# Effect of Methanolic Leaf Extract of Carissa Edulis on Alloxan Induced Diabetic Albino Rats

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***Specialty Section:***
*This article was submitted to Sciences, a section of NAPAS.*

***Submitted:*** *25 April2022*
***Accepted:*** *4 October 2022*
***Published:*** *15 November 2022*

***Citation:***
*M Zubairu, N Taila, K. M. Rabiu, A Zubairu , J. A. Abdullahi. (2022). Effect of Methanolic Leaf Extract of Carissa Edulis on Alloxan Induced Diabetic Albino Rats. Nig Annals of Pure & Appl Sci. 5(2):353-358. DOI: 10.5281/zenodo.7338448*

***Publisher:***
*cPrint, Nig. Ltd*
***E-mail:*** *cprintpublisher@gmail.com*

*Access Code*

*http://napas.org.ng*

## ABSTRACT

Diabetes mellitus is the third leading cause of death after heart disease and cancer. It is a growing global problem and places a heavy financial burden on health care services due to cost of orthodox medicine and associated toxicities. Thus, the demand for cheaper and safer alternatives is on the increase. Carissa edulis is widely used traditionally to cure diabetes and it was found to be relatively safe. This research is aimed to evaluate the effect of methanolic leaf extract of Carissa edulis on the glucose level and the body weight of alloxan induced diabetic rats. Carissa edulis leaves were harvested fresh in a garden at Chikun Local Government Area of Kaduna State. Cold maceration was used for the extraction using methanol as solvent. Diabetes mellitus was induced by the administration of alloxan hydrate (150 mg/kg body weight). The extract was administered orally at 75 mg/kg, 150 mg/kg and 300 mg/kg body weight while a standard drug, metformin was administered at 100 mg/kg body weight. All treatments lasted for 14 days. There was significant decrease in blood glucose concentration in the groups treated with 150 mg/kg and 300 mg/kg methanolic leaf extract of Carissa edulis compared to the negative control group. The group treated with 300 mg/kg b.w had lower glucose level compared to the normal control group. A significant increase in body weight was observed in the extract control group compared to the remaining groups. It can be inferred that the methanol leaf extract of Carissa edulis has antihyperglycemic effect and it reduces the level of blood glucose in a dose depended manner and can potentially be explored in the search for new anti-diabetic agents.

**Key words**: Carissa edulis, Metformin, Alloxan, Antihyperglycemic.

## 1.0 INTRODUCTION

Diabetes mellitus is a metabolic disorder characterized by raised plasma glucose level (Inzucchi *et al.,* 2004). An important feature of diabetes mellitus is that the body cells are starved of glucose despite its very

high concentration in the body system (Akinloye *et al*., 2007). Prolonged hyperglycemia leads to problems in the breakdown of carbohydrates, fats and proteins causing extensive microvascular and macrovascular difficulties (Edem *et al*., 2021). Blood glucose levels are mostly regulated by insulin which is a hormone produced by the pancreas. Insulin is released when the blood glucose elevates for example after meal. When the blood glucose elevates (for example, after eating food), Insulin is normalizes it by promoting the uptake of glucose into body cells. The absence or insufficient production of insulin or lack of response to insulin causes hyperglycemia. High blood sugar (hyperglycemia) affects people who have diabetes. Several factors can contribute to hyperglycemia in people with diabetes, including food and physical activity choices, illness, nondiabetes medications, or skipping or not taking enough glucose-lowering medication. The global prevalence of diabetes mellitus is on the rise and at the current rate the estimates for the year 2000 through 2030 show that this global epidemic will have an increase from 177 to 366 million patients (Wild *et al*., 2004). Synthetic anti-diabetic drugs are very costly and not easily affordable by the majority of Nigeria populations *(*Michael *et al*., 2005).

In recent times, researchers focus more on plants with medicinal values. *Carissa edulis* is one of the widely studied plants (Jyoti *et al*., 2021). *Carissa*, a genus of the Apocynaceae family, consists of evergreen species, such as shrubs as well as small trees that are native to Asia, Africa, and Oceania's subtropical and tropical regions. The *Carissa* fruits are rich in fibres, lipids, proteins, carbohydrates, and macro- and micro-nutrients, which are essential to build and maintain strong bones and to retain normal functioning of the heart, kidney, muscles, and nerves (Musinguzi, 2007). Traditionally, *Carissa* plants have been used for the treatment of a variety of diseases, such as headache, syphilis, chest discomfort, gonorrhea, malaria, arthritis, and rabies, since time immemorial (Yau *et al*., 2008). Research on *Carissa* species to distinguish several compounds from the leaves, stems, roots, and wood of the *plant* revealed a total of 93 compounds from *C. spinarum* (27 polyphenols, 27 lignans, 23 terpenoids, 8 steroids, 2 coumarins, and 6 cardiac glycosides), 28 from *C. carandas* (2 polyphenols, 4 lignans, 20 terpenoids, and 2 steroids), and 11 (6 polyphenols and 5 terpenoids) from *C. macrocarpa* (Jyoti *et al*., 2021). Studies conducted on fruits of *Carissa edulis* revealed its therapeutic use in the treatment of diabetes and anaemia (Elfik *et al*., 1996). The inclusion of herbal medicines in pharmacovigilance systems is becoming increasingly important given the growing use of herbal products and herbal medicines globally. *Carrissa edulis* is relatively safe as the oral LD50 of the extract was estimated to be > 5000 mg/kg body weight of rats (Ya'u *et al*., 2013).

This research work is aimed at evaluating the antihyperglycemic effect of *Carissa edulis leaf against alloxan induced diabetic albino rats.*

## 2.0 MATERIALS AND METHODS

### 2.1 Collection and Preparation of Plant Sample

The plant, *Carissa edulis* was collected from Maraban Rido, a community in Chikun Local Government Area of Kaduna State. It was identified and authenticated in the herbarium unit of the Department of Biological Science Ahmadu Bello University (A.B.U) Zaria. A voucher number 900086 was deposited.

The leaves were removed from the stem and properly washed using a clean water to remove debris; it was then dried under the shade at room temperature for seven days and then pulverized using mortar and pestle. Finally, the coarse powders were separated by sieving using Mesh and stored in an air tight container for further use.

**2.2 Preparation of Crude Extracts of *Carissa edulis Leaves***

The extraction was carried out according to Sarla *et al.,* 2011. Electronic Ohms balance was used to weigh 40 g of the powdered *Carissa edulis Leaves*. It was then transferred into a beaker (600 ml). Using a measuring cylinder, 500 ml of analytical grade methanol (Sigma Aldrich) was transferred into the beaker and carefully stirred for proper mixing. The solution was kept in a refrigerator at 4 °C for a period of 72 h with regular shaking. A muslin cloth was used after the 72 h to obtain a filtrate from the solution, which was further subjected to filtration using a whatman filter paper 1. The filtrate was then evaporated by using a Water bath at 65 °C . The extract was then stored in a refrigerator at 4 °C till further use.

**2.3 Acclimatization and Weighing of the Albino Rats**

Twenty eight (28) male albino rats weighing 100-120g where obtained from the Nigerian Institute for Trypanosomiasis and Onchocerciasis Research (NITR). No_ 1 Surami Road, U/Rimi G.R.A, Kaduna North Local Government Area of Kaduna State. They were acclimatized for a period of two (2) weeks, in the Department of Pharmacology, Faculty of Pharmaceutical Sciences Kaduna State University (KASU) Kaduna State. The rats were given normal pellets and water *ad libitum* throughout the study period. The weight of the rats were measured using an Electronic Ohms balance (Max-2000g, d=0.01).

Weight of the rat = weight of the plastic container and the rat – weight of the plastic container (Shibib *et al*., 1993).

**2.4 Experimental Design**

The experimental period was 15 days. The first day was for the base line data and the induction of the diabetes in the rats and the following 14 days were used for the administration of the methanolic leaf extract of the *Carissa edulis.*

The rats were divided into seven groups (four rats per group) as follows:

**1st Group:** 0.9 % w/v saline (Normal control)
**2nd Group:** 150 mg / kg Alloxan (Negative control)
**3rd Group:** 150 mg / kg leaves extract (Extract control)
**4th Group:** 150 mg / kg Alloxan + Metformin 100 mg / kg **(**Positive control)
**5th Group:** 150 mg / kg Alloxan + 75 mg / kg leaf extract (Extract treated diabetic rats)
**6th Group:** 150 mg / kg Alloxan + 150 mg / kg leaf extract **(**Extract treated diabetic rats)
**7th Group:** 150 mg / kg Alloxan + 300 mg / kg leaf extract **(**Extract treated diabetic rats).

**2.5 Induction of Diabetes**

The rats were made to fast 12 h before the induction of diabetes. Thereafter they were injected intraperitoneally with freshly prepared alloxan monohydrate (150 mg / kg body weight). Two days after injection, the rats with fasting blood glucose higher than 9.5 mmol / L were considered diabetic and used for the experiment. Feeding was stopped 12 h before blood sampling (Baishanab and Das, 2012).

**2.6 Collection of Blood Samples and Analysis**

The albino rats fasted for 12 h and Fasting blood glucose was determined using an On Call Plus Blood Glucose Monitoring System (REF G113-111). A drop of blood was obtained from the tip of conscious rat's tail and placed on the strip. The reading on the meter was noted and recorded as the blood glucose concentration.

**2.7 Statistical Analysis**

Data collected were reported in triplicate and results were expressed as mean ± standard deviation. Differences between two means were analyzed using Analysis of Variance (ANOVA

version 16). Post-hoc mean separations was performed by Turkey- Kramer Multiple Test and Values of $p \leq 0.05$ were considered to be significantly different.

## 3.0 RESULTS AND DISCUSSION

Results showed increase in blood glucose level in all the groups administered with alloxan at day four (Table 1). Compared to the negative control group, significant decrease in blood glucose level was observed in groups treated with 150 mg/kg and 300 mg/kg methanolic leaves extract of *Carissa edulis* in subsequent days. No significant difference in blood glucose level was observed in groups treated with 100 mg/kg Metformin, 150 mg/kg methanolic leaves extracts of the *Carissa edulis* and 300 mg/kg methanolic leaves extracts of the *Carissa edulis* respectively. Decrease in blood glucose level in groups treated with extracts could be as a result of the antiglycemic properties of *Carissa edulis* extract to stimulate or regenerate β cells of the pancreas for insulin secreation. This conforms with the findings of Abdallah *et al*., 2017 .This work revealed that the antihyperglycemic activity of *Carissa edulis* is dose dependent as the blood glucose level decreases with increase in the concentration of the extracts. This supports the work that was carried out by Babu *et al.,* 2002. A decrease in body weight was observed in day in all the groups except the extract control group negative control group compared to the normal control group (figure 1). Significant increase was observed in body weights of the albino rats in subsequent days following the treatment with 100 mg/kg metformin, 150 mg/kg and 300 mg /kg methanolic leaf extract of *Carissa edulis* . The loss in body weight of negative control group may be due to muscle destruction or degradation of structural proteins which conforms with the findings of Salahuddin, and Jalalpure (2010). Tissue wasting is a characteristic of poor glycemic control in diabetes and this usually foster protein and fat mobilization (Cotran *et al.,* 1999). Atangwho *et al*., 2007 also reported significant weight reduction in untreated diabetic rats. This was also observed in the present study with untreated diabetes as body weight of the albino rats was shown to have significantly reduced at the 13$^{th}$ day. The reduction in body weight of the untreated diabetic rats indicated the deterioration in glucose control mechanism. This observation shows that as the weight of rat decreases, the blood glucose levels increases over the experimental period, hence establishing inverse relationship between blood glucose and weight changes in untreated diabetes. This also supports the work that was carried out by Hegde and Joshi (2010).

The groups having different superscript vertically are considered to have significant difference ($p \leq 0.05$). Significant differences were analyzed between groups using one-way analysis of variance (ANOVA). Post-hoc mean separations was performed by Turkey- Kramer Multiple

**Table 1: Effect of Methanolic Leaf Extract of *Carissa edulis* on Glucose Level of Alloxan Induced Diabetic Rats**

| Treatment/Days | 0$^{th}$ | 4$^{th}$ | 7$^{th}$ | 10$^{th}$ | 13$^{th}$ |
|---|---|---|---|---|---|
| Group 1 | 4.48±0.25$^{a}$ | 4.48±0.25$^{a}$ | 4.53±0.28$^{a}$ | 4.55±0.26$^{a}$ | 4.53±0.22$^{a}$ |
| Group 2 | 4.13±0.36$^{a}$ | 12.38±0.87$^{b}$ | 11.38±0.29$^{b}$ | 9.15±0.29$^{b}$ | 7.23±0.22$^{b}$ |
| Group 3 | 4.30±0.29$^{a}$ | 4.33±0.28$^{a}$ | 4.23±0.17$^{a}$ | 3.98±0.05$^{c}$ | 3.65±0.24$^{c}$ |
| Group 4 | 4.80±1.07$^{a}$ | 11.55±1.09$^{bc}$ | 9.38±0.64$^{c}$ | 7.70±0.14$^{d}$ | 5.33±0.22$^{d}$ |
| Group 5 | 4.88±0.83$^{a}$ | 12.33±1.08$^{b}$ | 9.33±0.34$^{c}$ | 9.33±0.34$^{b}$ | 8.35±0.13$^{e}$ |
| Group 6 | 4.60±0.14$^{a}$ | 11.45±0.42$^{c}$ | 9.33±0.33$^{c}$ | 7.18±0.13$^{d}$ | 4.65±0.91$^{a}$ |
| Group 7 | 4.65±0.31$^{a}$ | 11.98±0.21$^{bc}$ | 8.05±02$^{d}$ | 5.35±0.17$^{e}$ | 3.98±0.09$^{c}$ |

*Blood Glucose Level mmol/L*

***Group 1:*** *Normal control (Normal saline)*
***Group 2:*** *Negative control (150 mg/kg Alloxan)*
***Group 3:*** *Extract treated (150 mg/kg Leaves Extract)*
***Group 4:*** *Positive control (150 mg alloxan+100 mg/kg Metformin)*
***Group 5:*** *Extract treated (150 mg alloxan+75 mg/kg Leaves Extract)*
***Group 6:*** *Extract treated (150 mg alloxan+150 mg/kg Leaves Extract)*
***Group 7:*** *Extract treated (150 mg alloxan+300 mg/kg Leaves Extract)*

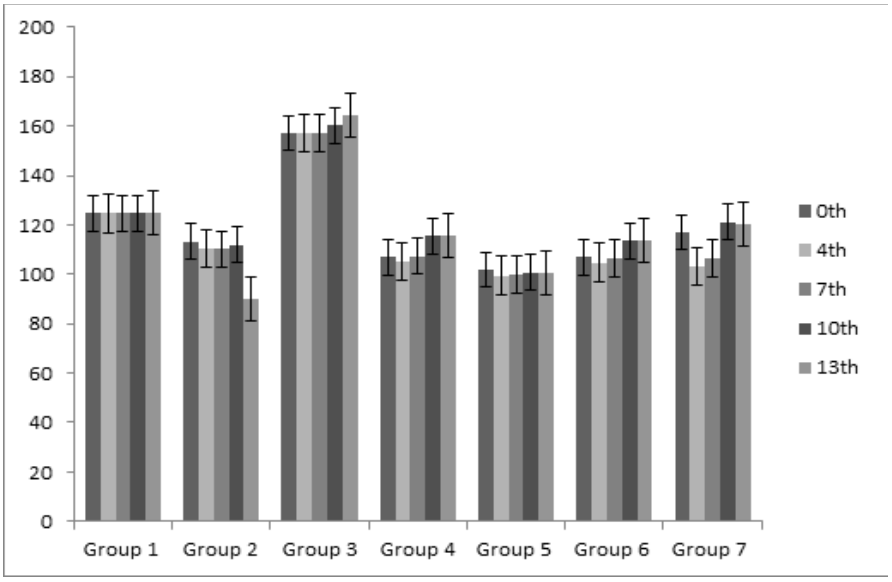


**Fig 1: Effect of Methanolic Leaf Extract of Carissa *edulis* on the Body Weight of Alloxan Induced Diabetic Rats.**

## CONCLUSION

In conclusion, the methanolic leaf extract of the *Carissa edulis* shows an antiglycemic activity, which support the use of the plant by the traditional healers in the treatment of diabetes mellitus. However, the potent component with the antiglycemic activity was not identified in this study. Fractionation and purification of the most active component involved in the antiglycemic activity of the plant extract should be carried out.

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