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Biochemical Indices of Macrovascular Complication in Diabetic Rat Model: Compared Effects of Vernonia amygdalina, Catharanthus roseus and Chlorpropamide

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Abstract: Hypoglycemic and biochemical effects of herbal extracts of Vernonia amygdalina Del. (Compositae), Catharanthus roseus (L.) G. Don (Apocynaceae) and chlorpropamide were compared and status of macrovascular complications evaluated using biochemical indices in normal and diabetic rats. The phytochemistry of the herbs was also assessed. Hypoglycemic activity of the herbs and chlorpropamide was evaluated on diabetic and non-diabetic rats but biochemical effects of the treatments was evaluated only on diabetic rats, assigned into four study groups (n = 8). Group I (control) received placebo (30% ethanol), treatment group II was gavaged with chlorpropamide in dose 14.28 mg kg⁻¹ body weight, while groups III and IV were administered extracts of V. amygdalina (400 mg kg⁻¹ body weight) and C. roseus (400 mg kg⁻¹ body weight), respectively in 30% ethanol vehicle for 21 days. Results of the phytochemistry assessment identified alkaloids, cardiac glycosides, saponins, flavonoids, tannins in V. amygdalina and C. roseus. Triterpenes were identified only in V. amygdalina and anthroquiones only in C. roseus. All three treatments produced hypoglycaemic activity in normal and diabetic rats and significantly (p<0.05 to <0.01) reduced triglyceride and total Cholesterol relative to controls. C. roseus alone significantly (p<0.01) elevated HDL-Cholesterol. Serum protein significantly (p<0.05) increased in all treatments compared with controls. Urea levels decreased in all the treatments but more dramatic with chlorpropamide. Aminotransferase activity was not altered except serum ALT which was reduced in treated rats. Electrolyte profile showed dilutional hyponatremia with chlorpropamide treatment, which was absent in C. roseus treatment but mild in V. amygdalina. These changes in biochemical indices of toxicity and macrovascular complications are discussed with respect to the comparative therapeutic benefits of the three treatments.

Keywords: Biochemical-indices, Catharanthus roseus, diabetes, chlorpropamide, hyponatremia, macrovascular complications, Vernonia amygdalina

INTRODUCTION

Hyperglycemia, the major feature of diabetes mellitus alters the normal metabolism of proteins and lipids, thereby giving rise to immediate symptoms and latter long term complications. The immediate symptoms of the endocrine disorder include glucosuria, ketoacidosis, hypertriglyceridemia and hypercholesterolemia with weight loss and caloric deficits (Grammer, 2000). These prolonged metabolic abnormalities, further more, give rise to life threatening long term complications in which

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glucose reacts with collagen components of arterial wall leading to hardening of arteries and this combined with the free radical oxidation of LDL-cholesterol induces atherosclerosis and vascular damage (Zheng and Wang, 2001).

According to Ramsey (1986), electrolyte imbalance, depression of CNS, uremia and hypovolemic shock eventually lead to diabetic coma.

Diabetes not only constitutes an economic burden, but the scourge over the years has drastically reduced life expectancy. In Africa and the world over chemo and phytotherapeutic approaches have been employed in its management alongside with lifestyle modification and dietary control. In this regard, chlorpropamide also known by trade name chlorpropamide - a sulphonyl urea which acts by synergizing with insulin release by the pancreas and its utilization by target tissues has remained the mainstay of oral hypoglycemic anti diabetic therapy (Luna and Feigl, 2001). Also secondary metabolites present in Vernonia amygdalina Del. (compositae), Catharanthus roseus (L.) G. Don (Apocynaceae) and other herbs have been employed in natural remedy of diabetes (Haywood, 1993; Joshi and Kaul, 2001).

Complications of diabetes mellitus highlighted above resulting from metabolic abnormality and build up of free radicals is compounded by toxicity of chemical drugs used in its management. Herbs which are rich sources of vitamin C and E (antioxidants) and also such secondary metabolites like flavonoids, which have antioxidants activity, may alleviate these toxic side effects. The present study compares the hypoglycemic efficacy and biochemical effect of V. amygdalina, C. roseus with chlorpropamide in normal and diabetic rats in order to establish which treatment may protect against macrovascular complications.

MATERIALS AND METHODS

Collection of Plant Materials

Mature leaves of Catharanthus roseus were harvested from the Cross River University of Technology Campus in Calabar, while whole fresh leaves of Vernonia amygdalina Del. were collected from the endocrine Research farm University of Calabar, Calabar both in Cross River State. Specimens of both leaves were collected in the month of April 2006 and authenticated by Dr. Mike Eko of Botany Department, Faculty of Biological Sciences, University of Calabar, Calabar where a voucher specimen (code No. MUE 2006) has been deposited, at the Department of Botany- Herbarium, University of Calabar.

Preparation of Plants Extracts

The leaves sample of both V. amygdalina and C. roseus were rinsed with distilled water, shade-should dried and the dried leaves of each were separately ground into powder with an electric blender. A quantity 133 g of each sample was macerated in 700 mL 80% ethanol, agitated for 10 min with electric blender and left over night in a refrigerator at 4°C. The mixture was filtered with a cheese cloth and the filtrate obtained concentrated in vacuo to about 10% of their original volume at 4°C. The C. roseus and V. amygdalina concentrates were further allowed in water bath for complete dryness at 37°C yielding 51.7 and 41.6 g of sticky greenish paste, respectively.

A fraction of the ethanol extracts obtained were subjected to phytochemical analysis based on Sofoorowa (1982) to test for alkaloids, sterols, polyphenols, triterpenes etc. the remaining part was reconstituted in 30% ethanol prior to administration to experimental animals. The standard anti diabetic drug, Chlorpropamide was supplied by KAMEL pharmacy, 106 Goldie Street, Calabar, Nigeria. The tablets were also crushed into powder and suspended in 30% ethanol preparatory to administration to experimental animals.
Animals
Wistar albino rats of both sexes (140-240 g) were obtained from the disease free stock of the animal house, Biochemistry department and used for study. The animals were housed in plastic cages with plastic bottom and wire screen top (North Kent Co. Ltd.) under standard conditions of temperature (28±2°C) and relative humidity (60±5%) with a 12 h light-dark cycle and adequate ventilation. The animals were maintained on rat chow, which was provided with water ad libitum throughout the duration of the experiment. Permission for use of animals and animal protocols in the present study was obtained from the college of medical sciences Animal Ethics Committee, University of Calabar prior to experimentation.

Induction of Diabetes (Hyperglycaemia)
After two weeks of acclimatization, diabetes was induced on a group of 70 rats by intra-peritoneal injection of 150 mg kg⁻¹ body weight of alloxan monohydrate (Sigma, St Louis MO, USA) using distilled water as vehicle. The administration was done in the morning and repeated in the evening of the same day. Diabetes was confirmed three days later in alloxan induced animals showing Random Blood Glucose (RBG) level ≥ 200 mg dL⁻¹ (11.1 mmol L⁻¹) as monitored in the blood from tail vein using glucometer.

Animal Grouping and Experimental Protocol
Hypoglycemic activity was assessed in both normal (non-diabetic) and diabetic rats. While changes in biochemical indices indicative of toxicity and macrovascular complication were evaluated in diabetic rats only. The experimental design for the hypoglycemic activity assessment consisted of 48 rats of which 24 rats were drawn from diabetic pool while the other 24 rats were drawn from the non-diabetic pool. The animals were assigned into four study groups of six rats per group. The administration of both the drug and plant extract was done by oral gavage, with group I (control) animals receiving 0.14 mL of 30% ethanol, test group II animals, 400 mg kg⁻¹ body weight of Vernonia amygdalina extract, test group III animals, 400 mg kg⁻¹ body weight of Catharanthus roseus extract and test group IV animals 14.29 mg kg⁻¹ body weight of chlorpropamide, respectively. The dose of chlorpropamide used corresponded with the therapeutic dose of 500 mg kg⁻¹ for a 70 kg adult human. While 400 mg kg⁻¹ body weight of the plant extract is the effective median dose established from our preliminary studies. The 32 rats for assessment of changes in biochemical indices of toxicity were assigned into four groups (group I (control) and test groups I-IV) of eight rats each and treated same as animals of the hypoglycemic activity assessment, except that all the animals used were diabetic.

Treatment was administered twice a day by gastric intubations in a 12 h cycle (7.00 am and 7.00 pm) daily for 21 days. Body weights of the animals were monitored throughout the period of administration.

Collection of Blood Sample for Analysis
Twenty four hours after the last dose administration, the animals were anaesthetized under chloroform vapour and dissected. A fraction of whole blood collected by cardiac puncture was immediately used for haematological analysys while the remaining fraction collected in plain sample tubes was allowed to clot for 2 h and further spin at 4000 g revolution per minute for 10 min to separate serum from the blood cells. The blood serum obtained was used for biochemical assays.

Hypoglycemic Activity and Biochemical Assays
Analysis of fasting glucose in serum was by the use of dialab Kits based on Bartham and Trinder (1972).

Serum cholesterol, triglycerides and HDL-cholesterol were determined using dialab kits based on Richmond (1973), Cole et al. (1997) and Difal and Warrick (1994), respectively.
Serum electrolytes were determined photometrically for potassium and sodium by use of Human Kit after Tietz (1976) and Trinder (1951), respectively, while serum chloride was by mercuric thiocyanate method as employed in dialab kits. Aspartate and alanine aminotransferase activities in serum were determined by Reitman and Frankel (1957) as applied in random kits, while serum urea, albumin and total protein were determined by dialab kits method.

Statistical Analysis

Data obtained were expressed as mean±standard deviation and analyzed using ANOVA and the standard students t-test. Significance was checked at p<0.05.

RESULTS

Alkaloids, saponins, polyphenols, tannins, cardiac glycosides and flavonoids were identified in both herbs with relative presence of polyphenols more in C. roseus but not in V. amygdalina while terpenes were present in V. amygdalina but not in C. roseus (Table 1). The three treatments displayed significant (p<0.05) hypoglycemic effect in normal, non-diabetic and fasted diabetic animals relative to their respective controls (Table 2). Some plant extracts have hypoglycemic activity in normal but not in diabetic rats. This may be due to different mechanism of hypoglycemic action. The three treatments produced significant (p<0.05 to >0.1) decrease in total cholesterol and triacylglycerol. Whereas, chlorpropamide and V. amygdalina treatment decrease HDL-cholesterol levels, C. roseus produced an increase (Table 3). The C. roseus extract therefore had a good cholesterol effect. Exception of the decrease in ALT observed with C. roseus treatment there were no significant changes in amino transferase activities in the three treatments as the mean values in treatments compared well with the controls.

Table 1: Comparative phytochemical components in alcoholic extracts of Vernonia amygdalina and Catharanthus roseus

<table>
<thead>
<tr>
<th>Components</th>
<th>V. amygdalina</th>
<th>C. roseus</th>
</tr>
</thead>
<tbody>
<tr>
<td>Alkaloids</td>
<td>++</td>
<td>++</td>
</tr>
<tr>
<td>Cardiac glycosides</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Saponins</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Tannins</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Flavonoids</td>
<td>++</td>
<td>++</td>
</tr>
<tr>
<td>Polyphenols</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Triterpenes</td>
<td>+</td>
<td>ND</td>
</tr>
<tr>
<td>Anthroquinones</td>
<td>ND</td>
<td>+</td>
</tr>
</tbody>
</table>

+ Slight presence; ++: Medium presence; +++: Heavy presence; ND: Not Detected

Table 2: Comparative hypoglycemic effect of V. amygdalina, C. roseus and chlorpropamide on normal and diabetic rats

<table>
<thead>
<tr>
<th>Treatments</th>
<th>Non-diabetic (mg dL⁻¹)</th>
<th>Diabetic (mg dL⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control (30% ethanol)</td>
<td>107.88±7.38</td>
<td>247.25±8.83*</td>
</tr>
<tr>
<td>Chlorpropamide treated (14.29 mg kg⁻¹)</td>
<td>81.31±3.23*</td>
<td>163.05±3.75*</td>
</tr>
<tr>
<td>V. amygdalina treated (400 mg kg⁻¹ body weight)</td>
<td>76.60±5.55*</td>
<td>142.29±4.54*</td>
</tr>
<tr>
<td>C. roseus treated (400 mg kg⁻¹ body weight)</td>
<td>78.64±6.47*</td>
<td>105.61±3.62*</td>
</tr>
</tbody>
</table>

Values are mean±SD, n = 6, *: p<0.05; Statistically significant compared with controls

Table 3: Comparative effect of V. amygdalina, C. roseus and chlorpropamide on serum enzymes and serum lipids of diabetic rats

<table>
<thead>
<tr>
<th>Treatment groups</th>
<th>Total cholesterol (mg dL⁻¹)</th>
<th>Triglycerides (mg dL⁻¹)</th>
<th>HDL (mg dL⁻¹)</th>
<th>AST (IU L⁻¹)</th>
<th>ALT (IU L⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control (30% ethanol)</td>
<td>69.98±9.50</td>
<td>142.19±40.13</td>
<td>43.10±4.00</td>
<td>110.13±44.26</td>
<td>15.73±1.75</td>
</tr>
<tr>
<td>Chlorpropamide treated (14.29 mg kg⁻¹)</td>
<td>63.62±16.11*</td>
<td>88.16±38.99*</td>
<td>39.99±9.25</td>
<td>101.00±46.42</td>
<td>14.62±0.90</td>
</tr>
<tr>
<td>V. amygdalina treated (400 mg kg⁻¹)</td>
<td>59.44±12.17*</td>
<td>62.96±18.92*</td>
<td>39.99±8.25</td>
<td>102.67±10.62</td>
<td>15.50±3.60</td>
</tr>
<tr>
<td>C. roseus treated (400 mg kg⁻¹)</td>
<td>60.80±8.22</td>
<td>86.50±27.76*</td>
<td>60.05±7.87**</td>
<td>102.60±8.22</td>
<td>13.13±1.31</td>
</tr>
</tbody>
</table>

Values represent mean±SD, n = 8, *: p<0.05; Statistically significant compared with controls, **: p<0.01. Statistically significant compared with controls
Table 4: Comparative effect of treatment on selected serum electrolytes

<table>
<thead>
<tr>
<th>Treatment groups</th>
<th>Chloride (mmol L(^{-1}))</th>
<th>Potassium (mmol L(^{-1}))</th>
<th>Sodium (mmol L(^{-1}))</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control (3.9% ethanol)</td>
<td>95.79±0.44</td>
<td>6.24±0.46</td>
<td>113.36±0.11</td>
</tr>
<tr>
<td>Chlorpropamide treated (14.29 mg kg(^{-1}))</td>
<td>94.79±0.02</td>
<td>4.48±0.01*</td>
<td>59.0±0.01*</td>
</tr>
<tr>
<td><em>V. amygdaлина</em> treated (400 mg kg(^{-1}))</td>
<td>90.93±1.06</td>
<td>4.30±0.71*</td>
<td>69.64±1.68*</td>
</tr>
<tr>
<td><em>C. roseus</em> treated (400 mg kg(^{-1}))</td>
<td>91.9±0.96</td>
<td>3.81±0.37*</td>
<td>97.34±0.01</td>
</tr>
</tbody>
</table>

Values represent mean±SD, n=8, *: p<0.05; Statistically significant compared with controls

The electrolyte profile showed significant (p<0.05) decrease in potassium for all three treatments compared with controls but no significant changes with the chloric. However, there was significant decrease in sodium (hyponatremia) in both Chlorpropamide and *V. amygdaлина* treatments (Table 4). The *C. roseus* treatment did not produce hyponatremia.

**DISCUSSION**

Unpleasant side effects associated with use of conventional anti-diabetic drugs in management of diabetes-hyperglycemia is a common occurrence. The evolving toxicity of the conventional drugs instigated the desire for alternative treatments through the use of plant based products. In the present study, the comparative effects *C. roseus, V. amygdaлина* and chlorpropamide as anti-hyperglycemic agents in regards to biochemical factors which contribute to the development of macrovascular complications was carried out in diabetic rat models.

Diabetes induced with alloxan, which destroys beta cells of the islets of langerhans was monitored by assessment of hyperglycemic state in this case the increase in blood glucose up to 247.25± 4.83 mg dl\(^{-1}\).

Chlorpropamide and ethanol herbal extracts (*C. roseus* and *V. amygdaлина*) all produced prompt reduction in glucose levels. These results agree with those of Chatopadhyay et al. (1991) for *C. roseus* and Akah and Okafor (1992) for *V. amygdaлина*. The hypoglycemic activity was also demonstrated in normal non-diabetic rats by chlorpropamide and the herbal extracts. Some herbal extracts which have hypoglycemic activity in normal rats may not do so in diabetic rats due to different mechanism of hypoglycemic action. Chlorpropamide serves as a model drug with known hypoglycemic activity for comparison of its effect with that of herbal extracts. The hypoglycemic activity of the herbal extracts of *C. roseus* and *V. amygdaлина* compared with that of the standard drug in both normal and diabetic rats implies that the three treatments were potent and efficient with respect to hypoglycaemic activity and may have a similar mechanism of action.

Chlorpropamide produce hypoglycemia by acting on beta cells to increase production of insulin. The extracts of *C. roseus* and *V. amygdaлина* act in similar fashion. Enhanced response to glucose by glucose obligatory tissues such as brain, nervous tissue and red blood cells amongst others may not be ruled out as possible mechanism of action of the herbal extracts. The blood glucose lowering effect was more pronounced with the herbal extracts than with chlorpropamide.

Cardiovascular diseases and stroke are major macrovascular complications in diabetes. These complications are triggered off when advanced glycation products cross link with collagen to increase arterial wall stiffness. This alongside with raised LDL-cholesterol which further undergoes oxidative modification in the presence of free radicals leads to atherosclerosis and vascular damage. A decrease in triacylglycerol, LDL-cholesterol with a raised HDL-cholesterol, play a protective role against the occurrence of macrovascular complications. The result of this study showed that treatment with *C. roseus, V. amygdaлина* and the standard drug each produced a decrease in total cholesterol and triacylglycerol concentrations. However, only *C. roseus* additionally elevated HDL-cholesterol concentration whereas chlorpropamide and *V. amygdaлина* fail to do so. Lancert (1998) has reported that intensive control of hyperglycemia with sulphonylureas did not significantly reduce risk of myocardial infarction or stroke in diabetes, a report which agrees with the observation of the present study. The *C. roseus* extracts may therefore, protect against macrovascular complications owing to
its positive impact on lipid fractions. Assay of the activities of AST and ALT did not produce any significant changes in mean values of treatment compared with the controls. The electrolyte profile indicated that tendency towards hypotension observed with chlorpropamide and V. amygda lina was not found in C. roseus treatments.

The phytochemistry results which showed rich flavonoids contents in both V. amygda lina and C. roseus extracts may enhance their anti-oxidant status. Flavonoids are known to mop up free radicals thereby preventing oxidation of LDL-cholesterol. Also tannins have been reported to lower glucose level by Nimenibo-Uadia (2003). Saponins also have been reported to exert cholesterol lowering activity (Nimenibo-Uadia, 2003). Electrolyte homeostasis is usually a function of the kidney. Diabetic glucosuria imposes dehydration via osmotic diuresis. This alters electrolyte balance. Treatment with chlorpropamide, V. amygda lina and C. roseus each, respectively produced a reduction in sodium levels. The extent of reduction was extreme for chlorpropamide, which is a usual side effect, but this was not for C. roseus. There was no profound change in potassium ion concentration. The study objective was not only to compare the hypoglycemic efficacy but also to evaluate using biochemical indices which treatment protects better against macrovascular complications. The decrease in total cholesterol and triacylglycerol by all treatments but with the elevation of HDL-cholesterol (good cholesterol) only by C. roseus, coupled with the electrolyte profile which showed absence of hypotension in C. roseus treatment indicated that of the three treatments, C. roseus may provide better protection against macrovascular complications compared with V. amygda lina and chlorpropamide.

In conclusion, anti-hypoglycemic and hypoglycemic activity has been demonstrated by C. roseus and V. amygda lina treatments in comparison with the standard drug-chlorpropamide, with the herbal extracts possessing a more pronounced effect. Therefore, macrovascular complications associated with diabetes appear to be ameliorated by the herbal extracts compared with the conventional drug (chlorpropamide). Of the herbal extracts studied, C. roseus appeared to protect better against macrovascular complications than V. amygda lina on account of its good cholesterol effect and absence of hypotension.

REFERENCES