**In Vitro A- Glucosidase Inhibitory and In Vivo Glucose Digestion Activities of Ethanol Leaf Extract of Acalypha Wilkesiana in Normoglycaemic Rats**

Anthony O Iyamu¹, Harrison O Otamere² and Uwaifoh Akpamu*³

¹Department of Medical Biochemistry, Faculty of Basic Medical Sciences, College of Medicine, Nigeria

²Department of Physiology, Faculty of Basic Medical Sciences, College of Medicine, Nigeria

³Gastrointestinal Secretory and Inflammatory Unit, Department of Physiology, Faculty of Basic Medical Sciences, College of Medicine, Nigeria

*Corresponding author: Akpamu Uwaifoh, Department of Physiology, Faculty of Basic Medical Sciences, College of Medicine, Nigeria

**ARTICLE INFO**

Received: 﨑 June 26, 2020
Published: 﨑 July 06, 2020

Citation: Anthony O Iyamu, Harrison O Otamere, Uwaifoh Akpamu. *In Vitro A- Glucosidase Inhibitory and In Vivo Glucose Digestion Activities of Ethanol Leaf Extract of Acalypha Wilkesiana in Normoglycaemic Rats* Biomed J Sci & Tech Res 28(4)-2020. BJSTR. MSID.004688.

**Keywords:** Acalypha wilkesiana; Normoglycaemic Rats; In vitro α-glucosidase inhibition; In vivo glucose digestion

**ABSTRACT**

We observed the ethanol leaf extract of Acalypha wilkesiana to be a rich source of phytochemicals and vitamins of therapeutic benefits. Considering the search for medicinal plants and products with hypoglycemic potentials, we conducted in vitro and *in vivo* studies to respectively investigate α-glucosidase inhibitory and glucose digesting activities of *A. wilkesiana* extract. The α-glucosidase inhibitory activity was determined following previously described methods at concentrations of 0.1-0.9μg/mL using acarbose as standard drug. Oral glucose tolerance test was determined in 24 normoglycemic adult male Wistar rats of (140-160g) weight, assigned to four groups (n=6). At 0 minute, Group1 (control) and 2 (glucose treated) received 10ml/kg mL distilled water while 3 (standard hypoglycemic treated) and 4 (*A. wilkesiana* extract treated) received glimepiride (0.1mg/kg) and *A. wilkesiana* extract (250mg/kg) respectively. 30 minutes after, group 1 received distilled water (10ml/kg mL) while groups 2, 3 and 4 received 5% glucose water (10ml/kg) and blood glucose level measured at 30 minutes interval for 180 minutes. The results showed a dose dependent % inhibition of α-glucosidase activity from 51.12±0.14 to 58.01±0.11 with acarbose and 50.48±0.29% to 56.14±0.56% with ethanol leaf extract of *A. wilkesiana* extract. The α-glucosidase inhibitory activity was determined following previously described methods at concentrations of 0.1-0.9μg/mL using acarbose as standard drug. Oral glucose tolerance test was determined in 24 normoglycemic adult male Wistar rats of (140-160g) weight, assigned to four groups (n=6). At 0 minute, Group1 (control) and 2 (glucose treated) received 10ml/kg mL distilled water while 3 (standard hypoglycemic treated) and 4 (*A. wilkesiana* extract treated) received glimepiride (0.1mg/kg) and *A. wilkesiana* extract (250mg/kg) respectively. 30 minutes after, group 1 received distilled water (10ml/kg mL) while groups 2, 3 and 4 received 5% glucose water (10ml/kg) and blood glucose level measured at 30 minutes interval for 180 minutes. The results showed a dose dependent % inhibition of α-glucosidase activity from 51.12±0.14 to 58.01±0.11 with acarbose and 50.48±0.29% to 56.14±0.56% with ethanol leaf extract of *A. wilkesiana*. *A. wilkesiana* (69.50±4.71mg/dl; p<0.05) and glimepiride (74.33±5.10mg/dl; p>0.05) administrations concealed the rise in blood glucose following glucose loading (97.67±3.25mg/dl) and significantly lowered blood glucose levels compared with the glucose treated group at 30 through 180 minutes. These findings suggest that ethanol leaf extract of *A. wilkesiana* possesses hypoglycemic potentials by inhibiting α-glucosidase activity and increasing glucose digestion or inhibiting absorption in normoglycemic rats.

**Introduction**

Metabolic syndrome is a cluster of several metabolic abnormalities, including central obesity, insulin resistance, hypertension, dyslipidemia, and hyperglycemia, that has become a major public health challenge [1]. Though different types of oral hypoglycemic agents are available along with insulin for the treatment of diabetes, there is an increase demand by patients to use the natural products with anti-diabetic activity [2]. Interestingly, medicinal plants are now increasingly used in most parts of the world as hypoglycemic [3-7]. The high cost, low availability, uncertainty of use during pregnancy and undesirable side effects of synthetic drugs have been some of the factors leading to a strong preference for hypoglycemic drugs of plant origin, which are believed to be suitable for hyperglycemia treatments [8]. Herbal products can improve glucose metabolism and the overall
condition of individuals with diabetes, not only by hypoglycemic effects, but also by improving lipid metabolism, antioxidant status and capillary function [9]. In our recent investigation, we observed the ethanol leaf extract of Acalypha wilkesiana to be a rich source of phytochemicals, macro-elements and vitamins known to have several therapeutic benefits [10]. Though this plant is not edible, it is found to contain alkaloids, tannins, resins [11] as well as plenty of terpenoids and vitamin C and moderate level of flavonoids [10]. The plant has wide uses in the traditional treatment of bacterial and fungal skin infections, neonatal jaundice, gastrointestinal disorders and malaria [12,13], antiparasitic and analgesic [14], possess antimicrobial [15, 16], antihypertension [17,18], anticarcinogenic properties [19]. Its antihyperglycemic, antihyperlipidemic and ameliorative role on electrolytes disturbances have been demonstrated in diabetic mice [20]. According to El-Khateeb et al. [21], not much pharmacological research has been carried out on A. wilkesiana despite its importance in traditional medicine. Therefore, this study investigates the in vitro and in vivo α-glucosidase inhibitory and glucose digestion activities of ethanol leaf extract of A. wilkesiana using normoglycemic adult male Wistar rats.

Materials and Methods

Collection, Processing and Extraction of Plant Materials

Samples of Acalypha wilkesiana leaves were collected from Benin City, Nigeria and authenticated at the Herbarium Unit of Forestry Research Institute, Ibadan, Nigeria. The leaves were sorted, air-dried for 7 days and then pulverized and packaged in polythene air tight bags. 200 g of the powder leave was added into a container containing 1.5 L of 70% ethanol and used to prepare the ethanolic extract as described in Majekodunmi and Nubani [22] with few modifications.

In Vitro Anti-Diabetic Activity of Ethanolic Leaves Extract of A. Wilkesiana

The α-glucosidase inhibitory activity of the ethanol leaf extract of A. wilkesiana was determined as described by Shai et al. [23] with slight modifications. Briefly, ethanol leaf extract of A. wilkesiana or acarbose at different concentrations (0.1-0.9 µg/mL) was incubated with 500 µL of porcine pancreatic amylase (2 U/mL) in phosphate buffer (100 mM, pH 6.8) at 37°C for 20 minutes. 250 µL of 1% starch dissolved in 100 mM phosphate buffer (pH 6.8) was added to the reaction mixture and incubated at 37°C for 1 hour. Dinitrosalicylate colour reagent (1 mL) was added and boiled for 10 minutes. The absorbance of the resulting mixture was read at 540 nm and the inhibitory activity was expressed as percentage of a control without the inhibitors. All assays were carried out in triplicate. The inhibitory activities of the fractions on the α-glucosidase were calculated using the following formula:

\[
\text{Inhibitory activity } \% = \left(1 - \frac{\text{AS}}{\text{AC}}\right) \times 100
\]

Whereas is the absorbance in the presence of the sample and AC is the absorbance of the control.

Experimental Animals

Twenty-four (24) adult male Wistar rats with average weight of 140-160g were obtained from the Animal House of the College of Medicine, Ambrose Alli University, Ekpoma. They were housed at room temperature on a 12 hour dark-light cycle and acclimatized for a 14-day period with ad libitum access to clean water and food (rat chow; Vital Feed Nig. Ltd, Jos, Nigeria).

Oral Glucose Tolerance Test in Normoglycemic Rats

The 24 rat which were normoglycemic were used for the study after acclimatizing for two weeks. They were fasted overnight for 12 hours before the experiment. Their fasting blood glucose levels were determined using Accu-check glucometer by Roche Diagnostic and then divided to four groups (n = 6) as follows:

a. Group 1: Negative control group; received 10ml/kg of distilled water and another 10ml/kg mL of distilled water after 30 minutes.

b. Group 2: Positive control group; received 10ml/kg of distilled water and 10ml/kg of 5% glucose water after 30 minutes.

c. Group 3: Standard drug treated group; received 0.1mg/kg of Glimepiride; a standard anti-diabetic drug, and 10ml/kg of 5% glucose water after 30 minutes.

d. Group 4: Extract treated group; received 250mg/kg ethanol leaf extract of Acalypha wilkesiana and 10ml/kg of 5% glucose water after 30 minutes.

The blood glucose levels were measured at 0, 30 min, 60 min, 90 min, 120 min, 150 min and 180 min after glucose loading. The mean increment of glycemia in each group was calculated as the sum of increases in blood glucose divided by the number of animals after each period according to the method of Madar [24]. Data were expressed in means ± SEM. Paired sample “t”-test and one-way analysis of variance (ANOVA) was used to compare the group means between treatments in the in vitro and in vivo studies respectively. Differences were considered significant when P ≤ 0.05.

Results

Figure 2 compares the α-glucosidase inhibitory activities of varying concentrations of the ethanol leaf extract of A. wilkesiana and acarbose. There was dose dependent % inhibition of α-glucosidase activity from 51.12±0.14% to 58.01±0.11% with acarbose and from 50.48±0.29% to 56.14±0.56% with the ethanol leaf extract of A. wilkesiana. The percentage α-glucosidase inhibitory activities was significantly higher (p<0.05) in the acarbose compared with the ethanol leaf extract at all doses but was similar at doses of 0.01 µ/ml (51.12±0.14% vs. 50.48±0.29%; p > 0.05) and 0.09µ/ml (58.01±0.11% vs. 56.14±0.56%; p > 0.05).
Figure 3 demonstrates the time course of oral glucose tolerance in normoglycemic rats treated with ethanol leaf extract of *A. wilkesiana* compared with standard drug and controls after glucose loading. Baseline glucose levels were similar in all the groups 30 minutes before glucose loading. Blood glucose level increased in the distilled water + glucose (97.67±3.25mg/dl) treated 30 minutes after glucose loading but the administration of both 250mg/kg ethanol leaf extract of *A. wilkesiana* (69.50±4.71mg/dl; p<0.05) and 0.1mg/kg glimepiride (74.33±5.10mg/dl; p>0.05), concealed the rise in glucose level. Compared to the control (83.33±2.22mg/dl) treated with only distilled water, blood glucose level was also lower in the extract and standard drug treated groups. Compared with distilled water + glucose treated group, blood glucose levels were significantly lower in the extract and standard drug treated groups by 30 through 180 minute after glucose loading. Although not significant, blood glucose was lower in the extract treated compared with the standard drug by 30 through 180 minutes.
Figure 3: Time course of oral glucose tolerance in the controls, standard drug and ethanolic leaves extract of *A. wilkesiana* treatments in normoglycaemic rats.

Values are Mean±SEM, (n = 6 rats), * indicates significant difference at p<0.05 compared with untreated control (water treated). H2O = water, Dist = distilled, Glim = Glimepiride treated, 250mg/kg = Acalypha wilkesiana treated.

Discussion

The major goal in diabetes management is the near normal maintenance of fasting and postprandial blood glucose levels [25]. In fact, Matsui et al. [26] and Wassmann and Nickenig [27], suggested suppression of glucose absorption via inhibition α-amylase or α-glucosidase enzymes inhibition as therapeutic approach to postprandial hyperglycaemia. In the present study, varying concentrations of ethanol extract of *A. wilkesiana* leaves presented similar promising impact in in vitro inhibition of α-glucosidase with acarbose; a standard diabetic drug (Figure 2). This finding suggests the ethanol leaf extract of *A. wilkesiana* to have digestibility action on glucose similar to acarbose. This assertion is based on the fact that α-glucosidase is involve in the digestion of starch into various oligosaccharides, and disaccharides to produce glucose and other monosacharides [28]. In support of this finding, Fonkoua et al. [29] has reported similar findings with hydroethanolic extract of *A. wilkesiana* but reported no effect with aqueous extract. Both murine model [30] and clinical [31] studies have shown inhibitors of α-amylase and α-glucosidase suppress the production and absorption of glucose in the small intestine. Acarbose is a complex oligosaccharide that binds competitively to the α-glucosidases at the brush border of the small intestine to delay sucrrose and starch breakdown and the absorption of glucose and fructose [32]. This drug has proven efficacious in reducing post-prandial increases in glucose and insulin [33].

Thus, the observed finding from the present study indicates the ethanolic leaves extract of *A. wilkesiana* can be a promising drug for postprandial glucose suppression through delaying sucrrose and starch breakdown or inhibiting absorption of glucose. In the in vivo study, 250mg/kg ethanolic leave extract of *A. wilkesiana* cause a decrease in postprandial blood glucose level after glucose loading in normoglycemic rats. The observed suppression of glucose level after glucose loading by 250mg/kg ethanolic leave extract of *A. wilkesiana* in normoglycemic indicates the extract to have glucose reducing properties. Martin et al. [29] has reported hypoglycaemic effect of 400mg/kg hydroethanolic extract of *A. wilkesiana* in streptozotocin diabetic rats. However, Al-Attar [20] reported orally aqueous *A. wilkesiana* leaves extract to cause a duration dependent decrease in blood glucose in streptozotocin induced diabetic rats.

Conclusion

Overall, this study showed that ethanolic leave extract of *A. wilkesiana* possesses hypoglycemic activity in normaglycemic state. Since a similar dose dependent difference was observed between *A. wilkesiana* leaves extract and acarbose in suppressing α-glucosidase, *A. wilkesiana* leaves extract therefore showed hypoglycemic potential via the inhibition of α-glucosidase and stimulation of glucose digestion mechanisms.

References

1. Alberti KG, Zimmet P, Shaw JIDF (2005) Epidemiology Task Force Consensus Group The metabolic syndrome-a new worldwide definition. Lancet 366(9491): 1059-1062.
2. Venkateswaran S, Pari L, Suguna L, Chandrakasan G (2003) Modulatory Effect of Coccinia indica on Aortic Collagen in Streptozotocin-Induced Diabetic Rats. Clinical and Experimental Pharmacology and Physiology 30(3): 157-163.
3. Osadebe PO, Okide GB, Akabogu IC (2004) Study on antidiabetic activities of crude methanolic extracts of Loranthusmicranthus (Linn) sourced from five different host trees. J Ethnopharmacol 95(2-3): 133-138.
4. Ezugwu GO, Odoh UE, Ajali U (2005) Blood sugar lowering effect of gum extract of Mucuna skanell Fawc. and Randle (Fam. Papillionaceae) seed. Biol Res 3(2): 49-51.
5. Patel VS, Chitra V, Lakshmi Prasanna P, Krishnanju V (2008) Hypoglycemic effect of aqueous extract of Parthenium hysterophorus L. in normal and alleoxan induced diabetic rats. Indian J Pharmacol 40(4): 183-185.
6. Yadav JP, Saini S, Kalia AN, Dangi AS (2008) Hypoglycemic and hypolipidemic activity of ethanolic extract of Salvadora oleoides in normal and alloxan induced diabetic rats. Indian J Pharmaco 40: 23-27.

7. Odoh UE, Enugu CO, Ezeliofo M (2013) Anti-diabetic and toxicological studies of the alkaloids of Acanthus montanus (Acanthaceae) leaf. Int J Curr Res S(12): 4249-4255.

8. Okigbo RN, Mmeka EC (2006) An appraisal of phytomedicine in Africa. KMITL Sci Tech J 6(2): 83-94.

9. Chen CH, Ngho GC, Yusoff R (2012) A brief review on anti diabetic plants: Global distribution, active ingredients, extraction techniques and acting mechanisms. Pharmacogn Rev 6(11): 22-28.

10. Rahmatullah M, Das AK, Mollik MAH, Jahan R, Khan M, et al. (2009) Am Eur Jasiac J Sustain Agric 3(4): 881-888.

11. Akinde BE, Odeyemi OO (1987) Extraction and microbiological evaluation of the oils from the leaves of Acalypha wilkesiana. Nig Med J 17: 163-165.

12. Ogundaini AO (2005) An Inaugural Lecture Delivered at Oduduwa Hall, Obafemi Awolowo University Ile-Ife, Nigeria. Inaugural Lecture Series 176, OAU Press Limited, Ile-Ife, Nigeria p 12-15.

13. Udobang JA, Nwafor PA, Okokon JE (2010) Analgesic and antimalarial activities of crude leaf extract and fractions of Acalypha wilkesiana. J Ethnopharmacol 127(2): 373-378.

14. Akininyi KD, OK Oluwaans, EO Onomigbehin (2006) Antimicrobial activity of crude extracts of three medicinal plants used in south-western Nigerian folk medicine on some food borne bacterial pathogens. Afr J Tradit Compl Altern Med 3(4): 13-22.

15. Gote JP, Agda GOA, Gbiee DS, Chollom S (2010) Antibacterial activity of ethanolic extract of Acalypha wilkesiana leaves growing in Jos, Plateau State, Nigeria. Malaysian J Microbio16(2): 69-74.

16. Nworgu ZAM, Ameachina FC, Owolabi J, Otokiti I, Ogidu U (2011) Cardiovascular effects of aqueous extract of Acalypha wilkesiana Hoffmannian leaves in rabbits and rats. Nig J Pharm Sci 10(2): 45-50.

17. Ikewuchi JC (2013) Moderation of hematological and plasma biochemical indices of sub-chronic salt-loaded rats, by an aqueous extract of the leaves of Acalypha wilkesiana "Godeffiana” Muel Arg (Euphorbiaceae). Asian Pac J Trop Med 6(1): 37-42.

18. Lim SW, Loh HS, Ting KN, Bradshaw TD, Zeenathul NA (2013) Acalypha wilkesiana ethyl acetate extract enhance the in vitro cytotoxic effect of alpha-tocopherol in human brain and lung cancer cells. Int J Biosci Biochem Bioinforma 3(4): 335-340.

19. Al Attar AM (2010) Physiological Study on the Effect of Acalypha wilkesiana leaves extract on streptozotocin-induced experimental diabetes in male mice. Am Med J 1(1): 51-58.

20. El Khateeb YA, Eid Azzaz AN, Mahmoud IH (2014) Phytochemical constituents, hypoglycemic and haematological effects of methanolic Acalypha wilkesiana leaves extract on streptozotocin-induced diabetic rats. European Journal of Chemistry 5(3): 430-438.

21. Majekodunmi SO, Nabani SE (2014) Formulation of Acalypha wilkesiana Muel Arg Ethanol leaf extract into creams for the treatment of microbial microbial skin infections. International Journal of Pharma Pharmacological Science 31(10): 45-53.

22. Madar Z (1989) The effect of acarbose and miglitol (BAY-M-1099) on postprandial glucose levels following ingestion of various sources of starch by non diabetic and streptozotocin induced diabetic rats. J Nutr 119(June): 2023-2029.

23. Bailey C (2009) Potential new treatments for type 2 diabetes. Trends in pharmacological sciences 21 (7): 259-265.

24. Matsui T, Tanaka T, Tamura S, Toshima A, Miya Y, et al. (2007) Alpha glucosidase inhibitory profiles of catechins and theaflavins. Journal of Agricultural and Food Chemistry 55: 99-105.

25. Wassmann K, Nickenig G (2004) Modulation of oxidant and antioxidant enzyme expression and function in vascular cells. Hypertension 44(4): 381-386.

26. Bhandari MR, Jong Anuradkun N, Hong G, Kwabata J (2008) Alpha glucosidase and alpha amylase inhibitory activities of Nepalese medicinal herb Pakhanbhed (Bergenia ciliate, Haw.). Food Chemistry 106: 247-252.

27. Fonkoua M, Ngahiu J, Edoun EFL, Mbab NL, Mbouobda HD, et al. (2017) Screening of antioxidant and antidiabetic properties of aqueous and hydroethanolic extracts of the leaves of Acalypha wilkesiana Muel Arg. American Journal of Pharmacy & Health Research 5(6): 78-88.

28. Kaur C, Kapoor H (2002) Antioxidant activity and total phenolic content of some Asian vegetables. International Journal of Food Sciences and Technology 37: 153-161.

29. Smith C, Marks AD, Lieberman M (2005) Marks’s Basic Medical Biochemistry: A Clinical Approach, 2nd (Edn.). Lipincott Williams & Wilkins, Baltimore, Maryland 21201-2436 USA.

30. Salam Z, Rafie E, Goodarzi TM, Ghaffari M (2016) Effect of Metformin, Acarbose and Their Combination on the Serum Visfatin Level in Nicotinamide/Streptozocin-Induced Type 2 Diabetic Rats. Iran Red Crescent Med J 18(3): e23814.

31. Hanefeld M, Fischer S, Schulze J, Spengler M, Wargenau U, et al. (1991) Therapeutic potentials of acarbose as first-line drug in NIDDM insufficiently treated with diet alone. Diabetes Care 14(8): 732-737.

---

ISSN: 2574-1241
DOI: 10.26717/BJSTR.2020.28.004688

V Abduazizova. Biomed J Sci & Tech Res

This work is licensed under Creative Commons Attribution 4.0 License
Submission Link: https://biomedres.us/submit-manuscript.php

Assets of Publishing with us
- Global archiving of articles
- Immediate, unrestricted online access
- Rigorous Peer Review Process
- Authors Retain Copyrights
- Unique DOI for all articles

https://biomedres.us/

Copyright® V Abduazizova | Biomed J Sci & Tech Res | BJSTR. MS.ID.004688.