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Hypoglycemic Potential of Combined Ethanol Extracts of *Gongronema latifolia* and *Vernonia amygdalina Leaf* in Alloxan-induced Diabetic Albino Wistar Rats

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ABSTRACT

The effect of *Gongronema latifolia* (GL) and *Vernonia amygdalina* (VA) leaf extracts on glucose level in alloxan-induced diabetic rats was studied. Sixty albino rats (170g-200g) were divided into twelve groups with five animals per group. Group 1 and 12 served as normal and diabetic controls respectively. Groups 2, 3 and 4 were normal rats and received 150mg/kg of GL, VA and combined 100mg/kg each of GL and VA respectively. Groups 5, 6 and 7 were treated as Groups 2, 3 and 4 respectively before diabetes induction. Groups 8, 9 and 10 were treated as Groups 2, 3 and 4 respectively while Group 11 was treated with insulin after diabetes induction. Extract administration lasted for 14 days. Glucose level was significantly ($P < 0.01$) lowered in normal and post-diabetic treated rats as well as Groups 5 and 6. Combine extract of *Gongronema latifolia* and *Vernonia amygdalina* have good glucose lowering potential.

Keywords: *Vernonia amygdalina, Gongronema latifolia,* Diabetes, Blood Glucose, Insulin.

INTRODUCTION

Plants have been used in traditional medicine for the treatment of ailments for thousands of years and there is still increasing interest in the study of medicinal plants and their uses in different parts of the world.[1,2] Reports have stated that about 80% of the world's population still depend on traditional medicine for their primary health care and there are considerable economic benefits in the development of indigenous traditional remedies for the treatment of various disease[3].

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Diabetes is a multifaceted disease in terms of its pathogenicity and has been identified as a group of metabolic disorders with hyperglycemia, polynuria, polydipsia, polyphagia and glucosuria as the major characteristics[4]. The hyperglycemia is a manifestation resulting from several metabolic anomalies and depicts a complete breakdown in metabolic activities rather than a pathogenic factor[5]. The pathological scenario in diabetes presents multiple therapeutic targets that may require a range of different agents to address the different features of the disease in different stages of its development. Individual therapeutic agents act only on part of the pathogenic process and only to a partial extent hence cannot holistically address the problem[6]. This contributes significantly to the increased morbidity and mortality of diabetes despite the detailed understanding of the condition and the availability of several pharmacological agents for its management.

Several orthodox medications exist for the management of diabetes, however, evidence has also shown that various herbal therapy has been proven useful in the management of diabetes especially in the underdeveloped and developing world.[7] *Gongronema latifolia* and *Vernonia amygdalina* are medicinal plants frequently consumed in Nigeria for its nutritional and pharmacological effects.[8,9,10] The plants have been widely studied as their anti-bacterial, anti-diabetic, anti-fungal, anticytotoxic, anti-pyretic, anti-inflammatory and anti-oxidant potentials have been documented.[11,12,13] Phytochemical screening of *Vernonia amygdalina* and *Gongronema latifolia* have been reported and the plants are rich in polyphenols, tannins, flavonoids, terpenoids, saponins, alkaloids, cardiac glycosides as well as steroids.[14,15] These phytochemicals (singly or in synergy) are responsible for the pharmacological and medicinal effects attributed to these plants.

The search for appropriate agents in the management of diabetes is therefore focused on traditional medicinal plants due to leads that have been provided by traditional plants and the presence of several medicinal phytochemicals in plants that have the potential to act on variety of targets by various modes and mechanisms.[16,17] Moreover, the combination of various agents from different plant sources have been used to enhance the efficacy of polyherbal formulations. Polyherbal therapies have the synergistic, agonist/antagonist pharmacological properties that can promote maximum therapeutic efficacy with minimum adverse effects.[17]

The present study was aimed at evaluating the glucose lowering potential of combined *Gongronema latifoli*a and *Vernonia amygdalina* leaf extract in alloxan-induced albino Wistar rats in view of formulating a polyherbal therapy for the management of diabetes.

METHODOLOGY

Plant Materials

Fresh Leaves of *Gongronema latifolia* and *Vernonia amygdalina* were obtained from Uyo metropolis, Akwa Ibom State, Nigeria. The plants were authenticated by Dr (Mrs) Margaret E. Bassey, a taxonomist at the Department of Botany and Ecological Studies, University of Uyo, Uyo, Nigeria. *Vernonia amygdalina* and *Gongronema latifolia* were assigned voucher number UUH2083 and UUH2084 respectively. The leaves were washed with clean water and air dried at room temperature. Separately, the leaves were blended into powdered form and were macerated in 80% ethanol for 48 hours. The filtrate from each extract was obtained and concentrated in a water bath at 45°C to obtain the crude extract of both leaves. The crude extracts were preserved in a refrigerator at -4°C until used.

Experimental Animal and Design

Sixty (60) albino rats weighing between 170 – 200 grams were used for the study and were divided into twelve (12) groups with five (5) animals in each group. The animals were obtained from the Department of Biochemistry Animal House, University of Calabar, Calabar, Nigeria. The rats were maintained under standard laboratory conditions and allowed access to food and clean water *ad libitum*. Group 1 and 12 served as normal and diabetic controls respectively. Groups 2, 3 and 4 received 150mg/kg of *Gongronema latifolia*, 150mg/kg of *Vernonia amygdalina* and combined 100mg/kg of *Gongronema latifolia* and 100 mg/kg of *Vernonia amygdalina* respectively without induction of diabetes. Groups 5, 6 and 7 received 150mg/kg of *Gongronema latifolia*, 150mg/kg of *Vernonia amygdalina* and combined 100mg/kg of *Gongronema latifolia* and 100 mg/kg of *Vernonia amygdalina* respectively before induction of diabetes. Groups 8, 9, 10 and 11 were treated with 150mg/kg of *Gongronema latifolia,* 150mg/kg of *Vernonia amygdalina*, combined 100mg/kg of *Gongronema latifolia* and 100 mg/kg of *Vernonia amygdalina* and insulin respectively after induction of diabetes. The extracts were orally administered daily for 14 days.

Induction of Diabetes with Alloxan

Alloxan selectively destroys the insulin-producing islet of Langerhans in the pancreas when administered to rodents resulting in insulin dependent diabetes mellitus which is similar to Type 1 diabetes in humans [18]. The body weight and blood glucose levels of the animals to be made diabetic were determined before the administration of alloxan to induce diabetes. Diabetes was induced by intraperitoneal administration of 150mg of alloxan per kilogram body weight of the experimental animal. After administration of alloxan, the animals were observed

for signs of diabetes which include polyuria, polydipsia, polyphagia and hyperglycemia. Blood sample was obtained through the tail of the experimental animals to check for blood glucose levels using a glucometer and glucose test strips as confirmatory test for hyperglycemia.

Determination of Blood Glucose Levels

At the end of the experiment, the animals were sacrificed under chloroform anesthesia. Blood sample was obtained through cardiac puncture and centrifuged at 3000rpm for 15 minutes to obtain the serum which was used for assay of glucose concentration. Randox-assay kit (GOD-PAP) method based on method described by Barham and Trinder was used for the assay.[19] The principle involves the enzymatic oxidation of glucose in sample by the enzyme glucose oxidase which generates hydrogen peroxide and gluconic acid. The concentration of H_2O_2 released is proportional to initial amount of glucose in the sample and it reacts under catalysis of peroxidase, with phenol and 4-amino phenazone to form a red violet quinoneimine dye whose colour intensity reflects the concentration of glucose in the sample.

Statistical Analysis

The data obtained were expressed as Mean ± SEM. SPSS software, Version 20.0 was used for statistical analysis of the data. One-way analysis of variance (ANOVA) was used for comparison and results were subject to post hoc test using Tukey multiple comparison tool. Test values of $p < 0.01$ were considered significant.

RESULTS

The result of the combined effect of *Gongronema latifolia* and *Vernonia amygdalina* on the blood glucose in the study is presented in Table 1. A significant decrease in the blood glucose level was observed in the non-diabetic rats administered the extracts when compared with the normal control. Similarly, a significant decrease in blood glucose levels was seen in post-diabetic treated groups compared to both the normal and diabetic control. Only the blood glucose of the *Gongronema latifolia* treated group in the pre-diabetic treated groups was significantly decreased when compared to the control.

Table 1. Effect of Combined Administration of *Gongronema latifolia* and *Vernonia amygdalina* on the Blood Glucose Level of Alloxan Induced Diabetic Rats.

GROUPS	BLOOD GLUCOSE (mg/dl)
Group 1 – Normal Control	60.60 ± 1.71
Group 2 – *Gongronema latifolia*	54.90 ± 1.79*
Group 3 – *Vernonia amygdalina*	50.60 ± 0.22*
Group 4 – *G. latifolia and V. amygdalina*	50.20 ± 0.86*

Group 5 - *Gongronema latifolia* before Alloxan	47.90 ± 0.57*#
Group 6 - *Vernonia amygdalina* before alloxan	58.30 ± 0.62#
Group 7 - *G. latifolia and V. amygdalina* before alloxan	69.00 ± 0.71*
Group 8 – Alloxan before *Gongronema latifolia*	57.40 ± 0.50*#
Group 9 – Alloxan before *Vernonia amygdalina*	41.30 ± 0.40*#
Group 10 – Alloxan before *G. latifolia and V. amygdalina*	40.20 ± 0.49*#
Group 11 – Alloxan before Insulin	60.80 ± 1.02#
Group 12 – Alloxan alone	68.80 ± 0.74*

*Data are presented as Mean ± Standard Error of Mean (SEM). Values are considered significantly different at p < 0.01. * = Significantly different from normal control (Group 1). # = significantly different from diabetic control (Group 12).*

DISCUSSION

Glucose concentration in the blood at any given time is determined by metabolic processes in the liver, muscle and adipose tissue which is under regulation by insulin and glucagon.[20] The target of diabetic treatment is to restore the normal metabolic processes and regulation resulting in normal glycemic level in the host. The administration of *Gongronema latifolia* and *Vernonia amygdalina* singly and in combination has been shown to demonstrate hypoglycemic result in normoglycemic rats. This is suggestive of the fact that the extracts may stimulate insulin production from the pancreatic β-cells or increase the activity of glucose transporters on target tissue membrane.[21]

However, when the extracts were administered prophylactically (pretreatment before inducing diabetes), the blood glucose of the *G. latifolia* and *V. amygdalina* treated groups tend towards that of the normal control. The combined extract treated group still had glucose level similar to the diabetic control group. This suggests that the combined extract had no protective effect against the destruction of insulin producing islet of Langerhans cells by alloxan. Alloxan selectively destroys the insulin producing islet of Langerhans in the pancreas when administered to rodents resulting in insulin dependent diabetes mellitus which is similar to Type 1 diabetes in humans.[18]

Mfon *et al.,* had earlier reported the effect of combined administration of *Vernonia amygdalina* and *Gongronema latifolia* on the pancreatic β-cells of streptozotocin induced diabetic rats[21]. They reported a regeneration and proliferation of islet cells of diabetic rats which were destroyed by streptozotocin during the induction of diabetes. The glucose lowering potential observed in the present

study may be due to the fact that there was regeneration of the islet cells that were destroyed by alloxan which was used to induce diabetes in the animals. Regeneration of pancreatic beta cells have been reported as a probable mechanism of hypoglycemic action of *Vernonia amygdalina*.[21] Regeneration of the islet cells restores the synthesis of insulin which then facilitate the uptake of glucose from the blood into the cells. Mfon *et al.,* also reported that the extracts of these plants have insulin mimetic effects in addition to regeneration of the islet of Langerhans which enhances endogenous insulin production.[22]

It is believed that phytochemicals such as tannins, flavonoids, glycosides and phytosterols present in *Vernonia amygdalina*[23] and alkaloids, glycosides and saponins present in *Gongronema latifolia*[24] may contribute to the hypoglycemic potential of the plants singly and in combination. Reports have shown that polyherbal therapies have the synergistic, agonist/antagonist pharmacological properties that can promote maximum therapeutic efficacy with minimum adverse effects.[17]

It can therefore be concluded that *Gongronema latifolia* and *Vernonia amygdalina* have good hypoglycemic potentials that is even better in combination in the treatment of hyperglycemia induced by alloxan in albino Wistar rats.

ETHICAL APPROVAL

Ethical approval for the study was obtained from the Research Ethical Committee of Faculty of Basic Medical Sciences, University of Uyo, Uyo, Nigeria.

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