Original Research Article

Tropical Journal of Chemistry, January 2025; 1(1): 37 – 49
Available online at http://www.tjchem.org

Changes in Pancreatic Antioxidant Biomarkers Activities Following the Ingestion of *Abelmoschus Esculentum* in *Streptozotocin-Induced* Diabetic Male Wistar Rats

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Article information

Article history: Received December 2024
Revised December 2024
Accepted December 2024
Published online January 2025

ABSTRACT

Type 2 Diabetes Mellitus (T2DM) is a metabolic condition involving high blood sugar and increased oxidative stress, with the pancreas being a primary target of the disease. This study aimed to investigate the significant changes in pancreatic antioxidant activities in diabetic rats treated with ethanol extract of *Abelmonschus esculentum.* Forty adult male Wistar rats were randomly divided into non-diabetic and diabetic units, each having four groups. Diabetes was induced in rats by a single intraperitoneal injection of *streptozotocin (STZ)* (600mg/kg). While Group 1 of both units was not treated, Group 2 received *A. esculentum ethanol* (300mg/kg) extract, Group 3 received *N-acetyl Cysteine (NAC)* (300mg/kg) and Group 4 received *A. esculentum* + *NAC* (300mg/kg). After 21 days of treatment, rats were euthanized and the pancreas were excised for biochemical analysis. Generated data were analyzed using GraphPad prism version 8.0, and results were expressed as Standard Error of Mean (SEM). The mean variations between groups were compared using a two-way analysis of variance (ANOVA), followed by the Turkey post hoc test, with p-values of 0.05 considered statistically significant. Results obtained showed a significantly decreased body weight, blood glucose, *Xanthine oxidoreductase (XOR)* and *Malondialdehyde* (*MDA)* levels with significantly increased *Glutathione reductase (GR) and Nitric Oxide (NO)* levels in treated groups indicating a positive mechanism in the management of oxidative stress induced by STZ. These findings suggest that *A. esculentum* extract has antioxidant properties that can mitigate oxidative stress in the diabetic pancreas, indicating its potential therapeutic value in management of T2DM.

Keywords: *Diabetes mellitus, Glutathione reductase, Malondialdehyde, Nitric Oxide, Xanthine oxidoreductase*.

1.0 INTRODUCTION

The American Diabetes Association [1] defines diabetes mellitus (DM) as a long-term metabolic condition characterized by high blood glucose levels or hyperglycemia due to abnormalities in insulin secretion, insulin action, or both. DM can lead to death or disability and affect the quality of life of Diabetics.

Diabetes requires attention and awareness as it is a prevalent global health concern which has caused 1.5 million deaths per year [2, 3, 4], and an elevated blood glucose level, higher than optimal, has also led to an additional 2.2 million deaths globally by increasing the likelihood of cardiovascular and other diseases with forty-three percent of cases occurring before the age of 70 years [5, 6]. A report from the International Diabetes Federation confirms that the number of people affected by diabetes is expected to rise significantly in 2035 [7]. Therefore, these statistics serve as a wake-up call to take preventive measures and promote a healthier lifestyle. Raising awareness and implementing effective strategies can reduce the prevalence of diabetes and improve global health [8]. Since DM poses a significant global health challenge as a chronic metabolic disorder [9] because of insufficient insulin secretion and/or function resulting in extended periods of high blood sugar levels and disturbances in the metabolism of carbohydrates, lipids, and proteins [3, 10], these metabolic disruptions in diabetes play a role in the emergence of various complications, affecting both major and minor blood vessels [11, 12, 13]. DM is majorly classified into Type 1 (T1DM) and Type 2 (T2DM) [13]. People with T1DM need an external insulin, which is a condition where the body or tissues

do not respond effectively to insulin [13, 15, 16, 17, 18, 19]. Disruption of insulin production and/ or function caused by DM increases blood sugar levels (hyperglycemia) and consequently increases blood pressure [20]. Prolonged hyperglycemia will result in arteriosclerosis, namely the accumulation of fat, cholesterol, and other substances in the arteries, thickening of the basement membrane, and changes in peripheral nerves that block blood flow [16, 20]. This impacts every organ in the body and generates a wide range of symptoms, hence DM is known as the silent killer [21]. Diabetes can also lead to a number of conditions, such as poor vision, cataracts, heart disease, kidney disease, erectile dysfunction, non-healing wounds, lung infections, blood vessel problems, stroke, and more [13]. Polyphagia (eating a lot), polydipsia (drinking a lot), polyuria (frequent nighttime urination), increased hunger yet rapid weight loss of up to 5-10 kg in 2-4 weeks, and fatigue are the most prevalent symptoms of diabetes mellitus [22, 23]. Numerous studies have shown a connection between diabetes and increased oxidative stress, which leads to damage to important cell components [17, 24, 25, 26]. This occurs when there is an imbalance between the production of oxygen-derived radicals and the body's ability to counteract them with antioxidants [27, 28]. The connection between oxidative stress and diabetes has been the subject of various studies, but the findings have been inconsistent. F2-isoprostanes, compounds similar to prostaglandins formed during the peroxidation of arachidonic acid, have shown potential as markers of oxidative stress [29]. In diabetes, oxidative stress levels rise due to autoxidation of glucose, which generates free radicals [30]. Additionally, there are imbalances in cellular oxidation/reduction processes and a decrease in antioxidant defenses [31]. This includes lower levels of cellular antioxidants and reduced activity of enzymes responsible for neutralizing free radicals [32].

Elevated levels of F2-isoprostanes have also been observed in the plasma of individuals with T2DM and the urine of both T1DM and T2DM diabetic patients [33]. Some studies suggest a possible association between poor glycemic control and increased lipid peroxidation [17, 34]. However, other research has found no correlation between glycemic control and levels of 8-iso-PGF2α or MDA [35, 36]. Diets high in antioxidants are crucial for maintaining general health and lowering the risk of oxidative stress-related chronic diseases such as cancer, dementia, and cardiovascular disease [37]. The therapeutic potential of natural products or plant extracts in the treatment of T2DM has gained significant attention globally [3, 16, 19, 38 39, 40, 41]. *A. esculentum*, (Okra) also known as ladyfinger has been extensively studied for its anti-diabetic properties. Bioactive compounds found in this plant (Okra) include polyphenols, flavonoids, polysaccharides, and other antioxidants, anti-inflammatory, and anti-glycemic properties [42]. *A. esculentum* ethanol extract has been shown to improve glucose metabolism and improve insulin sensitivity in diabetes experimental models [43]. While there is a growing interest in ethanol extract of *A. esculentum* for T2DM due to its potential therapeutic benefits [44], its effect on pancreatic function remains largely unknown, therefore this study was aimed to investigate the Pancreatic antioxidants biomarkers activities in T2DM diabetic rats treated with *A. esculentum* ethanol extract.

2.0 MATERIALS AND METHODS

Chemicals and drug

Streptozotocin (Batch no: 1378) was purchased from Aldrich sigma company, Spruce Street, St Louis U.S.A, ***N-acetyl cysteine*** (Batch no: 37188) was purchased from Wonder Laboratories, White House, U.S.A, **Sodium Citrate** (C_3H_4 (OH) $(COONa)_3$ $2H_2O$) (Batch no: 5425/18/58) was purchased from BDH chemicals LTD Pools England.

Phytochemical Study

Plant Material

Fresh *A. esculentum* (Okra) fruits were purchased from Obinomba local Market in Ukwuani Local Government of Delta State, Nigeria. The plant was identified and authenticated with a voucher specimen number DELSU/FOS/BOT 510430 at the Department of Botany, Faculty of Science, Delta State University, Abraka, Nigeria. Thereafter, the plant was transported to the Faculty of Pharmacy, Delta State University, Abraka for the extraction process.

Plant Extraction Process

The ethanol extraction of *A. esculentum* was carried out according to the method adopted by Ossai and others [3]. The extract was allowed to air dry into fine paste which was then stored in a universal bottle and kept in the fridge to be used for the experiment.

Experimental Animal

Handling of Animals

Forty (40) Adult male Wistar rats weighing between 118-180 g were purchased from Adewuyi Research Animal Foundation Ibadan, Oyo State, and transported to Delta State University, Abraka, Nigeria. The Wistar rats were housed in a clean and well-ventilated cage at the animal house in the Faculty of Basic Medical Science, Delta State University, Abraka and were acclimatized for a period of seven (7) days.

Ethical Consideration

The experiment protocol in this study was sorted and examined for approval by the Research, Ethics and Grant Committee of the Faculty of Basic Medical Sciences, Delta State University, Abraka, Nigeria (RBC/FBMC/DELSU/24/369). The research was performed following the ethical standard on the care and use of animals as laid down by Helsinki [19].

Preparation of Chemicals and Drugs

Sodium Citrate
Two grams (2.0g) of Sodium Citrate was dissolved in 100ml of water to yield 2% of citrate buffer.

Streptozotocin (STZ)
Streptozotocin (0.6g) was dissolved in 10ml of citrate buffer to give a stock solution (diabetic agent).

N-acetyl cysteine (NAC)
NAC (0.6g) was dissolved in 10 ml of water to give a stock solution (anti-oxidant drug).

Induction of Diabetes
Diabetes was induced in rats by a single intraperitoneal (IP) injection using the freshly prepared solution of the diabetic agent *(Streptozotocin)* at a dose of 60mg/kg body weight.

Confirmation of Diabetes Mellitus (DM)
The onset of diabetes was checked by collecting blood from the tail of the rats and measuring blood glucose level after 72 hours of diabetic injection with a glucometer (Accu-check active, Amazon, Inc. 1996, Germany). DM was confirmed by elevated fasting blood glucose of 200mg/dl and above [3, 19].

Determination of Blood Glucose Level
Weekly blood glucose level assessments were carried out using an accku check glucometer (Accu Check active German) and Accu Check active strip. Blood was collected from the tip of the rat's tail. Values obtained were rerecorded and expressed in mg/dl [3, 18, 19].

Experimental Design
The experimental study (Table 1) was conducted over 21 days to evaluate the effects of different treatments on non-diabetic and diabetic Wistar rats. The groups and treatments were as follows:

Table 1: Experimental Grouping of Non-Diabetic and Diabetic Rats Based on Treatments

Group	Non-diabetic Rat	Diabetic Rat
1	RC	RC
2	RC+AE	RC+AE
3	RC+NAC	RC+NAC
4	RC+AE+NAC	RC+AE+NAC

RC: Rat Chow (control diet).
AE: *Abelmoschus esculentus* extract (administered at 300 mg/kg body weight).
NAC: N-Acetylcysteine (administered at 300 mg/kg body weight).

Determination of Body Weight
The rats' body weight was checked weekly during bench work with a digital weighing balance (2674ER, Duack, Germany) until the last day before sacrifice.

$$\textbf{Percentage weight change } (\%) = \frac{final - intialbodyweight\ (g)}{intialbodyweight\ (g)} X \frac{100}{1}$$

Euthanizing of Animals and Sample Collection
Wistar rats were euthanized via cervical dislocation. Blood samples were collected through cardiac puncture into a plain sample bottle and centrifuged at 12,000 rpm for 10 minutes. A laparotomy was done and the pancreas was excised for histology examination.

Biochemical Analysis

Determination of *Nitric Oxide*
The *Nitric Oxide* level was determined by a microplate reader by a method described by Ossai and Ojieh [16]

Determination of *Glutathione reductase (GR)*
To estimate glutathione reductase (GR) activity, the harvested cells were lysed, and the measurements were recorded as adopted by Ossai and Ojieh [19], using NADPH as a cofactor for the enzymatic reaction

Determination of Xanthine oxidoreductase (XOR)
The activity of Xanthine oxidoreductase was determined in the tissue homogenates by the method adopted by Ossai and Ojieh [19]

Determination of Lipid Peroxidation
Lipid peroxidation was estimated in terms of thiobarbituric acid reactive species (TBARS), using *Malondialdehyde (MDA)* as standard by the method of Ossai and Ojieh [19]; Odeghe and others [41]

Statistical Analysis
The collected data were expressed using the Standard Error of Mean. GraphPad Prism version 8.0 (GraphPad Software, San Diego, CA, U.S.A) was used to analyze the data. A Turkish post hoc test was used after a two-way analysis of variance (ANOVA) to compare mean differences across groups. A p-value of less than 0.05 was deemed statistically significant for every test.

3.0 RESULTS AND DISCUSSIONS
Diabetes mellitus (DM) is a complex condition characterized by chronic hyperglycemia due to issues with insulin production, utilization, or both [19, 45]. Type 2 diabetes mellitus (T2DM), the most prevalent form globally, is associated with overweight, physical inactivity, and a family history of the disease [46]. Chronic hyperglycemia in diabetes leads to increased oxidative stress and the production of reactive oxygen species (ROS), contributing to complications such as pancreatic dysfunction, cardiovascular disease, nephropathy, neuropathy, and retinopathy.
Abelmoschus esculentus (okra) is a medicinal plant known for its potential to manage hyperglycemia, a hallmark of diabetes [47]. Its extract, rich in flavonoids and polyphenols, exhibits antioxidant properties that help mitigate oxidative stress and may aid in managing diabetes and its complications [48, 49].

Diabetes also impacts body weight and metabolism [50]. Weight fluctuations are common in diabetes, with overweight being a significant risk factor for T2DM [51].

3.1 Effect of Okra Ethanol Extract Alone and in Combination with N-Acetyl Cysteine on Body Weight in Non-Diabetic and Streptozotocin-Induced Diabetic Male Wistar Rats.

Our findings (Figure 1) demonstrate a significant decrease in body weight in both diabetic and non-diabetic rats treated with Okra ethanol extract, N-acetylcysteine (NAC), or their combination compared to diabetic controls. This weight reduction may be attributed to the hypoglycemic properties of Okra and NAC, which can enhance insulin sensitivity and glucose utilization. By improving glucose metabolism and reducing oxidative stress, as noted by Khanum et al. [52], these treatments likely shift the body's energy balance from fat storage to glucose utilization, contributing to weight loss in both diabetic and non-diabetic rats.

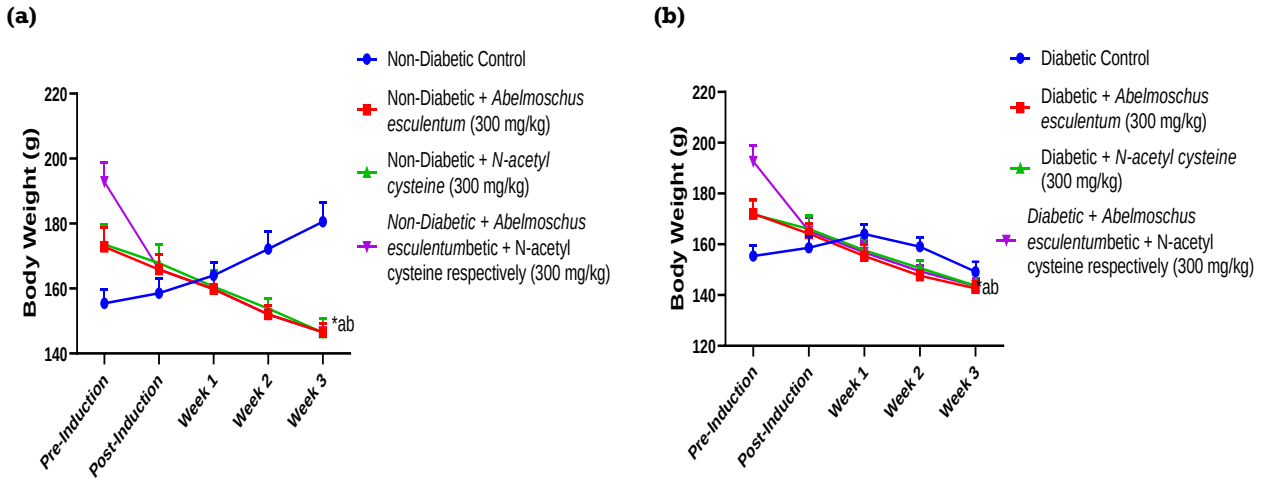

Figure 1: Effect of *Abelmoschus esculentum* ethanol extract alone and in combination with NAC on body weight (BW) in (a) non-diabetic and (b) diabetic male Wistar rats.
Bars represent Mean ± S.E.M. (n = 5). Statistical analysis was performed using one-way ANOVA followed by Tukey's post hoc test. *$p < 0.05$ relative to controls; $^{a}p < 0.05$.

3.2 Effect of Abelmoschus esculentum Ethanol Extract Alone and in Combination with N-Acetyl Cysteine on Blood Glucose in Non-Diabetic and Streptozotocin-Induced Diabetic Male Wistar Rats

Monitoring blood glucose levels is essential for diabetes management, as elevated levels indicate poor glycemic control [53]. Weekly assessments of blood glucose levels evaluated the effects of A. esculentum ethanol extract alone and in combination with NAC.

The results showed a significant reduction in blood glucose levels in both non-diabetic and diabetic rats treated with A. esculentum, NAC, or their combination compared to controls. Notably, the combination of A. esculentum and NAC produced greater hypoglycemic effects than either treatment alone (Figure 2). This may be attributed to mechanisms such as improved insulin sensitivity, reduced oxidative stress, regulation of glycogen metabolism, inhibition of carbohydrate-digesting enzymes [3], and decreased inflammation.

Previous studies have shown that A. esculentum and NAC lower fasting blood glucose in diabetic rats by enhancing glucose tolerance, delaying glucose absorption due to high fibre content, and protecting insulin-producing cells from oxidative stress [54, 55]. Our findings align with those of Sharma et al. [56], who reported reductions in oxidative stress and improvements in pancreatic histology with A. esculentum extract. However, including NAC in our study enhanced the observed hypoglycemic effects.

While earlier research supports the role of A. esculentum in managing diabetes and its complications, our study highlights its enhanced efficacy when combined with NAC, reinforcing its antioxidant and pancreatic protective properties.

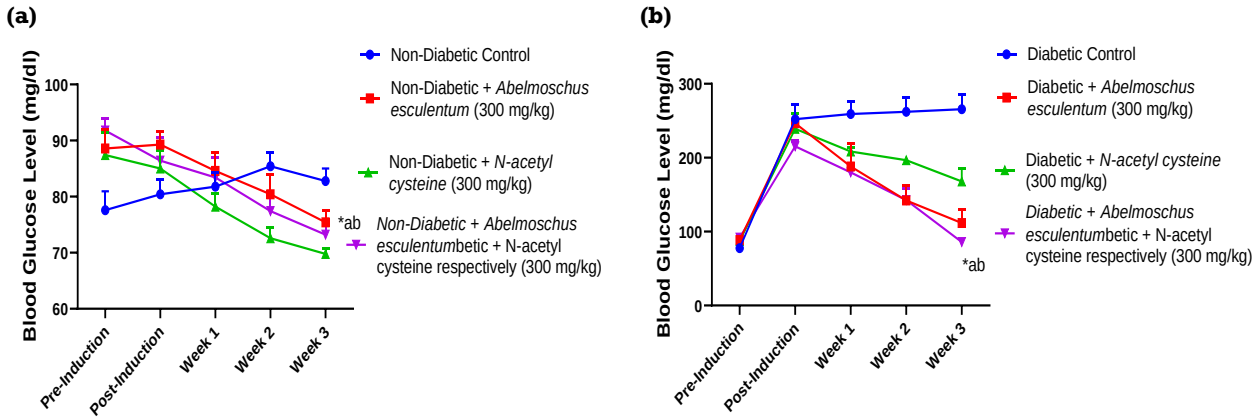

Figure 2: Effect of *Abelmoschus esculentum* ethanol extract on blood glucose levels in (a) non-diabetic and (b) diabetic male Wistar rats co-treated with N-Acetyl Cysteine.
Bars represent Mean ± S.E.M. (n = 5). Statistical analysis was performed using one-way ANOVA followed by Tukey's post hoc test. *p < 0.05 relative to controls; ᵃp < 0.05.
Blood glucose levels in both non-diabetic and diabetic rats treated with *A. esculentum*, NAC, and the combination of *A. esculentum* + NAC were significantly reduced compared to the respective control groups.

3.3 Effect of *Abelmoschus esculentum* on Pancreatic Antioxidant Biomarkers in Non-Diabetic and Streptozotocin-Induced Diabetic Male Wistar Rats Co-Treated with *N-Acetyl Cysteine*

Antioxidant biomarkers are crucial for countering oxidative stress and protecting cellular components. Key antioxidants such as GSH, thioredoxin, ascorbic acid, SOD, GPx, and CAT play vital roles in neutralizing ROS and preventing cellular damage [19, 26, 57]. In diabetes, excessive ROS production increases oxidative stress, exacerbating the condition [3, 16, 17, 26]. Monitoring these biomarkers can help assess oxidative damage and the effectiveness of treatments to reduce oxidative stress [58, 59]. MDA is a marker of lipid peroxidation and oxidative stress, while NO is essential for pancreatic health and insulin secretion [60]. Elevated MDA levels in diabetes indicate increased oxidative stress and lipid breakdown, contributing to pancreatic dysfunction [26].
As shown in Figure 3, this study observed a significant decrease in pancreatic MDA levels in both non-diabetic and diabetic rats treated with *A. esculentum*, NAC, or their combination compared to their respective control groups, which showed significantly higher MDA levels. The combination of *A. esculentum* and NAC further reduced MDA levels compared to treatment with *A. esculentum* alone. This suggests that *A. esculentum* contains bioactive compounds, such as flavonoids and phenolic substances, that neutralize free radicals and prevent lipid oxidation [61].
The reduction in MDA levels indicates a protective mechanism by *A. esculentum* and NAC, which shields pancreatic cells from oxidative stress, preserving their function and health. The elevated MDA levels observed in the diabetic control group align with previous findings, emphasizing the role of oxidative stress in pancreatic dysfunction in diabetes. The decrease in MDA levels following *A. esculentum* treatment further supports its antioxidant potential in protecting the pancreas from oxidative damage.

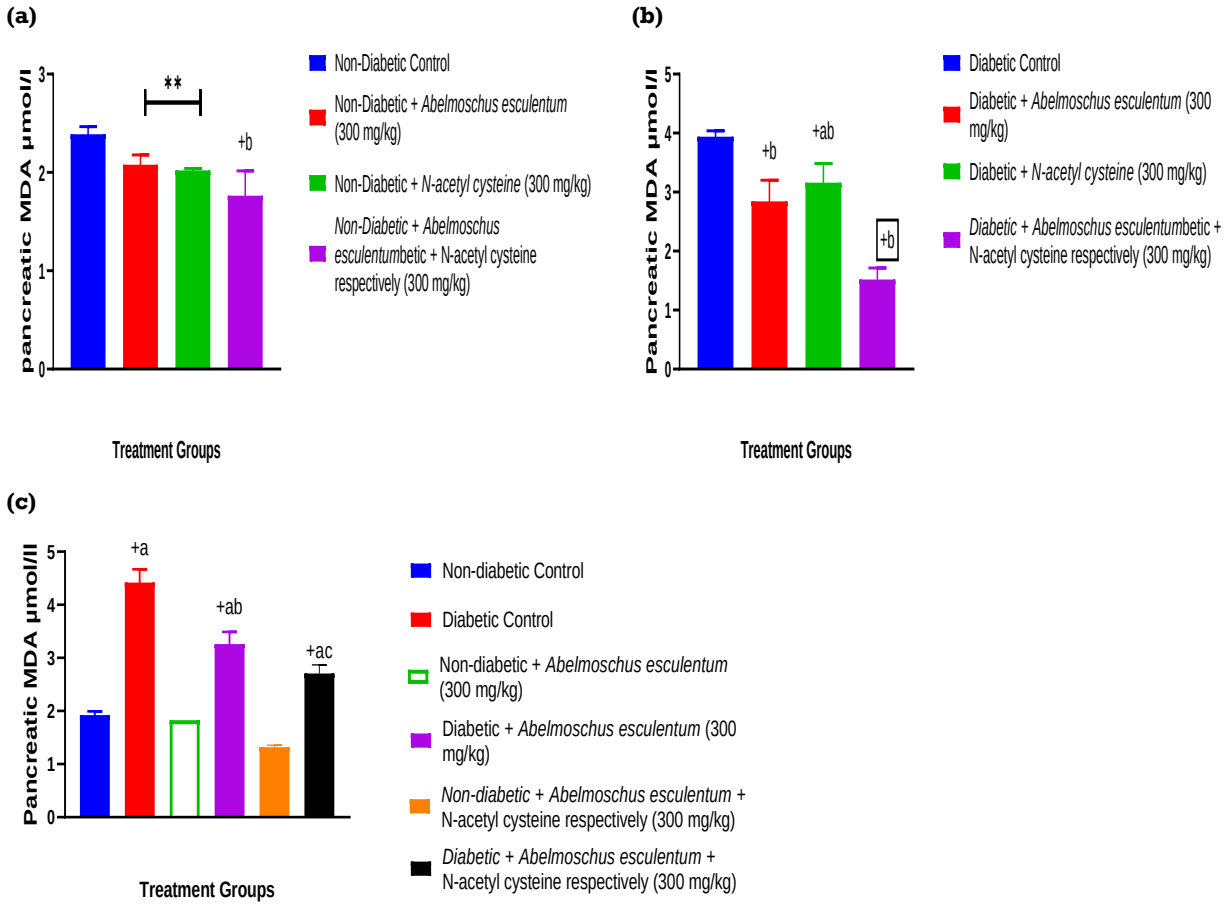

Figure 3: Effect of *Abelmoschus esculentum* ethanol extract on pancreatic MDA levels in (a) non-diabetic and (b) streptozotocin-induced diabetic male Wistar rats co-treated with N-Acetyl Cysteine, and (c) combined analysis of non-diabetic and diabetic rats.
Bars represent Mean ± S.E.M. (n = 5). Statistical analysis was performed using one-way ANOVA followed by Tukey's post hoc test. *$p < 0.05$ relative to controls; $^{a}p < 0.05$.

3.4 Effect of *Abelmoschus esculentum* Ethanol Extract Alone and in Combination with N-Acetyl Cysteine (NAC) on Pancreatic Nitric Oxide (NO)

Nitric oxide (NO) is a key signalling molecule for pancreatic function and insulin release [62]. As shown in Figure 4, this study observed a significant increase in pancreatic NO levels in both non-diabetic and diabetic rats treated with *A. esculentum* or NAC compared to their respective control groups. The combination of *A. esculentum* and NAC further elevated NO levels compared to treatment with *A. esculentum* alone in both non-diabetic and diabetic rats.
The increase in NO levels induced by *A. esculentum* may enhance pancreatic β-cell function, leading to improved insulin production and release, which helps regulate blood sugar levels in type 2 diabetes. This suggests that *A. esculentum* extract could stimulate NO production in a manner beneficial to pancreatic health.
Higher pancreatic NO levels indicate that *A. esculentum* may influence NO-regulated pathways critical for pancreatic function and insulin secretion. NO is vital for various processes, including pancreatic blood flow, β-cell growth, and insulin release, while impaired NO production is linked to diabetes development [63]. These findings highlight the potential of *A. esculentum* in modulating NO levels to support normal pancreatic function, warranting further investigation.

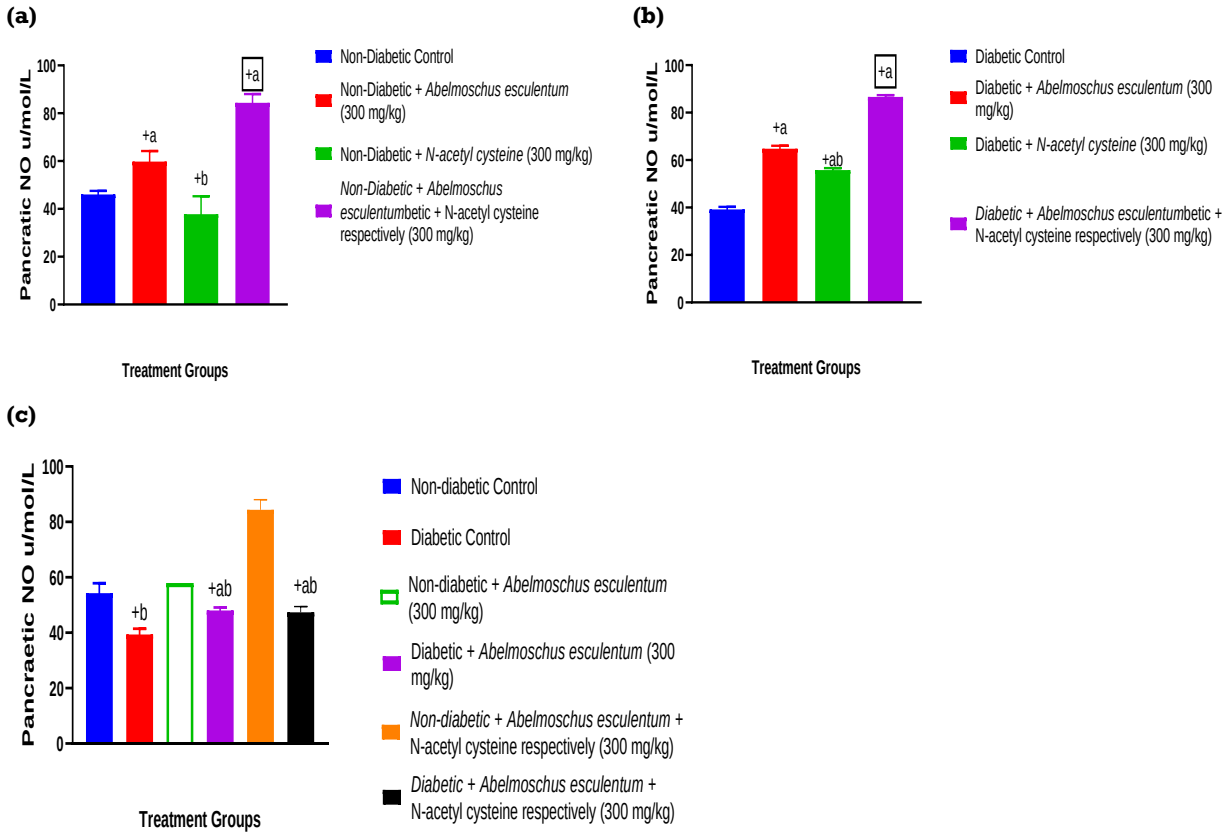

Figure 4: Effect of *Abelmoschus esculentum* ethanol extract alone and in combination with N-Acetyl Cysteine (NAC) on pancreatic Nitric Oxide (NO) levels in (a) non-diabetic male Wistar rats, (b) streptozotocin-induced diabetic male Wistar rats, and (c) combined analysis across groups.
Bars represent Mean ± S.E.M. (n = 5). Statistical analysis was performed using one-way ANOVA followed by Tukey's post hoc test. *p < 0.05 relative to controls; ªp < 0.05.

3.5 Effect of *Abelmoschus esculentum* Ethanol Extract Alone and in Combination with N-Acetyl Cysteine (NAC) on Pancreatic GR Levels

Figure 5 highlights the effects of *A. esculentum*, NAC, and their combination on pancreatic GR levels in non-diabetic and diabetic rats. Both *A. esculentum* and NAC treatments independently increased pancreatic GR levels in non-diabetic and diabetic rats compared to their respective controls. The combination of *A. esculentum* and NAC further elevated GR levels beyond those observed with *A. esculentum* alone.

In diabetic control rats, pancreatic GR levels were significantly reduced compared to non-diabetic controls. Treatment with either *A. esculentum* or its combination with NAC significantly restored GR levels in both groups. These findings align with previous studies by Alblihb et al. [64] and Hamed et al. [66, 67].

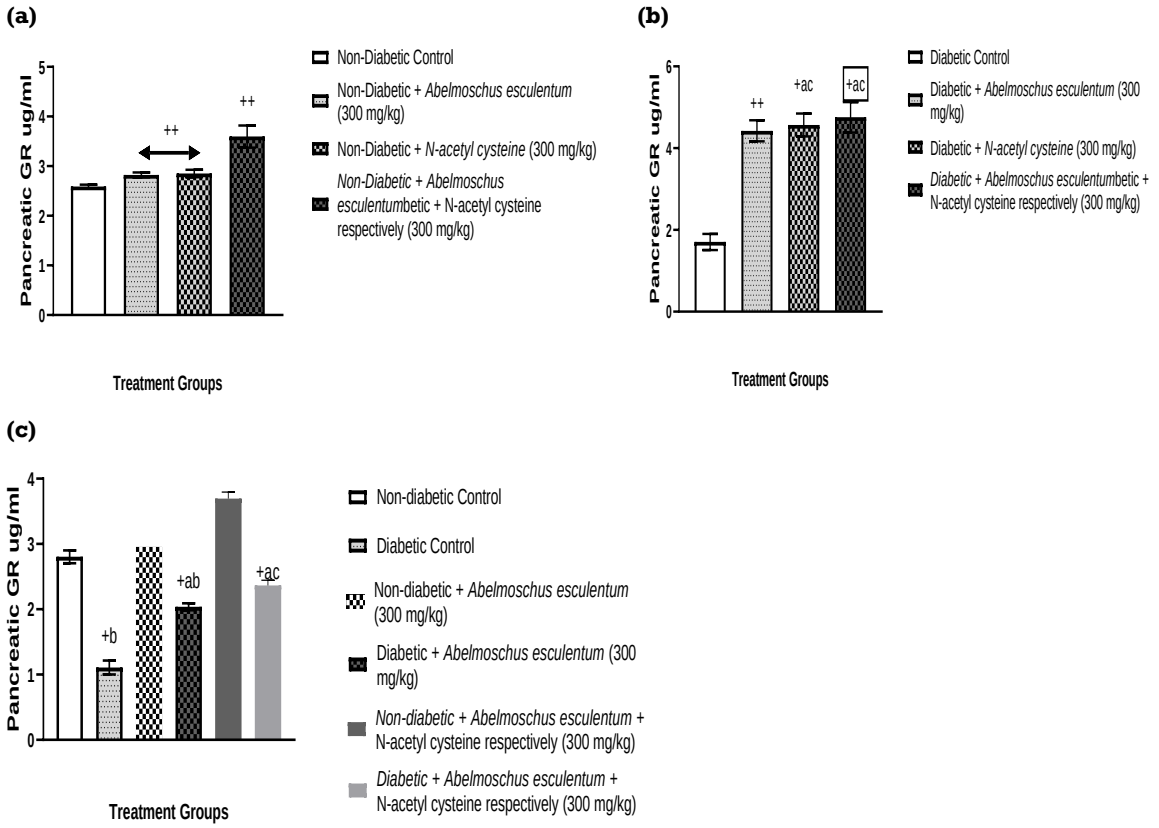

Figure 5: Effect of *Abelmoschus esculentum* ethanol extract alone and in combination with N-Acetyl Cysteine (NAC) on pancreatic GR levels in (a) non-diabetic male Wistar rats, (b) streptozotocin-induced diabetic male Wistar rats, and (c) combined analysis across groups.
Bars represent Mean ± S.E.M. (n = 5). Statistical analysis was performed using one-way ANOVA followed by Tukey's post hoc test. *$p < 0.05$ relative to controls; $^{a}p < 0.05$.

3.6 Effect of *Abelmoschus esculentum* Ethanol Extract Alone and in Combination with N-Acetyl Cysteine (NAC) on Pancreatic XOR Levels

Figure 6 highlights the effects of *A. esculentum*, NAC, and their combination on pancreatic XOR levels in non-diabetic and diabetic Wistar rats. Treatment with *A. esculentum* or NAC alone significantly decreased pancreatic XOR levels, which were elevated in both non-diabetic and diabetic control rats, demonstrating their antioxidant properties. The combination of *A. esculentum* and NAC further reduced XOR levels in both groups, indicating enhanced therapeutic efficacy in managing diabetes.

Diabetic rats exhibited significantly higher pancreatic XOR levels compared to non-diabetic controls (Figure 3.6c). However, treatment with *A. esculentum*, either alone or in combination with NAC, significantly decreased XOR levels in diabetic rats. Similarly, in non-diabetic rats, treatment with *A. esculentum*, NAC, or their combination significantly reduced XOR levels, with the combination therapy showing the greatest reduction.

Overall, these findings confirm the potential of *A. esculentum*, particularly when combined with NAC, to mitigate oxidative stress through reductions in pancreatic XOR levels in diabetic and non-diabetic conditions.

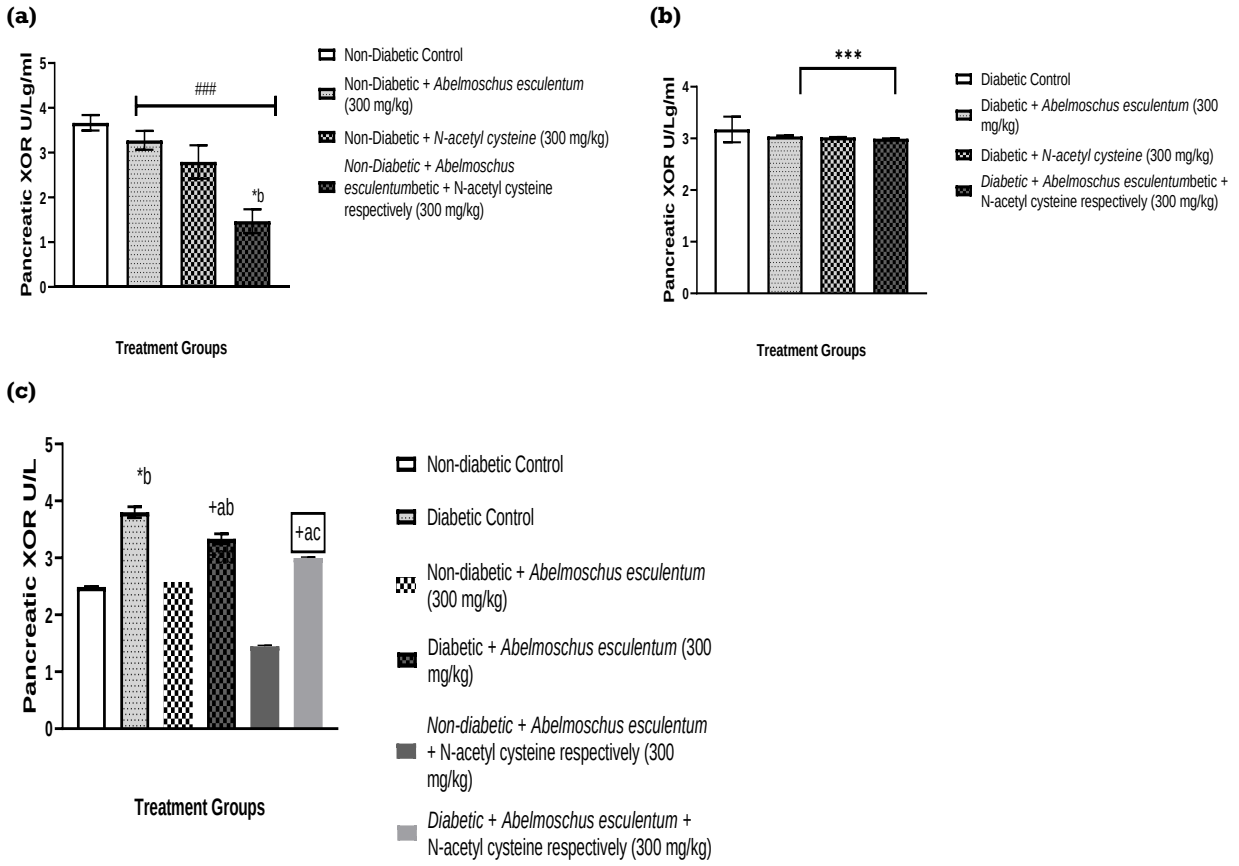

Figure 6: Effect of *Abelmoschus esculentum* Ethanol Extract Alone and in Combination with *N-Acetyl Cysteine (NAC)* on Pancreatic XOR Levels in (a) Non-Diabetic Male Wistar Rats, (b) Streptozotocin-Induced Diabetic Male Wistar Rats, and (c) Across Both Groups

Bars represent Mean ± S.E.M. (n = 5) (One-way ANOVA followed by Tukey's post hoc test). *$p < 0.05$, relative to controls; $^{a}p < 0.05$.

4.0 Conclusion

This study demonstrated that A. *esculentum* ethanol extract significantly reduced body weight and blood glucose levels in both non-diabetic and diabetic rats compared to their respective controls. It also decreased MDA and XOR levels while increasing pancreatic NO and GR levels in diabetic rats. These findings confirm A. *esculentum* as a hypoglycemic agent and a potent antioxidant, capable of mitigating oxidative stress induced by STZ. Its ability to modulate oxidative biomarkers highlights its potential as a therapeutic option for managing diabetes mellitus and its complications.

Acknowledgements

We sincerely thank everyone who contributed to the success of this study. Your support and efforts have been invaluable, and we are eternally grateful to you all.

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