https://dx.doi.org/10.4314/dujopas.v11i3d.12
ISSN (Print): 2476-8316
ISSN (Online): 2635-3490
Dutse Journal of Pure and Applied Sciences (DUJOPAS), *Vol. 11 No. 3d September 2025*

Methanolic Extract of *Calotropis Procera* Flower has Antihyperglycemic Effect in Streptozotocin-Induced Diabetic Wister Rats

Adda'ullahi Mohammed[1], *Misbahu Sambo[2], Abdulkadir Yunusa[3]

[1]Department of Biochemistry,
Modibbo Adama University,
Yola,
Nigeria.

[2]Department of Biochemistry,
Abubakar Tafawa Balewa University,
Bauchi,
Nigeria.

[3]Department of Science Laboratory Technology,
Federal Polytechnic Mubi,
Nigeria.

Email: misbahusambo@gmail.com

Abstract

Diabetes mellitus is a condition characterized by hyperglycemia as a result of impairment in insulin secretion, insulin action, or both. It causes long-term damage and failure of various organ systems (heart, blood vessels, eyes, kidneys and nerves), leading to disability and premature death. The available conventional drugs have not been doing enough in its management. Moreover, the side effects that some of these drugs present are of great health concern. Therefore, searching for alternative drugs is necessary. The aim of this study was to investigate the anti-hyperglycemic effect of methanol extract of the flower of Calotropis procera in wister rats induced with diabetes using streptozotocin (STZ). The extract was first assayed for the presence of phytochemicals using standard methods. Oral glucose tolerance test was then performed using 2 mg/kg body weight of glucose at various time interval (0, 30, 60, 120, 240 minutes) after glucose load. The positive control groups were treated for four weeks, and were compared with the normal and negative control group. The Results showed that the methanol extract contained alkaloids, flavonoids, saponins, tannins, cardiac glycosides and phenols. The level of the fasting blood glucose was found to be significantly ($P < 0.05$) lowered by the 200 and 400 mg/kg extract doses after two weeks of administration. This study ascertained that the assayed Calotropis procera flower had antihyperglycemic effect, and suggests further exploration of the plant as an antihyperglycemic agent.

Keywords: Antihyperglycemic, Diabetes, Calotropis procera, Streptozotocin, Wister rats.

INTRODUCTION

Diabetes mellitus (DM) is a condition characterized by hyperglycemia as a result of impairments in insulin secretion, insulin action, or both (Murea, 2021). It causes long-term damage and failure of various organ systems (heart, blood vessels, eyes, kidneys and nerves),

*Author for Correspondence

leading to disability and premature death (Salsali *et al.,* 2021). Several symptoms such as thirst, polyuria, blurring of vision, and weight loss accompany diabetes (Sparling and Taal, 2022).

There are two main types of diabetes; type 1 (T1DM) and type 2 (T2DM). Type 1 is primarily due to pancreatic islet beta cell destruction and is characterized by deficient insulin production in the body (Sperling and Taal, 2022). The majority of T1DM occurs in children and adolescents (Salsali *et al.,* 2021). On the other hand, T2DM results from the body's ineffective use of insulin and accounts for the vast majority of people with diabetes around the world. (Spellman *et al.,* 2021). T2DM diabetes occurs nearly entirely among adults but, in these days, it is increasing in children (Salsali *et al.,* 2021). It is estimated that 25% of the world population is affected by diabetes (Arumugam and Abou-Donia, 2022).

The availability of conventional drugs has not been doing enough in curtailing the increasing rate in diabetes cases and its consequences. Moreover, the side effects that these drugs present are of great health concern (Khan, 2016). Therefore, searching for alternative drugs (drugs from plants) is necessary. From ancient times, man has been relying heavily on plant-based medicine for treatment of varying diseases. The World Health Organization (WHO) estimated that about 80% of Africa's population use traditional medicine for their primary health care (Sharabana *et al*., 2022). Diabetes mellitus is among the conditions that are managed traditionally in Africa using varieties of plants including *Calotropis procera* (known as Sodom apple in English) (Khan, 2016).

Sodom apple is a plant that grows mainly in Northern land throughout Nigeria. It is called *Tunfafiya* in Hausa language. It is a shrub that can grow up to 5 meters in height. Its flowers are attractive and have been reported to have numerous medicinal uses (Kaur *et al.,* 2021). It has been reported as a traditional folkloric medicine for treatment of asthma in the Indian literature (Varier *et al*., 2020). Also different parts of the plant were used for the treatment of several diseases such as stem for skin disease, intestinal worms, leprosy, leucoderma; the roots are used for the treatment of leprosy, asthma, cough, elephantiasis and rheumatism; latex and leaves are used for swelling and joint pain (Evans and Ashwood, 2022). Its anticonvulsant effect was seen as there was a delay in the onset of pentylenetetrazole-induced convulsions as well as decrease in its sternness (Agral and Pathak, 2022). It also has anti-diarrhoeal activity (Chandra *et al.,* 2021), anti-inflammatory activity and antifungal activity (Namrata *et al*., 2022). These uses are all in addition to it been used as antidiabetic (Khan, 2016). This research was aimed at ascertaining the traditional antidiabetic effect of the plant.

Plate 1*:* Picture of *Calotropis procera* flowers (Kaur *et al.,* 2021)

MATERIALS AND METHODS

Plant Material
The flower of *Calotropis procera* was collected within the Modibbo Adama University in the morning (7:30am) during dry season. A staff of the Department of Plant Science, Modibbo Adama University (MAU), Yola, identified and authenticated the plant.

Experimental Animals
Male Wistar rats weighing 100 g ± 20 g were used as the experimental animal. They were purchased from the Department of Pharmacology, University of Jos, Nigeria. They were allowed to acclimatize to the environment before commencement of the experiment.

Equipment, chemical and reagents
The equipment used include micropipette (Witeg Labortecnik GmbH, Germany), incubator, oven, spectrophotometer (Surgifriend Medicals, England), centrifuge (Techmel and Techmel, USA), rotary evaporator (Genser Scientific Instruments, Germany) and portable glucometer (VivaChek Biotech Co., Ltd, China). Methanol, chloroform, ethyl acetate, n-Hexane, and butylated hydroxytoluene (BHT) are some of the chemicals used and were purchased from Sigma Chemical Co. (St. Louis, MO, USA).

METHODS
Extract Preparation
Eighty percent (80%) methanol solution of *Calotropis procera* flower (500 g in 1 liter) was prepared and allowed to stay for two days with frequent agitation. The solution was filtered, and evaporated using rotary evaporator. The residue was regarded as the extract (Trease and Evans, 1996).

Analysis of Phytoconstituents
Alkaloids, cardiac glycosides, flavonoids, saponins, tannins, terpenoids, steroids, phenols and resins were analyzed using standard methods (Harborne *et al.,* 1973; Sofowara *et al.,* 1993; Trease and Evans, 1996).

Induction of diabetes
Animals selected for induction were fasted for twelve hours, and then injected intraperitoneally with streptozotocin (STZ) at a dosage of 50 mg/kg body weight (Al-yahiya *et al.,* 2020). They were then administered 5% glucose solution overnight. Blood glucose concentration was measured 48 hours after injection, and rats with blood glucose levels > 200 mg/dL were considered diabetic. The treatment started 7 days after injection.

Animals grouping
The rats were divided into six groups and treated for a period of four weeks as follows:
Group I: Non diabetic rats given normal rats' pellets.
Group II: Diabetic rats not given any drug.
Group III: Non diabetic rats administered 400 mg/kg body weight of the extract.
Group IV: Diabetic rats administered 200 mg/kg body weight of the extract.
Group V: Diabetic rats administered 400 mg/kg body weight of extract.
Group VI: Diabetic rats administered 80 mg/kg body weight Metformin (standard drug).

Blood Collection

At the end of the experimental period, animals were sacrificed by cardiac puncture under anesthesia in accordance with guidelines of European convection for the protection of vertebrate animals. Blood was collected in well labelled tubes.

Oral glucose tolerance test (OGTT) in the Wistar rats.

Selected animals were fasted overnight, after which were administered 2 g/kg body weight of glucose. Blood glucose levels were measured at 0, 30, 60, 120, and 240 minutes after glucose load.

Statistical Analysis

Values were presented as mean ± standard error of mean. One-way analysis of variance (ANOVA) was used to assess differences between mean values. Tukey HSD was used for post hoc test. Group means were considered to be significantly different at $p < 0.05$.

RESULTS

Qualitative and Quantitattive Phytochemical Screening of *Calotropis procera* flower

Table 1 shows the results of the phytochemical screening of *Calotropis procera* flower extract. It shows that the extract contained alkaloids, tannins, saponins, phenols, flavonoids and cardiac glycosides, but resins was found to be absent. Quantitatively, phenols were found to be the highest.

Table 1: Phytochemical Screening of *Calotropis procera* flower.

Phytochemical	Inference	Quantity
Alkaloids	+	92.71 ± 0.28
Flavonoids	+	106.19 ± 2.17
Saponins	+	164.08 ± 1.25
Tannins	+	121.1 ± 0.47
Cardiac glycosides	+	89.43 ± 0.91
Resins	-	-
Phenols	+	293.51 ± 1.32

Values are expressed as mean ± Standard Error Mean (SEM)

Oral Glucose tolerance test (OGTT)

After administration of 2 mg/kg body weight of glucose to the Wistar rats, at 0, 30, 60 90,120 and 240 minutes, the serum glucose level was 290 ± 1.82, 310.75 ± 1.80, 295.25 ± 1.19, 255.75 ± 2.56, 220.00 ± 1.84, 193.75 ± 0.96 mg/dL respectively (Figure 1). This shows that the blood glucose level was at the highest level at the first 30 minutes, then started dropping down and reached normal serum glucose level after 240 minutes.

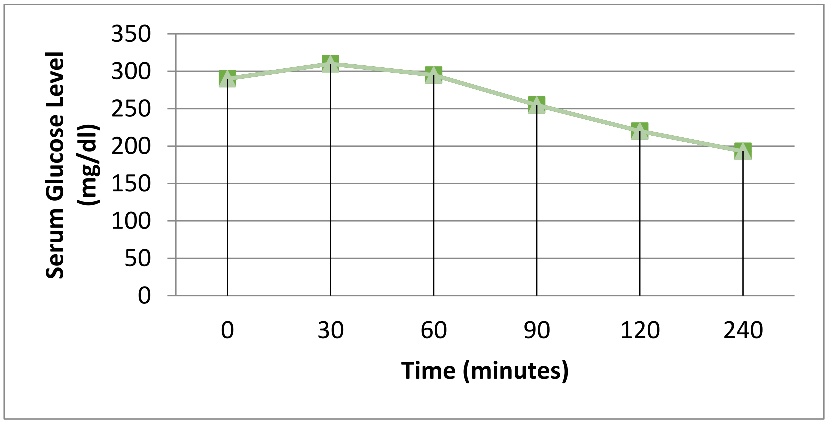

Figure 1: Glucose level in Wistar Rats after 2 g/kg/b.wt. Glucose Load

Antihyperglycemic effect of the extract

The results of antihypeglycemic effect of the extract in rats induced with diabetes using STZ are shown in Table 2. The results show that glucose level was significantly ($P < 0.05$) reduced by the administration of 200 mg/kg and 400 mg/kg of the extract to 182.80 ± 7.51 and 151.60 ± 7.26 mg/dL respectively compared to that in the diabetic rats (279.20 mg/dL). This represent a percentage decrease (in blood glucose level) of 34.6% and 45.8% respectively as compared to that in the diabetic rats.

Table 2: Antihyperglycemic effect of the extract

Group	Glucose level (mg/dL) Before treatment	After 4 weeks treatment	% Difference
Group I	93.80 ± 4.60	93.60 ± 4.50	0.21
Group II	281.80 ± 2.23	272.00 ± 2.57	3.2
Group III	102.00 ± 5.22	94.20 ± 1.86	7.6
Group IV	279.20 ± 3.74^{a}	182.80 ± 7.51^{b}	34.6
Group V	279.80 ± 3.51^{a}	151.60 ± 7.26^{b}	45.8
Group VI	278.80 ± 3.70^{a}	95.40 ± 4.57^{b}	65.82

Values are expressed as mean ± (SEM)
Values bearing the same superscript in the same column were significantly different ($P < 0.05$) with Group II.

DISCUSSION

There are about 387 million people living with diabetes worldwide and this number is expected to rise by 55% in 2035 (International Diabetes Foundation (IDF), 2020). World Health Organization (WHO) projects that diabetes will be the 7th leading cause of death in 2030 (WHO, 2020). In 2020, an estimated 4.9 million people died from consequences of diabetes (IDF, 2020). The African continent accounts for approximately 21.5 million people with diabetes (IDF, 2020). Estimates for Africa for 2035 are likely to more than double and reach 41.5 million (IDF, 2020). In Africa, Nigeria has the highest number of people with diabetes with approximately 3.7 million people affected (IDF, 2020).

Oral chemotherapeutic agents may be effective for glycemic control, but they come with their attendant side effects such as liver disorders, flatulence, abdominal pain, renal tumors, hepatic injury, abdominal fullness and diarrhea (Meenakshi *et al.*, 2020; Suzuki and Arnold, 2020). This calls for the need of alternative drugs (drugs from plants) (Khan, 2016). There are various plants across the world, particularly in Nigeria that have been shown to have anti-diabetic potentials.

Calotropis procera is among the plants that are locally used in Nigeria as an antihyperglycemic agent. Its antihyperglycemic effect in rats induced with diabetes using STZ so as to ascertain this traditional claim was assessed in this study. The results show the assayed flower of the plant contained alkaloids, flavonoids, tannins, cardiac glycosides, saponins and phenols. In support of our findings, Vishnu *et al.* (2013) in their preliminary qualitative and quantitative phytochemical analysis made for the flower of *Caloptropis procera* revealed the presence of alkaloids, cardiac glycosides, flavonoids, phenols, saponins, steroids and tannins.

The oral glucose tolerance test (OGTT) is widely used to evaluate apparent insulin release and insulin resistance in various clinical settings (Stuvoll *et al.*, 2020). The results of this study show that the serum glucose level was 290 ± 1.82, 310.75 ± 1.80, 295.25 ± 1.19, 255.75 ± 2.56, 220.00 ± 1.84, 193.75 ± 0.96 mg/dL respectively at 0, 30, 60 90,120 and 240 minutes respectively, following the administration of 2 mg/kg b.w of glucose to the Wistar rats. This means that the level of glucose continued to decrease with increase in time up to the 240th minute after administration, at which point it became stable. This shows that the Wistar rats were not diabetic prior to streptozotocin administration.

The results of the effect of the various concentrations of the extract on the glucose level show that both the 200 mg/kg and 400 mg/kg extract significantly ($P < 0.05$) decrease the level of glucose when compared to the diabetic group. *Calotropis procera* has earlier been reported to have antihyperglycemic effect (Khan, 2016). The mechanism by which the flower extract of this plant has antihyperglycemic effect is not yet revealed, but might be because of the flavonoids it contains, as there are many reports that associate flavonoids with antihyperglycemic effect (Khan, 2016).

CONCLUSION

Diabetes is one of the metabolic syndromes whose morbidity rate is alarmingly increasing. The available conventional drugs have not been doing enough in curtailing its increase. Moreover, the side effects that these drugs present are of great health concern. Therefore, searching for alternative drugs is necessary. This study was however conducted with a view to examining the traditional claim of antihyperglycemic effect of *Caloptropis procera.* The results we obtained in this study have confirmed this claim to be true. Thus, it is suggested that the flowers of *Caloptropis procera* should be further explored systematically towards the development of a natural novel antihyperglycemic agent.

REFERENCES

Agral, A. and Pathak, A. K. (2022). CNS activity of *Calotropis gigantea* roots. NCBI, Public.Medicine, 3015; 106 (1): Epub.

Al-yahiya, A. A., Mohajeri, D., Rezaie, A. and Mehrdad Nazeri, M. (2020). Protective effects of green tea extract against hepatic tissue injury in streptozotocin-induced diabetic rats. *Evidence-Based Complementary and Alternative Medicine*, 2020 (2012): 1-10.

Aramugan, A. A. and Abou-Donia, M. A. (2022). Heavy metals in Egyptian species and medicinal plants and the effect of processing on their levels. *Journal of Agricultural and Food Chemistry*, 48 (6): 2300-2304.

Chandra, A., Munoz, M. F. and Arguelles, S. (2021). Lipid peroxidation: Production, metabolism and signalling mechanisms of malondialdehyde and 4-hydroxy-2-neaonal. *Oxidative Medicine and Cellular Longevity*, 1-31.

Evans, C. A., and Ashwood, E. R. (2022). Tietz *Textbook of Clinical Chemistry. 3rd Edition*. Saunders, Philadelphia. pp. 1056-1072.

Harborne, M., Shlipak, M. G., Katz, R., Sarnak, M. J., Newman, A. B., Siscovick, D. S., Kestenbaum, B., Carney, J. K and Fried, L. F. (1973). Relationship of uric acid with progression of kidney disease. *American Journal of Kidney Disorder*, 50: 239-247.

IDF Diabetes Atlas. (2020). Fifth Edition. pp 23-40 improved the free fatty-acid- induced insulin resistance in cultured C2C12 cells. Diabetes Metabolic Research. Reveiw2020; 24 (6):459–464.

Kaur, A., Batish, D. R., Kaur, S. and Chauhan, B. S. (2021). Overview of the characteristics and potentials of *Calotropis procera* from Botanical, Ecological and Economic perspectives. *Frontiers in Plant Science*, 12: 690806. doi: 10.3389/fpls.2021.690806

Khan, M. A. (2016). Antihyperglycemic anti dyslipidemic effect of Calotropis flowers in experimental diabetic rats. *Pakistan Journal of Medicine and Dentistry, 5 (01)*: 28 - 35

Meenakshi, P. G., Leonarduzzi, G. and Biasi, F. (2020). Oxidative stress and cell signaling. *Current Medicinal Chemistry*, 11: 1163–1182.

Murea, (2021). Terpenoids as plant antioxidants. *Vitamins and Hormones*, 72:505-535.

Namrata, P., Dobashi, K. and Greene, J. K. (2022). Studies on hepatic injury and antioxidant enzyme activities in rat sub-cellular organelles following *in vivo* ischemia and reperfusion. *Molecular and Cellular Biochemistry*, 176: 337–347.

Salsali, B., Sahidi, F. and Naczk, M. (2021). Extraction and analysis of phenolics in food. *Journal of Chromatography A*, 1054(1-2): 95-111.

Sharabana, T., Nakamura, K., Ikai, H., K. Kikuchi, K., Sasaki, K. and Niwano, Y. (2022). Literature review of the role of hydroxyl radicals in chemically-induced mutagenicity and carcinogenicity for the risk assessment of a disinfection system utilizing photolysis of hydrogen peroxide. *Journal of Clinical Biochemistry and Nutrition*, 51 (1): 9–14.

Suzuki, D. and Arnold, L. D. (2020). Natural products: Drug discovery and therapeutic medicine. Humana Press, Totowa, New Jersy. pp. 384.

Sofowara, H., Hogg, N. and Frejaville, C. (1993). Characterization of sulfur-centered radical intermediates formed during the oxidation of thiols and sulfite by peroxynitrite-ESR-SPIN trapping and oxygen uptake studies. *Journal of Biological Chemistry*, 271: 6000–6009.

Sparling, A. and Taal, M. W. (2022). Renal risk scores: Progress and prospects. *Kidney International*, 73: 1216-1219.

Spellman, A., Trease, G. E. and Evans, W. C. (2021). *Pharmacognosy. 15th Edition. Saunder Publishers, London* pp. 42 - 44, 221 -229, 246 – 249, 404 -306, 331-332, 391-393.

Stuvoll, M., Jarvien, H. Y., Mitrakou, A., Haeften, T.V., Pimenta. W. et al. (2020). Use the oral glucose tolerance test to assess insulin release and insulin sensitivity. *Diabetes Care*. 23(3):295-301. http://dx.doi.org/10.2337/ diacare.23.3.29

Trease, G. E. and Evans, W. C. (1996). *Pharmacognosy. 15th Ed. Saunder Publishers, London pp*. 42 - 44, 221 -229, 246 – 249, 404 -306, 331-332, 391-393.

Varier, A., Chakravarty, S., Ghosh, P. and Bodhankar, S. L. (2020). Rutin alleviates diabetic cardiomyopathy a rat model of type 2 diabetes. *Journal Research Plant Science*. 2020; 9:451–55.

Vishnu R, Nisha R, Jamuna S, Paulsamy S. (2013). Quantification of total phenolics and flavonoids and evaluation of *in vitro* antioxidant properties of methanolic leaf extract of *Tarenna asiatica* - an endemic medicinal plant species of Maruthamali hills, Western Ghats, Tami Nadu. *Journal Research Plant Science,* 2 (2):196–204.

WHO Expert Committee on Diagnosis and Classification of Diabetes Mellitus (2020). "Follow-up report on the Diagnosis of Diabetes Mellitus" *Diabetes care*, 26:3160.