# Estimation of Serum Glutathione Peroxidase in Streptozotocin Induced Diabetic Rat Treated with Bitter Leaf Extract

**Arvin Nwakulite[1], Emmanuel Ifeanyi Obeagu[1,2*], Richard Eze[1], Valerie Esame Ugochi[3], C.C.N. Vincent[4], Chukwuma J. Okafor[5], Ejike Felix Chukwurah[6], Bright Chukwuebuka Unaeze[7], Chukwudi Ofodile Amaechi[7], Chetachi Blessing Okwuanaso[7], Ufuoma Chukwuani[8] and Bart I. Ifionu[9]**

[1]*Department of Medical Laboratory Science, Madonna University, Elele, Rivers State, Nigeria.*
[2]*Department of Medical Laboratory Science, Imo State University, Owerri, Nigeria.*
[3]*Department of Medical Laboratory Science, University of Calabar, Cross River State, Nigeria*
[4]*Department of Nursing Science, Imo State University, Owerri, Imo State, Nigeria.*
[5]*Department of Pathology and Biochemistry, State University of Zanzibar, Tanzania.*
[6]*Department of Haematology and Immunology, Faculty of Clinical Medicine, Ebonyi State University, Abakaliki, Nigeria.*
[7]*Department of Medical Laboratory Science, Nnamdi Azikiwe University, Nnewi Campus, Anambra State, Nigeria.*
[8]*Department of Medical Laboratory Science, Igbinedion University Okada, Edo State, Nigeria*
[9]*Department of Chemical Pathology and Immunology, Olabisi Onabanjo University, Ogun State, Nigeria.*

*Authors' contributions*

*Article Information*

*Original Research Article*

*\*Corresponding author: E-mail: emmanuelobeagu@yahoo.com;*

## ABSTRACT

Diabetes mellitus (DM) is a metabolic disease in which there are high blood sugar levels. Type 2 is due to the cells of the body not responding properly to the insulin produced. The aim of this study was to evaluate the enzyme activity in glutathione peroxidase in type 2 diabetic rats induced streptozotocin Wister rats. Enzyme linked immunosorbent assay (ELISA) methods was used. Thirty-two (32) adult rats of Wister strain weighing between 120 g – 200 g of both sexes equally were used. Streptozotocin was used to induce diabetes after high fat diet. The rats were randomly grouped into 4 groups of 8 rats; group 1 are rats fed with only feed and water, group 2 were given 37 mg/kg of streptozotocin with feed and water, group 3 had 37 mg/kg of streptozotocin, feed, water and treated with 2ml of freshly prepared bitter leaf extract daily, group 4 had feed, water, 37mg/kg of streptozotocin and treated with 5 mg/kg of glibenclamide (anti diabetic drug). Severity of the induced diabetic state was assessed by daily and weekly monitoring of body weights and blood glucose levels. The result of fasting blood sugar shows a significant difference (P<0.05) at group 3(7.72±0.99) compared to group 4(9.93±1.22) in week 2.There is also a significant decrease (p<0.05) at group 3(7.72±0.99) compared to group 4(9.90±1.24) in week 3.There is also a significant decrease (p<0.05) at group 3(6.22±1.20) compared to group 46.50±0.70) in week 5. There is a significant increase (p<0.05) at group 1(7.63±0.71) compared to group 4(5.78±1.40), group 2(7.45±0.87) compared to group 4(5.78±1.40)in week 4.There is also a significant decrease in GPX activity in group at group 1(424.59±102.65) compared to group 2(307.34±75.66). There is no significant difference (p>0.05) at group 2(307.34±75.66) compared to group 3(204.31±46.51). There is also no significant difference (p>0.05) at group 2(307.34±75.66) compared to group 4(206.12±55.37). No significant difference (p>0.05) at group 3(204.31±46.51) compared to group 4(206.12±55.37). In conclusion, the result of this study suggest that bitter leaf extract reduced glucose level and has no damage effect on the liver.

## 1. INTRODUCTION

Antioxidant is a molecule that inhibits the oxidation of other molecules. Oxidation is a chemical reaction that transfers electron or hydrogen from substances to an oxidizing agent. Oxidation reactions can produce free radicals. In turn, these radicals can start chain reactions, when the chain reactions occur in a cell, it can cause damage or death to the cell. Antioxidants terminate these chain reactions by removing free radical intermediates and inhibit other oxidative reactions. They do so by being oxidizing themselves. Antioxidants are often reducing agents such as, thiols, ascorbic acid or polyphenols.

Under physiological conditions, hydrogen peroxide, superoxide and hydroxyl radicals, collectively called reactive oxygen species, are continuously produced and kept under strict control by many enzymes and antioxidants within the cells [1]. Clinical and experimental studies have shown that disturbing of oxidant-antioxidant balance system is involved in the pathogenesis of chronic diseases such as cancer, coronary heart disease, diabetes and many diabetic complications [2].

Chemical drugs have many side effects; therefore, looking for new antidiabetic drugs from natural antioxidants sources is still attractive because they are safe and good alternative for treatment of diabetes mellitus. A growing body of research indicates that nutritional deficiencies of antioxidants contribute to the development of diabetes [3].

Among antioxidant micronutrients, selenium (Se) is an essential dietary trace element, which plays an important role in a number of biological processes in humans and other species. Deficiency of this element induces some pathological conditions, such as cancer, coronary heart disease, and liver necrosis. Researchers have shown selenium and zinc efficacy on immune system and increase response to influenza and HBV vaccine [4]. Also researchers have shown sodium selenite decrease levels of lipid peroxidation (LPO) and NOPs (nitric oxide products) and increase activities of superoxide dismutase, GR (glutathione reductase), and GPX (glutathione peroxidase) in heart diabetes-induced rats. Selenium is an essential component of several enzymes such as GPX, TR (thioredoxin reductase) and SeP

(selenoprotein P), which contains Seas selenocysteine.

Diabetes-related dysfunctions are the major causes of mortality and morbidity for diabetic patients. Although the precise mechanism by which hyperglycemia induces organ dysfunction is not fully understood, one of the hypothesis to explain this phenomenon is mainly focused on the role of free radicals in these disease states [5]. Various type of diabetes mellitus include the following.

Type 1 DM results from the body's failure to produce enough insulin. This form was previously referred to as "insulin-dependent diabetes mellitus" (IDDM) or "juvenile diabetes". The cause is unknownType 2 DM begins with insulin resistance, a condition in which cells fail to respond to insulin properly. As the disease progresses a lack of insulin may also develop. This form was previously referred to as "non insulin-dependent diabetes mellitus" (NIDDM) or "adult-onset diabetes". The primary cause is excessive body weight and not enough exercise.
Gestational diabetes mellitus: refers to glucose intolerance with onset or first recognition during pregnancy.

The study was done to estimate enzymatic antioxidant activity; serum glutathione peroxidase in streptozotocin induced diabetic rat treated with bitter leaf extract.

## 2. MATERIALS AND METHODS

### 2.1 Study Area

This experiment was carried out in Madonna University Teaching Hospital Animal Farm, Elele, Nigeria.

### 2.2 Preparation of Plant Material

The fresh leaves of *Vernonia amygdalina* (bitter leaf) was collected from the school surroundings daily, well sorted and only fresh leaves were used in preparation of extract juice.

### 2.3 Materials/Reagents

Includes steel cage with steel wire, syringe, feeds, feeding plates, reagent bottles, so dust, gloves, laboratory coat, beaker, weighing balance, markers, reagents such as Disodium citrate, citrate acid, deionized distilled water, Hydrochloric acid (HCl), sodium hydroxide (NaOH), Deionized water, pH meter, spatula.

### 2.4 Animal Handling

Thirty two (32) adult rats of Wister strain weighing between 120 g – 200 g of both sexes equally were used. They were allowed to acclimatize for two weeks a room of suitable temperature in an animal house in the animal farm in Madonna University Elele. After which they were fed with high fat diet for seven weeks to induce obesity. They were further grouped into four groups with each group having 8 rats. The animals were given 37mg/kg of streptozotocin to induce diabetes.

### 2.5 Experimental Design

Rats were grouped into four (4) groups:

Group 1 (Negative control): The animal in this group were fed with only animal feed and water throughout the experiment.
Group 2 (Positive control): Were given 37mg/kg of Streptozotocin in addition to food and water.
Group 3: In addition to food and water, received 37mg/kg of streptozotocin and 2ml of bitter leaf extract.
Group4: In addition to food and water, were given 5mg/kg of glibenclamide (anti-diabetic).

### 2.6 Sample Collection

At the end the acute feeding with normal feed, high fat feed and then treatment with glibenclamide and bitter leaf extracts, cardiac puncture was used to collect blood samples from the diabetic rat.

### 2.7 Quantitative Determination of Rat Glutathione Peroxidase

#### 2.7.1 Methods

The samples were analyzed using Enzyme linked immunosorbent assay (ELISA) as modified by Bioassay technology laboratory, (2017) Cat number- E1759Ra.

#### 2.7.2 Procedure

50u/l of standard was added to standard well. 40u/l of sample was added to sample well and

then add 10u/l of anti GSH-PX antibody to sample well. Then 50u/l of streptavidin-HRP was added to sample wells and standard well (not to blank control well) and mixed well and covered the plate with a sealer and incubated for 60 minutes at 37c.The sealer was removed and washed plate 5 times with wash buffer. The wells were soaked with at least 0.35ml of washed buffer to 30seconds for each wash. 50u/l of substrate solution A was added to each well and then add 50u/l of substrate solution B to each well and incubated plate covered with sealer for 10minutes at 37c in the dark. 50u/l of solution was added to each well, the blue colour changed into yellow immediately.

## 2.8 Statistical Analysis

Data generated from this study was analyzed using statistical package for social sciences (SPSS version 20.0) windows 19. The results were presented in tables and expressed as mean ± standard deviation. ANOVA was used to compare means, and values were considered significant at p<0.05.

## 3. RESULTS

Table 1 shows a significant difference (p<0.05) in weights of rats across weeks. There is a significant decrease (p<0.05) of rats in group1 (121.33±7.56) compared to group 2(152.67±12.88) and group 1(121.33±7.56)

compared to group 3(167.00±31.67) in week 3.There is also a significant decrease (p<0.05) in group 2(159.67 ±18.3) when compared to group 4(131.33±12.43).

Table 2 shows no significant difference (p>0.05) in BMI of rats across the weeks. There is no significant difference (P>0.05) in group 1(0.95±0.75) compared to group 2(0.94±0.71), group 3(0.82±0.10) compared to group 4(0.89± 0.08) of week 1.There is no significant difference (p>0.05) in group 1(0.80±0.09) compared to group 3(0.82±0.05),group 3(0.82±0.05) compared to group 4(0.79± 0.05) of week 3.There is also a no significant difference (p>0.05) in group 1(0.91±0.06) compared to group 3(0.86±0.1),group 2(0.84±0.07) compared to group 3(0.86±0.13),group 3(0.86±0.13) compared to group 4(0.83± 0.08) of week 5.

Table 3 shows a significant difference (P<0.05) in FBS level of rats across weeks. There is a significant decrease (P<0.05) at group 3(7.72±0.99) compared to group 4(9.93±1.22) in week 2.There is also a significant decrease (p<0.05) at group 3(7.72±0.99) compared to group 4(9.90±1.24) in week 3.There is also a significant decrease (p<0.05) at group 3(6.22±1.20) compared to group 46.50±0.70) in week 5. There is a significant increase (p<0.05) at group 1(7.63±0.71) compared to group 4(5.78±1.40), group 2(7.45±0.87) compared to group 4(5.78±1.40) in week 4.

**Table 1. The mean ± standard deviation of weight (g) of all the rats treated throughout experimental period**

| GROUPS | WEEK 1 | WEEK 2 | WEEK 3 | WEEK 4 | WEEK 5 |
|---|---|---|---|---|---|
| GROUP 1 | 151.67±13.16 | 163.33±11.98 | 121.33±7.56 | 167.67±17.31 | 189.00±22.44 |
| GROUP 2 | 159.67 ±18.3 | 165.67±11.41 | 152.67±12.88 | 185.33±27.38 | 188.33±32.18 |
| GROUP 3 | 140.33±12.29 | 171.33±24.87 | 167.00±31.67 | 176.00±40.00 | 180.33±32.18 |
| GROUP 4 | 33±12.43 | 179.00±22.37 | 157.33±28.23 | 191.33±28.44 | 191.33±28.44 |
| P values | 0.015 | 0.489 | 0.013 | 0.493 | 0.924 |

*KEY: Group 1 negative control; Group 2 positive control; Group 3 treatment with bitter leaf extract; Group 4 treatment with glibenclamide*

**Table 2. Shows the mean ± standard deviation of BMI of all the rats treated throughout experimental period**

| GROUPS | WEEK 1 | WEEK 2 | WEEK 3 | WEEK 4 | WEEK 5 |
|---|---|---|---|---|---|
| GROUP 1 | 0.95±0.75 | 0.94±0.11 | 0.80±0.09 | 0.84±0.06 | 0.91±0.06 |
| GROUP 2 | 0.94±0.71 | 0.95±0.07 | 0.87±0.04 | 0.89±0.07 | 0.84±0.07 |
| GROUP 3 | 0.82±0.10 | 0.86±0.07 | 0.82±0.05 | 0.90±0.90 | 0.86±0.13 |
| GROUP 4 | 0.89± 0.08 | 0.87± 0.09 | 0.79± 0.05 | 0.91± 0.06 | 0.83± 0.08 |
| P values | 0.045 | 0.166 | 0.149 | 0.288 | 0.491 |

*. KEY: Group 1 negative control; Group 2 positive control; Group 3 treatment with bitter leaf extract; Group 4 treatment with glibenclamide*

**Table 3. The mean ± standard deviation of FBS of all the rats treated throughout experimental period**

| GROUPS | WEEK 1 | WEEK 2 | WEEK 3 | WEEK 4 | WEEK 5 |
|---|---|---|---|---|---|
| GROUP 1 | 6.93±080 | 8.78±0.56 | 8.65±0.65 | 7.63±0.71 | 7.62±0.30 |
| GROUP 2 | 6.75±1.35 | 8.43±1.52 | 8.43±1.52 | 7.45±0.87 | 6.57±0.64 |
| GROUP 3 | 7.00±0.52 | 7.72±0.99 | 7.72±0.99 | 7.82±0.98 | 6.22±1.20 |
| GROUP 4 | 6.93±1.19 | 9.93±1.22 | 9.90±1.24 | 5.78±1.40 | 6.50±0.70 |
| P values | 0.977 | 0.021 | 0.027 | 0.009 | 0.028 |

*KEY: Group 1 negative control; Group 2 positive control; Group 3 treatment with bitter leaf extract; Group 4 treatment with glibenclamide*

**Table 4. Multiple comparism of groups of fasting blood sugar of all rats used within experimental period**

|  | Week 1 | Week 2 | Week 3 | Week 4 | Week 5 |
|---|---|---|---|---|---|
| Grp 1vs 2 | 0.989 | 0.949 | 0.987 | 0.989 | 0.123 |
| Grp 1vs 3 | 0.999 | 0.381 | 0.506 | 0.989 | 0.026* |
| Grp 1vs 4 | 1.000 | 0.318 | 0.262 | 0.025* | 0.093 |
| Grp 2vs 3 | 0.989 | 0.693 | 0.702 | 0.924 | 0.863 |
| Grp 2vs 4 | 0.989 | 0.131 | 0.152 | 0.048* | 0.999 |
| Grp 3vs 4 | 0.999 | 0.014* | 0.017* | 0.01* | 0.921* |

*KEYS: Grp – group; Vs – Against; Fbs - fasting blood sugar*
*\* - significant difference; Group 1 negative control; Group 2 positive control; Group 3 treatment with bitter leaf extract; Group 4 treatment with glibenclamide*

**Table 5. Estimation of mean glutathione peroxidase activity (U/L) in rats of various groups**

| GROUP | GPX |
|---|---|
| Group 1 | 424.59±102.65 |
| Group 2 | 307.34±75.66 |
| Group 3 | 204.31±46.51 |
| Group 4 | 206.12±55.37 |
| P-value | 0.000 |

*KEY: Group 1 negative control; Group 2 positive control; Group 3 treatment with bitter leaf extract; Group 4 treatment with glibenclamide*

**Table 6. Shows multi comparism glutathione peroxidase in groups of rats used during experimental period**

| Groups | GPX |
|---|---|
| Group 1 vs 2 | 0.084* |
| Group1 vs 3 | 0.001 |
| Group 1 vs 4 | 0.001 |
| Group 2 vs 3 | 0.150* |
| Group 2 vs 4 | 0.161* |
| Group 3 vs 4 | 1.000* |

*KEYS: Grp – group; Vs – Against; GPX - Glutathione peroxidase*
*\* -No significant difference; Group 1 negative control; Group 2 positive control; Group 3 treatment with bitter leaf extract; Group 4 treatment with glibenclamide*

Table 4 shows no significant difference (P>0.05) of GPX level of rats across groups. There is no significant difference (p>0.05) at group 1(424.59±102.65) compared to group 2(307.34±75.66).There is no significant difference (p>0.05) at group 2(307.34±75.66) compared to group 3(204.31±46.51). There is also no significant difference (p>0.05) at group 2(307.34±75.66) compared to group 4(206.12±55.37). No significant difference (p>0.05) at group 3(204.31±46.51) compared to group 4(206.12±55.37).

## 4. DISCUSSSION

Type 2 diabetes mellitus is the most common form of diabetes mellitus characterized by hyperglycemia, insulin resistance and relative insulin deficiency.

The effects of bitter leaf extract on induced streptozotocin diabetic rats of both sexes were evaluated. The values of fasting blood sugar level, body mass index and glutathione peroxidase activities were checked and recorded before and after administration of treatment.

Table 1 shows a significant difference (p<0.05) in weights of rats across weeks. There is a significant decrease (p<0.05) of rats in group1 (121.33±7.56) compared to group 2(152.67±12.88) and group 1(121.33±7.56) compared to group 3(167.00±31.67) in week 3.There is also a significant decrease (p<0.05) in group 2(159.67 ±18.3) when compared to group 4(131.33±12.43)).

Table 2 shows no significant difference (p>0.05) in BMI of rats across the weeks. There is no significant difference (P>0.05) in group 1(0.95±0.75) compared to group 2(0.94±0.71), group 3(0.82±0.10) compared to group 4(0.89± 0.08) of week 1.There is no significant difference (p>0.05) in group 1(0.80±0.09) compared to group 3(0.82±0.05),group 3(0.82±0.05) compared to group 4(0.79± 0.05) of week 3.There is also a no significant difference (p>0.05) in group 1(0.91±0.06) compared to group 3(0.86±0.1),group 2(0.84±0.07) compared to group 3(0.86±0.13),group 3(0.86±0.13) compared to group 4(0.83± 0.08) of week 5. This result is in line with finding by [6] who states that Through life style and diet modification, that there was significant reduction in the incidence of type 2 DM with a combination of maintenance of body mass index of 25 kg/m2, eating high fiber and unsaturated fat and diet low in saturated and trans-fats and glycemic index.

Table 3 shows a significant difference (P<0.05) in FBS level of rats across weeks. There is a significant decrease (P<0.05) at group 3(7.72±0.99) compared to group 4(9.93±1.22) in week 2.There is also a significant decrease (p<0.05) at group 3(7.72±0.99) compared to group 4(9.90±1.24) in week 3.There is also a significant decrease (p<0.05) at group 3(6.22±1.20) compared to group 46.50±0.70) in week 5.There is a significant increase (p<0.05) at group 1(7.63±0.71) compared to group 4(5.78±1.40), group 2(7.45±0.87) compared to group 4(5.78±1.40) in week 4. Crude chloroform

extract of bitter leaf has an antidiabetic effect on rats with type 2 diabetes mellitus under laboratory conditions. Similar result was stipulated [7] on observation of bitter leaf extract reducing glycemic level significantly having peripheral action similar to insulin or glucose metabolism which is attributed to the bioactive molecule contained in the vegetables. The result above is also in line with [8] who stated that the nutrient composition also revealed that bitter leaf contains moisture and fibers which contribute less sugar to blood sugar pool, this is because study revealed that food rich in fiber content induces less glucose response. A similar finding also concur with the above [9] showing great evidence that a high intake of dietary fiber associates with enhanced insulin sensitivity and therefore may have a role in prevention and control of type 2 diabetes[10-12].

Table 5 shows no significant difference (P>0.05) of GPX level of rats across groups. There is no significant difference (p>0.05) at group 1(424.59±102.65) compared to group 2(307.34±75.66).There is no significant difference (p>0.05) at group 2(307.34±75.66) compared to group 3(204.31±46.51). There is also no significant difference (p>0.05) at group 2(307.34±75.66) compared to group 4(206.12±55.37).No significant difference (p>0.05) at group 3(204.31±46.51) compared to group 4(206.12±55.37). No work was seen on this therefore it could be said that level of glutathione peroxidase were reduced showing no antioxidant damage on the body and liver.

## 5. CONCLUSION

The result of this study revealed that the levels of glutathione peroxidase and level of fasting blood sugar were decreased in streptozotocin induced diabetic rats treated with bitter leaf extract. Due to its decrease in Glutathione peroxidase activity level, there no damage effect on liver.

## CONSENT

It is not applicable.

## ETHICAL APPROVAL

The experimental protocols were according to our Institutional Animal Ethics Committee guidelines as well as internationally accepted practices for use and care of laboratory animals as stated in US guidelines.

## COMPETING INTERESTS

Authors have declared that no competing interests exist.

## REFRENCES

1. Sueishi Y, Hori M, Ishikawa M, Matsu-Ura K, Kamogawa E, Honda Y, et al. Scavenging rate constants of hydrophilic antioxidants against multiple reactive oxygen species. Journal of Clinical Biochemistry and Nutrition. 2014;13-53.
2. Tabak O, Gelisgen R, Erman H, Erdenen F, Muderrisoglu C, Aral H, et al. Oxidative lipid, protein, and DNA damage as oxidative stress markers in vascular complications of diabetes mellitus. Clinical and Investigative Medicine. 2011;E163-E171.
3. Bonnefont-Rousselot D. The role of antioxidant micronutrients in the prevention of diabetic complications. Treatments in Endocrinology. 2004;3(1):41-52.
4. Janbakhsh A, Mansouri F, Vaziri S, Sayad B, Afsharian M, Rahimi M, Salari F. Effect of selenium on immune response against hepatitis B vaccine with accelerated method in insulin-dependent diabetes mellitus patients. Caspian Journal of Internal Medicine. 2013;4(1):603.
5. Lucchesi AN, Freitas NTD, Cassettari LL, Marques SFG, Spadella CT. Diabetes mellitus triggers oxidative stress in the liver of alloxan-treated rats: A mechanism for diabetic chronic liver disease. Acta Cirurgica Brasileira. 2013;28(7):502-508.
6. Fuentes O, Arancibis A, Alarcon H. Hypoglycemic activity of *Bauhinia candican* in diabetic induced rabbits: Fitoterapis. 2004;6:527–532.
7. Willi C, Bodenmann P, Ghali WA, Faris PD, Cornuz J. Active smoking and the risk of type 2 diabetes: A systematic review and meta-analysis. Jama. 2007;298(22): 2654.
8. Okolie UV, Okeke CE, Oli JM, Ehiemere IO. Hypoglycemic indices of *Vernonia amygdalina* on postprandial blood glucose concentration of healthy humans. African Journal of Biotechnology. 2008;7(24).
9. Yionen K, Saloranta C, Kronber C, Kronberg C, Leif C, Antti A, et al. Associations of Dietary Fiber with Glucose metabolism in Non diabetic relatives of subjects with Type 2 diabetes. Diabetes Care. 2003;26:1979–1985.
10. Obeagu EI, Obeagu GU. Utilization of Antioxidants in the Management of Diabetes Mellitus Patients. J Diabetes Clin Prac.2018; 1: 102.
11. Obeagu EI. .An update on Diabetes Mellitus.Int. J. Curr. Res. Med. Sci. 2018;.4(6):71-81.DOI:http://dx.doi.org/10.22192/ijcrms.2018.04.06.012
12. Chinwe IA, Obeagu EI, Chukwudi A, Priscilla EU. Prevalence of Urinary Tract Infection in Diabetic patients attending Umuahia Health care facilities J Bio Innov. 2016;5(1):68-82.

---

*Peer-review history:*
*The peer review history for this paper can be accessed here:*
*http://www.sdiarticle4.com/review-history/68742*

---