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Original Research Article

BLOOD GLUCOSE LOWERING EFFECT OF ETHANOL LEAF EXTRACT OF *HYMENODICTYON FLORIBUNDUM* (RUBIACEAE)

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ABSTRACT

In developing countries, and some developed nations, diseases are managed using complementary medicine with the aid of preparations from a single or mixture of herbs. Diabetes mellitus, described by persistently high blood glucose levels, owing to abnormalities in insulin secretion, is among such disorders. *Hymenodictyon floribundum* has been reported to be used in ethnomedicine to manage diabetes. This study aimed to investigate the blood glucose-lowering activity of the ethanol extract of *Hymenodictyon floribundum* leaves in adult Wistar rats. Elemental analysis, preliminary phytochemical screening, and acute toxicity test were carried out. Oral glucose tolerance test (OGTT) was performed using five groups of adult Wistar rats administered graded doses (375, 750, and 1500 mg/kg) of the extract, distilled water (1 mL/kg) as negative control, and metformin (500 mg/kg) as positive control. Measurement of glucose levels in blood was performed at 0, 30, 60, and 120 minutes of extract administration, respectively. The result of the elemental analysis indicated the presence of Ca, Mg, K, Na, Fe, Mn, Cu, Cr, Ni, and Pb. Preliminary phytochemical screening revealed tannins, steroids, triterpenes, saponins, alkaloids, anthraquinones, and flavonoids. The Median lethal dose (LD_{50}) of the extract was estimated to be higher than 5000 mg/kg orally. The animals treated with the extract showed a significant ($p<0.05$) decrease in blood glucose levels compared to the negative control. Blood glucose levels dropped below baseline in the animals treated with the highest dose (1500 mg/kg) of the extract. The study therefore revealed that *Hymenodictyon floribundum* has the potential to produce a blood glucose-lowering effect and corroborates the claim of antidiabetic activity of the plant.

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INTRODUCTION

Diabetes, characterized by hyperglycemia is regarded as one of the most prevalent public health problems, and optimal regulation remains unattainable [1]. Uncontrolled diabetes characterized by persistent hyperglycemia can lead to major complications including renal disease, loss of vision, cardiovascular disease, foot ulcers, and gangrene leading to amputation of the lower limbs, all of which increase the morbidity and mortality rate associated with diabetes [2]. The

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incidence of diabetes mellitus is rapidly growing in Nigeria, in line with the global trend. For instance, more than 180 million people around the world are currently suffering from diabetes, and it is projected that the prevalence will more than double by the year 2030 [3]. The incidence of prediabetes in Nigeria was estimated to be 13.2% according to the criteria by the American Diabetes Association (ADA) and 10.4% based on the World Health Organization (WHO), as reported in a systematic review and meta-analysis [4]. This is a strong indicator of the significant burden of prediabetes and diabetes in the country, highlighting the need for effective prevention and management strategies. The use of traditional medicine in developed and third-world countries as a source of cure for many diseases has existed for millennia and their significance has undoubtedly been widely recognized [5]. Traditional and complementary medicine have used medicinal plants to cure, manage, or prevent a wide range of illnesses [6]. Several common herbs were known to lower blood glucose concentration, as such, the promise of achieving improved glycemic regulation or becoming less dependent on injection of insulin through the use of natural medications is unarguably attractive [7].

Hymenodictyon floribundum as a common African shrub has been used ethnobotanically for managing many diseases [8] among which include diabetes, by the indigenous society of Kuregu, Zaria Local Government of Kaduna State, Nigeria on which this research was based. Moreover, several pharmacological actions of the plant have been reported - significant analgesic and anti-inflammatory potential [9,10], anticancer and apoptotic action [11], and activity against mycobacterium species [12].

MATERIALS AND METHODS

Drugs and Chemicals

Metformin (Inventia Health Care, India), distilled water, D-Glucose anhydrous powder (BDH Chemicals Ltd, England), and ethanol prepared as 70% v/v (BDH Chemicals Ltd, England).

Animals

Adult Wistar rats of the same sex (male) weighing 150-200 g acquired within the Animal House Unit, Department of Pharmacology and Therapeutics, Ahmadu Bello University, Zaria, Nigeria were utilized in the study. They were housed in a safe well-ventilated room in their cages and fed with normal laboratory rodent pellets and water *ad libitum* until the end of the study. Ethical approval for the use of laboratory animals was sought from the institutional committee on the care and use of experimental animals and an approval number was issued ABUCAUC/2020/011.

Plant Collection and Identification

The leaves of the plant together with other aerial parts were collected from Kargi(a hill located around Birnin Gwari way, Zaria Local Government Area), Kaduna state in autumn of 2019. Identification and authentication of the plant was done at the taxonomy lab of the Botany Department, Faculty of Life Sciences, Ahmadu Bello University, Zaria, by Malam Namadi Sanusi (a taxonomist) and a voucher number (ABU900124) was given.

Plant Extraction

The extraction was performed by the method described by Kupchan *et al*. [13]. *Hymenodictyon floribundum* leaves were shade-dried and complete dryness was ascertained by regular weighing until the achievement of a steady weight. The dried leaves were then pulverized in a mortar until powdery. To ensure thorough extraction, the powdered leaves were steeped in continuous (70% v/v) ethanol for 72 hours in a Soxhlet machine. The solvent used for the extraction was removed from the extracted material using a water bath heated to 50℃. This produced an ethanol leaf extract of *Hymenodictyon floribundum* (EEHF), which was then preserved for future uses. For each investigation, fresh extract solutions were made using double distilled water.

Qualitative Phytochemical Analysis

According to the established technique laid out by Evans [14], phytochemical screening was carried out on EEHF. Secondary phytochemicals including flavonoids, alkaloids, saponins, tannins, cardiac glycosides, carbohydrates, steroids, triterpenes, and anthraquinones, were evaluated using specific test tube-based chemical tests.

Elemental Analysis

The wet digestion method as described by Muhammad *et al.*, was used [15]. Two (2) g of the powdered plant material was placed in a 50 mL falcon tube. 6.5 mL of mixed acid solutions containing nitric acid (HNO_3), sulfuric acid (H_2SO_4), and perchloric acid ($HClO_4$) (5:1:0.5) were added. After a few drops of distilled water were added and the mixture was allowed to cool, this was boiled in a fume hood on a hot plate until the digestion was complete, as evidenced by white fumes emerging from the flask. Thereafter, it was put into a 50 mL volumetric flask and filled with distilled water to the desired volume. This was passed through filter paper (Whatman No. 42), and the resulting product (filtrate) was retained in a plastic bottle with a label. A model AA 500 PG Atomic Absorption Spectrometer was used to analyze the solution for the relevant elements. The appropriate standard calibration curves, which were produced using standard analytical grade solutions of the elements, were used to calculate the concentrations of the various elements in milligrams/gram of the sample. The elements analyzed include Magnesium (Mg), Calcium (Ca), Iron (Fe), Manganese (Mn), Zinc (Zn), Cadmium (Cd), Copper (Cu), Lead (Pb), Chromium (Cr), and Nickel (Ni),

Acute Toxicity Testing

The median lethal dose (LD_{50}) of EEHF following oral administration was determined according to the Organization for Economic Co-operation and Development (OECD, 425) procedure, using five (5) rats that were fasted for 16 to 18 hours before dosing with EEHF at 5000 mg/kg orally. The oral administration of the extract was achieved using an oral cannula in a single dose. The initial body weights of the fasted animals were recorded to determine the appropriate dosage of the extract. First, one (1) rat was t dosed and food was withheld for 3-4 hours. It was observed and monitored for the first 24 hours for the signs and symptoms of toxicity such as salivation, urination, lacrimation, changes in mucous membrane and skin, and other clinical symptoms or even death. The remaining four (4) rats were also dosed with EEHF and observed for two (2) weeks. After the test, the surviving animals were weighed, and they were euthanized using chloroform [16].

Oral Glucose Tolerance Test (OGTT)

Rats that were fasted overnight for 18 hours were used for the OGTT according to the method by Santosh *et al.* [17]. Initially, a glucometer (Roche Diagnostics, Germany) was used to determine the baseline blood glucose (BBG) of all fasted rats at 0 minutes. The animals were allotted into five (5) groups of five (5) rats each using a random method and were treated as follows: distilled water (1 ml/kg) as the negative control, three groups were administered EEHF at doses of 375, 750, and 1500 mg/kg, respectively, and fifth group received metformin 500 mg/kg as the positive control group. Thirty (30) minutes later, oral glucose solution (2 g/kg body weight) was administered to the animals. Thereafter BGL was measured at 30, 60, and 120 minutes.

Statistical Analysis

The data were analyzed using parametric repeated measure analysis of variance (ANOVA). For data with significant p values, Bonferroni's post-hoc test was used for multiple comparisons to determine where the significant difference lies. SPSS (version 25) was used for the analyses. The results were reported as mean ± standard error of the mean. Statistical significance was considered at $P \leq 0.05$.

RESULTS

Phytochemicals and Elements in EEHF

Phytochemical screening on EEHF revealed the presence of tannins, steroids, triterpenes, saponins, alkaloids, anthraquinones, and flavonoids. Anthraquinones were absent. Elemental content analysis of the EEHF shows the presence of potassium, sodium, magnesium, calcium, manganese, iron, zinc, chromium, copper lead, and nickel as shown in Table 1. Iron was the most abundant element while lead was found to be the least abundant. Both experimental phases yielded no detectable signs of toxicity or fatality, and the median lethal dosage (LD50) of EEHF was calculated to be above 5000 mg/kg**.**

EEHF Lowered Blood Glucose Levels at all Doses

To check the effect of our extract on glucose levels. We administered graded doses of the extract to three groups of rats and compared the results with untreated rats and rats treated with metformin, a standard antidiabetic agent. We observed that the baseline blood glucose levels in rats across all test groups were comparable ($p>0.05$). The blood glucose in the distilled water group rose gradually up to one hour and then decreased to a level similar to the baseline value by the end of 2 hours. However, the group of rats that received the extract at all doses experienced a fall in blood glucose level after 60 minutes and the decline continued to 120 minutes. Like in the metformin-treated rats, the group of rats that received extract (EEHF) at all tested doses also had blood glucose levels lower than their baseline value by the end of 120 minutes, as presented in Figures 1a and 1b.

DISCUSSION

In this experiment, we set out to primarily validate the ethnomedicinal use of *Hymenodictyon floribundum* as an antidiabetic herbal preparation. To this end, we examined, in addition to its effect on blood glucose, its acute toxicity and phytochemical profiles. To ascertain the possible negative consequences of drugs that might arise from inadvertent or intentional short-term exposure, an assessment of their acute toxicity potential is necessary [18]. Acute toxicity test results are used as a reference to determine the appropriate dosage for long-term toxicity investigations and other animal-based research [19]. The median lethal dose (LD_{50}) of EEHF was found to be greater than 5000 mg/kg indicating that the extract is essentially or practically non-toxic following administration.

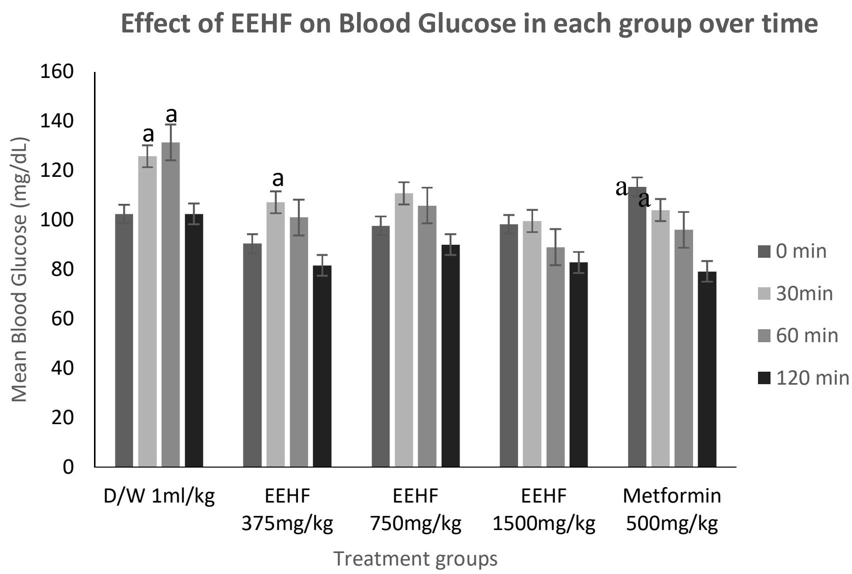
The glucose tolerance test (GTT) is applied to individuals with postprandial and fasting glucose levels at the borderline, to confirm or rule out the diagnosis of diabetes mellitus [20]. Postprandial hyperglycemia that occurs as a result of insufficient secretion or resistance to insulin is a sign of impaired glucose tolerance. In this study, the EEHF considerably reduced the postprandial rise in blood glucose level and greatly improved glucose tolerance in hyperglycemic rats evidenced by a significant fall in glucose level in the treated rats when compared to distilled water treated group. Also, at the highest test dose, The EEHF caused blood glucose levels to drop below baseline.

According to several studies [5,21,22], phytochemicals such as flavonoids, alkaloids, triterpenes, and saponins possess anti-hyperglycemic effects. The anti-hyperglycemic activity of phenolic compounds has been linked to the regulation of carbohydrate metabolism, improvement of glucose uptake, protection of pancreatic ß-cells, inhibition of end products in advanced glycation, as well as enhancement of insulin action [23–25].A family of phyto-constituents known as phenolic compounds exhibits high antioxidant activity [26]. In addition to enhancing antioxidant status, flavonoid compounds also produce considerable blood glucose-lowering effects by increasing the proliferation of pancreatic β-cells, as shown by the decrease in the concentration of glucose and the increase in serum concentration of C-peptide [27]. Alkaloids are yet another valuable plant secondary metabolite used in the management of dyslipidemia and diabetes mellitus [28]. They

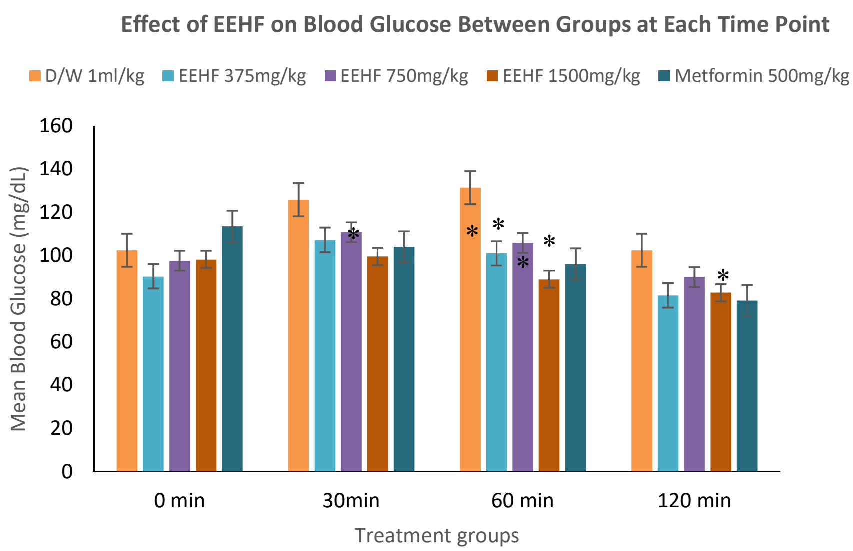
Table 1: Elemental Constituent of *Hymenodictyon floribundum*

Elements	Mg	Fe	Cd	Ni	Ca	Cu	Mn	Pb	Zn
Concentration(ppm)	1410	5342.7	5.42	2636.3	433.3	172.42	709.58	5.5	171.42

Key: ppp = parts per million. Mg: Magnesium, Fe: Iron, Cd: Cadmium, Ni: Nickel, Ca: Calcium, Cu: Copper, Mn: Manganese, Pb: Lead, and Zn: Zinc.



1a.



1b

Figure 1: Effect of Ethanol leaf Extract of *Hymenodictyon floribundum* on Blood Glucose Levels in Adult Wistar Rats.

*Blood glucose level is expressed as Mean ± SEM. Data were analyzed using repeated measure ANOVA followed by Bonferroni's post hoc test; [a] is $p \leq 0.05$ compared to baseline values and * is $p \leq 0.05$ compared to distilled water. N = 5*
Key: SEM = standard error of the mean; D/W Distilled Water,EEHF = Ethanol leaf Extract of Hymenodictyon floribundum; MET = Metformin, N = Number of rats in each group.

have been reported to cause a reduction in body weight and a significant enhancement in glucose tolerance [29]. Additionally, terpenes have been shown to have anti-hyperglycemic effects, reduce glycosylated hemoglobin, and simultaneously, promote insulin secretion from islet cells by blocking nitric oxide (NO) expression and oxidative stress [30,31]. Our experiment revealed the presence of some of these constituents in EEHF suggesting their probable role in the observed blood glucose-lowering effect.

Moreover, several mineral elements play a vital role in the treatment of hyperglycemia and they are considered potential and reliable sources of different required elements for patients with diabetes mellitus [32]. Studies have shown a strong relationship between some of these elements and insulin action [33], for example, magnesium is an important element in insulin action [34]. Potassium increases insulin sensitivity and reactivity while maintaining a normal blood glucose level [35]. Zinc has a protective effect against B-cell destruction [36]. Calcium is required for optimal insulin-mediated functioning and reduces the risk of developing type 2 diabetes due to enhanced insulin sensitivity [37]. Manganese is essential for glucose metabolism and its deficiency may cause glucose intolerance and altered carbohydrate and lipid metabolism [34]. Iron is found to influence glucose metabolism and insulin action [38]. Nickel is mostly found in the pancreas where it plays a significant role in the production of insulin [39]. All of these components play important roles in controlling a rise in the concentration of blood glucose. The presence of lead in considerably low quantities might be due to environmental factors like air pollution, the season in which samples were collected, and the age of the plant [40]. Although we did not compare the blood levels of these elements in the various treatment groups, their presence in the crude extract still provides a probable cause for the observed pharmacological activity. Further studies are needed to confirm their actual role.

We used metformin as the positive control because of its unique use as an oral hypoglycaemic agent. Metformin belongs to the biguanide class of hypoglycaemic agents and it is thought to act by reducing gluconeogenesis in the liver and increasing glucose uptake in skeletal muscle tissue [41]. In the present study, EEHF decreased blood glucose levels which follows the same pattern in the group treated with metformin. The significant decrease in blood glucose concentration justifies the anti-hyperglycemic activity which may be directly associated with the presence of the phytochemicals and elements in the plant.

CONCLUSION

This study, though simplistic in approach, shows that the EEHF possesses blood glucose-lowering potential and offers a scientific basis for its ethno-medicinal use in the management of diabetes.

CONFLICT OF INTEREST

The authors have no conflict of interest to declare.

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