

Effect of Ethanolic Extract of *Gongronema latifolium* Leaves on Malondialdehyde Level and Antioxidant Enzymes activities in Tissue samples of Streptozotocin induced Diabetic Male Wistar Rats

ABSTRACT

Background: The study evaluates the Effect of Ethanolic Extract of *Gongronema latifolium* Leaves (GLE) on Malondialdehyde Level and Anti-oxidant Enzymes in Tissue samples of Streptozotocin (STZ) induced Diabetic Male Wistar Rats

Methodology: Forty (40) Male Wistar Rats weighing between 100-138g were used for the study. The rats were divided into five groups of eight animals in each. Animals in group one were not induced while those in groups two to five were induced diabetes with single intraperitoneal injection of streptozotocin (STZ) at a dose of 65 mg/kg body weight. Group one (Normal control) and two (Diabetic control) were fed with rat chow and Water for eight weeks. Groups three, four and five received oral gavage of 200mg/kgbw/day, 400mg/kgbw/day ethanol extract of GLE and 100mg/kgbw/day Metformin respectively, and Rat Chow and Water for eight weeks. After Eight weeks, the rats were sacrificed and the Heart, liver and kidney were dissected out. The tissues were rinsed immediately with 0.9% ice-cold normal saline to remove blood and 0.5g of each was homogenized in 5ml of phosphate buffered saline (PBS) (pH 7.4) using a Mortar and Pestle homogenizer and the homogenate was centrifuged using TGL-20M Ultra Refrigerated Centrifuge at 12,000g for 20 minutes at 4°C to get the post mitochondrial supernatant. The supernatants were used for the estimation of Malondialdehyde, Total Antioxidant Capacity, Glutathione Peroxidase, Catalase and Superoxide Dismutase using standard Laboratory Methods.

Results: Result showed that the Liver, kidney and Heart Malondialdehyde (nmol/ml) were significantly increased while the Total antioxidant Capacity ($\mu\text{mol/l}$), Glutathione Peroxidase (U/mL), Catalase (kU/L) and Superoxide Dismutase (U/ml) were significantly reduced in Group two compared with Groups one, three, four and five ($P < 0.05$).

Conclusion: Ethanolic Extract of *Gongronema latifolium* Leaves significantly reduced the liver, kidney and heart Malondialdehyde concentration and increased the antioxidant enzymes activities in the treated groups.

Key words- *Gongronema latifolium*, Malondialdehyde, Antioxidant Enzymes, Tissue Homogenate

INTRODUCTION

Diabetes mellitus (DM), commonly known as diabetes, is a chronic metabolic disorder that occurs either when the pancreas does not produce enough insulin or the cells of the body not responding properly to the insulin produced. It is characterized by elevated levels of blood glucose which leads over time to serious damage to the heart, blood vessels, eyes, kidneys and nerves (WHO, 2021). Diabetes is a serious, long-term condition with a major impact on the lives and well-being of individuals, families, and societies worldwide. It is among the top 10 causes of death in adults, and was estimated to have caused four million deaths globally in 2017

Comment [U1]: In this part should give the rational of this research, why this research is conducted, not the objective of research.

Comment [U2]: Should be effect of ethanolic extract of *Gongronema latifolium* leaves (GLE) on malondialdehyde level and anti-oxidant enzymes in tissue samples of streptozotocin (STZ) induced diabetic male wistar rats

Comment [U3]: Should be male wistar rats

Comment [U4]: Should be five were

Comment [U5]: Should be metformin respectively

Comment [U6]: Should be rat chow and water

Comment [U7]: Should be eight

Comment [U8]: Should be heart

Comment [U9]: Should be mortar and pestle

Comment [U10]: Should be malondialdehyde, total antioxidant capacity, glutathione peroxidase, catalase and superoxide dismutase

Comment [U11]: Should be laboratory methods

Comment [U12]: Should be liver, kidney and heart malondialdehyde (nmol/ml) were significantly increased while the total antioxidant capacity ($\mu\text{mol/l}$), glutathione peroxidase (U/mL), catalase (kU/L) and superoxide dismutase (U/ml) were significantly reduced in group two compared with groups one, three, four and five ($P < 0.05$)

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(International Diabetes Federation, 2017) and is seen as the third highest risk factor for worldwide premature mortality due to hyperglycaemia and hyperglycaemic-induced oxidative stress and inflammation (Oguntibeju, 2019). There is a strong link between hyperglycaemia, hyperglycaemic-induced oxidative stress, inflammation and the development and progression of type 2 diabetes mellitus (Oguntibeju, 2019). In 2014, 8.5% of adults aged 18 years and older had diabetes. In 2016, diabetes was the direct cause of 1.6 million deaths and in 2012 high blood glucose was the cause of another 2.2 million deaths (International Diabetes Federation (IDF), 2015). Between 2000 and 2016, there was a 5% increase in premature mortality from diabetes. In high-income countries the premature mortality rate due to diabetes decreased from 2000 to 2010 but then increased in 2010-2016. In lower-middle-income countries, the premature mortality rate due to diabetes increased across both periods. By contrast, the probability of dying from any one of the four main noncommunicable diseases (cardiovascular diseases, cancer, chronic respiratory diseases or diabetes) between the ages of 30 and 70 decreased by 18% globally between 2000 and 2016. In 2017, global health expenditure on diabetes was estimated to be USD 727 billion (IDF, 2017). According to the 2017 IDF estimates, about 425 million adults have DM. This figure is projected to increase to 629 million adults by 2045, which is a 48% increase. Africa is estimated to have 15.9 million adults living with DM which is a regional prevalence of 3.1%. The African continent has the greatest proportion of people with undiagnosed DM and global projections show that it will experience the greatest future increase in the burden of DM of about 156% by 2045 (Zhou *et al.*, 2006).

Total adult population in Nigeria is 91,560,500, total cases of diabetes in adults in Nigeria are 2,743,800 and the current prevalence of DM in Nigeria is 3% (IDF, 2020).

Diabetes tends to damage cell membranes which results in elevated production of reactive oxygen species (ROS). The generation of ROS during cellular metabolism, and by certain

Comment [U17]: There no reference for Zhou et al., 2006 in Reference List. Please check again the cited references in this manuscript!

environmental factors, including life style, appears to play a critical role in the pathogenesis of diabetes mellitus (Harnett *et al.*, 2000). Hyperglycemia associated with diabetes increases the production of ROS and affects antioxidant enzymes and reactions (Haskins *et al.*, 2004). Defects in ROS scavenging enzyme system have been reported in diabetes mellitus (Kesavulu *et al.*, 2000).

Oxidative stress leads to protein or enzyme inactivation such as SOD, GPX, CAT and reduced glutathione and reduction in these proteins promote oxidative stress.

Lipid peroxidation in which Malondialdehyde is generated has been linked to impairment of the kidney and liver (Nnodim *et al.*, 2010) and antioxidants have been observed to play prominent beneficial role in the prevention of lipid peroxidation and the generation of free radicals (Valko *et al.*, 2006).

Comment [U18]: Should be malondialdehyde, first letter is not capital

A medicinal plant is any plant which, in one or more of its organs, contains substances that can be used for therapeutic purposes or which are precursors for the synthesis of useful drugs. When a plant is designated as medicinal, it is implied that the said plant is useful as a drug or therapeutic agent or an active ingredient of a medicinal preparation (Yudharaj, 2016). This description makes it possible to distinguish between medicinal plants whose therapeutic properties and constituents have been established scientifically, and plants that are regarded as medicinal but which have not yet been subjected to a thorough scientific study.

Oxidative stress is reported to associate with the development of several metabolic, chronic disorders (Aminjan *et al.*, 2019; Ogbodo *et al.*, 2019) and it has also been implicated in the pathogenesis of diabetes, liver damage, nephrotoxicity, inflammation, cancer, cardiovascular disorders, and neurological disorders, as well as in the process of aging (Marx, 1987). It is the imbalance between the generation of oxidative chemical species (OCS) and the body defense mechanisms.

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However, medicinal plants have been exploited for their antioxidant properties in prevention and treatment of human diseases like diabetes and cardiovascular disorders in which oxidative stress has been implicated in their pathogenesis. In recent times, food supplements have increasingly become attractive alternatives to prevent or treat various types of disease. Therefore, this study aims to evaluate the effects of ethanolic extract of *G. latifolium* leave on malondialdehyde and some antioxidant enzymes in Tissue samples of Streptozotocin (STZ) induced Diabetic Male Wistar Rats

Comment [U20]: Should be tissue samples of streptozotocin (STZ) induced diabetic male wistar rats

MATERIALS AND METHODS

Preparation of Ethanol Extract of *Gongronema latifolium* Leaves (Owu *et al*; 2012)

Fresh and apparently uninfected leaves of *Gongronema latifolium* were collected from the farm in Nnewi, in Anambra State, Nigeria. Specimen of the leaves was authenticated by Mrs. Amaka Onwuyili of the Department of Pharmacognosy and Traditional Medicine, Nnamdi Azikiwe University, Awka.

The leaves collected were washed with clean water and finally rinsed with distilled water and air dried under shade for two weeks. The dry leaves were grounded into powder in a grinding machine after which the powder was subjected to complete extraction by cold maceration method as described by (Owu *et al*; 2012) with some modifications. 954.3g of the powder was suspended in 4L of 80% ethanol in a stoppered container and allowed to percolate for five days at room temperature with intermittent agitation. This is to enable softening and breaking of the plant cell wall to release the soluble phytochemicals. At expiration of the fifth day, the mixtures were first filtered with cheese cloth, and then filtered with Whattman No. 1 filter paper. The filtrate was then evaporated in the hot air oven at 45 °C to a thick, dark brown gummy crude extract yielding 128.35g (13.4%). The extracts were stored in a refrigerator from where aliquots were used for the administration. The extract was reconstituted in 10% Tween 80 in distilled water to Stock solutions of 250 mg/ml which was further diluted to working concentrations of

100mg/ml and 200mg/ml from which final volume to obtain doses of 200 mg/kg weight (Nnodim *et al.*, 2012) and 400 mg/kg were administered to the experimental animals.

Experimental Animals

Forty (40) Male Wistar Rats weighing between 100-138g were used for the study. The rats were divided into five groups of eight animals in each. Animals in group one were not induced while those in groups two to five were induced diabetes with single intraperitoneal injection of streptozotocin (STZ) at a dose of 65 mg/kg body weight (Molehin, Oloyede and Adefegha, 2018). Group one (Normal control) and two (Diabetic control) were fed with rat chow and Water for eight weeks. Groups three, four and five received oral gavage of 200mg/kgbw/day, 400mg/kgbw/day ethanol extract of GLE and 100mg/kgbw/day Metformin respectively, and Rat Chow and Water for eight weeks.

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Comment [U25]: Should be metformin

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Induction of Experimental Diabetes

Prior to diabetes induction, the animals were fasted for 12 hours but have access to water. Body weight and baseline blood glucose levels of the animals were measured just prior to induction of diabetes and after the test period using laboratory electronic scale and One Touch Ultra 2 Blood Glucose Monitoring System (Lifescan, Europe) respectively.

Eighty (40) wistar rats were used for the study. Thirty two (32) of the animals were induced diabetes with single intraperitoneal injection of streptozotocin (STZ) at a dose of 65 mg/kg body weight (Molehin, Oloyede and Adefegha, 2018). The STZ (lot # SLBJ7785V obtained from Sigma Aldrich Co, St Lois MO, USA) was freshly dissolved in citrate buffer (0.01 M, pH 4.5) and kept on ice prior to use. The remaining uninduced control rats received only the buffer. Four days after injection, fasting blood samples were collected from the animals through the tail vein to check level of blood glucose using One Touch Ultra 2 Blood Glucose Monitoring System (Lifescan, Europe). Animals with fasting blood glucose greater than 10mmol/L were considered diabetic and selected for the study.

Comment [U27]: There no reference for Molehin, Oloyede and Adefegha, 2018 in Reference List. Please check again the cited references in this manuscript!

Preparation of tissue homogenates

After eight weeks, the rats were sacrificed and the heart, liver and kidney were dissected out. The tissues were rinsed immediately with 0.9% ice-cold normal saline to remove blood and 0.5g of each was homogenized in 5ml of phosphate buffered saline (PBS) (pH 7.4) using a Mortar and Pestle homogenizer and the homogenate was centrifuged using TGL-20M Ultra Refrigerated Centrifuge at 12,000g for 20minutes at 4°C to get the post mitochondrial supernatant. The supernatants were used for the estimation of malondialdehyde, Total antioxidant capacity, glutathione peroxidase, catalase and superoxide dismutase using standard laboratory methods.

Determination of Malondialdehyde (MDA) Level and Antioxidant Enzymes activities

MDA level was determined by the colorimetric method of Gutteridge and Wilkins, (1982). Total antioxidant activity was estimated by Ferric Reducing Ability of Plasma (FRAP) method by Benzie and Strain, 1996. The activity of glutathione peroxidase was determined by the method of Rotruck *et al.* (1973). SOD activity was assayed by colorimetric method of Misra and Fredovich, (1972). The activity of catalase was determined by the method of Hadwan and Abed, (2016).

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Comment [U29]: There no reference for Rotruck *et al.* (1973) in Reference List. Please check again the cited references in this manuscript!

Comment [U30]: There no reference for Misra and Fredovich, (1972) in Reference List. Please check again the cited references in this manuscript!

Ethical Consideration

Ethical approval was obtained from Ethics Committee, Nnamdi Azikiwe University Teaching Hospital, Nnewi.

Statistical analysis

The data obtained was analyzed using SPSS statistics tool version 23.0 software to compare the changes in tissue MDA levels and antioxidant enzymes activities using t-test. $P < 0.05$ was taken as significant.

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RESULTS

Effects of Eight Weeks administration of GLE on mean±SD of MDA of Liver, Kidney and Heart Tissue Samples of Diabetic Male Wistar Rats.

Liver MDA

The mean activity of liver MDA in the Diabetic Control (2.36±0.07), 200mg/kg GLE (1.25±0.07), 200mg/kg TCE (1.30±0.07) and 100mg/kg Metformin (1.65±0.05) were significantly increased when compared with Normal Control (1.02±0.06), ($p < 0.05$) while, there was no significant difference in mean activity of the 400mg/kg GLE (1.05±0.05) when compared

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with the **Normal Control** (1.02 ± 0.06), $p > 0.05$. However, the mean activity of liver MDA in the **Normal Control** (1.02 ± 0.06), 200mg/kg GLE (1.25 ± 0.07), 400mg/kg GLE (1.05 ± 0.05), and 100mg/kg **Metformin** (1.65 ± 0.05) were significantly reduced when compared with **Diabetic Control** (2.36 ± 0.07), ($p < 0.05$).

Kidney MDA

The mean activity of **Kidney** MDA in the **Diabetic Control** (2.34 ± 0.06), 200mg/kg GLE (1.35 ± 0.06) and 100mg/kg **Metformin** (1.71 ± 0.06) were significantly increased when compared with **Normal Control** (1.14 ± 0.06), ($p < 0.05$) while, there was no significant difference in mean activity of the 400mg/kg GLE (1.20 ± 0.06) when compared with the **Normal Control** (1.14 ± 0.06), $p > 0.05$. However, the mean activity of **Kidney** MDA in the Normal Control (1.14 ± 0.06), 200mg/kg GLE (1.35 ± 0.06), 400mg/kg GLE (1.20 ± 0.06), and 100mg/kg Metformin (1.71 ± 0.06) were significantly reduced when compared with **Diabetic Control** (2.34 ± 0.06), ($p < 0.05$).

Heart MDA

The mean activity of **Heart** MDA in the **Diabetic Control** (2.06 ± 0.07), 200mg/kg GLE (1.22 ± 0.07), 400mg/kg GLE (1.06 ± 0.07) and 100mg/kg Metformin (1.29 ± 0.05) were significantly increased when compared with **Normal Control** (0.88 ± 0.06), ($p < 0.05$). Conversely, the mean activity of **Heart** MDA in the **Normal Control** (0.88 ± 0.06), 200mg/kg GLE (1.22 ± 0.07), 400mg/kg GLE (1.06 ± 0.07), and 100mg/kg **Metformin** (1.29 ± 0.05) were significantly reduced when compared with **Diabetic Control** (2.06 ± 0.07), ($p < 0.05$).

Table 1: Show mean \pm SD of MDA (nmol/ml) of Tissue Samples from Liver, Kidney and Heart of Diabetic Wistar Rats after eight weeks administration

Groups	Treatment	Liver	Kidney	Heart
1	Normal Control	1.02 ± 0.06	1.14 ± 0.06	0.87 ± 0.06
2	Diabetic Control	2.36 ± 0.07	2.34 ± 0.06	2.06 ± 0.07
3	200mg/kg GLE	1.25 ± 0.07	1.35 ± 0.06	1.22 ± 0.07
4	400mg/kg GLE	1.05 ± 0.05	1.20 ± 0.06	1.06 ± 0.07

5	100mg/kg Metformin	1.65±0.05	1.71±0.06	1.29±0.05
	F value	597.275	448.797	316.540
	P value	0.001*	0.001*	0.001*
	Post Hoc			
	1 vs 2, 3	P=0.001, P=0.001	P=0.001, P=0.001	P=0.001, P=0.001
	1 vs 4, 5	-----, P=0.001	-----, P=0.001	-----, P=0.001
	2 vs 3, 4	P=0.001, P=0.001	P=0.001, P=0.001	P=0.001, P=0.001
	2 vs 5,	P=0.001,	P=0.001	P=0.001
	3 vs 4, 5	P=0.001, P=0.001	P=0.001, P=0.001	P=0.001, -----
	4 vs 5	P=0.001	P=0.001	P=0.001

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Comment [U36]: It is better to put this statistical analysis results in supplementary information.

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The Effects of Eight Weeks Administration of GLE on Mean±SD of TAC of Liver, Kidney and Heart Tissue Samples of Diabetic Male Wistar Rats.

Liver TAC

The mean activity of liver TAC in the **Diabetic Control** (782.41±25.54), 200mg/kg GLE (914.75±24.05), and 100mg/kg **Metformin** (840.48±23.14) were significantly reduced when compared with Normal Control (1093.62±20.90), (p<0.05) while, there was no significant difference in mean activity of the 400mg/kg GLE (1085.38±21.46) when compared with the **Normal Control** (1093.62±20.90), p>0.05. However, the mean activity of liver TCA in the **Normal Control** (1093.62±20.90), 200mg/kg GLE (914.75±24.05), 400mg/kg GLE (1085.38±21.46), and 100mg/kg **Metformin** (840.48±23.14) were significantly increased when compared with **Diabetic Control** (782.41±25.54), (p<0.05).

Kidney TAC

The mean activity of **Kidney** TCA in the **Diabetic Control** (816.57±25.18), 200mg/kg GLE (944.66±24.09), 400mg/kg GLE (1199.83±23.80) and 100mg/kg **Metformin** (895.23±24.92) were significantly reduced when compared with **Normal Control** (1056.49±24.10), (p<0.05). However, the mean activity of **Kidney** TCA in the **Normal Control** (1056.49±24.10), 200mg/kg GLE (944.66±24.09), 400mg/kg GLE (1199.83±23.80) and 100mg/kg **Metformin** (895.23±24.92) were significantly increased when compared with **Diabetic Control** (816.57±25.18), (p<0.05).

Heart TAC

The mean activity of Heart TCA in the Diabetic Control (593.86±21.56419), 200mg/kg GLE (742.04±22.63185), 400mg/kg GLE (908.02±23.98399) and 100mg/kg Metformin (696.68±24.53666) were significantly reduced when compared with Normal Control (950.67±22.85143), (p<0.05). Conversely, the mean activity of Heart TCA in the Normal Control (950.67±22.85143), 200mg/kg GLE (742.04±22.63185), 400mg/kg GLE (908.02±23.98399), and 100mg/kg Metformin (696.68±24.53666) were significantly increased when compared with Diabetic Control (593.86±21.56419), (p<0.05).

Table 2: Show mean±SD of TAC of Tissue Samples from Liver, Kidney and Heart of Diabetic Wistar Rats after eight weeks administration

Groups	Treatment	Liver	Kidney	Heart
1	Normal Control	1093.62±20.91	1056.49±24.10	950.67±22.86
2	Diabetic Control	782.41±25.54	816.59±25.18	593.88±21.56
3	200mg/kg GLE	914.75±24.04	944.66±24.09	742.04±22.63
4	400mg/kg GLE	1085.38±21.46	1199.83±23.80	908.02±23.98
5	100mg/kg Metformin	840.48±23.14	895.23±24.92	696.68±24.54
	F value	257.702	249.288	281.836
	P value	0.001*	0.001*	0.001*
	Post Hoc			
	1 vs 2, 3	P=0.001, P=0.001	P=0.001, P=0.001	P=0.001, P=0.001
	1 vs 4, 5	-----, P=0.001	P=0.001, P=0.001	P=0.010, P=0.001
	2 vs 3, 4	P=0.001, P=0.001	P=0.001, P=0.001	P=0.001, P=0.001
	2 vs 5	P=0.001	P=0.001	P=0.001

3 vs 4, 5	P=0.001, P=0.001	P=0.001, P=0.008	P=0.001, P=0.011
4 vs 5	P=0.001	P=0.001	P=0.001

Effects eight weeks administration of GLE on mean \pm SD of GPx of Liver, Kidney and Heart Tissue Samples of Diabetic Male Wistar Rats.

Liver GPx

The mean activity of liver GPx in the **Diabetic Control** (0.40 \pm 0.04), 200mg/kg GLE (0.53 \pm 0.06) and 100mg/kg **Metformin** (0.57 \pm 0.05) were significantly reduced when compared with **Normal Control** (0.69 \pm 0.04726), ($p < 0.05$) while, there was no significant difference in mean activity of the 400mg/kg GLE (0.70 \pm 0.06) when compared with the **Normal Control** (0.69 \pm 0.04726), ($p > 0.05$). However, the mean activity of liver GPx in the **Normal Control** (0.69 \pm 0.04726), 200mg/kg GLE (0.53 \pm 0.06), 400mg/kg GLE (0.70 \pm 0.06) and 100mg/kg **Metformin** (0.57 \pm 0.05) were significantly increased when compared with **Diabetic Control** (0.40 \pm 0.04), ($p < 0.05$).

Kidney GPx

The mean activity of **Kidney** GPx in the **Diabetic Control** (0.88 \pm 0.06), 200mg/kg GLE (1.24 \pm 0.04), and 100mg/kg **Metformin** (0.98 \pm 0.04) were significantly reduced when compared with **Normal Control** (1.55 \pm 0.06), ($p < 0.05$), while, there was no significant difference in mean activity of the 400mg/kg GLE (1.42 \pm 0.05) when compared with the **Normal Control** (1.55 \pm 0.06), ($p > 0.05$). However, the mean activity of **Kidney** GPx in the **Normal Control** (1.55 \pm 0.06), 200mg/kg GLE (1.24 \pm 0.04), 400mg/kg GLE (1.42 \pm 0.05) and 100mg/kg **Metformin** (0.98 \pm 0.04) were significantly increased when compared with **Diabetic Control** (0.88 \pm 0.06), ($p < 0.05$).

Heart GPx

The mean activity of **Heart** GPx in the **Diabetic Control** (0.45 \pm 0.04), 200mg/kg GLE (0.66 \pm 0.04) and 100mg/kg **Metformin** (0.59 \pm 0.06) were significantly reduced when compared with **Normal Control** (0.88 \pm 0.06), ($p < 0.05$) while, there was no significant difference in mean activity of the 400mg/kg GLE (0.85 \pm 0.05) when compared with the **Normal Control** (0.88 \pm 0.06), ($p > 0.05$). Conversely, the mean activity of Heart GPx in the **Normal Control** (0.88 \pm 0.06), 200mg/kg GLE (0.66 \pm 0.04), 400mg/kg GLE (0.85 \pm 0.05) and 100mg/kg **Metformin** (0.59 \pm 0.06) were significantly increased when compared with **Diabetic Control** (0.45 \pm 0.04), ($p < 0.05$).

Table 3: Show mean \pm SD of GPx (U/mL) of Tissue Samples from Liver, Kidney and Heart of Diabetic Wistar Rats after eight weeks administration

Group	Treatment	Liver	Kidney	Heart
1	Normal Control	0.69 \pm 0.05	1.55 \pm 0.06	0.88 \pm 0.06
2	Diabetic Control	0.40 \pm 0.04	0.88 \pm 0.06	0.44 \pm 0.04
3	200mg/kg GLE	0.53 \pm 0.06	1.24 \pm 0.04	0.66 \pm 0.04
4	400mg/kg GLE	0.70 \pm 0.06	1.42 \pm 0.05	0.85 \pm 0.05
5	100mg/kg Metformin	0.57 \pm 0.05	0.98 \pm 0.04	0.59 \pm 0.06
	F value	39.870	203.463	81.394
	P value	0.001*	0.001*	0.001*
	Post Hoc			
	1 vs 2, 3	P=0.001, P=0.001	P=0.001, P=0.001	P=0.001, P=0.001
	1 vs 4, 5	-----, P=0.001	P=0.001, P=0.001	-----, P=0.001
	2 vs 3, 4	P=0.001, P=0.001	P=0.001, P=0.001	P=0.001, P=0.001
	2 vs 5,	P=0.001,	P=0.017	P=0.001, P=0.001
	3 vs 4, 5	P=0.001, -----	P=0.001, P=0.001	P=0.001, -----
	4 vs 5	P=0.001	P=0.001	P=0.001

Effects of Eight Weeks administration of GLE on mean±SD of Catalase of Liver, Kidney and Heart Tissue Samples of Diabetic Male Wistar Rats.

Liver Catalase

The mean activity of **Liver Catalase** in the **Diabetic Control** (67.54±2.42), 200mg/kg GLE (81.05±2.50) and 100mg/kg **Metformin** (75.88±2.81) were significantly reduced when compared with **Normal Control** (92.94±2.34), ($p < 0.05$) while there was no significant difference in mean activity of the 400mg/kg GLE (97.36±3.65) when compared with the Normal Control (92.94±2.34), ($p > 0.05$). However, the mean activity of liver **Catalase** in the **Normal Control** (92.94±2.34), 200mg/kg GLE (81.05±2.50), 400mg/kg GLE (97.36±3.65), and 100mg/kg **Metformin** (75.88±2.81) were significantly increased when compared with **Diabetic Control** (67.54±2.42), ($p < 0.05$).

Kidney Catalase

The mean activity of **Kidney Catalase** in the **Diabetic Control** (44.17±5.80), 200mg/kg GLE (60.47±3.74) and 100mg/kg **Metformin** (57.49±4.24) were significantly reduced when compared with Normal Control (73.41±4.40), ($p < 0.05$), while, there was no significant difference in mean activity of the 400mg/kg GLE (75.67±4.27), when compared with the **Normal Control** (73.41±4.40), ($p > 0.05$). However, the mean activity of **Kidney Catalase** in the **Normal Control** (73.41±4.40), 200mg/kg GLE (60.47±3.74), 400mg/kg GLE (75.67±4.27) and 100mg/kg **Metformin** (57.49±4.24) were significantly increased when compared with **Diabetic Control** (44.17±5.80), ($p < 0.05$).

Heart Catalase

The mean activity of **Heart Catalase** in the **Diabetic Control** (40.46±4.95), 400mg/kg GLE (75.90±0.05), were significantly reduced when compared with **Normal Control** (66.89±5.36), ($p < 0.05$) while, the mean activity of **Heart Catalase** in the 400mg/kg GLE (75.90±6.24) was significantly increased when compared with **Normal Control** (66.89±5.36), ($p < 0.05$). Nevertheless, there was no significant difference in mean activity of the 200mg/kg GLE (63.95±4.19) and 100mg/kg **Metformin** (59.44±5.25) when compared with the **Normal Control** (66.89±5.36), ($p > 0.05$). Conversely, the mean activity of **Heart Catalase** in the **Normal Control** (66.89±5.36), 200mg/kg GLE (63.95±4.19), 400mg/kg GLE (75.90±6.24), and 100mg/kg **Metformin** (59.44±5.25) were significantly increased when compared with **Diabetic Control** (40.46±4.95), ($p < 0.05$).

Table 4: Show mean \pm SD of Catalase ((kU/L)) of Tissue Samples from Liver, Kidney and Heart of Diabetic Wistar Rats after eight weeks administration

Groups	Treatment	Liver	Kidney	Heart
1	Normal Control	92.94 \pm 2.34	73.41 \pm 4.40	66.89 \pm 5.36
2	Diabetic Control	67.54 \pm 2.42	44.17 \pm 5.80	40.46 \pm 4.95
3	200mg/kg GLE	81.05 \pm 2.50	60.47 \pm 3.74	63.95 \pm 4.19
4	400mg/kg GLE	97.36 \pm 3.65	75.67 \pm 4.27	75.90 \pm 6.24
5	100mg/kg Metformin	75.88 \pm 2.81	57.49 \pm 4.24	59.44 \pm 5.25
	F value	127.950	53.605	39.823
	P value	0.001*	0.001*	0.001*
	Post Hoc			
	1 vs 2, 3	P=0.001, P=0.001	P=0.001, P=0.001	P=0.001, -----
	1 vs 4, 5	P=0.035, P=0.001	-----, P=0.001	P=0.019, -----
	2 vs 3, 4	P=0.001, P=0.001	P=0.001, P=0.001	P=0.001, P=0.001
	2 vs 5,	P=0.001	P=0.001	P=0.001
	3 vs 4, 5	P=0.001, P=0.018	P=0.001, -----	P=0.002, -----
	4 vs 5	P=0.001	P=0.001	P=0.001

Effects of Eight Weeks Administration of GLE on mean±D of SOD of Liver, Kidney and Heart Tissue Samples of Diabetic Male Wistar Rats.

Liver SOD

The mean activity of Liver SOD in the Diabetic Control (13.35±1.63), 200mg/kg GLE (18.03±1.69) and 100mg/kg Metformin (15.18±1.66) were significantly reduced when compared with Normal Control (26.56±1.76), ($p < 0.05$) while, there was no significant difference in mean activity of the 400mg/kg GLE (25.05±1.81) when compared with the Normal Control (26.56±1.76), ($p > 0.05$). However, the mean activity of liver SOD in the Normal Control (26.56±1.76), 200mg/kg GLE (18.03±1.66) and 400mg/kg GLE (25.05±1.81) were significantly increased when compared with Diabetic Control (13.35±1.63), ($p < 0.05$) while, there was no significant difference in mean activity of the 100mg/kg Metformin (15.18±1.66) when compared with the Diabetic Control (13.35±1.63), ($p > 0.05$).

Kidney SOD

The mean activity of Kidney SOD in the Diabetic Control (10.09±1.59), 200mg/kg GLE (13.60±1.46), and 100mg/kg Metformin (12.92±1.48) were significantly reduced when compared with Normal Control (17.69±1.67), ($p < 0.05$), while, there was no significant difference in mean activity of the 400mg/kg GLE (16.28±1.41) when compared with the Normal Control (17.69±1.67), ($p > 0.05$). However, the mean activity of Kidney SOD in the Normal Control (17.69±1.67), 200mg/kg GLE (13.60±1.46), 400mg/kg GLE (16.28±1.41), and 100mg/kg Metformin (12.92±1.48) were significantly increased when compared with Diabetic Control (10.09±1.59), ($p < 0.05$).

Heart SOD

The mean activity of Heart SOD in the Diabetic Control (7.42±1.49), 200mg/kg GLE (10.36±1.62) and 100mg/kg Metformin (12.67±1.53) were significantly reduced when compared with Normal Control (15.28±1.45), ($p < 0.05$) while, there was no significant difference in mean activity of the 400mg/kg GLE (15.34±1.45), 400mg/kg TCE (13.44±1.62) and 400mg/kg GLE+TCE (14.94±1.48) when compared with the Normal Control (15.28±1.45), ($p > 0.05$). Conversely, the mean activity of Heart SOD in the Normal Control (15.28±1.45), 200mg/kg GLE (10.36±1.62), 400mg/kg GLE (15.34±1.45) and 100mg/kg Metformin (12.67±1.53) were significantly increased when compared with Diabetic Control (7.42±1.49), ($p < 0.05$).

Table 5: Show mean \pm SD of SOD (U/ml) of Tissue Samples from Liver, Kidney and Heart of Diabetic Wistar Rats after eight weeks administration

Group	SOD	Liver	Kidney	Heart
1	Normal Control	26.56 \pm 1.76	17.69 \pm 1.67	15.28 \pm 1.45
2	Diabetic Control	13.35 \pm 1.63	10.09 \pm 1.59	7.42 \pm 1.49
3	200mg/kg GLE	18.03 \pm 1.69	13.60 \pm 1.46	10.36 \pm 1.62
4	400mg/kg GLE	25.05 \pm 1.81	16.21 \pm 1.41	15.34 \pm 1.45
5	100mg/kg Metformin	15.18 \pm 1.66	12.92 \pm 1.48	12.67 \pm 1.53
	F value	81.032	25.727	33.660
	P value	0.001*	0.001*	0.001*
	Post Hoc			
	1 vs 2, 3	P=0.001, P=0.001	P=0.001, P=0.001	P=0.001, P=0.001
	1 vs 4, 5	-----, P=0.001	-----, P=0.001	-----, P=0.025
	2 vs 3, 4	P=0.001, P=0.001	P=0.002, P=0.001	P=0.012, P=0.001
	2 vs 5,	-----	P=0.025,	P=0.001
	3 vs 4, 5	P=0.001, P=0.043	P=0.021, -----	P=0.001, -----
	4 vs 5	P=0.001	P=0.004	P=0.026

DISCUSSION

The finding in this study showed that the tissue (heart, kidney and liver) Malondialdehyde (MDA) levels were significantly increased while the tissue antioxidant enzymes activities (SOD, GPX, CAT and TAC) of the untreated diabetic control rats were significantly reduced compared with the diabetic rats treated with 200mg/kgbw and 400mg/kgbw GLE which showed that the untreated diabetic control rats were subjected to oxidative stress as indicated by significantly reduced level of their antioxidant enzyme, and significantly increased serum MDA when compared with normal control group. This oxidative stress caused an increase in lipid peroxidation which manifested in the tissues as increase in MDA levels and reduction in antioxidant enzymes activities. The ethanolic extract of *G. latifolium* leaves reversed this situation dose dependently as shown by significant increase in the activity of antioxidant enzymes and reduced levels of serum Malondialdehyde in the treated rats. The 400mg/kgbw GLE exhibited a significantly higher effect compared with the 100mg/kgbw GLE Metformin which is a standard drug for management of diabetes mellitus. This finding in this study is in line with the work of Ugochukwu and Babady, (2002); Ugochukwu *et al.*, (2003) and Ugochukwu and Cobourne, (2003); Akpan and Ekpo (2015) and Nwanjo *et al.*, (2006).

This decrease in MDA level in the tissues could be attributed to the increase in GPx activity in rats treated with the ethanolic extract since GPx has been known to inactivate lipid peroxidation reactions (Levy *et al.*, 1999). GLE contain some phytochemicals with known antioxidant properties which have been shown by previous researcher, (Usoh *et al.*, 2015) and the action of these phytochemicals and nutrients might have potentiated against the free radical generation process or moped up the circulating radicals responsible for diabetic complications, thereby reversing the derangement observed in the MDA levels and antioxidant enzymes activities. SOD scavenges the O_2^- whereas CAT and GPX remove H_2O_2 (Mehdi *et al.*, 2020). CAT is a

Comment [U38]: What kind of phytochemicals, phenolic or flavonoid compounds? What are the total contents?

Comment [U39]: There no reference for Usoh *et al.*, 2015 in Reference List. Please check again the cited references in this manuscript!

Comment [U40]: There no reference for Mehdi *et al.*, 2020 in Reference List. Please check again the cited references in this manuscript!

tetrameric ferrihemeoxidoreductase, which catalyzes H₂O₂ dismutation to water and gaseous oxygen (Grigoras, 2017). GPX is a selenium-dependent oxidoreductase, which uses H₂O₂ or organic hydroperoxide as the oxidant (Cardoso *et al.*, 2017)

The antioxidant activities of these plants resulted in increased activities of the enzymes and reduction in MDA thereby causing a reduction in lipid peroxidation and these may possibly reduce the risks associated with diabetes and cardiovascular diseases.

In recent years, the awareness on the use of plant-derived bioactive molecules as extract or plant parts (stem and leaves) have increased tremendously, because of their therapeutic benefits in prevention and treatment of various diseases of man including diabetes and cardiovascular diseases, therefore their use as foods supplements should be given more consideration as an effective way of managing these diseases.

CONCLUSION

The results of this study show that ethanolic extract of *G latifolium* leaves have antioxidant potential as shown by the reduction in the liver, kidney and heart malondialdehyde levels and increase on the activities of Total antioxidant capacity, glutathione peroxidase, catalase and superoxide dismutase. Therefore, they can be regarded as natural antioxidant supplement that can increase the antioxidant concentration thereby contributing to protect the cellular membranes against oxidative stress and damage.

Comment [U41]: Should be in italic

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Comment [U42]: This reference should be before Ugochukwu NH, Babady NE (2002)