AQUEOUS FRACTION OF *CHLOROPHYTUM ALISMIFOLIUM* LOWERS BLOOD GLUCOSE LEVEL IN WISTAR RATS

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Abstract

Diabetes mellitus contributes to one in nine deaths among adults aged 20–79 years globally. This research investigated the blood lowering effect of the aqueous fraction of *Chlorophytum alismifolium* (AFCA) in normoglycaemic and alloxan-induced hyperglycaemic rats. The tubers of *C. alismifolium* were processed and extracted with various solvents to obtain the aqueous fraction of *C. alismifolium*. Phytochemical screening was conducted using established protocol. The oral median lethal dose was established using the OECD 425 guideline. AFCA (150, 300 and 600 mg/kg) was administered to normal and alloxan-induced hyperglycaemic rats. Blood glucose levels were monitored at 0, 1st, 3rd and 5th hours. The LD_{50} was estimated to be greater than 5000 mg/kg. Administration of AFCA at the doses of 300 and 600 mg/kg significantly ($p<0.01$) reduced the blood glucose level in the 5th hour when compared to the normal control. In the antihyperglycaemic study, AFCA significantly ($p<0.05$) lowered the blood glucose level compared to the hyperglycaemic control at all the doses tested. Phytochemical screening revealed the presence of alkaloids, glycosides, triterpenes, flavonoids and saponins. In conclusion, AFCA lowered the blood glucose levels in normal and alloxan-induced hyperglycaemic rats.

Key words: Alloxan, *Chlorophytum alismifolium*, hyperglycaemia, hypoglycaemia, rats

Introduction

Diabetes mellitus (DM) is made up of a group of heterogeneous complicated metabolic disorders of the endocrine system characterized by persistent hyperglycaemia due to defects in insulin secretion, insulin action, or both (Harreiter and Roden, 2019). Symptoms of DM include; hyperglycaemia, polyuria, polydipsia, weight loss sometimes with polyphagia and blurred vision (Satoskar *et al*., 2015). Type 1 DM accounts for about 5-10% of all cases of diabetes and it is attributed to autoimmune destruction of pancreatic β-cells which leads to deficiency in insulin secretion and thus triggering several metabolic derangements (Godoy-Matos, 2014; Saeedi *et al*, 2019). Type 2 DM accounts for about 90-95% of all cases of

diabetes and linked to peripheral resistance to insulin action, especially in the muscles, altered pancreatic insulin secretion and increased production of glucose by the liver (Satoskar *et al*., 2015).

Recent reports by the International Diabetes Federation (IDF) estimated 5 million Nigerians to have DM (IDF, 2019). Globally, people living with DM have been estimated to be about 464 million; Sub-Saharan Africa (19 million), Europe (59 million), Middle East and North Africa (55 million), North America and Caribbean (48 million), South and Central America (32 million), South and East Asia (88 million) and Western Pacific (163 million) and this alarming figure is projected to rise to approximately 578 million by the year 2030 (IDF, 2019).

Although insulin is the main therapy for type 1 DM, there are several drawbacks to its use which include hypoglycaemia, hypokalaemia, hypomagnesaemia, neuroglycopenic symptoms and abdominal bloating (Satoskar *et al*., 2015). Sulphonylureas, biguanides and thiazolidinediones are the most commonly used oral antidiabetic agents for type 2 DM but limited by adverse effects like hypoglycaemia, lactic acidosis and hepatotocity respectively (Wang *et al*., 2017; Kennedy and Masharani, 2018). Due to the aforementioned adverse effects of the conventional antidiabetic agents, medicinal plants have gained wide acceptance and are being used traditionally for the management of DM and its complications (Rafe *et al*., 2017; Rahimi *et al.*, 2020). Many compounds obtained from plant sources have long been known to possess biological activities and therefore utilized in the management of several diseases including diabetes (Che and Zhang, 2019).

Chlorophytum alismifolium (Baker) belonging to the Liliaceae family is a widely utilized medicinal plant in the management of several ailments. It has been reported to possess antidiabetic activity (Abubakar *et al*., 2018; 2021a; 2021b), analgesic activity (Abubakar *et al*., 2020) and its sub-acute toxicological profile had also been established (Abukakar *et al*., 2019). This study focused on the blood glucose lowering effect of the aqueous fraction of the tubers of *C. alismifolium* in normoglycaemic and alloxan-induced hyperglycaemic Wistar rats.

Materials and Methods

Drugs and Chemicals

Alloxan-monohydrate (Sigma chemical Company St Louis, USA), pioglitazone (Micro Laboratories Limited, India), glimepiride (Sanofi Aventis, Frankfurt, Germany), normal saline and 10 % dextrose (Dana pharmaceuticals, Niger State, Nigeria), hexane, ethylacetate and butanol solvents (JHD, China).

Equipment

Glucometer (Accu-check Active), Roche, Germany, test strips (Accu-check Active), weighing balance, cotton wool, scissors, syringes (2 mL), spatula, animal cages, pestle and mortar.

Experimental Animals

Wistar rats weighing 130-170 g were obtained from the Animal House of the Faculty of Pharmaceutical Sciences, Ahmadu

Bello University Zaria, Nigeria. They were maintained under standard laboratory conditions and given free access to pelletized animal feed and water *ad libitum*. The studies were conducted with the approval of the Ahmadu Bello University Committee on Animal Use and Care (ABUCAUC) with the Ethical Approval Number: ABUCAUC/2020/31.

Plant Material

The whole plant of *C. alismifolium* was collected from Tilden Fulani River in Toro Local Government Area of Bauchi State. It was identified and authenticated by Mallam Musa Muhammed of the Herbarium Unit of the Department of Botany, Ahmadu Bello University Zaria, Nigeria. The plant was issued a voucher specimen number (No. 6785) after comparing with an existing reference voucher specimen.

Extraction of Plant Material

The tubers were washed, chopped into smaller sizes and then air-dried under shade until constant weight was obtained. The dried plant was then crushed into fine powder using pestle and mortar. The powdered plant (1 kg) was extracted sequentially with solvents of different polarities using a soxhlet extractor until the aqueous fraction was obtained (James *et al*., 2014; Dehgan *et al*., 2016). The fraction was coded; 'aqueous fraction of *Chlorophytum alismifolium'* (AFCA) and stored in a desiccator until further use.

Percentage Yield and Phytochemical Screening

The percentage yield of AFCA was calculated as (dry extract weight/dry starting material weight) × 100 and it was subjected to standard phytochemical screening for the presence or absence of phytochemicals including; alkaloids, flavonoids, saponins, glycosides, tannins, anthraquinones and triterpenes as described by Evans (2009).

Acute Toxicity Study

The median lethal dose (LD_{50}) of AFCA was determined in accordance with the method of the Organization for Economic Co-operation and Development (OECD, 425) guideline (2001) using five (5) rats which were fasted overnight for 12 hours before dosing with AFCA at the dose of 5 000 mg/kg orally. One (1) rat was first dosed and food was withheld for 4 hours. It was observed for the first 24 hours for signs and symptoms of toxicity such as hyperactivity, salivation , urination, changes in mucous membranes, skin, fur and eyes, circulatory, respiratory, somato-motor activity and mortality. The remaining four (4) rats were also dosed with AFCA and observed for two (2) weeks and thereafter, the LD_{50} was estimated.

Acute Hypoglycaemic Study

The hypoglycaemic effect of AFCA was investigated using the method described by Florence *et al*. (2014) with slight modification. Twenty five (25) rats were randomly divided into five (5) groups of five (5) rats each and treated orally as follows: Group I served as the negative control and received the vehicle only (distilled water, 10 mL/kg). Groups II to IV received graded doses of AFCA (150, 300 and 600 mg/kg respectively) while Group V served as the positive control and received glimepiride (1 mg/kg). Blood samples were drawn from the

tail vein prior to treatment at (0 hour) and at 1st , 3rd and 5th hours after treatment. Blood glucose levels were measured using a glucometer.

Induction of Hyperglycaemia

Forty (40) Wistar rats were injected with alloxan monohydrate dissolved in sterile 0.9% normal saline at a dose of 200 mg/kg *i.p.* (Federiuk *et al*., 2004). The rats were then kept for the next 24 hours on 10% dextrose solution in their cages to prevent hypoglycaemia. Seventy-two (72) hours post administration of alloxan, the animals were examined for the development of hyperglycaemia and those with fasting blood glucose levels of 190 mg/dL and above were considered hyperglycaemic and selected for the study.

Acute Antihyperglycaemic Study

The antihyperglycaemic activity of AFCA was evaluated according to the method described by Yusoff *et al*. (2015), with slight modification. Thirty (30) Wistar rats were divided into six (6) groups of five (5) rats each (normoglycaemic rats in the first group and hyperglycaemic rats in the other groups. Group I rats served as the normal control and received distilled water (1 mL/kg), while group II served as the hyperglycaemic control which was also administered distilled water (1 mL/kg), groups III to V received graded doses of AFCA (150, 300 and 600 mg/kg respectively) while group VI served as the positive control and received pioglitazone (5 mg/kg). Blood samples were drawn from the tail vein prior to treatment (0 hour) and then 1st, 3rd, and 5th hours after treatment. Blood glucose levels were measured using a glucometer.

Statistical Analysis

The data obtained were analyzed by repeated measure analysis of variance (ANOVA) followed by Bonferroni's post hoc test for multiple comparisons using statistical package for social sciences (SPSS) software (Version 20). The differences between means were considered significant at $p< 0.05$. All values were expressed as Mean ± Standard Error of the Mean (S.E.M.) and presented as figures.

Results

Percentage Yield and Phytochemical Composition

The Percentage yield of AFCA was calculated to be 1.9 % w/w. The preliminary phytochemical screening revealed the presence of alkaloids, flavonoids, glycosides, saponins and triterpenes and the absence of anthraquinones, steroids and tannins (Table 1).

Table 1: Phytochemical Constituents of Aqueous Fraction of *Chlorophytum alismifolium*

Constituents	Test	Observation	Inference
Glycosides	Kella-killani	A Purple ring at the interface	Present
Saponins	Frothing	Honey comb froth	Present

Flavonoid	Shinoda	Red coloration of the solution	Present
Alkaloids	Dragendorff	Red to brownish precipitate	Present
Tannins	Goldbeaters skin	The skin turns dark brown	Absent
Steroids	Salkowski	Cherry red coloration at the interphase	Absent
Triterpenes	Liebermann-Burchard	A reddish brown ring	Present
Anthraquinones	Bontrager	Cherry red/pink coloration	Absent

Acute Toxicity

Oral administration of AFCA at the dose of 5000 mg/kg in rats did not produce any visible sign of toxicity or mortality over a period of 14 days. The oral median lethal dose (LD_{50}) was therefore estimated to be above 5,000 mg/kg.

Effect of Aqueous Fraction of *Chlorophytum alismifolium* on Blood Glucose Levels in Normoglycaemic Rats

Administration of AFCA at the dose of 300 mg/kg significantly ($p<0.01$) reduced the blood glucose level in the 5th hour when compared to the normal control. Similarly, AFCA at the dose of 600 mg/kg significantly ($p<0.05$, $p<0.01$) reduced the blood glucose level in the 3rd and 5th hours respectively when compared to the normal control (Figure 1).

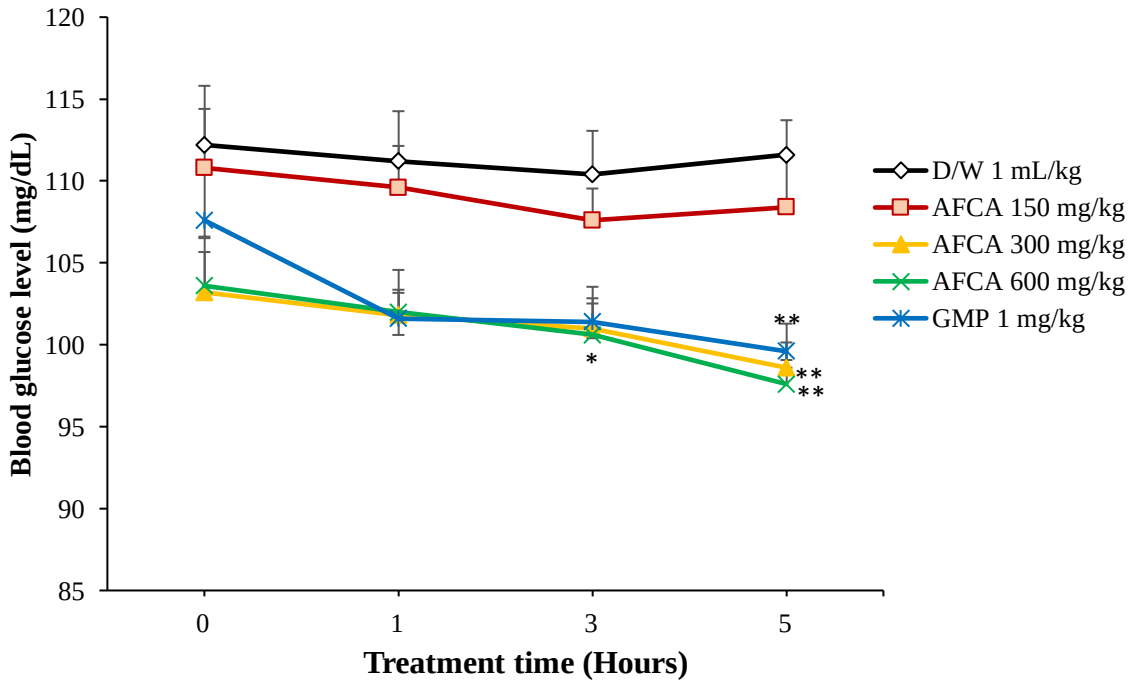

Figure 1: Effect of Aqueous Fraction of *Chlorophytum alismifolium* on Blood Glucose Levels in Normoglycaemic Rats

Values are presented as Mean ± S.E.M., * = p<0.05, ** = *p*<0.01 when compared to D/W group, Repeated measure ANOVA followed by Bonferroni's post hoc test, n =5, D/W = Distilled water, AFCA= Aqueous fraction of *Chlorophytum alismifolium*, GMP= Glimepiride

Effect of Aqueous Fraction of *Chlorophytum alismifolium* on Blood Glucose Levels in Alloxan-Induced Hyperglycaemic Rats

Induction of hyperglycaemia produced a significant (*p*<0.05) increase in blood glucose level in the hyperglycaemic control when compared to the normal control. Treatment with AFCA at the dose of 150 mg/kg significantly (*p*<0.05) lowered the blood glucose level in the 3rd and 5th hours when compared to the hyperglycaemic control. Similarly, treatment with AFCA at the doses of 300 and 600 mg/kg significantly (*p*<0.01) lowered the blood glucose levels in the 3rd and 5th hours relative to the hyperglycaemic control. On comparison over time, AFCA at the dose of 300 mg/kg significantly (*p*<0.05) reduced the blood glucose level in the 5th hour when compared to 0 hour. AFCA also significantly (*p*<0.01) reduced the blood glucose level at the dose of 600 mg/kg in the 3rd and 5th hours when compared to 0 hour (Figure 2).

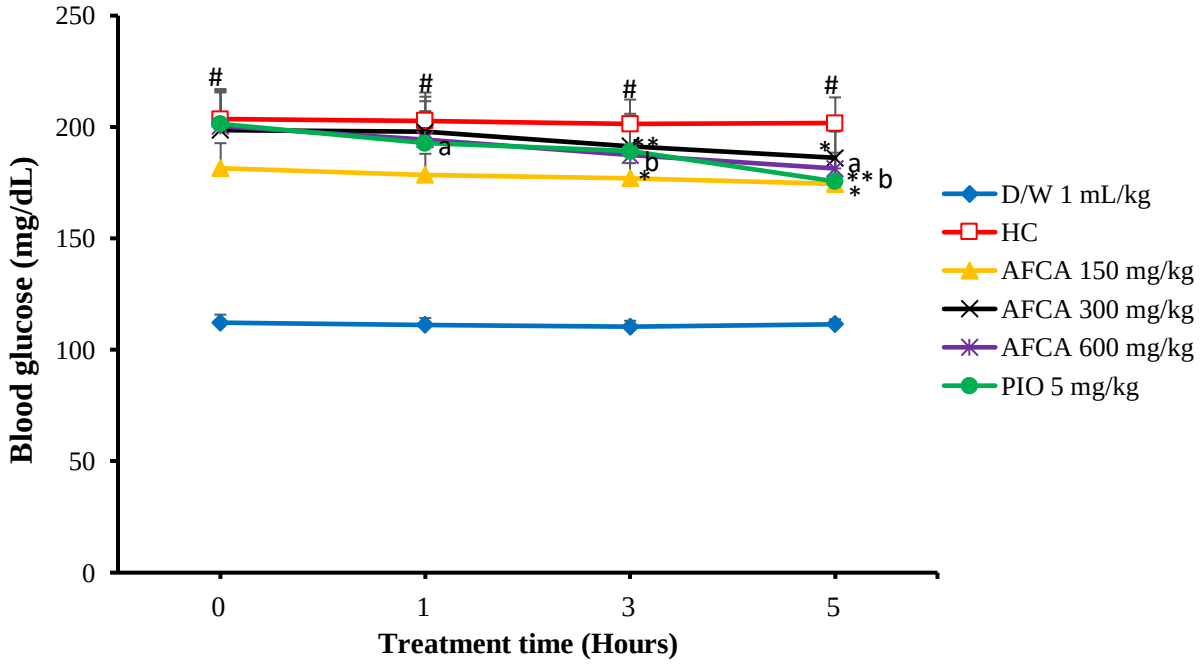

Figure 2: Effect of Aqueous Fraction of *Chlorophytum alismifolium* on Blood Glucose Levels in Alloxan-Induced Hyperglycaemic Rats

Values are presented as Mean ± S.E.M., # = *p*<0.05 when compared to D/W group, * = *p*<0.05, ** = *p*<0.01 when compared to HC group, a = *p*<0.05, b = *p*<0.01 when compared to 0 Hr – Repeated

measure ANOVA followed by Bonferroni's post hoc test, n =5, D/W = Distilled water, HC = Hyperglycaemic control, AFCA= Aqueous fraction of *Chlorophytum alismifolium,* PIO= Pioglitazone

Discussion

This research investigated the antihyperglycaemic activity of the aqueous fraction of *Chlorophytum alismifolium* tubers in Wistar rats. Phyto-constituents play a significant role as therapeutic agents (Das *et al.,* 2015). The percentage yield is a measure of the solvent's efficiency to extract specific components from the original plant material (Adam *et al.,* 2019). The low percentage yields are attributed to the part of the plant used because the tubers unlike the stem barks or leaves are associated with less yield.

Toxicological investigations are performed in experimental animals to establish safety and to provide a rationale for choosing non-toxic doses in humans (Ekeanyawu and Njoku, 2014). Acute toxicity evaluation is generally the first step examination to be carried out in experimental studies and it provides relevant information on the safety margin and the relative toxicity expected to emanate from a single exposure for a short period of time (Ekeanyawu and Njoku, 2014; Colerangle *et al.*, 2017). The acute oral toxicity profile of AFCA was found to be greater than 5000 mg/kg in this study which indicates that it is relatively non-toxic on acute exposure (Erhirhie *et al*., 2018).

In the hypoglycaemic study, administration of AFCA at the doses of 300 and 600 mg/kg significantly lowered the blood glucose levels in the rats which is an indication that it can trigger hypoglycaemia. Alloxan is a urea derivative which causes selective necrosis of the pancreatic β-cells and thus producing experimental diabetes in animals including rats (Etuk, 2010). In the antihyperglycaemic study, administration of alloxan triggered hyperglycaemia which was improved following the administration of AFCA at all doses when compared to the hyperglycaemic control and over time.

The observed antihyperglycaemic activity of AFCA could be attributed to the existence of secondary metabolites such as alkaloids, flavonoids, saponins and triterpenes in the plant extract. Alkaloids represent a diverse group of natural products with an array of therapeutic properties and in the last two decades, research on their anti-diabetic activity has tremendously increased (Christodoulou *et al.,* 2019). Naturally occurring flavonoids and polyphenols possess anti-diabetic effect elicited through the glucose transporter, hepatic enzymes, tyrosine kinase, PPAR-ɤ, lipid profile and antioxidant systems (Al-Ishaq *et al*., 2019; Mukhtar *et al.,* 2020). Saponins have potential therapeutic benefits and are theorized as an alternative medication in decreasing serum blood glucose levels in the people suffering from diabetes (Choudhary *et al.,* 2020). Terpenoidal compounds have also been reported to elicit ɑ-glucosidase inhibitory activity and observed to improve outcomes in diabetics (Assefa *et al*., 2019). The secondary metabolites present in the AFCA used in this study are most probably responsible for its observed antidiabetic activity. It is possible that the observed effect is as a result of the combined antidiabetic

activity of one or more secondary metabolites detected in the AFCA. There is no guarantee that further purification of this fraction will increase the antidiabetic potency at the similar doses.

Conflict of Interest

The authors have no conflict of interest to declare.

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