EFFECTS OF *VERNONIA AMYGDALINA* AND CHLORPROPAMIDE ON BLOOD GLUCOSE

ABRAHAM A.A. OSINUBI*

SUMMARY: Despite significant achievements in treatment modalities and preventive measures, the prevalence of diabetes has risen exponentially in the last decade. Because of these limitations there is a continued need for new and more effective therapies. An increasing number of people are using dietary and herbal supplements, even though there is a general lack of evidence for their safety and efficacy. Consequently, science-based medical and governmental regulations are needed for more randomized clinical studies to provide evidence of efficacy and safety. The aim of this study was therefore to subject one such promising Vernonia amygdalina (VA), to agents to further investigate the potential function of VA for treatment of diabetes mellitus as potentially emerging alternative therapy for type 2 diabetes.

Sixty adult male Sprague-Dawley rats weighing 180-220g were used for the experiment. Half of the animals were randomly rendered diabetic by administering alloxan (150 mg/kg). Equal numbers (20) of the rats were variously administered aqueous leaf extract of VA (500 mg/kg), chlorpropamide (250 mg/kg) and distilled water (2 ml/kg).

Aqueous leaf extract of VA produced significant ($p < 0.05$-0.001), reductions in the blood glucose concentrations of normal (normoglycemic) and diabetic (hyperglycemic) rats 1 to 12 hours after acute treatment compared with distilled water-treated control animals. Its blood-glucose-lowering potential in both normoglycemic and alloxan-induced diabetic male Sprague-Dawley rats compared favourably to that of chlorpropamide.

Administration of the aqueous extract of VA at a concentration of 500 mg/kg of body weight significantly decreased the levels of blood glucose. The hypoglycemic efficacy was comparable with that of chlorpropamide, a standard hypoglycemic drug.

Key words: Vernonia amygdalina, chlorpropamide, diabetes mellitus, hypoglycemic effect.

INTRODUCTION

Diabetes is a major health problem in the world, reaching presently to epidemic proportions. This increase in incidence of diabetes has occurred in spite of major inroads in understanding the pathophysiology and treatment of this insidious disease. Current therapies seem to be insufficient to prevent diabetic complications in type 2 diabetes, with a two- to four-fold likelihood for developing cardiovascular complications (1). Because of these limitations, there is a continuous need for the development of novel health promotion strategies and therapeutic modalities. Despite the large armamentarium presently available, the progressive deterioration of diabetes control is such that treatment is still insufficient, with the majority of type 2 diabetes patients eventually requiring insulin therapy to achieve targeted glycemic levels (2), and an esti-

*From Department of Anatomy, College of Medicine, University of Lagos, Idi-Araba, Lagos, Nigeria.

mated 75% dying of diabetes- related complications from cardiovascular disease (3).

The value of current therapies is unequivocal, yet inadequate. While physicians advocate aggressive use of drugs to tighten glucose control and attenuate cardiovascular disease risk factors, many patients are more inclined toward use of alternative therapies that include diet, food supplements and herbal medicine. When considering new recommendations for treatment of diabetes (4), major health agencies and governmental authorities have largely ignored the role of diet, especially dietary fibre, and/or herbs with hypoglycemic characteristics, due to the paucity of data available. However, use of 'popular' diets, nutritional supplements and botanicals is increasing among consumers. Only a minority of patients has begun to approach their physician about these types of therapies, with over 60% of patients failing to report usage of these products to their physician (5). Insufficiency of current therapies for the treatment of diabetes, combined with both a lack of trust in conventional medical treatment and an inability of the economy to absorb the cost of pharmaceuticals, have created a growing public interest in dietary supplements and botanicals. The use of herbs has more than tripled over the last 10 years (5), and a whole new industry referred to as 'nutraceuticals' has evolved. Little scientific evidence exists to support the numerous herbs used to improve diabetes-related metabolic disorders (6). Since ancient times, plants have been an exemplary source of medicine. Ayurveda and other Indian literature (7) mention the use of plants in treatment of various human ailments. The use of herbal products for medicinal benefits has played an important role in nearly every culture on earth. Herbal medicine was practiced by ancient people in Africa, Asia, Europe, and the Americas (8). During the past decade, use of herbs and related products has increased from 34% in 1990 to 42% in 1995, with related out-of-pocket costs of about $27 billion (9). India has about 45000 plant species and among them, several thousands have been claimed to possess medicinal properties. West Africa has several thousands of such plants (10). The people of Western Nigeria have been using the leaves of *Vernonia amygdalina* (VA) to treat diabetes locally in Nigerian folk medicine. While a lot of plants have been studied (11) for their antihyperglycemic effect, little is known about the antidiabetic effect of VA, (a commonly used herb in Western Nigeria) except from indigenous folk medicines where it is being speculated to have antihyperglycemic effect. VA had, however, been reported to exert schistosomicidal, anti-plasmodial, leshmaniacidal and anti-cancer effects (12-15). In this present study, we compared the hypoglycemic and anti-hyperglycemic effects of aqueous extract of the leaves of VA with chlorpropamide in normoglycemic and alloxan-induced diabetes in adult male Sprague-Dawley rats, with a view to providing a pharmacological rationale for the folkoric use of mature, green leaves of VA by the Yoruba-speaking people of South-Western Nigeria.

MATERIALS AND METHODS

Plant material

Fresh, mature, green leaves of VA were harvested from the garden of the Anatomy Department of College of Medicine, University of Lagos. The leaves were identified to be those of VA by taxonomists in the Department of Botany of the University of Lagos. The Department keeps voucher specimens of the plant in its herbaria. The extraction process was carried out in the Pharmacognosy Department of the College of Medicine, University of Lagos. Briefly, the completely air-dried leaves of *Vernonia amygdalina* (500g) were reduced to powdery form, which was placed in distilled water and allowed to boil, simmering for one hour. The water extract was dialyzed and the internal solution lyophilized. The powder obtained (5.15 g, 1.13% yield) was stored at 4°C before use and was prepared in distilled water for pharmacological studies. The dose of 0.5 g/kg body weight of the extract administered to the rats was arrived at based on a previous study that evaluated the antimalarial activity of similar extract of VA in mice (15).

Animal material

Sixty adult male Sprague-Dawley rats weighing 180-220g were used for the experiments. They were procured from the Animal House of the College of Medicine, University of Lagos. They were allowed to acclimatise and maintained under standard photoperiodic condition in the Rat Room of the Department of Anatomy for two weeks. The rats were weighed, and randomly divided into two main groups of 30 rats each. The animals in the 2 main groups were subdivided into 6 subgroups of five rats each. All animals were observed for clinical signs of drug toxicity (tremors, weakness, lethargy, refusal of feeds, weight loss, falling-off of hair, coma and death) throughout the duration of the experiment and four weeks thereafter. All procedures involving animals in this study conformed to the guiding principles for research involving animals as recommended by the Declaration of Helsinki and the Guid-

ing Principles in the Care and Use of Animals (16) and were approved by the Departmental Committee on the Use and Care of Animals.

Experimental procedure

Diabetes was induced (in the group of diabetic rats) with alloxan. 0.4 g of crystalline powdered alloxan purchased from Nafco Nigeria Limited was taken and dissolved in 10 ml of distilled water to yield a concentration of 40 mg/ml. 150 mg/kg body weight of alloxan was administered intraperitoneally (21) to 30 of the animals, after an overnight fast (access to only water) of 12 hours to make them more susceptible to developing diabetes (17,18). Normal (normoglycaemic) rats (with blood glucose levels < 100 mg/dl) were treated with intraperitoneal injections of distilled water (2 ml/kg) only. All animals were maintained under the same laboratory conditions of temperature (ambient temperature maintained between 26-28°C), humidity and light (L:D; 12:12) and were allowed free access to food (rat chow from Pfizer, Nigeria) and water. The alloxan-treated rats, also allowed unrestricted access to water and food, were left undisturbed for 48 hours, during which time diabetes developed and reached a steady state in the animals. Alloxan-treated rats with blood glucose levels ≥400 mg/dl were considered to be diabetic, and were used in this study. Chlorpropamide (250 mg/kg, *per oral*) was used as the standard antidiabetic agent for comparison. All the rats used in this study were fasted for a period of 12 hours (but still allowed free access to drinking water) before they were treated with either distilled water, the plant extract or chlorpropamide. The test compounds (VA and chlorpropamide) were orally administered to the animals by gastric intubation. Immediately before and 1, 2, 4, 6, 8, 10 and 12 hours after acute administrations of the test compounds, blood samples were taken from the tail vein of each animal for blood glucose analysis. Blood glucose level was assayed by allowing a single drop of fresh whole blood from the vein to drop on the strip provided with the glucose monitor. The blood glucose monitor used was One Touch Basic made by Lifescan (Johnson and Johnson Company) and the results were read off on the meter 45 seconds after application of samples to the strips. The technical performance of the glucometer used was evaluated by comparison with standard laboratory method of blood glucose estimation (spectrophotometer) at the beginning, midway and at end of the experment as previously described by Ajala *et al.* (18, 19).

In order to minimize the effects of circadian rhythm on our results, the experiment was structured in such a way that the serial blood glucose estimation of half of the rats were commenced at 0800h (8 a.m.) 1 hour after administration of test compounds) and subsequently at 0900h (9 a.m.), 1100h (11 a.m.), 1300h (1 p.m.), 1500h (3 p.m.), 1700h (5 p.m.) and 1900h (7 p.m.), while those of the other half were commenced at 2000h (8 p.m.) and subsequently at 2100h (9 p.m.), 2300h (11 p.m.), 0100h (1 a.m.), 0300h (3 a.m.) and 0500h (5 a.m.). The serial blood glucose levels taken were the means of these two sub-groups. This was done to minimize the effects of circadian rhythm on our results. We have previously described a circadian rhythm for blood glucose concentration in male Sprague-Dawley rats in Lagos, with peak at 1900h and nadir at 1100h (20, 21). The experiment was carried out in the month of March.

Data analysis

Blood glucose data obtained from the blood samples of the plant extract- and chlorpropamide-treated rats, as well as those obtained from the distilled water-treated control animals were pooled and expressed as mean±SD (standard deviation). The difference between distilled water-treated test means was analysed statistically by Student's *t*-test. Values of $p < 0.05$ were taken to imply statistical significance.

RESULTS

Aqueous leaf extract of VA produced significant ($p<0.05$-0.001), reductions in the blood glucose concentrations of normal (normoglycaemic) and diabetic (hyperglycaemic) rats, 1 to 12 hours after acute treatment (compared with distilled water-treated control animals). The maximal rate of decline in blood glucose concentrations was found 8 hours after administration of the extract in both the normo- and hyperlycaemic subjects. Chlorpropamide also induced significant ($p<0.05$-0.001) reductions in the blood glucose concentrations of normal and alloxan-treated diabetic rats 1 through 12 hours following acute treatment. Distilled water had no significant effect ($p>0.05$) on the blood glucose concentrations of both normoglycaemic and alloxan-induced diabetic rats after acute treatment. Tables 1 and 2 summarize the hypoglycaemic and anti-hyperglycaemic effects of aqueous leaf extract of VA (compared with chlorpropamide and distilled water) in normal and alloxan-treated diabetic rats, respectively. There were no obvious signs of toxicity observed in any of the animals throughout the duration of our observation.

DISCUSSION

It is clear from the results of this experimental animal study that the tested aqueous leaf extract of VA induced significant, reductions in the blood glucose concentrations of normoglycaemic and alloxan-induced diabetic rats. The findings of this investigation may, therefore, suggest that

the plant extract could, at least in part, stimulate insulin production and glucose utilization, like chlorpropamide, to bring its hypoglycaemic effect in the mammalian experimental model used. Although the present findings suggest the presence of hypoglycaemic compound/s in leaf extract of VA, the precise mechanism of its hypoglycaemic action is still speculative and requires further studies for appropriate elucidation. It is, however, interesting to note that the plant extract, like chlorpropamide, is more effective in reducing the blood glucose concentrations of diabetic rats than in reducing the blood glucose concentrations of normoglycemic rats.

Major constituents of the extract from the leaves of VA include sesquiterpene lactones (vernodalin, vernolide, hydroxyvernolide), and steroid glucosides (vernonioside A1-A4: for bitter tasting constituents and vernonioside B1-B3; for nonbitter related constituents) (22, 23). It has been reported that a sesquiterpene lactone isolated from the extract of *Ambrosia Maritima* is an effective hypoglycemic agent. It has been suggested that the hypoglycemic effect of aloes and its bitter principle may be mediated through stimulating synthesis and/or release of insulin from the beta-cells of Langerhans (24). VA, containing both sesquiterpene lactones and bitter principle may therefore act through stimulation of synthesis and/or release of insulin from the beta-cells of the pancreatic islets. Other mechanism of actions such through the inhibition of glucose absorption, increase sensitivity of receptors to insulin, insulinase inhibiting effect, and stimulation of peripheral tissues uptake of glucose cannot be ruled out.

CONCLUSION

Based on our current results, it appears that the aqueous extract of the leaves of VA shows a pronounced blood-glucose-lowering potential in both normoglycaemic and alloxan-induced diabetic male Sprague-Dawley rats comparable to that in chlorpropamide-treated rats and is thus provided with a pharmacological support of the folklore claims of anti-diabetic activity.

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Correspondence:
Abraham A. A. Osinubi
Department of Anatomy
College of Medicine,
University of Lagos,
Idi-Arabia, Lagos,
NIGERIA.
e-mail: abrahamosinubi@yahoo.co.uk