OPEN ACCESS
Advances in Animal and Veterinary Sciences

Research Article

Antidiabetic and Antioxidative Potentials of Aqueous Extract of Cola Acuminata Leaves in Alloxan-Induced Diabetes Mellitus in Male Albino Rats

Owolabi Olutunmise Victoria[1*], Saka Olusola Stephen[2], Olashinde Oluwaseun Ruth[1], Alli Smith Yemisi Rufina[3]

[1]Department of Medical Biochemistry, Afe Babalola University, Ado-Ekiti, Nigeria; [2]Department of Human Anatomy, Afe Babalola University, Ado-Ekiti; [3]Department of Biochemistry, Ekiti State University, Ado-Ekiti, Nigeria.

Abstract | Numerous body organs (eyes, liver, kidney and testes) eventually suffer damage as a result of elevated blood glucose levels, which are a defining feature of the metabolic disorder known as diabetes mellitus. Researchers are in search for safe drugs that can bring a total recovery from diabetes. Thirty (35) male wistar rats were divided into six (7) groups of five animals each to study the antidiabetic and antioxidative properties of young Cola acuminata leaves aqueous extract. Diabetes was induced using alloxan monohydrate.5 ml of normal saline was given to group I , group 2 received 5 mg/kg/ body weight of the standard drug glibenclamide, group 3 was given 140 mg/kg of alloxan while groups Young *C. acuminata* leaves aqueous extract was administered orally in graded doses to the diabetic animals (50mg/kg, 100mg/kg, 200mg/kg and 400mg/kg bodyweight) and glibenclamide. Results showed a significant reduction of blood glucose level in the extract-treated rats ($p < 0.5$) especially at 200 mg/kg and 400 mg/kg body weight. Samples were obtained for some biochemical (parameters) related to oxidant and antioxidants such as antioxidant enzymes, Reduced glutathione (GSH) activity, Superoxide dismutase (SOD) activity and Malondialdehyde (MDA) activity. When compared control and glibenclamide-treated rats, the antioxidant activity of the extract increases with advancing the dose of treatment. According to the findings, Cola acuminata young leaves have comparable anti-diabetic efficacy to that of a glibenclamide administration. This suggests a promising prospect in developing novel drugs for treating diabetes mellitus.

Keywords | Anti-diabetic, Cola acuminata, Antioxidant, Alloxan, Glibenclamide, Wistar rats, Experimental work

Received | September 02, 2022; **Accepted** | November 05, 2022; **Published** | January 31, 2023

***Correspondence** | Owolabi Olutunmise Victoria, Department of Medical Biochemistry, Afe Babalola University, Ado-Ekiti, Nigeria; **Email:** akintehinseoo@abuad.edu.ng

Citation | Victoria OO, Stephen SO, Ruth OO, Rufina ASY (2023). Antidiabetic and antioxidative potentials of aqueous extract of cola acuminata leaves in alloxan-induced diabetes mellitus in male albino rats. Adv. Anim. Vet. Sci. 11(2):305-309.
DOI | https://dx.doi.org/10.17582/journal.aavs/2023/11.2.305.309
ISSN (Online) | 2307-8316

INTRODUCTION

Insulin insufficiency leads to complicated metabolic dysfunction known as diabetes mellitus. Blood glucose levels are raised, which causes disability, hospitalization, and increased financial burden (Vats et al., 2002). It has an impact on industrialized, developing, and underdeveloped countries and does not make gender distinctions. The World Health Organization claims that 171 million individuals worldwide had diabetes mellitus in 2000 and it will double by 2030 (WHO, 2002). This report was corroborated by Wild et al. (2004). The management of diabetes mellitus is hard due to the deleterious effects and limited effectiveness of currently available antidiabetic drugs (Abdissa et al.,

2017). Medicinal plants with established traditional uses are source of novel antidiabetic drug. *Galega officinalis* L. yields Galengine, an anti-diabetic compound (Family: Leguminoseae), served as a blueprint for the manufacture of metformin, a commonly used anti-diabetic medication (Henrich, 2010).

Many West African societies consume kola nuts and also use them ceremonially. Kola nut (*Cola* spp.) belongs to the steruliacea plant family, which includes more than 20 species of trees that are indigenous to Africa's tropical rain forests (Olaniyan et al., 2016). *Cola acuminata* (*C. acuminata*) and *Cola nitida* (*C. nitida*) species are the common and important species found in Nigeria. These two species have a very high level of economic and social relevance because of their applications in traditional ceremonies. The diameter of a *C. acuminata* tree is approximately 30 cm, and its height can reach 30 m. The foliage is simple, sparse and confined to the tips of the branches. Traditional remedies for dysentery, coughing, diarrhea, and vomiting included a tonic made from the leaves, twigs, flowers, fruit follicles, and bark of *Cola nitida* and *C. acuminata* (Yalwa and Bello, 2017). Nuts can be used to help create new foods and medications (Acharibasam and McVittie, 2021). Chantal et al. (2019) reported that *Cola nitida* stem bark extracts may one day be used to regulate fertility naturally. Although this economically and socially important tree has been the subject of extensive research, relatively little has been done on its leaves, which is why this study is carried out.

MATERIALS AND METHODS

Preparation of extract

In May 2018, fresh young leaves of *C. acuminata* were procured in Igede-Ekiti, Irepodun/Ifelodun Local Government, Ekiti State. The leaves were carefully cleaned of dust and allowed to air dry at room temperature. They were later homogenized to powder using a grinder and then soaked with distilled water 1:3 w/v for 24 hours. It was then filtered using whatman No1 filter paper. The filtrate was subsequently evaporated to dryness using a rotary evaporator at 45°C. Before usage, the extract was collected into an air tight container and refrigerated (Airaodion et al., 2019).

Experimental animals

For this experiment, 35 male wistar rats whose weight is between (120-150g) were employed. They were maintained at the Experimental Animal House of the Faculty of Basic Medical Sciences, ABUAD. The rats were housed in cages with ventilation, fed pelletized food, and given water was provided *ad libitum*. The National Institute of Health's guidelines for handling and protocol compliance were followed in this experiment. The approval number for these committees is REC/FBMS/ABUAD/21/35. The animals were maintained in the Animal House of the Afe Babalola University's Department of Anatomy in Ado Ekiti.

Experimental design

The rats were divided into seven groups of five each at random. 5 ml of normal saline was given to group 1, group 2 received the 5 mg/kg standard drug which is glibenclamide, group 3 rats received 100 mg/kg of alloxan for diabetes control while groups, 4, 5, 6 and 7 received 50mg/kg, 100mg/kg, 200mg/kg and 400mg/kg of extract respectively (El-Missirya and El-Gindy, 2000). The diabetic state of the rats was verified after treatment with alloxan monohydrate using Accu-check glucometer before the commencement of treatment with the extract and glibenclamide. Blood samples were collected from different groups at 0, 7 and 14 days for determination of serum blood glues level. At the end of the experiment, cervical dislocation method was used to sacrifice the rats. For further processing and analysis, blood samples were obtained. Kidney, testis, and liver were also removed from the animals and stored at -4°C for further analysis.

Reduced glutathione (GSH) activity

GSH (reduced glutathione) activity was measured using the Ellman method (Ugar et al., 2018). Reduced Glutathione was evaluated by spectrophotometer to determine DTNB (Dithiobis-(2-nitrobenzoic acid) reduced by SH-groups, expressed as µg/mg wet tissue. 2.4 ml of a 0.02 M EDTA solution was applied to 0.1 ml of various tissue samples and left on ice for 10 minutes. Then, 0.5 ml of 50%w/v TCA and 2 ml of distilled water were added. This mixture was centrifuged for 15 minutes at 3000 g after being held on ice for 10 to 15 minutes. To 1 ml of supernatant, 2.0 ml of Tris buffer (0.4 M) was added. DTNB solution (Ellman's reagent; 0.01M DTNB in methanol) was then added and carefully vortexed at 0.05 ml. After adding DTNB, OD was measured using a spectrophotometer at 412 nm and compared to a blank for the reagent within 2–3 minutes.

Superoxide dismutase (SOD) activity

SOD activity was carried out using Kakkar et al., (1984) guidelines. The sodium pyrophosphate buffer concentration was 0.052 M in the final volume of 3 ml, 186 µMphenozinemetho-sulphate (PMS), 300 µMnitroblue tetrazolium (NBT), 780 µM NADH, sonicated enzyme preparation and water. NADH's addition to the reaction begin it, and it was then incubated at 37°C for 90 s. After the incubation period, the reaction was halted by adding 1.0 ml of glacial acetic acid, and the mixture was then vigorously shaken with 4.0 ml of n-butanol. After centrifuging the mixture and separating the butanol layer, the mixture was left for 10 minutes. Using a spectrophotometer,

chromogen's color intensity in butanol was evaluated at 560 nm versus butanol. As a control, a combination with cell suspension but no enzyme was used.

Malondialdehyde (MDA) activity

Malondialdehyde was evaluated according to the guidelines of Okhawa (Okhawa et al., 1979). To 1 milliliter of tissue homogenate, 1 ml of normal saline and 2.0 ml of 10% TCA were added and mixed well. Centrifuging the mixture for 10 mins at 3000g was done to separate the proteins. To create the pink-colored MDA, 2 ml of supernatant were taken, and 0.5 ml of 1.0% TBA were added. Then, for 60 minutes, this mixture was heated at 95 degrees Celsius. At 532 nm, the samples' optical density was measured.

Statistical analysis

The Statistical Package for Social Sciences (SPSS) version 10.0 for Windows was used to evaluate the results. Mean SEM (n = 5) is used to represent the whole set of data. When comparing means, the student's t-test was utilized, and results were deemed significant at p 0.05 (Ismail et al., 2020).

RESULTS AND DISCUSSION

As observed in Table 1, significant blood sugar level reductions were seen in the groups that were given 200mg/kg and 400mg/kg of the extract when compared with the control at 7 and 14 days. The beneficial effects of the extract also compared favorably with a standard antidiabetic drug glibenclamide. All of the compartments showed a noticeable rise in GSH levels. Animals receiving 100mg/kg and 200mg/kg of the drug showed a more dramatic increase than other animals. Compared to the control group, the treated animals' SOD levels significantly increased. The increase observed was not dose-dependent but the result showed that all the administered dose was able to increase the SOD levels in the treated rats. The treated groups showed a marked decline in MDA levels especially at 100mg/kg, 200mg/kg and 400mg/kg when compared with the control and glibenclamide treated rats.

Table 1: Effects of young *C. acuminata* leaves' aqueous extract on the blood sugar of rats with alloxan-induced diabetes.

Days	50mg/kg bodyweight	100 mg/kg bodyweight	200 mg/kg bodyweight	400 mg/kg bodyweight	Glibenclamide 5mg/kg bodyweight	Control 5ml/kg NS	Diabeti-control
0	109.12	107.67	113.18	109.25	106.28	112.66	141.55
7	118.36	115.56	92.67*	84.36*	87.57*	114.72	144.21
14	117.14	102.34*	84.75*	64.54*	60.88*	111.39	138.72

Values are Mean ± SEM; n = 5; *P < 0.05.

Table 2: Effects of young *C. acuminata* leaves' aqueous extract on the GSH levels in the liver, kidney and testis of rats with alloxan-induced diabetes.

Groups	Liver	Kidney	Testis	Plasma
1	207.53±16.12 [a]	203.05±10.44[a]	214.78±11.95[a]	178.25±15.14[a]
2	226.06±18.41[b]	223.98±10.47[b]	221.66±17.41[a]	192.42±15.32[b]
3	207.64±13.16[b]	203.63±12.52[b]	212.44±15.11[b]	179.36±15.14[b]
4	227.75±14.25[b]	232.79±17.54[c]	234.41±15.66[b]	206.71±16.12[c]
5	221.81±15.28[b]	225.85±19.28[b]	225.17±15.21[a]	208.85±15.59[c]
6	228.36±15.41[b]	231.36±19.32[c]	226.97±16.11[b]	203.53±14.88[b]
7	238±13.21[c]	237±16.11[c]	232±14.76[b]	218±14.26[d]

Values are Mean ±SEM; n = 5, numbers with different alphabets within a column shows that P < 0.05.

Table 3: Effects of young *C. acuminata* leaves' aqueous extract on the SOD levels in the liver, kidney and testis of rats with alloxan-induced diabetes.

Groups	Liver	Kidney	Testis	Plasma
1	60.21±5.23[b]	60.14±7.42[b]	70.26±7.21[b]	68.26±6.44[a]
2	56.64±4.32[b]	70.25±6.23[c]	72.34±8.25$_{b}$	75.55±6.63[b]
3	30.64±2.84[a]	30.25±4.33$_{a}$	60.34±7.23[a]	57.45±3.61$_{a}$
4	80.52±6.43[d]	80.58±6.21$_{d}$	78.89±7.41[b]	78.63±7.95[b]
5	73.22±7.12[c]	80.77±7.12$_{d}$	80.71±8.22$_{c}$	86.27±6.76$_{c}$
6	80.64±6.55[d]	80.69±8.04$_{d}$	83.26±8.46c	90.45±7.01[c]
7	75.38±6.21[c]	68.45±7.72$_{c}$	79.54±9.34c	80.62±5.74[b]

Values are Mean±SEM; n = 5, numbers with different alphabets within a column shows that P < 0.05

Table 4: Effects of young *C. acuminata* leaves' aqueous extract on the MDA levels in the liver, kidney, and testis of rats with alloxan-induced diabetes.

Groups	Liver	Kidney	Testis	Plasma
1	4.81±1.12[a]	4.62±1.24[a]	3.69± 0.98[a]	5.64±1.01[a]
2	4.98±1.01[a]	5.72±0.99[a]	5.13±1.25[a]	7.65±1.02[b]
3	7.65±1.48[a]	6.66±1.42[a]	7.16±0.12[a]	8.22±1.00[a]
4	4.5±1.02[a]	3.98±0.64[a]	4.34±0.22[a]	7.18±1.04[b]
5	1.41±1.11[b]	1.55±0.28[b]	1.12±1.14[b]	4.66±0.98[d]
6	1.09±0.10[d]	1.74±0.48[d]	1.04±0.02[d]	4.33±0.87[d]
7	3.71±1.01[c]	3.58±0.66[b]	3.13±1.25[c]	5.24±0.99[c]

Values are Mean ±SEM; n = 5, numbers with different alphabets within a column shows that P <0.05.

Alloxan monohydrate administered intraperitoneally to rats significantly raised blood sugar levels as compared to uninduced rats. The blood sugar levels increased from 100 to 546 mg/dl. Following oral administration of the extract at varying doses, the blood glucose level significantly reduced. Even though not every dose that was given shown this reduction. The reduction observed was dose-dependent as compared with the research of Saka et al. (2016) on the study of biochemical studies of aqueous extract of garlic on the myocardium of left ventricle of high salt fed adult wistar rats. Treatment of the diabetic rats with 200mg/kg and 400mg/kg for 14 days significantly reduced the blood when compared with diabetic untreated rats in the control and alloxan-induced diabetic rats (Table 1) which was accord with the research of Elgazer et al. (2013). Values obtained at 400 mg/kg body weight compared favorably well with that of glibenclamide treated group. Caffeine concentrations in Cola acuminata's young leaves have been found to be quite high (Umenwanne et al., 2021). According to (Van et al., 2005), caffeine has also been found to lower the chance of developing diabetes mellitus. As a result, the rats treated with graduated dosages of *C. acuminata* leaf extract may have had lower glucose levels because of the leaves' caffeine content. Compared to control and glibenclamide-treated rats, the GSH and SOD values in the rats' liver, kidney, testicles, and plasma were considerably higher in the extract-treated animals (Tables 2 and 3). Pothiraj et al. (2021) observed a high concentration of phenols, alkaloids, tannins, saponins, flavonoids and carotenoids in the young leaves of *C. acuminata*. The presence of phytochemicals in the leaves may be the cause of the extracts' capacity to lower the diabetic rats' blood glucose levels. According to the findings, the extract's potency was most pronounced at doses of 100 mg/kg and 200 mg/kg (Pothiraj et al., 2021). This could be seen in the results obtained in all the assay carried out and across all the compartments of the treated animals. In Malondialdehyde, an oxidative stress marker the treated groups have significantly lower than the control group and the glibenclamide treated rats especially in animals that received lower doses of the leaves extract (Table 4) which is accordance with the study of Edwin et al. (2008). The phytochemicals in the plant were able to scavenge some of the free radicals produced due to the diabetic state of the animals. Numerous synthetic chemical medications had been used in treating diabetes mellitus, but none has demonstrated the potential to fully cure the condition (Edwin et al., 2008), this leads to constant intake of these synthetic agents which might have other negative effects on the body system, hence the need to search for natural alternatives which are harmless and can bring a total recovery from the diabetic state. Antioxidant parameters levels were reported to be lower in diabetes mellitus patients, hence numerous studies have advised using phytochemicals with antioxidant and free radical-scavenging properties to increase insulin sensitivity (Bacanli et al., 2019). These findings had shown that the extract increased the antioxidant levels of the treated animal and also reduced the blood glucose of the diabetic rats.

CONCLUSIONS AND RECOMMENDATIONS

The result obtained showed that young leaves of *C. acuminata* possess antidiabetic ability, the phytochemicals in leaves may be responsible for this potential. This antidiabetic effect might lead to a further study to isolate the bioactive compounds from this plant and might serve as a novel drug in treating of diabetes.

ACKNOWLEDGEMENTS

The authors are grateful to Afe Babalola University Ado Ekiti for financial assistance.

NOVELTY STATEMENT

Little work has been done on the leaves of *C. acuminate* but none has been done on its anti-diabetic properties. The present research shows the anti-diabetic and anti-oxidative potentials of young *C. acuminate*. However, this could be a novel drug source in the treatment of diabetes mellitus.

AUTHOR'S CONTRIBUTION

OOV initiated the research and supervised various stages of the work. OOV was involved in the design of the experiment and supervised various stages of the work. OOR was involved in the bulk of the research work and writing of the article. OOV, OOR and ASYR were involved in the design of the experiment and proof-read the article. OOV and SOS were involved in the writing and editing of article.

Funding

All the experiment proceeded at the Afe Babalola University, Ado-Ekiti.

Conflicts of interest

The authors have declared no conflict of interest.

REFERENCES

Abdissa D, Geleta G, Bacha K, Abdissa N (2017). Phytochemical investigation of Aloe pulcherrima roots and evaluation for its antibacterial and antiplasmodial activities. PLoS One, 12(3): e0173882. https://doi.org/10.1371/journal.pone.0173882

Acharibasam JB, McVittie J (2021). I will use the left hand in school and the right hand at home: A two-eyed seeing approach. Int. Educ. J. Comp. Perspect., 20(1): 81-98.

Airaodion AI, Ekenjoku JA, Ogbuagu EO, Ogbuagu U, Airaodion EO (2019). Antihaemolytic effect of ethanolic leaf extract of Vernonia amygdalina in Wistar rats. Int. J. Bio-Sci. Bio-Technol., 11(7): 173-178.

Bacanli M, Dilsiz SA, Başaran N, Başaran AA (2019). Effects of phytochemicals against diabetes. Adv. Food Nutr. Res., 89: 209-238. https://doi.org/10.1016/bs.afnr.2019.02.006

Bacanli M, Dilsiz SA, Başaran N, Başaran AA (2019). Effects of phytochemicals against diabetes. Adv. Food Nutr. Res., 89: 209-238. https://doi.org/10.1016/bs.afnr.2019.02.006

Chantal NM, Désiré DD, Caude BD, Sandrine MN, Lohik MN, Francine MM, Larissa, DT, Mireille KP, Pierre K (2019). Neuroprotective effects of the Anthocleista schweinfurthii Gilg. (loganiaceae) stem bark extract in postmenopause-like model of ovariectomized wistar rats. J. Complement. Integr. Med., 16(1). https://doi.org/10.1515/jcim-2017-0137

Edwin J, Siddaheswar BJ, Dharam CJ, Sheeja E, Gupta VB, Jain DC (2008). Diabetes and herbal medicines. Iran. J. Pharmacol. Ther., 7(1): 97-106.

Elgazar AF, Rezq AA, Bukhari HM (2013). Anti-hyperglycemic effect of saffron extract in alloxan-induced diabetic rats. Eur. J. Biol. Sci., 5(1): 14-22.

El-Missirya MA, El-Gindy AM (2000). Amelioration of alloxan induced diabetes mellitus and oxidative stress in rats by oil of Eruca sativa seeds. Ann. Nutr. Metab., 44: 97-100. https://doi.org/10.1159/000012829

Heinrich M (2010). Ethnopharmacology in the 21st century-grand challenges. Front. Pharmacol., 28(1): 8. https://doi.org/10.3389/fphar.2010.00008

Ismail H, Rahmat A, Emzir E (2020). The effect of model e-learning material on EFL reading comprehension. Int. J. Multicult. Multirelig. Understandi., 7(10): 120-129. https://doi.org/10.18415/ijmmu.v7i10.2069

Kakkar P, Das B, Viswanathan PN (1984). A modified spectrophotometric assay of superoxide dismutase. Indian J. Biochem. Biophys., 21(2): 130-132.

Ohkawa H, Ohsini N, Yagi K (1979). Assay for lipid peroxides in animal tissues by thiobarbituric acid reaction. Anal. Biochem., 95: 351-358. https://doi.org/10.1016/0003-2697(79)90738-3

Olaniyan AB, Kolapo KA, Hammed LA (2016). Curing influences nut-germination and seedling growth of kolanut (*Cola nitida* Vent. (Schott and Endl.)). III All Afr. Hortic. Congr., 1225: 437-442. https://doi.org/10.17660/ActaHortic.2018.1225.62

Pothiraj C, Balaji P, Shanthi R, Gobinath M, Babu RS, Munirah AAD, Ashraf AH, Kumar KR, Veeramanikandan V Arumugam R (2021). Evaluating antimicrobial activities of *Acanthus ilicifolius* L. and *Heliotropium curassavicum* L against bacterial pathogens: An *in-vitro* study. J. Infect. Publ. Health, 14(12): 1927-1934. https://doi.org/10.1016/j.jiph.2021.10.013

Saka OS, Komolafe OA, Ogunlade O, Olayode AA, Akinjisola AA, Odukoya SA (2016). Biochemical studies of aqueous extract of garlic on the myocardium of left ventricle of high salt fed adult wistar rats. Fiziol. Physiol., 26: 9-12.

Ugar M, Tufan AN, Altun M, Guclu K, Ozyurek M (2018). Glutathione peroxidase activity of biological samples using a novel microplate-based method. Curr. Anal. Chem., 14(5): 512-518. https://doi.org/10.2174/1573411014666171204154653

Umenwanne CL, Ogugofor MO, Njoku OU (2021). Ethyl acetate fraction of Cola hispida leaf protects against doxorubicin-induced myocardial injury in male albino rats. Future J. Pharm. Sci., 7(1): 1-7. https://doi.org/10.1186/s43094-021-00207-5

Van Dam RM, Hu FB (2005). Coffee consumption and risk of type 2 diabetes: a systematic review. Jama, 294(1), 97-104.

Vats V, Grover JK, Rathi SS (2002). Evaluation of anti-hyperglycemic and hypoglycemic effect of trigonella foenum-graecum Linn, Ocimum sanctum Linn and Pterocarpus marsupium Linn in the normal and alloxanized diabetic rats. J. Ethnopharmacol., 79(1): 95-100. https://doi.org/10.1016/S0378-8741(01)00374-9

WHO (2002). Traditional Medicine Strategy 2002-2005. WHO Publications, pp. 1-6.

Wild S, Roglic G, Green A (2004). Globsal prevalence of diabetes mellitus; Estimates for year 2000 and projections for 2030. Diabetes Care, 27(5): 1047-1053. https://doi.org/10.2337/diacare.27.5.1047

Yalwa IR, Bello AM (2017). Determination of caffeine content in some varieties of kola nut (*C. acuminate*). Bayero J. Pure Appl. Sci., 10(1): 247-251. https://doi.org/10.4314/bajopas.v10i1.50S