Effects of *Cataranthus roseus* on Electrolyte Derangement Induced by Chlorpropamide (Diabinese)^R on Normoglycemic Albino Wistar Rat

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Abstract

This study was designed to evaluate the efficacy (or otherwise) of Cataranthus roseus extract in ameliorating electrolyte imbalance following treatment with anti-diabetic drug chlorpropamide (Diabinese)^R. A total of 32 rats of mixed sexes were randomly assigned into 4 treatment groups of 8 rats each. Group A (control) received placebo (0.14ml of 30% ethanol)/ kg body weight of rat. Group B received Diabinese in a dose of 1.6mg/kg body weight of rat while group C received 400mg /kg body weight of Cataranthus roseus (C. roseus) extract. Group D received Diabinese in a dose of 1.6mg/kg body weight plus 400mg/kg body weight C. roseus extract. The above dosages were administered twice daily via orogastric intubation in a 12 hour cycle. The administration was terminated at the end of 14 days, animal sacrificed, serum collected and electrolyte (Na^+, K^+ and CL^-) profile assayed. The result obtained showed no-significant (P>0.05) decrease in serum concentration of chloride ion in Group B-D relative to control. There is however, a significant (P<0.05) reduction in serum potassium ion and sodium which is well pronounced in serum of rats treated with Diabinese and mild in group C treated with C. roseus. However, a combined treatment of C. roseus with Diabinese significantly (P<0.05) elevated serum sodium ion concentration relative to control than Diabinese treated group alone. This implies a restoration of the seemingly hyponatremia associated with Diabinese therapy.

Keywords: *Cataranthus roseus*, Electrolyte derangement, Diabinese, Normoglycemic

Introduction

The non-insulin dependent diabetes mellitus (NIDDM) otherwise known as type 11 diabetes mellitus is age long chronic metabolic disease of humans (Bray *et al*., 1992). Although type 11 diabetic patients may have normal or elevated levels of insulin, yet they show decreased sensitivity to insulin receptors (Allison, 1992). Beside this, in about 90% of diabetics (aged over 40 years), the pancreas is not producing enough insulin the cause of which may be obesity and genetic factors (mutation of genes coding for enzymes e.g. glucokinase, hepatic nuclear factors amongst others).Consequent upon the inability of the cells to utilize glucose due to glucose inability to cross the cell membranes, this led to its accumulation in plasma(hyperglycemia) cells resulting to breaking down of lipids and proteins to source for fuel and spills into urine (Attah, 2002).

The passing out of sweat goes along with loss of large amount of solute resulting in osmotic diuresis. This in turn results to polyuria and polydypsia culminating in the loss of large amount of salt from the body (Bary *et al*., 1992). The use of sulfonylureas to bring down the blood glucose level is well documented in literature (Luna and Feinglous, 2001). The acute administration of sulfonylureas e.g. chlorpropamide (Diabinese)^R or tolbutamide to NIDDM patients increases insulin release from pancreas or decrease hepatic clearance of insulin (Stephen et al., 1996).

The use of Diabinese in the management of NIDDM is not without side effect and one of which is hypoglycemia which is dependent on the half –life, the longer the half-life, the more is its hypoglycemic effect (Ferner and Neil, 1988; Seltzer, 1989). Another undesirable effect of Diabinese is induction of hyponatremia by potentiating the effect of anti diuretic hormone (ADH) on the renal collecting duct (Paice et al., 1985). This leads to the loss of electrolytes, water intoxication and impairment of renal function (Bondy and Rosenberg, 1984). Hypoglycemia has been found to associate with alcohol ingestion (Charterjea and Shinde, 2002).

The use of plant extracts in management of Diabetes mellitus has been reported. Plakel (1997) reported the use of *Vernonia amygdalina* while Iwu et al., (1990) reported the hypoglycemic effect of biflavones of Garcinia kola seed extract. Madagascar periwinkle (*Catarnthus roseus*) is popular for its antineoplastic properties. Its products (Vincristine) is used for the treatment of leukemia (Farnsworth and Kass, 1981, Svoboda, 1967).

There is dearth of information regarding the electrolyte modulation effects of C. roseus or its derivatives exception of an only study by Noble et al., (1958) who investigated and reported the hypoglycemic effects of C. roseus extract and vinblastine an analog of vincristine alkaloid. The present study is therefore carried out to ascertain the effect of Cataranthus roseus on electrolyte derangement induced by Diabinese on normoglycemic albino wistar rats.

Materials and Methods

Preparation of ethanolic extract: Five hundred (500) grams of Cataranthus roseus leaves were harvested from the horticultural garden along Marian hill in Calabar South Local Government Council of Cross River State of Nigeria. The harvested C. roseus leaves were thoroughly washed with clean water, dried at room temperature to a constant weight and the dried leaves ground to powder using electric blender. The milled sample was macerated and extracted over night in 700 ml of 30% ethanol. The mixture was sieved using a chess cloth and the filtrate was subjected to evaporation in a hot water bath thereby yielding a greenish paste like semi-solid mass (crude extract of Cataranthus roseus). This was stored in the refrigerator until commencement of treatment. The tablets of anti-diabetic drug, chlorpropamide (Diabinese)^{R} obtained from KAMEL pharmacy were crushed to powder and reconstituted in 30% ethanol by the same ratio as that of the C. roseus crude extract.

Animal and animal treatment: Thirty two albinos Wister rats of mixed sexes weighing between 150 and 200 were obtained from animal house Department of Biochemistry, University of Calabar and were used in this study. The rats were assigned into four treatment groups of 8 rats each. Treatment A. (control) received 0.14ml of 30% ethanol as placebo. Treatment B received Diabinese in doses of 1.6mg 1kg body weight of rats. Treatment group C. on the other hand received Cataranthus roseus leaf extract in doses of 400mg /kg body weight while Group D received Diabinese in doses of 1.6mg/kg and 400mg/kg of *Cataranthus roseus* leaf extract. Our previous preliminary studies had established 400mg /kg body weight as the effective median dose of C. roseus in Wistar rats hence the basis of this dosage. Diabinese and *C*. *roseus* extract administration were done twice daily (i.e. morning and evening) via orogastric intubation in a 12 hour cycle every day for 14 days.

Collection of blood samples: The animals were anaesthetized in chloroform vapour 24 hours post treatment. The rats were dissected and blood was obtained by cardiac puncture from each animal using sterile syringe and needle into sterile screw capped tubes. The sample tubes were allowed to stand for one hour to clot and serum separated from the cells by centrifugation at 4000g for 10 minutes and immediately used for electrolyte assay.

Assay of serum electrolytes: The flame photometry method of Vogel (1960) was used for the determination of sodium ion (Na^+) and potassium ion (K^+) concentration in serum while the chloride ion (Cl^-) concentration was determined using the traditional methods of AOAC (1984). The result presented in Table 1 below was the outcome of serum electrolyte concentration of various treatment administered to the experimental rats. The result obtained showed non-significant (P>0.05) change in serum chloride ion level except for the rats that received *Diabinese* which revealed a significant (P<0.05) reduction. Serum potassium ion electrolyte concentration however decreased significantly (P<0.05) in all treatment relative to control with the highest reduction occurring in the group treated with C*atarnthus roseus* leaf extract. Sodium ion electrolyte concentration significantly (P<0.05) decreased in serum of rats treated with *Diabinese* but there was no-significant (P>0.05) change in serum sodium ion concentration of rats treated with *C. roseus* leaf extract. The combination of *Diabinese* with *C. roseus* leaf extract produced a significant (P<0.05) reduction in sodium ion relative to control but a significant increase when compared with the value obtained from *Diabinese* treated rats. This is an improvement on the seemingly hyponatremic condition associated with *Diabinese* therapy.

Results and Discussion

Electrolyte homeostasis is usually a function of the kidney. Abnormal loss of fluid via the kidney results in an abnormal loss in serum electrolytes (Bowmer and Yates, 2002). For instance, in diabetes mellitus, *Lucosuria* impose *dehydration* via osmotic diuresis, which to a large extent alter normal balance in electrolytes (Tripathi, 2001). The significant (P<0.05) reduction in serum sodium ion concentration of rats treated with *Diabinese* is in consonance with the findings of (Paice *et al.,* 1985) who reported an induction of *hyponatremia* by *chlorpropamide* via *potentiating* the effects of *antidiuretic* hormone (ADH) on the renal collecting duct. The non-significant (P>0.05) decrease in sodium ion concentration (130.28±5.42 mmol/L) caused by *C. roseus* leaf extract relative to control (138.33±4,60 mmol/L) and significant reduction by combined treatment with *C. roseus* extract. (116.85±3.19 mmol/L) suggest that *Cataranthus roseus* extract is capable of ameliorating the *hyponatremic* effect of *Diabinese* therapy. Serum potassium ion concentration was significantly (P<0.05) reduced (3.13±0.35; 2.06±0.05 and 2.37±0.09mmol/L) by all levels of treatment relative to control (4.68±0.37 mmol/L) with the lowest level (2.06±0.05 mmol/L) being observed in rats treated with extract of *Cataranthus roseus* leaves.

Naturally, potassium ion is more abundant in actively growing and secreting tissues than in the blood and supporting tissues (Rankin and Hiderath, 1966). The significant (P<0.05) serum potassium ion reduction in rats treated with *C. roseus* extract may not be unconnected with the positive sodium ion balance of *Cataranthus roseus.* Potassium ion is only conserved in the extra cellular fluid to maintain extra cellular fluid balance (Guyton and Hall, 2006). This may explain why the concentration of potassium ion in the *Diabinese* treated rats did not decrease much lower than the *Cataranthus roseus* treated rats as the case was with the sodium electrolyte. Many plant extracts contain cardiac glycosides which are capable of inhibiting Na^+ K^+ ATpase why both serum Na^+ and K^+ are decreased by *C. roseus* since most of the Na+ in the body is received in form of sodium chloride (NaCl), (Guyton and Hall, 2006).

Table 1: Serum electrolytes concentration of Wistar rats on different treatments

Groups	Na^+ mmol/L	K^+ mmol/L	CL mmol/L
A: (control)	138.33±4.60	4.68±0.37	103.20±4.13*
B: Diabinese	79.78±2.51*	3.13±0.35*	62.42±13.0
C: Cataranthus roseus	130.28±5.42	2.06±0.05*	100.23±3.30
D: diabinese + C. roseus	116.85±3.19*	2.37±0.09*	146.60±27.30

Values are expressed as mean ± SD and means on the same row with asterisk are significantly (P<0.05) different from control.

The concentration of chloride ion (CL^-) in serum of rats treated with *Diabinese* revealed a significant (P<0.05) reduction relative to control treatment. Although treatment with *C. roseus* extract alone did not produce any significant (P>0.05) change in serum chloride ion of rats, a combination of *Diabinese* with the same extract produced significant (P<0.05) increase in serum chloride ion concentration. This lends more credence to the earlier statement that *Cataranthus roseus* leaf extract ameliorates *hyponatremic* effect of *Diabinese* therapy (Seltzer, 1989; Paice *et al.,* 1985).

Conclusion: The electrolyte indices assay after 14 days revealed that a combination of *Diabinese* and *Cataranthus roseus* is better that *Dibinese* alone in enhancing serum sodium and chloride electrolyte levels. Sodium ion is significantly restored by combined treatment of *Diabinese* and *C. roseus* from (79.78±2.5 to 116.85±3.19 mmol/L). Serum potassium ion maintains an inverse relationship with sodium ion in all treatment for purposes of maintaining body electrolyte balance. The result therefore shows that *Cataranthus roseus* leaf extract is highly efficacious in ameliorating electrolyte derangement consequent upon *Diabinese* therapy. So the undesired or adverse effect of *Diabinese* can be restored by *Cataranthus roseus.*

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