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Research Article
Glutathione Reductase and Malondialdehyde Activity in Alloxan Induced Diabetic Rats Treated with *Costus lucanusianus*

[1]E. Jeroh, [2]E.P. Awhin and [2]E.A. Eyikimiaghan

[1]Department of Physiology, Faculty of Postgraduate Studies, Delta State University, Abraka, Nigeria
[2]Department of Medical Biochemistry, College of Basic Medical Sciences, Delta State University, Abraka, Nigeria

Abstract

Background and Objective: The use of medicinal plants has been known to have effect on diabetes which has been observed to cause high mortality and morbidity rate. The objective of this work was to carry out an analysis of Glutathione reductase (GSH) and malondialdehyde (MDA) activities in diabetic rats treated with *Costus lucanusianus*. **Materials and Methods:** Plant material was harvested from the rich forest of Abraka community. The plant was soaked in distilled water for a period of 48 h after which the resulting solution was oven dried to concentrate the extract which was used to treat the diabetic rats. ANOVA was used as statistical tool. **Result:** It was observed from this study that the activity of GSH in the serum of group B and group C increased moderately, while group D showed slight decrease, in comparison to control group A. Similarly, the activity of GSH in the liver of group B increased moderately, while group C and group D, decreased mildly compared to control group A. Also, the result of MDA activity in the serum showed mild decrease in group B and group C and an increase in group D, in comparison to control group A and MDA activity in the liver of group B and C showed mild increase, while group D showed slight decrease compared to the control group A. **Conclusion:** The result from this finding has shown that *Costus lucanusianus* may have an ameliorative effect on diabetes mellitus due to the increase activity of GSH and decrease activity of MDA.

Key words: *Costus lucanusianus*, glutathione reductase (GSH), malondialdehyde (MDA), alloxan, serum, liver, antioxidant, diabetes

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Corresponding Author: E. Jeroh, Department of Physiology, Faculty of Postgraduate Studies, Delta State University, Abraka, Nigeria Tel: +2348137936850

Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Diabetes mellitus has been observed to cause high mortality and morbidity rate in both developing and developed countries[1]. It is characterized by increase in the blood glucose level which progresses into frequent urination, increased thirst as well as increased hunger. Majority of the people with diabetes are known to live in low- and middle-income countries[2].

It has been stated that in the year 2010, Africa alone had about 12.1 million people who have been observed to live with diabetes mellitus[3]. Research has also shown that there would be an expected increase in the prevalence of diabetes mellitus[4] in Asia and Africa by 2030. This is because of increased urbanization and lifestyle changes that have changed the indigenous diet to a modern diet[5].

Diabetes mellitus is a serious lifelong condition that affects an estimated population of about 151 million[6] and a third of these go about undiagnosed until many years after the onset[7]. In diabetes, either the pancreas cannot secrete insulin (type 1 diabetes) or the body does not utilize the insulin effectively (types 2 diabetes). As the body is unable to convert glucose into energy, the glucose remains in the bloodstream causing high blood sugar levels, which has a very damaging effect on the body[8]. Diabetes is mainly due to oxidative stress and an increase in reactive oxygen species that can have major effects. Many plants contain different natural antioxidants, in particular tannins, flavonoids, C and E vitamins that have the ability to maintain β-cells performance and decrease glucose levels in the blood[9]. Attention is also paid to other health problems that may accelerate the deleterious effects of diabetes. These include elevated cholesterol levels, obesity, high blood pressure, and lack of regular exercise. Specialized footwear is widely used to reduce the risk of ulceration, or re-ulceration, in at-risk diabetic feet evidence for the efficacy of this remains equivocal[10].

Herbs also known as medicinal plants are common in the medicine of ancient India, where the principal treatment for diseases was diet. Many plants from different parts of the world have been investigated for antidiabetic effects[11]. It has been observed that medicinal plants have pharmaceutical outcome in diabetes[12].

Medicinal plants have been observed to play a golden role as traditional medicine and as a trade commodity which meets with the demand of distant markets for the development of new drugs[13]. It has also been observed that to realize the effective integration of plants into the medical system, it is expected that researchers and practitioners should be trained in modern and traditional medicine in the use of compounds derived from medicinal plant.

Costus lucanusianus is a perennial herbaceous plant species with stems and leaves whose fruit is an ellipsoid capsule and is used as an ornamental flower and as a medicinal plant[14]. It is a climbing herb which is mainly found in the Niger Delta region of Nigeria and has contributed to the healthcare system in rural communities[15].

The main organ that metabolizes xenobiotics and endogenous molecules to maintain metabolic homeostasis in the organism is the liver and it contains antioxidant that performs specialized function in protecting the liver against peroxidation reaction that leads to cellular damage[16]. The objective of this study was to carry out an analysis of glutathione reductase (GSH) and malondialdehyde (MDA) activity in alloxan induced diabetic rats treated with C*ostus lucanusianus*.

MATERIALS AND METHODS

Animal care ethics: Twenty wistar rats with average weight of 130 g and of mixed sexes used for this research were purchased from Obiaruku Delta State and transported to animal house, College of Health Science, Delta State University, Abraka, where they were allowed to acclimatize to environmental condition for a period of two weeks before being used for the research. After 8 weeks of confirmation of diabetes, the animals were divided into 4 different groups, namely A, B, C and D. Each group had five rats (n = 5). This research was conducted at the College of Health Science, Delta State University, Abraka between 12 December, 2016- 13th February, 2017 while the animals were now sacrificed on the 15th February, 2017.

Experimental design:

Groups	Experiment
A	Normal animal taking regular feed and water
B	Diabetic animal taking 2.5 mL of *Costus lucanusianus*
C	Diabetic animals taking 5 mL of *Costus lucanusianus*
D	Diabetic animal taking anti diabetic drug

Protocol of research design was adapted from Nwangwa[17]

Preparation of *Costus lucanusianus*: The protocol for the preparation of *Costus lucanusianus* was adapted from Baba and Onanuga[18] and Saliu and Fapohunda[19] adapted to suit this research purpose. The mature *Costus lucanusianus* (commonly called monkey sugarcane), was harvested from the

rich forest of Abraka community. Prior to the administration of the plant extract, the plant was soaked in distilled water for a period of 48 h after which the resulting solution was oven dried to concentrate the extract before using it to treat the diabetic animals.

Chemical and reagents: Alloxan, Distilled water Chloroform-may and baker, Dagenham England, Sodium citrate salt-kermal chemical, Sodium chloride salt-kermal chemical, Top feeds grower mash-premier feed mills co Ltd., Gentian violet paint-Glona-G-pharma Nigeria Ltd.

Induction of animals: The induction of diabetes was done using the protocol of Williamson *et al*[20]. The standard dosage for alloxan was 150 mg kg^{-1}. The rats were divided into four groups. Each animal in group B, C and D received a single intravenous injection of alloxan monohydrate into the lateral tail vein (according to their body weight following the standard dosage) after fasting them for 18-24 h. Accuchek active blood glucose monitor and lancing device (Roche diagnostics Germany) was used to check and confirm diabetes where animals with glucose level higher or equal to 180 mg dL^{-1} were considered diabetic.

Collection of liver and serum: The protocol adapted from of Williamson *et al*[20] was used in the collection of liver and serum before sacrificing the animals in order to collect the liver and serum needed for biochemical analysis, the animals were fasted for a period of 12 h and each anaesthetized in a chloroform saturated chamber. While under anesthesia, the abdominal region of each rat was cut open to expose the inferior vena cava and the liver. Blood of about 8 mL was collected immediately and stored in a plain container. Similarly, the liver of each animal was harvested and weighed. Thereafter, 0.5 g of the liver was weighed and used in preparing a homogenate in 4.5 mL of normal saline. The homogenate and the whole blood was then centrifuged separately, using a bucket centrifuge model 800 at 4000 rmp for 10 min each, in order to obtain tissue homogenate supernatant and serum, respectively. The resulting samples (supernatant and serum) were properly stored frozen in a refrigerator until analysis.

Biochemical assay: The measurement of enzyme activity was done using the spectrophotometer. Malondialdehyde (MDA) was determined by the method of Okhawa *et al*[21]. Reduced glutathione (GSH) was measured according to the method of Ellman[22].

Statistical analysis: The results were expressed as Mean±SD. The analysis of variance (ANOVA) method of statistical analysis was used for the evaluation of statistical significance. Differences were considered to be statistically significant *$p<0.05$.

RESULTS

Glutathione reductase (GSH) and malondialdehyde (MDA) activity in serum: The result from this research revealed that the activity of GSH in the serum of group B and group C increased moderately, when compared with the control group A but the activity of GSH in group D showed slight decrease. While results of MDA activity in the serum showed mild decrease in group B and group C in comparison to control group A while diabetic group D showed mild increase when compared to control group A. The changes seen with GSH and MDA activity in this studies was not statistically significant (*$p<0.05$) as determined by one way analysis of variance (ANOVA) (Table 1).

Glutathione reductase (GSH) and malondialdehyde (MDA) activity in liver: The result for liver revealed that the activity of GSH in the liver of group B increased moderately, when compared with that of the control group A, But in group C and group D the activity of GSH decreased mildly in comparison to control group A and group B While the result of MDA in the liver of group B and C showed mild increased level in comparison to the control group A. While the level of MDA in group D showed slight decrease in comparison to control group A (Table 2).

Table 1: Analysis of glutathione reductase (GSH) and malondialdehyde (MDA) in serum of wistar rats

Groups	GSH (mg dL^{-1})	MDA (mg dL^{-1})
A	$09.47\times10^{-3}\pm09.46\times10^{-3}$	$12.02\times10^{-3}\pm04.11\times10^{-3}$
B	$20.23\times10^{-3}\pm20.44\times10^{-3}$	$08.98\times10^{-3}\pm01.64\times10^{-3}$
C	$13.21\times10^{-3}\pm05.67\times10^{-3}$	$10.82\times10^{-3}\pm07.05\times10^{-3}$
D	$09.46\times10^{-3}\pm01.75\times10^{-3}$	$20.66\times10^{-3}\pm28.36\times10^{-3}$

Values are Mean±SD, n = 5, Group A: Normal animal taking regular feed and water, Group B: Diabetic animal taking 2.5 mL of *Costus lucanusianus*, Group C: Diabetic animals taking 5 mL of *Costus lucanusianus*, Group D: Diabetic animal taking anti diabetic drug

Table 2: Analysis of glutathione reductase (GSH) and malondialdehyde (MDA) in liver of wistar rats

Groups	GSH (mg dL^{-1})	MDA (mg dL^{-1})
A	$10.86\times10^{-3}\pm05.49\times10^{-3}$	$12.38\times10^{-3}\pm4.24\times10^{-3}$
B	$13.99\times10^{-3}\pm25.14\times10^{-3}$	$15.12\times10^{-3}\pm1.52\times10^{-3}$
C	$09.65\times10^{-3}\pm03.33\times10^{-3}$	$14.66\times10^{-3}\pm5.17\times10^{-3}$
D	$09.85\times10^{-3}\pm01.67\times10^{-3}$	$12.08\times10^{-3}\pm4.76\times10^{-3}$

Values are Mean±SD, n = 5, Group A: Normal animal taking regular feed and water, Group B: Diabetic animal taking 2.5 mL of *Costus lucanusianus*, Group C: Diabetic animals taking 5 mL of *Costus lucanusianus*, Group D: Diabetic animal taking anti diabetic drug

DISCUSSION

Costus lucanusianus, a perennial herbaceous plant species has been used as an ornamental flower as well as a medicinal plant[14] while Diabetes mellitus has been observed to cause high mortality and morbidity rate in both developing and developed countries[15]. The objective of this research therefore was to carry out an analysis of glutathione reductase (GSH) and Malondialdehyde (MDA) activities in diabetic rats treated with *Costus lucanusianus* with the view of knowing if the plant extract can be used as a medicinal remedy for the treatment of diabetes. The research findings from this study showed that there was an increase of GSH activity in group B treated with low dosage of the plant extract in the liver and group B and C of serum treated with the plant extract. This change indicated an ameliorative effect of the plant on the activity of GSH, especially in the blood in response to free radical. While the decrease associated with MDA activity in the serum in diabetic groups treated with *Costus lucanusianus* may also be an indicator of ameliorative effects of the plant extract. Findings from this study are in agreement with an earlier report that an increased level of MDA was associated with diseases due to oxidative injury of the liver[23] also in agreement with the report that the hepatotoxic effect of Bisphenol induced oxidative stress in rat model, resulted in decreased GSH level[24]. The implication of the findings from this research is that extracts of *Costus lucanusianus* can be used in the treatment of diabetic cases since it reduced blood glucose level. This finding is also in agreement with the earlier report that the administration of the extract of *Costus lucanusianus* normalised blood glucose level and that *Costus lucanusianus* extract has antihyperglycemic, hepatoprotective and renoprotective potentials thus making the plant a suitable candidate in the management of diabetes mellitus[19]. The research findings are in agreement with Christoper[14] Saliu and Fapounda[19] and Templar *et al.*[23]. It is hereby recommended that *Costus lucanusianus* is a medicinal plant which can be used in the treatment of diabetes.

SIGNIFICANCE OF STUDY

This study has shown that extracts of *Costus lucanusianus* has ameliorative effects on diabetic treated rats. The implication is that, with the emphasis on medicinal plants for treatment of certain diseases such as diabetes, the use of *Costus lucanusianus* helps the researcher to develop new therapeutic strategies for the treatment of diabetes.

CONCLUSION

Conclusively the result of this study showed that *Costus lucanusianus* may have an ameliorative effect on diabetes mellitus due to the increase activity of GSH and decrease activity of MDA as seen in this study. It could also be concluded that oxidative stress induced by diabetes may not be controlled by the anti diabetic drug used for this studies.

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