Journal of Intercultural Ethnopharmacology
www.jicep.com
DOI: 10.5455/jice.20140610054950

Original Research

Evaluation of methanol extract of *Gongronema latifolium* leaves singly and in combination with glibenclamide for anti-hyperglycemic effects in alloxan-induced hyperglycemic rats

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Received: May 22, 2014
Accepted: June 10, 2014
Published: June 26, 2014

ABSTRACT

Objective: This study evaluated the anti-hyperglycemic effect of the methanol extract of *Gongronema latifolium* leaves singly and in combination with an oral hypoglycemic agent; glibenclamide. **Materials and Methods:** The plant material was extracted with methanol for 48 h using cold maceration and concentrated *in vacuo* in a rotary evaporator. The methanol extract of *G. latifolium* at doses of 200, 300, 400, 500, and 800 mg/kg were studied for anti-hyperglycemic effect in alloxan-induced hyperglycemic rats. More so, the extract at doses of 400 mg/kg + 5 mg/kg glibenclamide and 500 mg/kg + 5 mg/kg glibenclamide were studied for possible additive effects. **Results:** The 300 mg/kg of the extract decreased blood glucose at 1 h post-treatment though not significantly ($P > 0.05$) compared with 5 ml/kg distilled water, but failed to lower the blood glucose at 3 and 6 h post-treatment. The 400 and 500 mg/kg decreased the blood glucose level from 1 to 6 h post-treatment. However, the decrease in blood glucose was only significant ($P < 0.05$) at 6 h post-treatment. The two combination protocol of the extract significantly ($P < 0.05$) decreased the blood glucose from 1 to 6 h post-treatment compared with 5 ml/kg distilled water. However, there was no significant ($P > 0.05$) difference between the effects of the combination protocol and glibenclamide 5 mg/kg alone though the effects of the combination protocol were better than that of glibenclmide 5 mg/kg alone. **Conclusion:** Our studies suggest that there is treatment benefit of combining extract of *G. latifolium* leaves and glibenclamide over *G. latifolium* or glibenclamide alone.

KEY WORDS: *Gongronema latifolium*, glibenclamide, combination, additive effect, treatment benefit

INTRODUCTION

Diabetes is a pathological and metabolic condition caused by inadequate insulin action with glucose intolerance being a common feature to all types [1]. Clinically, it is defined as a fasting plasma glucose level >7.8 mmol/l (140 mg/dl) or a 2 h post-prandial plasma glucose >11 mmol/l (200 mg/dl). Over 99% of cases of diabetes are caused by two diseases – Type 1 and Type 2 diabetes with Type 2 being 10 times more common than Type 1 [1]. Effective clinical management of diabetes relies on adequate control of blood glucose, which must take into consideration of the need to maintain adequate energy in the face of intermittent food intake along with variable exercise and thus variable demand [2]. The blood glucose concentration is controlled by a feedback system between liver, muscle, fat, and pancreatic islet cells and the overall pattern of control differing in basal and fed states. In insulin dependent diabetes mellitus (IDDM), insulin is the main form of treatment, while in the non-IDDM, diet is the cornerstone, often combined with oral hypoglycemic agent (metformin and the sulfonylureas) and/or insulin. Dietary management is essential in the management of both types of diabetes [2]. Various vegetables used for food in the Eastern part of Nigeria are known to have anti-hyperglycemic effect and therefore are consumed by diabetic patient even when on oral hypoglycemic agents or insulin. One of such vegetable is *Gongronema latifolium*. It is called amaranth globe in English; while it is known as *utazi* by the Igbo speaking parts of Eastern Nigeria. This is a herbaceous shrub of the tropical rain forest of the family *Asclepiadaceae*. It is a climbing shrub up to 5 m long. The leaves are commonly used either as vegetable or as a spice. Various pharmacological actions have been reported on this plant and include antioxidant potential [3], anti-asthmatic [4] antimalarial, anti-inflammatory and anti-sickling activities [5,6]. The plant has also been reported to cure cough, loss of appetite, and stomach disorders [7]. It is a popular remedy for diabetes [7]. This work was designed to evaluate the rationale for consumption *G. latifolium* leaves as hypoglycemic agent and again to verify if there is any treatment benefit or

order wise in combining the methanol extract of this plant with a standard oral hypoglycemic agent. This is because this plant is consumed by diabetic patients even when they are on an oral hypoglycemic agent.

MATERIALS AND METHODS

Animals

Albino rats between the ages of 10-12 weeks and weighing 120-140 g were used. They were kept in metal cages and fed and watered *ad libitum*. They were acclimatized for 14 days before the experiments. All animal experiments were in accordance with the guideline stipulated by the National Institute of Health for Care and use of laboratory animals (Pub. No. 85: 23 revised 1985).

Preparation of Plant Extract

The plant material was obtained from Orba in Udenu local Government Area of Enugu State Nigeria and identified as *G. latifolium* by a plant taxonomist at the Department of Botany University of Nigeria Nsukka. Fresh green leaves were dried under shed and ground to a coarse powder using hammer mill. A total of 400 g of the pulverized material was extracted in methanol using cold maceration for 48 h and filtered using Whatman No. 1 Filter Paper and concentrated *in vacuo* in a rotary evaporator.

Induction of Diabetes

The baseline 16 h fasting blood glucose level of each rat was determined using Accu-Chek Active Glucometer and Strips. Hyperglycemia was induced using a single intraperitoneal injection of alloxan monohydrate (160 mg/kg). Rats with fasting blood glucose >7.8 mmol/l or 140 mg/dl were considered hyperglycemic and were selected for the study.

Dose Response Effect of Methanol Extract of *G. latifolium* Leaves

Rats were grouped into 7 groups of 5. They were fasted for 16 h, but water was provided *ad libitum* before the experiment. The fasting blood glucose of each rat was determined before extract and drug were administered. Group 1 received 5 ml/kg distilled water and served as a negative control, Group 2 received 5 mg/kg glibenclamide and served as the positive control, while Groups 3-7 were treated with 200, 300, 400, 500, and 800 mg/kg, respectively. Change in blood glucose levels was accessed for each rat at 1, 3, and 6 h post-treatment. The percent change in blood glucose for each rat was calculated and average for each group determined.

Combination Effect of Methanol Extract of *G. latifolium* Leaves and Glibenclamide

20 rats grouped into 4 of 5 rats per group were used. They were fasted for 16 h before the experiment. The fasting blood glucose of each rat was determined before extract and drug administration. They were treated as follows: Group 1 (5 ml/kg distilled water), Group 2 (5 mg/kg glibenclamide), Group 3 (400 mg/kg extract + 5 mg/kg glibenclamide) and Group 5 (500 mg/kg extract + 5 mg/kg glibenclamide). Effects of these treatments on blood glucose were evaluated at 1, 3, and 6 h for each rat and average for each group determined.

Data Analysis

Data obtained were subjected to one-way analysis of variance and variant means separated *post-hoc* using least significant difference. Significance was accepted at $P < 0.05$.

RESULTS

Dose Response Effect

There was no reduction in blood glucose at extract doses 200 and 800 mg/kg. At 300 mg/kg of the extract, there was a reduction in blood glucose at 1 h post-treatment though not statistically significant ($P < 0.05$) compared with 5 ml/kg distilled water. The extract at doses of 400 mg/kg and 500 mg/kg caused a reduction in blood glucose at 1, 3, and 6 h post-treatment, but these effects were only significant at 6 h post-treatment when compared with 5 ml/kg distilled water. At 800 mg/kg, there was an increase in blood glucose (Figure 1).

Combination Effect of Methanol Extract of *G. latifolium* and Glibenclamide

There was significant ($P < 0.05$) reduction in blood glucose in the two combination protocol of the extract and glibenclamide at 1, 3, and 6 h post-treatment compared with 5 ml/kg distilled water. There was no significant ($P > 0.05$) difference between the two combination protocols, and between the protocol and glibenclamide alone, though the effects of the two combination protocol were higher than that of glibenclamide alone at 3 h post-treatment, while the combination of 400 mg/kg extract and 5 mg/kg glibenclamide was higher than glibenclamide (5 mg/kg) alone at 6 h post-treatment (Figure 2).

DISCUSSION

The anti-hyperglycemic effect of methanol extract of *G. latifolium* leaves was evaluated singly and in combination with glibenclamide to determine if there is a scientific basis for the traditional use of the plant as an anti-diabetic agent. More so to determine if there is potential herb-drug interaction (adverse effects) or treatment benefit of the combination (additive effect). This is because the leaves of *G. latifolium* are common component of most meals and diabetic patients on oral hypoglycemic agents equally consume it concurrently with these oral hypoglycemic agents. Alloxan-induced hyperglycemia is due to selective toxicity of alloxan on the pancreatic beta cells, generation of superoxide radicals and cytotoxic action mediated by generation of reactive oxygen species (ROS) [8-11]. The methanol extract of *G. latifolium* leaves did not decrease blood glucose of alloxan-

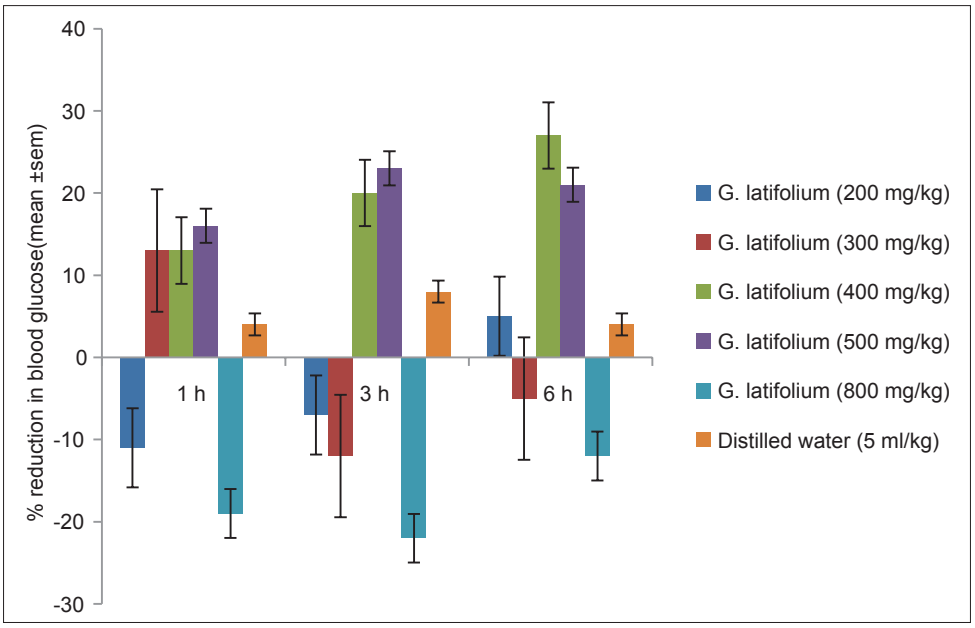

Figure 1: Dose response effect of methanolic leaf extract of *Gongronema latifolium* in alloxan-induced hyperglycemic rats

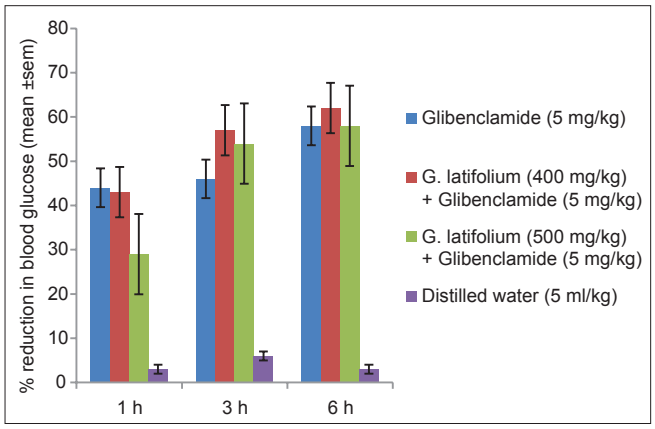

Figure 2: Effect of combination of different doses of methanolic leaf extract of *Gongronema latifolium* and glibenclamide in alloxan-induced hyperglycemic rats

induced hyperglycemic rats at doses of 200 and 300 mg/kg, but was able to decrease the blood glucose significantly ($P < 0.050$) at doses of 400 and 500 mg/kg 6 h post-treatment. This shows that the anti-hyperglycemic effect of the extract is dose-dependent. However, there was exacerbation of hyperglycemia at the dose of 800 mg/kg indicating that some phytochemical constituents of the extract or metabolic by-products of the extract contributed to elevated blood glucose. Some phytochemical constituents of methanol extract *G. latifolium* leaves include tannins, reducing sugars, flavonoids, saponins, and alkaloids [12]. These phytochemical constituents are known to have anti-hyperglycemic effects in other plants. Thus, could be responsible for the anti-hyperglycemic effect of *G. latifolium* leaves. These phytoconstituents are equally known to be antioxidant in other medicinal plant and therefore could have led to mopping up of ROS, therefore enhancing anti-hyperglycemic activity [13,14]. The combination of the methanol extract of *G. latifolium* and glibenclamide produced an anti-hyperglycemic effect that was higher than that of the extract or glibenclamide alone. However, this effect was not significantly better than that of glibenclamide alone. This effect of the combination protocol shows that there is treatment benefit in combining the extract and glibenclamide. This means that diabetic patients taking both *G. latifolium* leaves and glibenclamide simultaneously are likely going to have better glycemic control than those taking either glibenclamide or *G. latifolium* leaves alone. The combination protocol did not produce overt hypoglycemia showing that there is less risk of dangerous herb-drug interaction. In conclusion, our studies showed that there are basis for the use of *G. latifolium* as anti-diabetic agent traditionally and reveals a possible treatment benefit in combination of *G. latifolium* and glibenclamide in management of diabetes mellitus.

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Source of Support: Nil, Conflict of Interest: None declared.