



**EVALUATION OF EFFECT OF TIME OF COLLECTION ON HYPOGLYCEMIC
POTENTIAL OF MISTLETOES (*VISCUM ALBUM*) GROWN ON COCOA TREE**

Akinlami Omokehinde Oseni*

PhD Scholar, Department of Chemistry, Adeyemi College of Education, Ondo, Nigeria.

***Corresponding Author: Akinlami Omokehinde Oseni**

PhD Scholar, Department of Chemistry, Adeyemi College of Education, Ondo, Nigeria.

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ABSTRACT

This study was carried out to evaluate the effect of time of collection on hypoglycemic potential of mistletoes (*viscum album*) grown on cocoa tree. Aqueous leaves extracts of different time of collection of *Viscum album* were prepared and oral glucose tolerance test method was used to determine quantitatively the hypoglycemic potential of plant extracts in hyperglycemic rats. In the experiments, after 150 min of after post glucose challenge, the Sunrise aqueous extract of *V album* produced a maximum fall of 36.96%, Noon extract was 34.65%, also Sunset extract was 45.58% while maximum fall of 45.60% and 28.90% were recorded for Standard group and Control group respectively. The hypoglycemic values were significant ($P = 0.05$) when compared different time of plants collection. The results showed that the hypoglycemic potentials depend on the time of collection which was maximum when the leaves were obtained at sunset. It is therefore advisable that the use of this extract in herbal medicine should be encouraged and the plant should be plucked at sunset to maximize the hypoglycemic potential of the mistletoes.

KEYWORDS: *Viscum album*, Oral glucose tolerance test, hypoglycemic, collection.

INTRODUCTION

Today, most of the developing countries notable Nigeria has adopted traditional medical practice as an integral part of their culture and plant remedies are now being used as alternative medicines and they are proving to be very effective, more especially in the treatment of diabetes. This ailment has been treated successfully with the use of medicinal plants and herbal preparations.^[1] It is on this basis that researchers keep on working on the medicinal plants in order to produce the best medicines for physiological uses. The Oral glucose tolerance test (OGTT) is often referred to as physiological induction of diabetes mellitus because the blood glucose level of the animal is transiently increased with no damage to the pancreas. It indicates the body's ability to utilize a type of sugar named glucose that is the body's main source of energy. Oral glucose tolerance tests, a test of immense value and sentiment, in favour of using fasting plasma glucose concentration alone as a practical attempt to simplify the diagnosis of diabetes.^[2] Hyperglycemia is an important factor in development and progression of the complication of diabetes mellitus.^[3] *Viscum album* (mistletoe) is a hemi-parasitic shrub, frequently globular in shape. It grows on the branches of other trees, to which it is attached by a swelling called a haustorium. As a hemi-parasite it depends on its host for water and mineral nutrients but is able to photosynthesize (create its carbohydrates using

sunlight) because it has green leaves and stem. *Viscum album* species contain lectins (viscumin/agglutinin), protein toxins, alkaloids and polysaccharides. A number of biological effects, such as anticancer, antimicrobials, antiviral, apoptosis inducing and immunomodulatory activities have been reported for mistletoes.^[4] It was reported to be effective in the management of chronic metabolic disorder such as diabetes.^[5] Obatomi et al.^[5] also reported the antioxidants properties in mistletoes growing on cocoa and cashew trees in Nigeria. They found out that mistletoes growing on cocoa had more antioxidants properties than mistletoes grown on cashew tree. Also, Bussing and Schietzel^[6] reported that the biological activities of *Viscum album* are dependent on the host tree, manufacturing process, and time of collection. According to Akinlami^[7] the antioxidants values of *Viscum album* grown on orange tree are dependent on the time of collection of the plant from the host tree. Therefore, the present study was conducted to evaluate the effect of time of collection on hypoglycemic potential of mistletoes (*viscum album*) grown on cocoa tree.

MATERIALS AND METHODS

Chemicals: Glucose and metformin tablet respectively were obtained from Uche Care Pharmacy, Ondo. All other chemicals and reagent used were of analytical grade.

Experimental Plant: The fresh leaves of mistletoe plant (*Viscum album*) from host plants Cocoa tree were collected from a research farm of Adeyemi College of Education, Ondo, Ondo State, Nigeria in sunrise (6:00 AM), at noon (12:00 AM) and at sunset (6:00 PM). Authentication of the plants was done in the department of Biological Sciences of the institute.

Preparation of Aqueous Extract

The fresh leaves of mistletoe plant (*Viscum album*) were sorted out to remove extraneous material and rinsed with water to remove debris and dust particles. They were air dried for two weeks, packed in a paper bag stored and pulverised. A portion (50 g) of the powdered leaves was weighed into a beaker and 500ml of warm distilled water was added and stirred for 20 minutes. This was left to stand for 24 hours. It was then filtered using Whatman filter paper to obtain *Viscum album* aqueous extract and use for hypoglycemic analysis.

Test Animals: Male albino rats weighing 120-220 gm. of were procured from Covenant's feeds Care, Ibadan, Oyo State. All the animals were kept in standard polypropylene cages and maintained at $27^{\circ}\text{C} \pm 2^{\circ}\text{C}$ under 12 h. dark/light cycle. The animals were fed with standard rat feed and water was given ad libitum. Ethical clearance for handling the animals was strictly adhered to.

Oral Glucose Tolerance Test (OGTT)

This test was performed according to Shirwaikar & Rajendran.^[8] Rats were divided into five groups (n = 5). They were fasted overnight and accessed to water only. Blood was taken from the lateral veins of the tail and the blood sugar levels (0 min) were initially monitored with a glucometer (One touch Horizon). Fasting blood glucose greater than 50 mg/dL was included in the study. Sunrise group I, Noon group II, and Sunset group III treated with (500 mg/kg, p.o., each) of *V.album* leaves extracts. Standard group IV treated with 100mg/kg of metformin as a standard drug while Control group V received normal saline (10ml/kg). After 30 min, the animals were treated with 5% (wt/v) glucose orally. Blood glucose levels were monitored from lateral tail veins at 30, 90 and 150 min intervals after post glucose challenge.

STATISTICAL ANALYSIS

The data obtained were statistically analyzed using the Statistical Package for Social Sciences (SPSS) version 23. Blood glucose values were expressed in mg/dL as mean \pm SEM. The data were statistically analyzed using ANOVA with multiple comparisons by Turkey's method. The results were considered statistically significant if $P < 0.05$.

RESULT AND DISCUSSION

Figure i depict the fasting blood glucose level of normal healthy rats after fasted for 18 hours with only access to water. The blood glucose values range between 68 and

103. The effect of time of collection of *Viscum album* (mistletoes) aqueous extracts against it is hypoglycemic effect on blood glucose level of normal healthy rats after 30 min of after post glucose challenge was measured (Fig. ii). Experimental induction of hyperglycemia by intragastric ingestion of glucose resulted in a 1.5-fold increase in plasma glucose levels comparing bar groups of 0 minute (Fig, i). The Sunrise group increased by 52.3%, Noon group increased by 58.0%, Sunset group was 45.08% while 45.52% and 83.41% were recorded for Standard group and Control group respectively.

The effect of time of collection of *Viscum album* (mistletoes) aqueous extracts against it is hypoglycemic effect on blood glucose level of normal healthy rats after 90 min of after post glucose challenge was measured (Fig. iii). The dose of 500mg kg⁻¹ of Sunrise *V.album* aqueous produced a maximum the fall of 28.11%, Noon extract was 25.5%, also Sunset extract was 31.86% while maximum fall of 34.20% and 20.60% were recorded for Standard group and Control group respectively at 1 h after glucose administration.

The effect of time of collection of *Viscum album* (mistletoes) aqueous extracts against it is hypoglycemic effect on blood glucose level of normal healthy rats after 150 min of after post glucose challenge was measured (Fig. iv). The dose of 500mg kg⁻¹ of Sunrise *V.album* aqueous produced a maximum the fall of 36.96%, Noon extract was 34.65%, also Sunset extract was 45.58% while maximum fall of 45.60% and 28.90% were recorded for Standard group and Control group respectively at 2 h after glucose administration.

OGTT-induced hyperglycemia has been described as a useful experimental model to study the activity of hypoglycemic agents.^[9] The purpose of the present study was to investigate the effect of time collection on the hypoglycemic potential of Mistletoes (*Viscum album*) Grown on cocoa. All the aqueous extract of *V. album* leaves collected at different time improves glucose tolerance in hyperglycemic rats. The hypoglycemic activity of the aqueous leaves extract was better than all others when the leaves was collected at Sunset the result was in agreement with similar report by Akinlami,^[7] on the antioxidants potential of the plant collected at different time. Metformin was used as a standard reference drug. It is interesting to note that the extract collected at Sunset was almost effective as the standard drug. It is likely to be expected that the aqueous extract of leaves has some direct effect by increasing the tissue utilization of glucose^[10,11] by inhibiting hepatic gluconeogenesis or absorption of glucose into the muscles and adipose tissues.^[12]

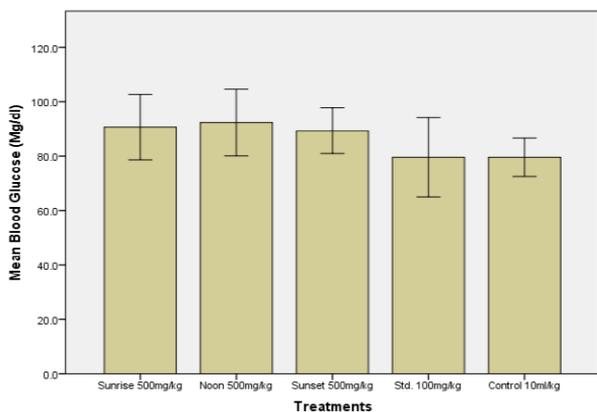


Fig. I. FBG level (0 min) of normal healthy rats. Each value shown in mean \pm S.D. (n = 5).

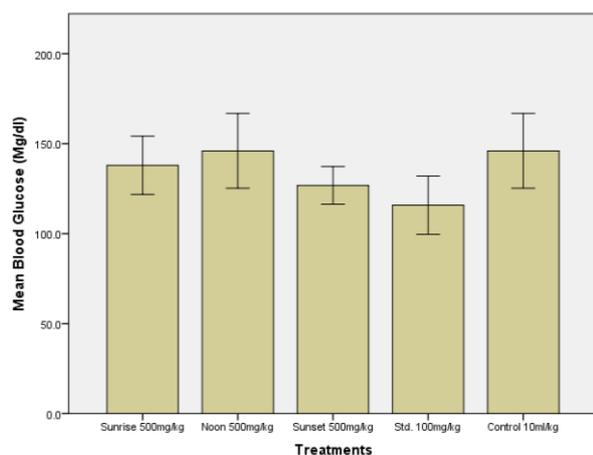


Fig. ii. Effect of time of collection of *Viscum album* (mistletoes) aqueous extracts against it is hypoglycemic effect on blood glucose level of normal healthy rats after 30 min of after post glucose challenge, each value shown in mean \pm S.D. (n = 5).

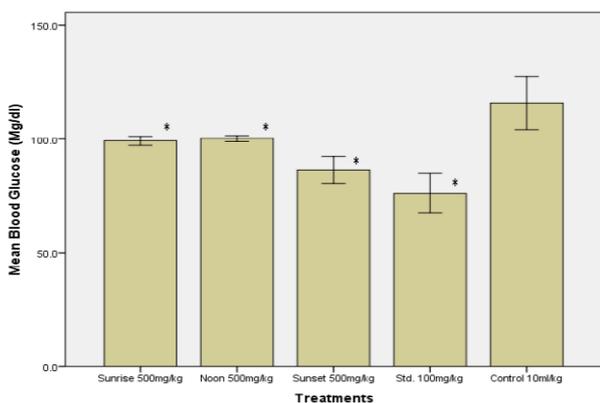


Fig.iii. Effect of time of collection of *Viscum album* (mistletoes) aqueous extracts against it is hypoglycemic effect on blood glucose level of normal healthy rats after 90 min of after post glucose challenge, each value shown in mean \pm S.D. (n = 5). *Significant change at P=0.05 with respect to the control.

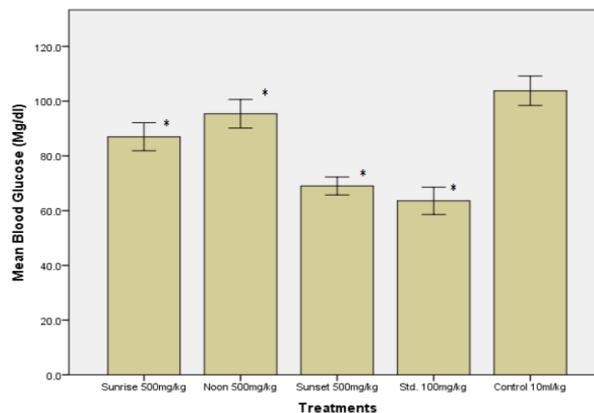


Fig. iv. Effect of time of collection of *Viscum album* (mistletoes) aqueous extracts against it is hypoglycemic effect on blood glucose level of normal healthy rats after 150 min of after post glucose challenge, each value shown in mean \pm S.D. (n = 5). *Significant change at P=0.05 with respect control.

The antioxidants studies of extracts of plant extract revealed that values of total phenol, flavonoids and DPPH all at maximum when the plant was collected at sunset.^[7] Flavonoids are the most common and widely distributed groups of plant phenolic compounds which are very effective antioxidants.^[13] Flavonoids possess hypoglycemic action.^[14] Effect of the flavonoids on pancreatic β -cells leading to their proliferation and secretion of more insulin have been proposed by Mahesh and Menon^[15] and Sri-Balasubashini et al.,^[16] as the mechanism by which they reduced hyperglycemia caused by streptozocin in diabetic rats. These secondary metabolites present in plant extract may also be acting similarly thereby decreasing the high blood glucose levels of normal healthy hyperglycemic rats.

CONCLUSION

The results showed that the hypoglycemic activities of aqueous leaves extracts of mistletoes grown on cocoa tree depend on the time of collection which was maximum when the leaves were obtained at dawn. It is therefore advisable that the use of this extract in herbal medicine should be encouraged and the plant should be plucked at dawn to maximize the antidiabetic potential of the mistletoes. These facts justified the medicinal use of the plants as a hypoglycemic plant.^[1]

ETHICAL APPROVAL

The authors declare that this work was not against public interest. Animal experiments were conducted in accordance with NIH guidelines for care and use of Laboratory animals (Pub. No.85-23, Revised).

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