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Effect of Methanolic Extract of *Passiflora foetida* on Glucose Kinetics in Alloxan-induced Diabetic Mice

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Author's contribution

The sole author designed, analyzed and interpreted and prepared the manuscript.

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ABSTRACT

Background: This study investigated the hypoglycemic potential of methanolic extract of *Passiflora foetida* (PF) in alloxan-induced diabetic albino mice. Diabetes is a metabolic disease characterized with high blood sugar levels over a prolonged period. Discovery of a new drug will greatly reduce the mortality and morbidity associated with the disease.
Methods: Diabetes was induced in albino mice by administration of 150 mg/kg body weight (b.w.) alloxan. Different concentrations of the methanolic extract of PF was prepared and administered orally to groups of alloxan-induced diabetic mice. Blood glucose was determined at different time points over 4 hrs.
Results: The extract reduced blood glucose levels in diabetic mice significantly ($P<0.001$) and the kinetic parameters such as, area under glucose concentration time curve ($AUC_{0\text{-}4hG}$) ($P<0.05$), glucose mean residence time (MRT_G), glucose $t_{1/2G}$ were significantly lower (<0.05) in PF treated groups when compared with the control groups. The rate of glucose clearance (CL_G) was high in the group treated with the extract.
Conclusion: The results of this study indicate presence of hypoglycemic constituent in the plant.

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Keywords: Antidiabetics; glucose kinetics; Passiflora foetida; plant.

1. INTRODUCTION

Diabetes mellitus (DM) is one of the leading causes of death and should not be left neglected. It is the most common endocrine disorder that occurs not only in the poor but also occurs greatly among the rich and all nations are suffering the impact of the diabetes epidemic [1]. The disease is worse in many low and middle income countries. The prevalence of diabetes was estimated to be 9% among adults ≥18 years as reported by WHO in 2014 and the total number of people with diabetes is projected to rise from 171 million in 2000 to 366 million in 2030. The prevalence of diabetes is higher in men than women, but there are more women with diabetes than men [2,3].

Diabetes is one of the causes of several other important and often lethal diseases, both non-communicable diseases such as cardiovascular disease and renal disease and communicable diseases such as pneumonia, bacteraemia and tuberculosis, which have considerable impacts on morbidity and mortality [4-9]. Despite the introduction of hypoglycemic agents from natural and synthetic sources, diabetes and its secondary complications continue to be a major medical problem. Many medicinal plants have found to be useful to successfully manage diabetes. One of the greatest advantages of traditional medicinal plants is that they are readily available and have no side effects [10]. Apparently, WHO has suggested the evaluation of the potential use of plants as effective therapeutic agents, especially in areas where there is lack of safe modern drugs [1].

The use of herbs and plant-derived products for treating various diseases has been a common practice since ages and there is need to investigate the use of some of these plants. *Passiflora* is the largest genus in the *Passifloraceae* family and comprises nearly 500 species [11]. *Passiflora foetida,* also called stinking Passion flower a member of the *Passifloraceae,* is a widely growing perennial climber which has been used in traditional medicine for treating many ailments. The species is distributed in the warm and tropical regions of the world which actually originated from South America. Traditionally, the plant has been used as antiproliferative, sedative, anti-anxiety, antibacterial, dressing for wounds and antiulcer [12-14]. However it is also important to investigate the anti-diabetic potential of methanolic extract of *Passiflora Foetida* in alloxan-induced diabetic mice.

2. MATERIALS AND METHODS

2.1 Plant Materials

The plants of PF were collected in the month of April and June 2011 from Lagelu Local Government area of Ibadan, South-west Nigeria. The plant was identified and its authenticity was confirmed by comparing the voucher specimen at the herbarium of Forest Research Institute (FRIN), Ibadan, Nigeria. A voucher specimen was kept in the herbarium for record.

2.2 Extraction

The freshly collected plant (except fruits) were washed well with water and dried under shade for two weeks. After complete drying, the plants were further chopped into small pieces and reduced to powder using electric blender. About 500 g of the blended plant were later extracted with 2 L of methanol for 7 days to obtain the crude methanolic extract. Methanol is an amphiphilic compound. It helps to extract all the various chemical groups, from plant material. The liquid filtrate was then concentrated and evaporated to dryness on a water bath. The plant extract yielded 8.62%. The dried crude extract was stored in a refrigerator at 4^{O}C until used for experiment.

2.3 Experimental Animals

Swiss albino mice (19-25 g) of both sexes were used for these experiments. The animals were obtained from the Animal House of Malaria Research Laboratories, College of Medicine, University of Ibadan, Nigeria. The animals were housed in standard cages, well-ventillated and were fed with standard pellet and water *ad libitum*. They were handled according to the standard protocols for the use of laboratory animals [15].

2.4 Chemicals

Alloxan and glibenclamide (Sigma–Aldrich Co., USA). Blood glucose was determined using Accu-Chek active glucometer (Roche Diagnostics) following the manufacturer's instructions. All chemicals and drugs used were of the highest purity and analytical grade.

2.5 EXPERIMENT

2.5.1 Normoglycemic mice

The blood glucose concentration was determined at zero time in four groups of 4 set of mice weighing between 20-25 gms. The groups were divided to control, and group administered with 200 mg/kg, 400 mg/kg of the aqueous extract (0.2 mL) administered orally and the blood glucose values were determined 30, 60, 90, 120, 180 and 240 min later. The values obtained were compared with the corresponding control studies from mice receiving water only. Similarly, glibenclamide 5 mg/kg body weight was given orally to fasted mice at the same intervals for the same duration.

2.5.2 Induction of diabetes mellitus

Swiss albino mice (20–25 g) were fasted overnight prior to injection with alloxan monohydrate dissolved in sterile distilled water. Diabetes mellitus was induced in all the animals by intraperitonial injections of alloxan dissolved in sterile water (200 mg/kg). Diabetes was allowed to develop and stabilize in these alloxan-treated mice over a period 7 days. The blood glucose level was monitored in randomly selected mice to know the progression to hyperglycemia.

2.5.3 Antidiabetic treatment

The animals were randomly divided into four groups of six animals per group. Before the commencement of the experiment all the mice (alloxan-treated) were fasted overnight but still allowed free access to water throughout. Fasted alloxan-treated mice with blood glucose concentrations ≥150 mg/dL were considered to be diabetic, and used in this study [16]. Those that their glucose level was less than this value were not included in the study. At the end of the overnight fasting period– taken as zero time (i.e., 0 hr), blood glucose levels (initial glycose level – *G*o) of the alloxan-treated diabetic mice were determined and recorded. Glibenclamide (5 mg/kg) was used as the reference antidiabetic (hypoglycemic) agent for comparison. The negative control (Group I, untreated diabetics) mice were administered with sterile distilled water (0.2 ml oral) only. The group II and III which were all diabetics administered orally with, 200 and 400 mg/kg b.w of *P. foetida,* methanol extract and group IV treated with 5 mg/kg b.w of glibenclamide (positive control). Blood glucose (G_b) were determined at 0, 0.5, 1, 2, 3 and 4 hr following administration of the extract to the mice. In each case, blood samples were collected from the tail tip vein of each mouse for blood glucose level determination. This experiment was performed thrice for consistency and better statistical analysis. All the experiment were carried out at the Department of Pharmacology, College of Medicine, Ekiti State University, Nigeria.

2.6 Data Analysis

The glycaemia kinetic was calculated to determine the glucose mean residence time (MRT_G), the area under-glucose-concentration time curve ($AUC_{0\text{-}4hG}$), rate of glucose clearance (Cl_G) and the time its take the glucose concentration to reduce to its half value ($t_{1/2G}$). All data were expressed as means (±S.D). Turbo Ken (Department of Clinical Pharmacology, Southampton University, UK) was used for the kinetic analysis. GraphPad Prism was used for the statistical analysis. Student's *t*-test was used to determine a significant difference between the control group and experimental groups. $P < 0.05$ was considered as significant compared to control.

3. RESULTS

The effect of the methanolic extract on normoglycemic mice was determined and this is shown in Fig. 1. The methanolic extract produced a significant decrease in the blood glucose level in the overnight fasted mice between 120 and 180 minutes after administration.

The effect of aqueous extracts of *Passiflora foetida* on the blood glucose levels of alloxan-induced experimental animals was evaluated at various time interval for over 4h after oral administration of 200 or 400 mg/kg body weight (Fig. 2 and Table 1). There was a significant elevation in the blood glucose level by 3-5 time during experimental time period in alloxan-induced diabetic mice, when compared to normal mice. However, the administration of methanolic extract caused the blood glucose to reduce significantly to 64 and 76% at 1h and 4hr after administration ($p<0.05$) and these was observed in the test group administered with 400 mg/kg compared to the group that was administered with 200 mg/kg and the control groups.

Studying the kinetics of glucose during exposure to anti-diabetics drug is important to know the rate of glucose reduction in order to avoid hypo

or hyperglycemia condition. However as this was monitored in this study, the glucose kinetic data (Table 1) shows that *Passiflora foetida* exhibited high glucose reduction as indicated by the glucose half-life (t1/2 G) when compared with the control group (p<0.05). In addition, the mean total blood glucose clearance was 0.30±0.0 dL/hr and 0.5±0.01 dL/hr in the groups of animal that were administered with 200 and 400 mg/kg of the extract respectively. In the control groups the rate of glucose clearance was 0.13±0.04 and 0.4±0.01 dL/hr for the group administered with distilled water and 5 mg/kg glibenclamide respectively. The rate of glucose clearance was high in the extract groups compared to the other control groups (p<0.001).

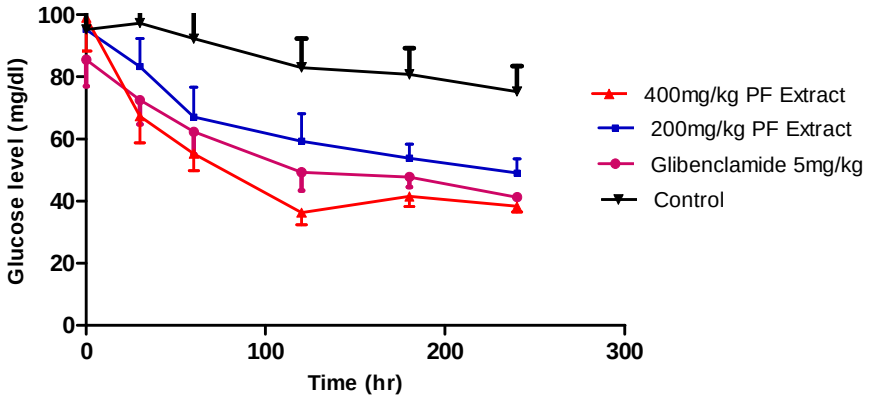

Fig. 1. Effect of methanolic extract of *Passiflora foetida* in normoglyceamic mice

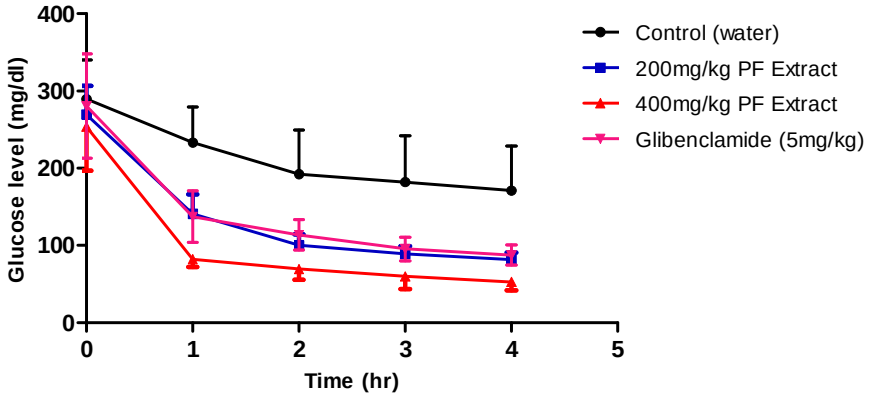

Fig. 2. Blood glucose levels over 4 hr after single oral administration of distilled water, 5 mg/kg glibenclamide and methanolic extract of *Passiflora foetida* (200 mg/kg and 400 mg/kg) in alloxan-induced diabetic mice

Values are expressed as means±sem

Table 1. Blood glucose kinetics over 4hr after single oral administration of distilled water, glibenclamide and methanolic extract of *Passiflora foetida* (200 mg/kg and 400 mg/kg) in alloxan-induced diabetic mice

Parameters	Weight (g)	$T_{1/2G}$ (hr)	Cl_G ($dLhr^{-1}$)	MRT_G (hr)	$AUC_{0\text{-}4hG}$ ($mghrdL^{-1}$)
Control (Dist. water)	22.1±4.2	$7.4±0.8^{+}$	$0.1±0.04^{+}$	$10.5±1.4^{+}$	$858.5±48.1^{+}$
200 mg/kg extract	19.9±1.2	$2.9±0.5^{*a}$	$0.3±0.1^{*b}$	$3.9±0.7^{*a}$	$534.1±64.9^{*a}$
400 mg/kg extract	21.2±2.1	$2.6±1.2^{\#a}$	$0.5±0.3^{*b}$	$4.0±1.8^{\#a}$	$310.2±66.4^{*a}$
Glibenclamide (5 mg/kg)	21.4±1.2	$3.0±0.0^{*}$	$0.3±0.1^{*}$	$4.3±1.1^{\#}$	$534.4±161.0^{\#}$

*Data are expressed as mean±s.d; *p<0.001, #p<0.05, treated vs control; [a]p>0.05, [b]p<0.05, treated vs glibenclamide; glibenclamide vs control [+]p<0.05,n=6 in all groups*

The exposure of glucose to the drug (extract) determined by the area under the glucose concentration time curve ($AUC_{0-\ 4hG}$) in the groups that were treated with the extract showed a significant reduction (p<0.005) especially in group that was treated with 400 mg/kg b.w. of the extract compared to the control groups but similar to group treated with glibenclamide. AUC_G reflect the actual exposure of glucose (diabetic) to the drug (extract) after administration. Similarly, this study showed that the extract lowered the glucose mean residence time (MRT_G) in the groups of mice administered with extract (3.9±0.7 hr and 4.0±1.8 hr) compared to the control group (10.5±1.4 hr) (P<0.05).

4. DISCUSSION

The use of medicinal plant in the treatment of disease is important to be considered. In spite of the presence of known antidiabetics medicine in the pharmaceutical market, more and more interest is now growing all over the world in the use of medicinal plant for the treatment of diabetics in patient successfully [17-21]. Not only because they are easily accessible but, plant or herbal formulations are frequently considered to be less toxic and free from side effects than synthetic one. In general, there is little biological knowledge on the specific mode of action in the treatment of diabetes, but most of the plants have been found to contain substances like glycosides, alkaloids, terpenoids, flavinoids that are frequently implicated as having antidiabetics effects [22]. Apparently, previous phytochemical study have shown the presence of these substances in *Passifloa foetida* [23]. It is found that the methanolic extract significantly reduced the alloxan-induced diabetes in a dose dependent manner. Even a 200 mg/kg dose of plant extract produced significant effect as compared to standard dose of glibenclamide. Toxicity test done in previous studies have shown that doses of *Passiflora foetida* crude extract is non-toxic and does not produced any mortality even at doses as high as 2000 mg/kg in mice [24]. In this study the extract did not alter the general behavior of the mice and the only mortality recorded was due to injury suffered during administration of alloxan induction.

Alloxan is a “chemical’ widely used for induction of experimental diabetes in variety of animal species by damaging the insulin secreting pancreatic β-cells, resulting in a decrease endogenous insulin release [25]. The cytotoxic action of this diabetogenic agent is mediated by reactive oxygen species, superoxide radicals, hydrogen peroxides and hydroxyl radicals [26]. These hydroxyl radicals are ultimately responsible for the death of the β-cells [27]. Several studies have shown the use of plant extract to reduce the alloxan-induced glucose level in animal model [28]. The hypoglyceamic action of the *Passiflora foetida* may be attributable to scavenging of the free radicals and antioxidant activities as shown by some plant [26,27]. In addition, it could also be as a result of protective effect on DNA damage caused by the hydroxyl radicals. Based on the above mentioned report, we suggest that the possible mechanism of *Passiflora foetida* methanolic extract could be related to antioxidant that aid the recovery from impaired metabolism of glucose. In another perspective, study have shown regeneration of β-cells in both human and rodent following diabetogenic injury if autoimmunity is blocked [29]. The antidiabetic effect of this plant could be attributed to increase release of insulin from β-cells of the pancreas as regeneration of β-cells take place when autoimmunity is blocked by the plant chemical constituents. The findings by Yessoufou et al. [30] have revealed that plant with good level of alkaloids exhibited a significant immunosuppressive action as this has been reported as one of the phytochemical component of PF [22,23].

The glucose AUC has been used as an index of glycemic excursions after meals in nutrition-related studies [31] because it accurately indicates the complete post-prandial increase in blood glucose. This is dependent on the rate of utilization of glucose in the body and the clearance is an indication of this effect. This is also dependent on the bioavailability of the extract. The AUC_G is inversely proportional to the clearance of the glucose. That is the higher the clearance, the less time the glucose spend in the systemic circulation and the faster the decline in the blood glucose level. In this study it shows that the rate of glucose clearance is significantly higher in all the groups treated with the extract and glibenclamide.

The limitation of this study is that the blood glucose level was not monitored for a long period of time to give an existing life representation of the disease in human. However further study is important to determine the chemical constituent that excerted the hypoglycemic activities of the plant observed in this study.

5. CONCLUSION

The results of the present study suggest that the plant, *Passiflora foetida* could be a good source of alternative or supplementary herbal remedy for treatment of diabetics. Further research is essential to identify active principle(s) and explore the mechanism involve for its antihyperglycemic activity.

CONSENT

It is not applicable.

ETHICAL APPROVAL

It is not applicable.

COMPETING INTERESTS

Author has declared that no competing interests exist.

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