

## Full Length Research Paper

# A study of the effect of the combined leaf extracts with chlorpropamide in alloxan diabetic rats

Samuel Ekanem<sup>\*1</sup>, Vincent Bassey Eduok<sup>2</sup> and Nkanga Ukpong<sup>2</sup><sup>1</sup>Departments of Biochemistry, College of Medical Sciences, University of Calabar, P. M. B. 1115, Calabar, Nigeria.<sup>2</sup>Department of Clinical Pharmacy and Biopharmacy, Faculty of Pharmaceutical Sciences, University of Uyo, P. M. B. 1017, Uyo, Nigeria.

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The comparative anti-hyperglycemic efficacy along with the impact on some biochemical parameters of alloxan-induced diabetic rats, following treatment with chlorpropamide and combined leaf extracts from *Vernonia amygdalina* (VA) and *Azadirachta indica* (AI) was evaluated in this study. Twenty four rats, 18 diabetic and 6 non-diabetic were assigned into four groups of 6 rats each, and respectively treated with one of the following combinations: saline (non-diabetic and diabetic controls), extracts of AI and VA combined (200 mg/kg b. w. in 1:1 ratio), and chlorpropamide (14.286 mg/kg b. w.), for a 21-day period. Measured blood glucose reductions relative to their initial values at the end of treatment were 71.05% and 75.83% for combined extracts and chlorpropamide respectively. Similarly the serum glucose respectively decreased by 58.74% and 59.97% relative to the diabetic control value of  $264.27 \pm 4.36$  mg/dl. The extents of decrease of both blood and serum glucose in the combined extracts-treated group, compared well ( $p > 0.01$ ) with the chlorpropamide test group. The 21-day treatment with chlorpropamide and the combined plant extracts significantly ( $p < 0.05$ ) reduced serum triglyceride and total cholesterol concentrations as well as aminotransferase activities to a similar extent, and the levels were both comparable to those of the normal control. Result of serum electrolytes and urea concentrations also showed comparable ameliorative effect on associated renal complications of diabetes. Therefore combined extracts of AI and VA has the potentials to replace sulfonylureas such as chlorpropamide in the management of diabetes.

**Keywords:** Polyherbal therapy, chlorpropamide, diabetes mellitus, blood and serum glucose, biochemical parameters.

## INTRODUCTION

Medicinal plants have formed the basis of health care throughout the world since the earliest days of humanity and are still widely used, and have considerable importance in international trade (Ahmed et al., 2006). In developed countries such as United States, it is estimated that plant drugs constitute as much as 25% of the total drugs, whereas, in developing countries including China and India, the contribution is as much as

80% (Joy et al., 1998). This underscores the increased research interest in medicinal plants and traditional medicine all over the world, particularly as it concerns chronic and debilitating diseases like diabetes mellitus.

Diabetes mellitus has yet remained an enigma in various respects, despite the volume of research information available to the medical community, especially in prevention and management of the disease. Chemotherapy which is presently in use employs a range of pharmacological agents and prominent among these is chlorpropamide, a typical sulfonylurea. Chlorpropamide (diabenese) has a half-life of 35 hours and is slowly meta-

\*Corresponding author E-mail: [samuel\\_ekanem@yahoo.com](mailto:samuel_ekanem@yahoo.com)

bolized in the liver thereby precipitating most commonly among others, the risk of hypoglycemic reactions particularly in the elderly patients (Ukwe, 2006). Also, there is increased risk of jaundice due to hepatic dysfunction when administered in high dose, since patients on this drug are usually monitored with special care (Ukwe, 2006).

However the goal of any diabetic management measure besides relief from hyperglycemic symptoms is optimal physical health and prevention of complications of hypoglycemia (Ukwe, 2006). Evidence from our previous investigation on the antidiabetic efficacy of combined extracts of *Vernonia amygdalina* and *Azadirachta indica* appear to achieve this goal (Ebong et al., 2008). The extracts when administered in combination were seen to protect the liver as well as exert optimum glucose control over the period of the experiment, through a synergistic action of the ingredients in the two plant extracts. The active ingredients in these plants were detected in our earlier investigation to include flavonoids, polyphenols, tannins and saponins (Atangwho et al., 2009). The other components also identified were antioxidant vitamins and mineral elements.

*A. indica* normally found in the Indian subcontinent and the dry forest areas of South and Southeast Asia including Pakistan, Sri Lanka, Thailand, Malaysia and Indonesia, but cultivated in most other countries of the world, belongs to the plant family Meliaceae (National Research Council, 1992). Biswas et al (2002) have reviewed the biological activities and medicinal properties of the plant, and articulated, the hypoglycemic effects of its leaves, stem and bark and seed oil. On the other hand, *V. amygdalina* is a compositae of both wild and domestic browse in Africa. The plant has a reputation in its use in traditional management of diabetes in Southern Nigeria (Abo et al., 2001). An earlier study in our laboratory compared the effect of extracts from these two plants separately on beta cell integrity in rats pretreated with alloxan, a beta cell destructive agent (Ebong et al., 2006).

The present study therefore compared the effect of the combined leaf extracts with chlorpropamide in alloxan diabetic subjects (rats). Studies such as this may become the basis for new drug discovery and development from natural products with relative advantages over the existing ones.

## MATERIALS AND METHODS

### Preparation of extracts

Matured leaves of *V. amygdalina* and *A. indica* were obtained from the Endocrine Research Farm of the University of Calabar, Calabar and University of Calabar staff village respectively. The plants were authenticated by Dr E. G. Amanke, of the Department of Botany, University of Calabar and voucher specimens deposited in a herbarium with numbers ERU/2006/011 and ERU/2006/012

respectively in the Department of Botany. Four hundred grammes (400 g) each of the leaf samples were homogenized in 80% ethanol. The homogenates were allowed in a refrigerator (4°C) for 48 hours, and thereafter filtered using a cheese cloth. The filtrates were concentrated at low temperature (37°C) using a rotary evaporator to one tenth the original volume and there after allowed to completely dry in a bath (37°C) yielding 36.48g (9.12%) and 24.84g (6.21%) respectively for *V. amygdalina* and *A. indica*. (Ebong et al., 2008) and reconstituted in normal saline prior to animal treatment.

### Diabetes induction and animal treatment

Twenty four albino Wistar rats (150- 180g) obtained from the animal house, Department of Zoology, University of Calabar, Calabar after due permission from the Faculty of Basic Medical Sciences Animal Ethics Committee, were used for the study. The rats were allowed one week to acclimatize in Biochemistry departmental animal house, where they were housed throughout the experiment using polycarbonated cages. The animal housing facility was maintained at standard conditions: temperature (28 ± 2°C), relative humidity (50 ± 5%) and a 12 h light/dark cycle. Water and palletized rat feed were available to the animals *ad libitum*, throughout the treatment period. Eighteen of the animals were induced with diabetes through intra-peritoneal injection of 150 mg/kg body weight of alloxan monohydrate (Sigma St. Louis, MO, USA) after an overnight fast. Four days after alloxan treatment, rats which confirmed diabetic (i.e. random blood glucose 200 mg/dl) were selected for the experiment. Prior to animal treatment, the extracts were reconstituted in normal saline (vehicle) and administered orally, via gastric intubation, at doses of 200 mg/kg each (1:1 ratio) for the extracts and 14.286 mg/kg body weight for chlorpropamide (Ebong et al., 2008). The controls received normal saline (placebo).

### Experimental design

The design consisted of 24 rats (18 surviving diabetic rats and 6 normal rats) divided into 4 groups of 6 rats each. Groups 1 and 2, normal and diabetic control rats given placebo treatment, Group 3: diabetic rats treated with combined extracts from *A. indica* and *V. amygdalina* (1:1 ratio) and Group 4: diabetic rats administered chlorpropamide. Administration of drugs was done twice a day, 12 hours apart (6: am and 6: pm) for 21 days. At the end of the 21-day treatment the animals were fasted for 12hrs, then anaesthetized with chloroform and dissected. Before then, *in vivo* measurement of blood glucose was done with blood obtained from tail vein of the rats using a Glucometer (Lifescan, Inc. 1995 Milpitas, California 95035, USA). Whole blood obtained by cardiac puncture into sterile plane tubes was allowed to clot for about 2 hours and thereafter centrifuged (3,000 g for 10 min) to remove cells and recover serum. The serum was used for the biochemical assays.

### Biochemical analysis

Alanine aminotransaminase (ALT) and Aspartate aminotranaminase (AST) activities in serum were determined by the method of Reitman and Frankel (1956) as applied in commercial assay kits obtained from Randox Laboratories Ltd., Admore Diamond Road, Crumlin, Co., United Kingdom, Bt294QY, whereas serum cholesterol and triglycerides were determined using commercial assay kits based on Difal and Warnick (1994) (DIALAB production, Gessellschaft m.b.H A-1160 Wien-panikengasse, Germany). Assay kits for determination of total protein, albumin and urea were also obtained from DIALAB, Germany. Whereas total protein was measured by the method based on biuret reaction

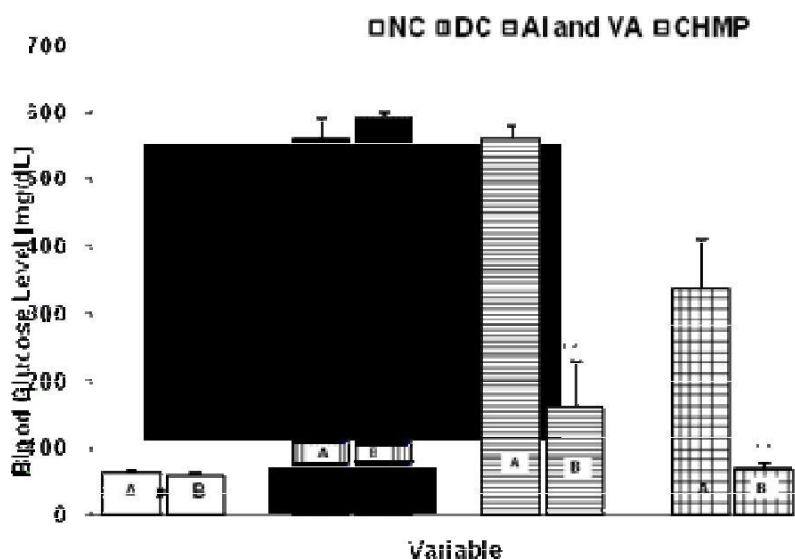


Figure 1. Compared effect of combined extracts of *A. indica* and *V. amygdalina* and chlorpropamide on Blood glucose: A, before and B, after the 21-day treatment. \*\*\*P<0.001 vs A.

**Table 1.** Effect of the combined extracts and chlorpropamide on serum glucose and lipid parameters.

	Serum glucose (mg/dl)	% Change	Serum triglyceride (mg/dl)	% Change	Serum total cholesterol (mg/dl)	% Change
NC	105.34 ± 20.24 <sup>c</sup>		72.83 ± 20.09 <sup>c</sup>		48.58 ± 15.67 <sup>c</sup>	
DC	264.27 ± 41.36	60.14*	165.96 ± 46.78	56.12*	73.00 ± 3.32	33.45*
VA/AI	109.04 ± 8.68 <sup>a, c</sup>	58.74	56.23 ± 13.76 <sup>a</sup>	66.12	37.10 ± 8.98 <sup>a, c</sup>	49.18
CHMP	105.80 ± 33.27 <sup>a</sup>	59.97	72.42 ± 14.30 <sup>a</sup>	56.36	40.92 ± 9.42 <sup>a</sup>	43.95

Mean ± SEM, n = 6, a = P<0.05 vs DC, c = p > 0.05 vs CHMP,

\* = % Increase wrt NC, = % reduction wrt DC, NC=Normal control, DC= Diabetic control, VA= *V. amygdalina*, AI= *A. indica* and CHMP= Chlorpropamide.

(Thomas 1998), that of albumin relied on the binding of bromocresol green (BCG) to albumin (Tietz., 1994). Urea determination was based on urea hydrolysis by urease enzyme (Tietz, 1994). Potassium was determined by photometric turbidometric test (Tietz, 1976) using analytical kits from TECO DIAGNOSTICS, 1268 N. Lakeview Ave. Anaheim, CA 92807 USA. Serum sodium and chloride were estimated by Mg-Uranylocetate method of HUMAN diagnostic kit (Human Gesellschaft fur Biochemica und Diagnostic m.b.H Max-Planck-Ring 21 D- 65205 Wiesbaden, Germany) and mercuric thiocyanate method of DIALAB diagnostic kit respectively, both based on Tietz (1976). Bilirubin total and Uric acid measurements were based on the method of Newman and Price (1999).

#### Statistical analysis

The results were analysed for statistical significance by one-way ANOVA using the SPSS statistical program and Post Hoc Test (LSD) between groups using MS excel program. All data were expressed as Mean ± SEM and p values < 0.01 and 0.05 were considered significant.

## RESULTS

Four days after alloxan-treatment, blood glucose of diabetic rats was significantly raised by 5.4 – 9 times the value of the normal control rats. However, at the end of 21-day treatment, blood glucose decreased by 71.05% and 75.83% of the initial values following treatment with combined extracts (*A. indica* and *V. amygdalina*), and chlorpropamide respectively (Figure 1). Similarly, serum glucose which increased by 60.14% in the diabetic control rats relative to normal control became reduced by 58.74% and 59.79% after the 21-day treatment with combined leaf extracts and chlorpropamide respectively (Table 1). These reductions were both significant at p < 0.01 and p < 0.05, and were not statistically different from the normal control.

Serum total cholesterol (TC) and triglyceride (TG) concentrations which respectively increased by 33.45%

**Table 2.** effect of the combined extracts and chlorpropamide on some indices of liver function

Group	ALT (Unit l <sup>-1</sup> )	AST (Unit l <sup>-1</sup> )	Total protein (g / dl)	Albumin (g / dl)
NC	4.22 ± 0.67	5.85±1.33	7.68 ± 0.15	2.88 ± 0.19
DC	15.50 ± 0.73 <sup>b</sup>	74.00±5.14 <sup>b</sup>	7.17 ± 0.24	2.84 ± 0.18
VA/AI	6.40 ± 0.70 <sup>a</sup>	13.88±2.88 <sup>c</sup>	7.15 ± 0.36	3.17 ± 0.11
CHMP	4.40 ± 0.25 <sup>a</sup>	24.10±8.37 <sup>a</sup>	7.77 ± 0.25	3.05 ± 0.23

Mean ± SEM, n = 6, a = p < 0.05 vs DC b = p < 0.05 vs. NC, c = p < 0.05 vs CHMP

**Table 3.** Effect of the combined extracts and chlorpropamide on some serum indices of kidney function

Group	K <sup>+</sup> (mEq/L)	Na <sup>+</sup> (mEq/L)	Cl <sup>-</sup> (mg/dl)	Total bilirubin (mg/dl)	Urea (mg/dl)	Uric acid (mg/dl)
NC	4.75 ± 0.52	101.26 ± 5.50	28.12 ± 0.15	0.42 ± 0.02	36.85 ± 4.11 <sup>a</sup>	5.34 ± 0.62
DC	5.24 ± 0.36	83.09 ± 2.31 <sup>b</sup>	27.34 ± 0.22	0.73 ± 0.02	118.98 ± 10.88	4.33 ± 0.31
AI/VA	4.91 ± 0.26	76.09 ± 10.12 <sup>b,c</sup>	29.18 ± 0.52	0.49 ± 0.02	73.88 ± 35.85 <sup>a,c</sup>	4.65 ± 0.50
CHMP	4.56 ± 0.21	76.55 ± 3.96 <sup>b</sup>	27.29 ± 0.40	0.43 ± 0.02	69.35 ± 40.89 <sup>a</sup>	4.19 ± 0.41

Mean ± SEM, n = 6, a = p < 0.05 vs DC b = p < 0.05 vs Nc c = p > 0.05 vs CHMP

and 56.12% in the untreated diabetic rats compared to the normal control, were reduced significantly (p < 0.05) by 49.18% and 66.12% in the combined extracts treated rats and 43.95% and 56.36% in the chlorpropamide treated. Although they both compared well with the normal control, the extent of reduction was more with combined extract treatment (Table 1).

Results of some indices of liver function including ALT and AST activities, protein total, and albumin and urea levels are shown in table 2. Serum ALT and AST activities of untreated diabetic rats which were significantly raised (p < 0.05) by 3.6 and 12.7 times respectively relative to the normal control became significantly reduced upon treatment. ALT activity significantly reduced (p < 0.05) to 2.4, and 3.5 times the diabetic control value in rats treated with the combined extracts, and chlorpropamide respectively. In the same order AST activity respectively reduced to 5.3 and 3.1 times the value of the diabetic control. However, when compared with normal control and the drug, the combined extracts showed non significant differences in the aminotransferase activities. Serum total protein and albumin levels indicated no significant changes.

Results of some indices of kidney function: potassium, sodium, chloride, urea, bilirubin and uric acid are shown in table 3. Levels of potassium, chloride, total bilirubin and uric acid indicated non-significant changes (p > 0.05) in the four treatment groups relative to the diabetic control. Serum urea which was 3.2 times high in diabetic control group compared to normal control was significantly reduced (p < 0.05) by the two treatments. Compared to the normal control, urea level in both test groups treated with combined extracts and chlorpropamide was non-significant (p > 0.05). Serum

sodium levels which were decreased significantly (p < 0.05) from 101.26 ± 5.50 to 83.09 ± 2.31 following diabetic induction, were observed to decrease further to 76.09 ± 10.12 upon treatment with combined extracts. This was however comparable with the levels in chlorpropamide treated group (76.55 ± 3.96).

## DISCUSSION

The anti-hyperglycemic effect of combined ethanol extracts of *A. indica* and *V. amygdalina* and chlorpropamide have been evaluated in this study. The individual anti hyperglycemic action of extracts of *A. indica* and *V. amygdalina* have been reported (Ebong et al., 2008). When combined, as shown in this study the effect on blood and serum glucose compares fairly well with that of chlorpropamide, a typical sulphonylurea, and returns the glucose to normoglycemic level in the study subjects. Within the treatment period, the two extracts have indicated a positive synergy on their action on blood glucose, since in an earlier study the administration of extract of *V. amygdalina* alone could not return the glucose level to normoglycemia after 14 days (Atangwho et al., 2007b). In the combined form, the extracts tend to compliment each other thereby producing the desired normoglycemia. This observation may buttress the proposition of Tiwari and Rao (2002) as per advantage of polyherbal therapies over monotherapy. Several target tissues/ mechanisms may have been exerted via the multiple of bioactive components in the two plants, to completely reverse the hyperglycemia.

Macrovascular complications engendered by altered lipoprotein metabolism are often evident in diabetes

mellitus. This was observed in this study, as serum total cholesterol and triglyceride concentration in untreated diabetic rats increased significantly by 33.45% and 56.12% respectively. This is in agreement with earlier reports (Atangwho et al., 2007a; Eteng et al., 2008; Ekaidem et al., 2008). The result of this study showed that treatment with the combined plant extracts and the standard drug each produced a decrease in cholesterol and triglyceride concentration.

Serum markers of hepatotoxicity – AST and ALT were also evaluated. Diabetic control rats showed significantly elevated enzyme activities in serum compared to normal control. This wholly agrees with Kim et al (2006) and an earlier report (Atangwho et al., 2007c). Upon treatment with the extracts and the drug, the activities of both enzymes become significantly reduced. Again, this agrees with hepatoprotective effect of *V. amygdalina* (Atangwho et al., 2007c ; Babalola et al., 2001) and *A. indica* (Kale et al., 2003; Chattopadhyay and Bandyopadhyay, 2005) reported in earlier studies. The protective effect of the combined extracts, from this work may be more efficient compared to chlorpropamide: reduction in AST activity by the combined extracts was almost 2-fold compared to that of chlorpropamide.

Serum total protein and albumin, markers of liver synthetic ability were also assessed during this period. Increase in albumin level of rats treated with combined extracts, although non- significant, support the hepatoprotective action of the extracts, given that albumin is exclusively synthesized in the liver. Evidence from this investigation indicates that combination of the two extracts may be promising and more desirable in the management of diabetes. Further studies to confirm this relative advantage of polytherapy is however suggested.

The present study also compared effect of the two treatments on kidney function of the test animals by measuring serum electrolytes and some kidney function indicators. The treatments ameliorated/modulated the potential risk diabetes posed to the kidneys such as decreased urea levels, which rose in the untreated diabetic group. This effect is similar to kidney protective action of *V amygdalina* extract earlier reported (Atangwho et al., 2007c). Most striking in this study is the fact that the action of the two treatments was similar with respect to kidney protection.

It is evident from the result of this investigation that, co-administration of extracts of *V. amygdalina* and *A. indica* can potentially replace chlorpropamide in the management of diabetes mellitus.

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