring, but can flower throughout the year. Traditionally this plant is taken for colds, digestive problems, gastro-intestinal troubles, indigestion, pulmonary affections. Natural habitat is North Africa & Endemic of Sahara. The dried plant, crushed and mixed with either olive oil, honey or crushed dates (to mask the bitterness) is good for colds.\[6\]

**Citrus maxima**

Citrus maxima have been recommended in traditional herbal medicine as the source of diabetic medication or remedy for diabetes. The plant Citrus maximum is well recognized for their various ethno medicinal uses. These properties are attributed to their various chemical constituents. Citrus fruits contain flavonoids and limonoids which are proven to possess anti-inflammatory and antitumor effects. The antihyperglycemic effect and anti-oxidant property of C. maxima leaf in established. Fruit juice of Shaddock (C.maxima) possess hypoglycemic and hypocholesterolemic activities.\[7\]

This study was designed to assess the effect of ethanolic extract of fruit peels of Citrus maxima & ethanolic extract of dried whole plant of Anvillea garcinii on streptozotocin-induced diabetic rats and glucose tolerance test.

**MATERIALS AND METHODS**

**Source of Data**
Experiment was performed as described in the standard bibliography, literatures and text books. The reputed journals and publications are obtained from college library and through web search.

**Preparation of crude extract: Citrus maxima crude extract**
The dried fruit peels of *Citrus maxima* were grinded to fine powder. 100 g powder mixed with 500 ml of distilled water, allowed to stand overnight at room temperature. Then mixture was filtered through (Whatman No. 1) and the filtrate was evaporated to dryness at 40°C in evaporator. The crude extract was prepared by redissolving the filtrate in distilled water at concentration of 0.2 g/ml.

**Anvillea garcinii crude extract**
The dried whole plant of *Anvillea garcinii* was grinded to fine powder. 100 g powder mixed with 500 ml of distilled water, allowed to stand overnight at room temperature. Then mixture was filtered through (Whatman No. 1) and the filtrate was evaporated to dryness at 40°C in evaporator. The crude extract was prepared by redissolving the filtrate in distilled water at concentration of 0.3 g/ml.

**Preparation of the ethanolic extracts**

**Ethanolic extract of Citrus maxima**
Mixture of absolute ethanol (80 ml) and 14 g dried crude extract kept overnight in dark place. The resultant mixture was filtered and filtrate was evaporated to dryness at 40°C. 2 g of dry material was found and redissolved in absolute ethanol (0.3 g/ml).

**Ethanolic extract of Anvillea garcinii**
Mixture of absolute ethanol (80 ml) and 14 g dried crude extract kept overnight in dark place. The resultant mixture was filtered and filtrate was evaporated to dryness at 40°C. 2 g of dry material was found and redissolved in absolute ethanol (0.3 g/ml).

**Animals**
Wistar albino rats (180-200 g) of either sex will be used for the study. They will be maintained under standard conditions (temperature 22 ± 2°C, relative humidity 50±5% and 12 h light/dark cycle) and have free access to standard pellet diet and water ad libitum. All experimental protocols will be reviewed and approved by the Institutional Animal Ethical Committee (IAEC) prior to the initiation of the experiment and the care of the laboratory animals will be taken as per the CPCSEA regulations.

**Chemicals**
Streptozotocin was procured from Loba chemie, Mumbai, India. Aventis pharma Ltd, Goa, India sourced Glibenclamide. Glucose oxidase-peroxidase strip (Accue-check® diagnostic kit) used for estimation of Blood Glucose level. Diagnostic kits (Erba Mannheim Cholesterol kit, Transasia Bio-Medicals Ltd., Daman) used for estimation of Serum total cholesterol (TC), triglyceride (TG) and High density lipoprotein (HDL)-cholesterol levels. All the chemicals used were of analytical grade.

**Induction of diabetes mellitus**
Being more selectively toxic to beta-cell than alloxan the streptozotocin (STZ) was used for induction of diabetes mellitus in experimental animals. STZ is unstable in solution, it was dissolved in distilled water (at 65 mg/ml) immediately before use and then injected intravenously (65 mg/kg) into the tail vein of the rat under...
halothane/nitrous oxide anaesthesia. The animals were replaced in ordinary cages and left for three days for recovery and development of the symptoms of diabetes mellitus before use. After 72 hours of STZ injection, blood samples will be taken and glucose estimation is done. Animals having fasting blood glucose level more than 250mg/dl will be chosen for studies. In spite of diabetes developed after 72h of STZ injection, the animal will be stabilized up to 6 days. The treatment will be started from 7th day of STZ injection once daily post orally for next 30 days. Glibenclamide (500 mcg/kg in 0.5 ml 5% tween 80) was used as reference standard. The dose of Glibenclamide was selected based on previous reports. A preliminary toxicity study was designed to demonstrate the appropriate safe dose range that could be used for subsequent experiments. Dose of Citrus maxima (200mg/Kg) & Anvillea garcinii (300mg/kg) was selected by carrying out the acute toxicity study (staircase method). Extracts were given once daily orally. Following five groups of rats (n = 6) were taken.

**Experimental Design:**
Animals will be randomly divided into 5 groups of 6 each and assigned as below.

- **Group 1:** Normal control (normal saline).
- **Group 2:** Diabetic control (Streptozotocin 65mg/Kg).
- **Group 3:** Diabetic + Citrus maxima(200mg/Kg) [D + CM 200mg/Kg]
- **Group 4:** Diabetic + Anvillea garcinii(300mg/Kg) [D + AG 300mg/Kg]
- **Group 5:** Diabetic + Glibenclamide (500mcg/Kg) [D + GB 500mcg/Kg]

Every 24 hours the Body weight was measured & biochemical parameters were measured as given below.

**Biochemical Estimation**
Blood glucose (FBG) concentration of all the four experimental groups was determined by glucometer during different phases of the experiment. Serum was isolated from the blood collected on 40th day of experimental work and serum total cholesterol (TC), triglyceride (TG) and HDL-cholesterol were estimated by using diagnostic kits. Very low density lipoprotein (VLDL) and low density lipoprotein (LDL) cholesterol were calculated as per Friedewald’s equation i.e.

\[ \text{VLDL - Cholesterol} = \frac{\text{Serum triglyceride}}{5} \]

And \[ \text{LDL-cholesterol} = \text{Serum total-cholesterol} - \text{VLDL cholesterol} - \text{HDL cholesterol}. \]

Results were expressed in mg/dl.

**Oral Glucose Tolerance Test (Oggt) In Rats**
The oral glucose tolerance test was performed in overnight fasted normal animals. Rats divided into four groups were administered 2% gum acacia solution, ethanolic extract of Citrus maxima (200 mg/kg), ethanolic extract of Anvillea garcinii(300mg/Kg) and glibenclamide (0.25 mg/kg), orally, respectively. Glucose (2 g/kg) was fed 30 minutes after the administration of samples. Blood was withdrawn from the retro-orbital sinus at 0, 30, 60, 90 and 120 min of samples administration.
glucose levels were estimated by glucose oxidase-peroxidase reactive strips.

**Experimental Design:**
The animals will be randomly divided into 4 groups of 6 each and assigned as below

- **Group 1:** Glucose control.
- **Group 2:** Glucose load + Citrus maxima (200mg/Kg) [Glc + CM 200mg/Kg]
- **Group 3:** Glucose load+ Anvillea garcinii (300mg/Kg) [Glc + AG 300mg/Kg]
- **Group 4:** Glucose load+ Glibenclamide (500mcg/Kg) [Glc + GB 500mcg/Kg]

**EVALUATION:**
The blood glucose concentration will be determined at 0, 30, 60, 90, and 120 min after dosing.

**STATISTICAL ANALYSIS:**
The data will be expressed as mean ± Standard Error of Mean (SEM). Statistical analysis was performed using one-way Analysis of Variance (ANOVA) and student’s ‘t’-test. The values of P < 0.05 were considered as statistically significant.

**Has Ethical Clearance Been Obtained From Your Institution?**
**Yes.** Ethical clearance has been obtained.
Ref: VIP/GVS/B.PHARM/2012-13/581.
Resolution No.: VIP/IAEC/2012-13/251

**RESULTS**
In the study of STZ induced diabetes mellitus in rats, oral OD treatment with *Citrus maxima* (200mg/Kg) & *Anvillea garcinii* (300mg/Kg) significantly (P< 0.05) improves body weight (Table 01 & Figure 01). Significant (P< 0.05) decrease in blood glucose level (Table 02 & Figure 02) was observed after oral OD treatment with *Citrus maxima* (200mg/Kg) & *Anvillea garcinii* (300mg/Kg) in STZ induced diabetes mellitus rats as well as significantly altered the serum lipid concentrations (TC= P< 0.001, TG= P< 0.01, HDL= P< 0.01, VLDL= P< 0.01) (Table 03 & Figure 03) were also observed. Oral glucose tolerance (OGTT) test in rats demonstrated significantly decreased blood glucose concentration after oral treatment with *Citrus maxima* (200mg/Kg) & *Anvillea garcinii* (300mg/Kg). All results compared with Glibenclamide (500mcg/Kg) as standard antidiabetic/hypoglycemic agents.

**DISCUSSION**
Being more selectively toxic to beta-cell than alloxan the streptozotocin (STZ) was used for induction of diabetes mellitus in experimental animals. Hence STZ induced diabetes mellitus in rats is a useful experimental model to study the activity of hypoglycemic agents. Free radical formation is enhanced by STZ affecting antioxidant defense system resulting in hyperglycemia due to destruction of beta cells of pancreas.[14] Ethanolic extract of *Citrus maxima* fruit peel & *Anvillea garcinii* demonstrated their antidiabetic potential in STZ diabetic rats by significantly decreasing blood glucose levels and significantly improving body weights in diabetic rats. In Oral glucose tolerance (OGTT) test in rats extract treatment was found to be significantly beneficial for decreasing blood glucose levels. Present study demonstrated serum lipid lowering activity of *Citrus maxima* & *Anvillea garcinii* which can be compared to the findings of Perez et al.,[15] who reported significant lipid
lowering activity of extract of *Ficus carica* (fig tree) leaves in STZ diabetic rats.

**CONCLUSION**

Though the ethanolic extracts demonstrated hypoglycemic and lipid lowering activity, still there is study warranted for correlating the mechanisms of glycemic control (involving receptor level) and phytochemical constituents (focusing on active antidiabetic contents).

**ACKNOWLEDGEMENT**

We thank Management of Veerayatan Institute of Pharmacy, Jakhaniya, Kutch, Gujarat, India, for providing required research work facilities. We specifically acknowledge to the contribution of Department of Pharmacognosy, Shivajirao S. Jondhle College of Pharmacy, Asangaon, Thane, Maharashtra, India, for preparation of plant extracts.

**REFERENCES:**

12. Bhavana S, Santosh K, Satapathy N. Hypoglycemic and hypolipidemic effect of Aegle marmelos leaf extract on STZ


Table 01: Effect of ethanolic extract of Citrus maxima (200mg/Kg) & Anvillea garcinii (300mg/Kg) treatment on body weight (Mean ± SEM) comparative data

<table>
<thead>
<tr>
<th>Group</th>
<th>Treatment</th>
<th>Body weight (g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Normal Control</td>
<td>193.28 ± 2.31</td>
</tr>
<tr>
<td>2</td>
<td>Diabetic Control</td>
<td>149.55 ± 0.90***</td>
</tr>
<tr>
<td>3</td>
<td>D + CM 200mg/Kg</td>
<td>205.09 ± 2.16*</td>
</tr>
<tr>
<td>4</td>
<td>D + AG 300mg/Kg</td>
<td>204.22 ± 2.99*</td>
</tr>
<tr>
<td>5</td>
<td>D + GB 500mcg/Kg</td>
<td>207.43 ± 2.57*</td>
</tr>
</tbody>
</table>

n=6, all the values are in MEAN ± SEM, all the data were subjected to one-way ANOVA followed by Students ‘t’ test, *P< 0.05, **P < 0.01, ***P < 0.001 as compared with diabetic control. D = diabetic, CM = Citrus maxima, AG= Anvillea garcinii, GB = Glibenclamide.
Table 02: Effect of ethanolic extract of Citrus maxima (200mg/Kg) & Anvillea garcinii (300mg/Kg) treatment on rat blood glucose level (mg/dl) in STZ induced diabetes mellitus Comparative data of blood glucose level (mg/dl) in rats under study

<table>
<thead>
<tr>
<th>Group</th>
<th>Treatment</th>
<th>Day 1</th>
<th>Day 10</th>
<th>Day 20</th>
<th>Day 30</th>
<th>Day 40</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Normal Control</td>
<td>90.33 ± 1.02</td>
<td>91.33 ± 0.84</td>
<td>89.66 ± 1.80</td>
<td>89.00 ± 1.29</td>
<td>90.33 ± 0.88</td>
</tr>
<tr>
<td>2</td>
<td>Diabetic Control</td>
<td>294.83 ± 2.63</td>
<td>392.00 ± 2.43</td>
<td>416.66 ± 2.33</td>
<td>384.00 ± 0.57</td>
<td>384.33 ± 1.05</td>
</tr>
<tr>
<td>3</td>
<td>D + CM 200mg/Kg</td>
<td>296.83 ± 3.91</td>
<td>266.50 ± 3.19*</td>
<td>238.00 ± 2.39*</td>
<td>214.00 ± 1.39*</td>
<td>200.16 ± 0.70*</td>
</tr>
<tr>
<td>4</td>
<td>D + AG 300mg/Kg</td>
<td>291.50 ± 2.98</td>
<td>264.66 ± 2.70*</td>
<td>240.66 ± 1.54*</td>
<td>213.66 ± 3.39*</td>
<td>203.66 ± 1.64*</td>
</tr>
<tr>
<td>5</td>
<td>D + GB 500mcg/Kg</td>
<td>292.66 ± 3.27</td>
<td>233.83 ± 2.98**</td>
<td>191.33 ± 2.30**</td>
<td>177.16 ± 0.60**</td>
<td>139.33 ± 0.80**</td>
</tr>
</tbody>
</table>

n=6, all the values are in MEAN ± SEM, all the data were subjected to one-way ANOVA followed by Students ‘t’ test, *P < 0.05, **P < 0.01, ***P < 0.001 as compared with diabetic control. D = diabetic, CM = Citrus maxima, AG= Anvillea garcinii, GB = Glibenclamide

Table 03: Effect of ethanolic extract of Citrus maxima (200mg/Kg) & Anvillea garcinii (300mg/Kg) treatment on rat serum lipid concentrations in STZ induced diabetes mellitus Comparative data of serum lipid concentration (mg/dl) in rats under study

<table>
<thead>
<tr>
<th>Group</th>
<th>Treatment</th>
<th>TC</th>
<th>TG</th>
<th>HDL</th>
<th>LDL</th>
<th>VLDL</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Normal Control</td>
<td>90.66 ± 1.17</td>
<td>77.83 ± 1.74</td>
<td>31.83 ± 0.79</td>
<td>50.16 ± 0.70</td>
<td>19.83 ± 1.13</td>
</tr>
<tr>
<td>2</td>
<td>Diabetic Control</td>
<td>219.73 ± 0.87</td>
<td>165.50 ± 1.83</td>
<td>16.16 ± 0.94</td>
<td>171.33 ± 2.55</td>
<td>37.83 ± 1.57</td>
</tr>
<tr>
<td>3</td>
<td>D + CM 200mg/Kg</td>
<td>101.50 ± 2.07***</td>
<td>84.33 ± 1.97**</td>
<td>42.16 ± 1.70</td>
<td>55.50 ± 2.48**</td>
<td>18.50 ± 0.92**</td>
</tr>
<tr>
<td>4</td>
<td>D + AG 300mg/Kg</td>
<td>106.33 ± 2.95***</td>
<td>82.00 ± 2.60**</td>
<td>37.16 ± 1.07</td>
<td>49.33 ± 1.33**</td>
<td>21.16 ± 1.13**</td>
</tr>
<tr>
<td>5</td>
<td>D + GB 500mcg/Kg</td>
<td>87.00 ± 2.12***</td>
<td>62.83 ± 2.18***</td>
<td>50.66 ± 3.08</td>
<td>21.66 ± 1.92***</td>
<td>16.00 ± 1.65**</td>
</tr>
</tbody>
</table>

n=6, all the values are in MEAN ± SEM, all the data were subjected to one-way ANOVA followed by Students ‘t’ test, *P *< 0.05, *P < 0.01, ***P < 0.001 as compared with diabetic control. D = diabetic, CM = Citrus maxima, AG= Anvillea garcinii, GB = Glibenclamide, TC = total cholesterol, TG = triglyceride, HDL = high density lipoproteins, LDL = low density lipoproteins, VLDL = very low density lipoproteins
Figure 01: Effect of ethanolic extract of Citrus maxima (200mg/Kg) & Anvillea garcinii (300mg/Kg) treatment on rat body weight in STZ induced diabetes mellitus, all the data were subjected to one-way ANOVA followed by Students ‘t’ test, *P< 0.05, **P < 0.01, ***P < 0.001 as compared with diabetic control. (D = diabetic, CM = Citrus maxima, AG= Anvillea garcinii, GB = Glibenclamide)
Figure 02: Effect of ethanolic extract of Citrus maxima (200mg/Kg) & Anvillea garcini (300mg/Kg) treatment on rat blood glucose level (mg/dl) in STZ induced diabetes mellitus, all the data were subjected to one-way ANOVA followed by Students ‘t’ test, *P< 0.05, **P < 0.01, ***P < 0.001 as compared with diabetic control. (D = diabetic, CM = Citrus maxima, AG = Anvillea garcini, GB = Glibenclamide)
Figure 03: Effect of ethanolic extract of Citrus maxima (200mg/Kg) & Anvillea garcinii (300mg/Kg) treatment on rat serum lipid concentrations (mg/dl) in STZ induced diabetes mellitus, all the data were subjected to one-way ANOVA followed by Students ‘t’ test, *P < 0.05, **P < 0.01, ***P < 0.001 as compared with diabetic control. (D = diabetic, CM = Citrus maxima, AG= Anvillea garcinii, GB = Glibenclamide)