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# Comparative study of the hypoglycemic and biochemical effects of C*atharanthus roseus* (Linn) g. apocynaceae (Madagascar periwinkle) and chlorpropamide (diabenese) on alloxan-induced diabetic rats

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## Abstract

The effect of the aqueous extracts of *Catharanthus roseus* and chlorpropamide (Diabenese) on the levels of serum cholesterol, total protein, lipid peroxidation, blood glucose and liver enzymes were compared in alloxan-induced diabetic rats. Four groups namely A, B, C and D comprising of nine rats each were used. A and B were administered with chlorpropamide and *C. roseus* extracts respectively, while C and D served as diabetic and non-diabetic controls respectively. The results showed comparatively significant reductions ($P \leq 0.05$) in the levels of glucose, protein, cholesterol, lipid peroxidation and liver enzymes in the groups administered *C. roseus* extracts and chlorpropamide relative to the controls. The reductions were higher in the groups treated with *C. roseus* extract than in the groups treated with diabenese.

**Key words**: *Catharanthus roseus,* Diabenese, alloxan-induced diabetes

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## INTRODUCTION

Diabetes is not a single disease but a syndrome that is characterized by a total or relative lack of insulin leading to persistent elevation of blood glucose as well as alteration in lipid and protein metabolism[1].This syndrome can occur as a result of some secondary causes such as pancreatectomy and iron overload of beta-cells resulting from haemachromatosis. Other causes include excess cortisol production in Cushing's syndrome and excess growth hormone secretion in acromegaly and insulin-resistant syndrome[2].Two basic types of diabetes are common namely type 1 or insulin-dependent and type 2 or non-insulin-dependent diabetes[3]. Type 1 commonly seen in juveniles is characterized by failure to produce insulin due to autoimmune destruction of beta-cells of the pancreas while type 2 is usually adult-onset and is associated with insufficient production of insulin and loss of responsiveness by cells to insulin[4,5]. Insulin is pivotal to carbohydrate metabolism and controls the concentration of glucose in the blood via a feedback mechanism.

Diabetes is characterized by symptoms such as weakness, polyuria, excessive thirst as well as ketonemia, ketouria and ketosis due to altered metabolism of lipids and proteins. It is associated with abnormalities such as kidney failure, nervous defect, impotence, blindness, stroke and heart diseases. Abnormalities in lipid metabolism may contribute to excessive hepatic glucose through gluconeogenesis as well as abnormal drive from the autonomic nervous system.

Diabetes especially type 2 is difficult to treat and will continuously get worse with time. The supplemental use of insulin in controlling blood sugar is complex and involves accurate and timed injection of the hormone as food is ingested and digested and usually leads to treatment failures. Commonly used oral antiglycemic drugs used in the treatment of diabetes include the older sulfonylurea which act by stimulating production of insulin and the newer ones that act by increasing sensitivity of cells to insulin and prevents additional release of glucose by the liver and intestines. Chlorpropamide (1-{p-chlorophenyl-sulfonyl-3-propylurea, MW; 276.74) commercially sold as Diabenese is a sulfonamide derivative that occurs as a white crystalline solid insoluble in water but soluble in alcohol. It is rapidly absorbed and metabolized and excreted unchanged with a half life of 36 hours[6]. Its metabolism contributes to its high antidiabetic activity and minimal side effects compared to other antidiabetic drugs. However its pronounced accumulation due to its long elimination period could be a serious problem. The sulfonylurea moiety is responsible for its distribution and binding to beta-cell surface and insulin producing actions[7].

Treatment failure and side effects associated with oral hypoglycemic drugs have led to alternative forms of treatment and management of diabetes. Plants such as *Catharanthus roseus, Azadiracta indica, Ficus racemosa, Trignonella foenum* etc are now used in alternative therapy for diabetes as a result of their large content of bioactive substances such as glycosides, sterols, flavonoids, alkaloids, tannins, etc[8-10]. *C.roseus*, formerly known as *Vinca rosea* is the common or Madagascar periwinkle. It is a perennial evergreen herb of the family Apocynaceae originally native to Madagascar[11,12]. It grows to a height of two feet and has dark green glossy leaves and pale pink or white flowers. The organic extracts of *C. roseus* is used in the folklore treatment of diabetes, malaria, leukemia wasp stings, sore throat, eye irritation, infections and to stop bleeding[13]. It is also used a as an astringent, diuretic and expectorant. The plant contains about seventy alkaloids some of which include cartharathine, lochnenine, vindoline vindolinenine, vincristine, vinblastine, vindoline, tetrahydroalstronine, reserpinne, serpentine, etc[14].

Diabetes is assuming an increasing epidemic trend affecting about 10% of the world's population[15]. The consequent death and economic cost associated with diabetes is enormous and its treatment needs to be reviewed and improved. There is perceived failure of treating diabetes using drugs and emphasis is gradually shifting to use of plant products, diet management and exercise[16,17]. The objective of this study is geared in this direction by comparing the antidiabetic effects of

chlorpropamide and aqueous extracts of *C. roseus* instead of the organic extracts on alloxan-induced diabetic rats. The results will further substantiate or deny claims of the use of plants as better alternative therapies to drugs in the treatment and management of diabetes.

## MATERIALS AND METHODS

### Plant

The plant used in this study was collected from the stand at the courtyard around the Dean's office, Faculty of Biological and Physical Sciences, Abia State University, Uturu, Nigeria. The specimens were authenticated by Dr. C. U Okeke, a botanist in the Department of Botany, Abia State University, Uturu, Nigeria.

### Laboratory animals

A total of thirty six apparently healthy, male albino rats of about eight weeks old and weighing between130 ±30.5grams were purchased from the animal house of the Faculty of Pharmaceutical Sciences, University of Nigeria, Nsukka, Nigeria. The Animals were housed in clean metallic cages and kept in a well ventilated room. Their cages were cleaned every two days.

### Preparation of plant extract

200g of the fresh, dried leaves, flowers and tender stems of *C. roseus* were ground using a Gallenkamp electric blender. The sample was soaked in 200mls of distilled water for 12hours and thereafter filtered with a cheese cloth. The filtrate was concentrated using a rotary evaporator to obtain 20g powdered extract.

### Animal grouping and treatment

Thirty six albino rats were randomly assigned into four groups namely A, B, C, and D comprising of nine animals each. Animals in A, B and C were each given single doses of 70mg/ml of alloxan intraperitonealy to induce diabetes. Animals in group D were not injected with alloxan and served as non-diabetic control. Diabetes induction was confirmed by increased blood glucose in excess of 300mg/ml which occurred after four days of alloxan administration. Twenty four hours following the induction of diabetes, treatment with the plant extract and chlorpropamide commenced. Animals in group A were orally given 25mg/ml of chlorpropamide while the animals in group B received 25mg/ml plant extract by oral gavages. Animals in group C and D were neither treated with chlorpropamide nor plant extracts and served as diabetic and non-diabetic controls respectively. All the animals were fed *ad libitum* with the normal rat chow and water for the period the treatments and experiments lasted.

### Collection of blood and serum preparation

Blood samples were collected from the animals anaesthetized with chloroform through cardiac puncture using a hypodermic needle and syringe. The blood was collected 24hours, 72hours and 7days after the induction of diabetes. The serum was separated by centrifuging approximately 5ml each of the blood samples with an MSE table centrifuge (Minor Gallenkamp) at 4,000×g for 10 minutes. The serum obtained was used immediately for the estimation of blood glucose, total serum protein, serum cholesterol, lipid per oxidation and activities of serum glutamic pyruvate transaminase (SGPT) and serum glutamic oxaloacetate transaminase (SGOT) .

### Determination of biochemical parameters

The concentration of blood glucose was determined using the o-toludine method as previously described[18,19]. The serum cholesterol was determined using the Ilca's method as previously described[19]. Lipid per oxidation was estimated using the method as previously described[20]. The serum total protein was estimated using the Biuret method as previously described[21] and the SGPT activity was determined using the method as previously described[22] while the SGOT activity was also determined using the method as previously described[22].

### Statistical analysis

Data were expressed as mean ± standard error of mean. Statistical analyses were done by using the student t-test. Significance was checked at $P \leq 0.05$

## RESULTS

### Effects of plant extract and chlorpropamide (diabenese) on blood glucose

The plant extracts and chlorpropamide (diabenese) caused a reduction in the blood

**Table 1:** Effects of plant extract and chlorpropamide (diabenese) on blood glucose

| | Blood glucose level (mM/L) | | | |
|---|---|---|---|---|
| **Time (Hours/Days)** | **Diabenese Treated Group (A)** | **Plant extract Treated Group (B)** | **Diabetic Control Group (C)** | **Non diabetic Control Group (D)** |
| 24 hours | 12.35±1.1 | 10.98±1.6 | 13.45±2.1 | 9.02±0.3 |
| 3 days | 11.77±3.2 | 10.40±1.3 | 13.98±0.5 | 9.20±0.1 |
| 7 days | 10.31±2.1 | 9.71±0.5 | 14.75±0.1 | 9.37±0.6 |

TABLE 2: Effects of plant extract and chlorpropamide (diabenese) on serum cholesterol level

| | Serum cholesterol level (mM/L) | | | |
|---|---|---|---|---|
| **Time (Hours/Days)** | **Diabenese Treated Group (A)** | **Plant extract Treated Group (B)** | **Diabetic Control Group (C)** | **Non diabetic Control Group (D)** |
| 24 hours | 9.40±3.4 | 8.70±2.7 | 10.40±1.0 | 6.80±0.2 |
| 3 days | 8.10±0.3 | 7.60±1.1 | 11.90±2.1 | 6.89±1.7 |
| 7 days | 7.90±1.6 | 7.00±1.6 | 12.06±0.8 | 6.98±1.7 |

glucose concentration of the rats. The plant extract reduced the concentration of glucose from 13.45±2.1 to10.98±1.6mM/L while chlorpropamide reduced it to 12.35±1.1mM/L after 24hours as shown in Table 1. Similar reductions in blood glucose were observed after 3 and 7 days. The results show that the plant extracts reduced blood glucose more than the chlorpropamide. These reductions were significant ($P \leq 0.05$).

**Effects of plant extract and chlorpropamide (diabenese) on serum cholesterol**

Table 2 shows that both chlorpropamide (diabenese) and the plant extracts caused significant ($P \leq 0.05$) reductions in serum cholesterol from 10.40±1.0mM/L to 9.40±3.4mM/L and 8.70 ±2.7mM/L respectively after 24 hours. A higher reduction in serum cholesterol was observed for both diabenese and the plant extracts after 3 and 7 days. The plant extracts caused more reductions in the serum cholesterol when compared to diabenese.

**Effects of plant extract and chlorpropamide (diabenese) on serum protein**

The total serum protein was also reduced by both chlorpropamide (diabenese) and the plant extracts with the plant extracts causing higher reductions when compared to diabenese as shown in Table 3.

Table 3: Effects of plant extract and chlorpropamide (diabenese) on total serum protein concentration

| | Serum protein concentration (mg/ml) | | | |
|---|---|---|---|---|
| **Time (Hours/Days)** | **Diabinese Treated Group (A)** | **Plant extract Treated Group (B)** | **Diabetic Control Group (C)** | **Non diabetic Control Group (D)** |
| 24 hours | 17.40±7.1 | 16.00±6.2 | 18.10±1.0 | 10.00±1.2 |
| 3 days | 17.60±4.7 | 14.80±9.1 | 18.98±3.6 | 10.40±6.1 |
| 7 days | 14.01±1.1 | 13.60±1.6 | 19.59±7.1 | 10.48±1.9 |

**Effects of plant extracts and chlorpropamide (diabenese) on serum lipid peroxidation**

Table 4 shows that both the plant extracts and chlorpropamide (diabenese) almost completely reduced the level of lipid peroxidation after 7 days from 13.40 ±1.5mM/L to 1.5± 0.8 mM/L and 1.98± 6.2mM/L respectively. Also higher reductions in lipid peroxidation were achieved with the plant extracts. These values were significant at P≤0.05.

**Effects of plant extracts and chlorpropamide (diabenese) on serum glutamate pyruvate transaminase (SGPT) activity**

The activity of serum glutamate pyruvate transaminase (SGPT) was significantly reduced (P≤0.05) after 7 days by both the plant extracts and chlorpropamide (diabenese) from 109.10± 0.8I.U/ml to 37.10± 0.1I.U/ml and 39.80± 1.7I.U/ml respectively. These values are shown in Table 5.

**Effects of plant extract and chlorpropamide (diabenese) on serum glutamate oxaloacetate transaminase (SGOT) activity**

Reductions in the activity of serum glutamate oxaloacetate transaminase (SGOT) occurred with both the plant extracts and chlorpropamide (diabenese) only after 24 hours and 3 days. The plant extracts caused more reductions than the diabenese especially after 3 days as shown in Table 4. These reductions were also significant at P≤0.05.

Table 4: Effects of plant extract and chlorpropamide (diabenese) on serum lipid peroxidation

**Serum lipid peroxidation (mM/L)**

| Time (Hours/Days) | Diabinese Treated Group (A) | Plant extract Treated Group (B) | Diabetic Control Group (C) | Non diabetic Control Group (D) |
|---|---|---|---|---|
| 24 hours | 5.40±3.7 | 3.60±1.1 | 7.20±0.7 | 1.60±2.1 |
| 3 days | 4.20±1.8 | 2.51±0.9 | 9.81±2.0 | 1.51±0.5 |
| 7 days | 1.98±6.2 | 1.50±0.8 | 13.40±1.5 | 1.45±1.4 |

Table 5: Effects of plant extract and chlorpropamide (diabenese) on serum glutamate pyruvate transaminase (SGPT) activity

**SGPT activity (I.U/ml)**

| Time (Hours/Days) | Diabinese Treated Group (A) | Plant Extract Treated Group (B) | Diabetic Control Group (C) | Non Diabetic Control Group (D) |
|---|---|---|---|---|
| 24 hours | 92.60±5.6 | 80.10±2.2 | 99.10±1.2 | 34.16±6.7 |
| 3 days | 72.10±3.0 | 55.22±1.7 | 106.20±3.1 | 33.08±0.2 |
| 7 days | 39.80±1.7 | 37.10±0.1 | 109.10±0.8 | 32.10±1.7 |

Table 6: Effects of plant extract and chlorpropamide (diabenese) on serum glutamate oxaloacetate transaminase (SGOT) activity

**SGOT activity (I.U/ml)**

| Time (Hours/Days) | Diabenese Treated Group (A) | Plant Extract Treated Group (B) | Diabetic Control Group (C) | Non Diabetic Control Group (D) |
|---|---|---|---|---|
| 24 hours | 105.10±2.5 | 100.80±6.0 | 109.10±1.1 | 70.10±3.2 |
| 3 days | 90.26±0.8 | 72.17±0.3 | 118.06±3.6 | 71.0±1.7 |
| 7 days | 73.07±1.2 | 73.07±1.2 | 66.12±2.9 | 71.98±0.6 |

## DISCUSSION

Diabetes characterized by deleterious hyperglycemia is one of the leading diseases in the world[23]. There is a high level of treatment failures and unpleasant side effects associated with oral anti-diabetic drugs generating an urgent need and desire for alternative treatments[24]. Folklore and the use of plant based products are becoming popular in the treatment and management of diabetes. *Catharanthus roseus* is used in the treatment of diabetes in India.

The effect of the organic extracts of the leaves of *C. roseus* on blood glucose has been studied in rats[25]. This study investigated the effects of the aqueous extracts of flowers, leaves and tender stems of *C.roseus* on blood glucose and some other associated parameters. In this present study, diabetes was successfully induced as seen in increase in blood glucose in excess of 300mg/ml in the rats administered alloxan. This is in accordance with the work done by Olajide *et al*[26]. Oral administration of aqueous extracts of *C.roseus* and diabenese caused a significant reduction ($P \leq 0.05$) in the blood glucose concentration of the albino rats. Data from other results[27] tallies with our observations on the reduction of blood glucose by the aqueous extract of *C. roseus*. Also the reduction in blood glucose by chlorpropamide (diabenese) which was not as much as that caused by aqueous extract of *C.roseus* is similar to the result obtained by Levine[28]. Alloxan induces diabetes by destroying the beta-cells of the Islets of Langerhans in the pancreas leading to reduction in synthesis and release of insulin[29]. Sulfonylureas such as chlorpropamide are known to produce hypoglycemia by acting on the beta-cells and thus increasing secretion of insulin.*C.roseus* acts in a similar fashion in alloxan-induced diabetes, but the enhanced reduction of blood glucose by aqueous extracts of the plant as compared to diabenese could be attributed to the action of *C.roseus* on multiple sites on the beta-cells so as to sustain increases in synthesis and release of insulin. Also enhanced tissue response to glucose cannot be ruled out as a possible mechanism of action by *C. roseus*. Further work is needed to substantiate this possibility.

Serum cholesterol concentrations following the treatments with diabenese and the plant extract were found to be decreased significantly ($P \leq 0.05$).High cholesterol levels is associated with coronary heart disease (CHD) observed in diabetic patients. The implication of this is that the use of aqueous extracts of *C. roseus* in the treatment of diabetes will also ameliorate the occurrence of CHD in addition to reducing glucose levels.

Assay of liver enzymes SGOT and SGPT indicated a decrease in their activities in both chlorpropamide (diabenese) and plant extract treated groups, eventhough the reductions were more in the *C.roseus* treated group. Increase in these liver enzymes is an indication of liver damage[30] and so from the results obtained in this study, the plant extract of *C. roseus* and diabenese had protective effects on the liver.

Oxidative stress is associated with diseases and occurs in alloxan-induced diabetic rats seen as an increase in malondialdehyde (MDA), an end product of lipid peroxidation[31,32].There was far more increase in lipid peroxidation in the negative control group than in the group treated with diabenese and *C. roseus* extract. This shows that both diabenese and *C. roseus* has anti-oxidant activity, an attribute required in the treatment of diseases.

There were also decreases in total serum protein in the groups treated with *C. roseus* and diabenese which could be attributed to increased binding of the drug and plant components to serum albumins.

Generally the significant effects of the aqueous extracts of the flowers, leaves and tender stems of *C. roseus* which were higher than those produced by chlorpropamide (diabenese) in alloxan-induced diabetic rats is attributable to its content of some bioactive phytochemicals notably alkaloids such as vindoline and vindolinenine which are known to reduce sugar levels[33]. Other bioactive alkaloids that could contribute to the high anti-diabetic actions of *C.*

*roseus* include reserpine, a tranquilizer and vincristine, an anti-carcinogen.

In conclusion, there was a profound reduction in glucose levels and other biochemical parameters assayed in the rats treated with aqueous extracts of *C. roseus* compared to those treated with chlorpropamide (diabenese) relative to the controls. Thus plants such as *C. roseus* may complement the use of such effective antidiabetic drugs as chlorpropamide (diabenese) in treatment of diabetes and also contribute to the development of new prescription drugs for treatment of diabetes as either biochemical models or templates for drug synthesis.

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