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Effect of Fig (*Ficus capensis*) and Ackee *(Blighia sapida*) Tea on Glucose Measurement and Antioxidant Potential in STZ-Induced Diabetic Rats

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Abstract

This study investigates the effects of Fig *(Ficus capensis*) and Ackee (*Blighia sapida*) tea on blood glucose levels and antioxidant activity in streptozotocin (STZ)-induced diabetic rats. Male Wistar rats were divided into ten groups: normal control, diabetic control, Fig tea-treated, Ackee tea-treated, Metformin-treated, Market sample-treated and combination-treated groups. Diabetes was induced using STZ (35 mg/kg, intraperitoneal). Tea infusions were administered orally for 16 days. Blood glucose levels were measured, and antioxidant activity was assessed using in vitro assays (DPPH, FRAP, F^+, ABTS and OH content). In vivo antioxidant markers were evaluated, including catalase activity and glutathione levels. Fig and Ackee tea significantly reduced blood glucose levels ($p<0.05$) and enhanced antioxidant defenses, as evidenced by increased in vitro antioxidant activity and higher catalase and glutathione levels Fig and Ackee tea demonstrated glucose-lowering and antioxidant effects in diabetic rats, suggesting their potential in diabetes management.

Keywords: Fig tea, Ackee tea, diabetes, antioxidants, blood glucose, oxidative stress

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1. Introduction

Diabetes mellitus is a chronic metabolic disorder characterized by hyperglycemia and oxidative stress. Diabetes is the most deadly disease globally, causing several complications (Aisyah *et al*., 2023). This condition occurs when the blood sugar level is >125 and >180 mg/dL during fasting and 2hrs postprandial, respectively (Alagbe *et al*., 2024). According to Alberti *et al*., (1998),the effects of diabetes mellitus include long-term damage, dysfunction and failure of various organs. Diabetes is majorly divided into Type I and Type II (Aisyah *et al*., 2023). The third known type of diabetes is Gestational diabetes which occurs during pregnancy

Medicinal plants have been widely explored for their potential in managing diabetes and its complications. Fig (*Ficus capensis*) and Ackee (*Blighia sapida*) contain bioactive compounds with antioxidant and anti-diabetic properties. They were used in traditional medicine for diabetes mellitus management and their ability to reduce oxidative stress (Jimoh *et al*., 2012; Kazeem *et al*.,2013; Oloyede *et al*., 2014).

Hence the primary objective of this study aims to assess the effects of Fig (*Ficus capensis*) and Ackee *(Blighia sapida*) Tea on glucose measurement and antioxidant potential in STZ-Induced diabetic rats.

2. Materials and Methods

2.1 Sources of Materials.

Ackee leaf (*Blighia sapida*) was obtained from Itamo, Akure, Ondo state, Nigeria. Fig leaf (*Ficus capensis*) was obtained from a local farm in Ilado, Akure ondo state, Nigeria. The plants were authenticated at the Department of Forestry and Wood Technology, Federal University of Technology Akure, Nigeria. The herbarium specimen was deposited at the Herbarium Unit of the Forestry and Wood Technology Department. Wister rats used for the experiment were obtained from Oshogbo, Qsun State, Nigeria and reared at animal house Federal University of Federal University of Technology, Akure, Nigeria. All laboratory works were carried out at Food Science and Technology Processing Laboratory Federal University of Technology, Akure, Nigeria. All chemical used

were of analytical grade and obtained from Sigma –Aldrich, London, United Kingdom.

2.2 Processing of Leaves into Flour and Tea Preparation.
The harvested leaves were weighed out with digital balance scale 8kg each of the samples. The Leaves were sorted by removing the extraneous materials, thoroughly washed with tap water for ten minutes and drained with plastic sieve then air-dried at room temperature for 14 days after which the dried leaves were milled into powder using an electric blender and stored in specimen bags and then bagged into a tea bag.
Fig and Ackee tea were prepared by steeping 1.25g, 2.5g and combination of Ackee and Fig tea bags in 100 mL of boiling water for 10 minutes. The infusions were administered orally at 5 mL/kg body weight daily for four weeks.

2.3 Experimental design for the Rat Study.
Male albino rats weighing 150g- 200g were purchased from a breeding colony. The rats were kept in clean cages, placed in good aerated housing conditions, they were given free access to rat pellets and water and acclimatized for a duration of two weeks. The rats were handled according to guidelines of the Ethical Committee of the Centre of Research and Development (CERAD) of the Federal University of Technology, Akure, Nigeria. The animals were given high fat diet for fourteen days. On the fifteenth day, Diabetes was induced using streptozotocin (STZ, 35 mg/kg, intraperitoneal). After 72 hours' blood samples were obtained by tail snipping and blood glucose was determined using an Accu-Check strip and glucometer. Afterwards, rats having fasting blood glucose (FBG) ≥ 200 mg/ld. were categorized diabetic Animal Model Male Wistar rats (n=30) were divided into Ten groups: normal control, diabetic control, Metformin -treated, Market sample-treated, Fig tea-treated, Ackee tea-treated, and combination-treated groups. As shown in the table1 below.

Table 1: Experimental design for the Rat study.

Group 1	Positive control fed with basal feed
Group 2	STZ- induced rat fed with basal diet
Group 3	STZ- induced rat treated with standard drug(metformin)
Group 4	STZ-induced rat treated with 1.25g fig leaf extract
Group 5	STZ-induced treated with 2.5g fig leaf extract
Group 6	STZ-induced rat treated with 1.25g Ackee leaf extract
Group 7	STZ-induced rat treated with 2.5g Ackee leaf extract
Group 8	STZ-induced rat treated with 1.25g Ackee + fig leaf (50:50) extract
Group 9	STZ-induced rat treated with 2.5g Ackee + fig leaf (50:50) extract
Group 10	STZ-induced rat treated with standard herbal tea

2.4 Biochemical Analysis
The rats were euthanized twenty-four hours after the final day of treatment, following laboratory protocols that adhered to the National Institutes of Health guidelines for the care and use of experimental animals. After an overnight fast, the animals were sacrificed by cervical dislocation and the required organ was removed.

2.4.1 Blood Glucose Measurement: Blood samples were collected weekly, and glucose levels were measured using a glucometer**.**

2.4.2 Determination in-vitro antioxidant ability
In Vitro Antioxidant Assays: DPPH and FRAP, F^{2+}, ABTS and OH^{+} content assays were used to assess free radical scavenging activity.

2.4.2.1 Determination of free radical scavenging ability (DPPH)
The free radical scavenging ability of the extract against DPPH (1, 1- diphenyl-2-picryhydrazyl) using (Gyamfi *et. al*., 1999) method. 1ml of the extract was mixed with 1ml of the 0.4mM methanolic solution of the DPPH the mixture was left in the dark for 30min before measuring the absorbance at 516nm.

$$DPPH\ inhibition = (Absorbance\ of\ standard - 2\ of\ Sample) \times 100$$

2.4.2.2 Determination of ABTS scavenging ability
2, 2'-azino-bis (3-ethylbenthiazoline-6-sulphonic acid) (ABTS) scavenging ability. The ABTS scavenging ability of the extract was determined according to the method describe by Re *et al*., (1999). The ABTS was generated by reacting an (7mM) ABTS aqueous solution with $K_2S_2O_8$ (2.45 mM/l, final conc.) in the dark for 16 hours and adjusting the absorbance at 734nm to 0.700 with ethanol 0.2 of the appropriate dilution of the extract was then added to 2.0ml of ABTS solution and the absorbance was read at 734nm after 15mins. The TROLOX equivalent antioxidant capacity was subsequently calculated.

2.4.2.3 Determination of ferric reducing antioxidant potential
The reducing property of the extract was determined by (Pulido *et al*., 2000), 0.25ml of the extract was mixed with 0.25ml of 200mM of Sodium phosphate buffer pH 6.6 and 0.25ml of 1% KFC. The mixture was incubated at 50oC for 20min, thereafter 0.25ml of 10% TCA was also added and centrifuge at 2000rpm for 10min, 1ml of the supernatant was mixed with 1ml of distilled water and 0.1% of $FeCl_3$ and the absorbance was measure at 700nm.

$$Concentration\ (m/g) = \frac{Absorbance\ of\ Sample}{Slope\ of\ standard}$$

2.4.2.4 Determination of OH Radical Scavenging Ability
The ability of the extract to prevent Fe^{2+}/H_2O_2 induced decomposition of deoxyribose was determined using the method of Halliwell and Gutteridge (1981). Freshly prepared extract (0-100µl) was added to the reaction mixture containing 120µl, 20mM deoxyribose, 400µl, 0.1M phosphate buffer pH 7.4, 40µl, 20mM hydrogen peroxide and 40µl, 500Mm $FeSO_4$, and the volume was made to 800µl with distilled water. The reaction mixture was incubated at 37°c for 30min and the

reaction was stopped by the addition of 0.5ml of 2.8% TCA, this was followed by the addition of 0.4ml of 0.6% TBA solution. The tubes were subsequently incubated in boiling water for 20min. The absorbance was measured at 532nm in spectrophotometer.

$$\%\ OH\ Scavenging\ activity = \frac{Absorbance\ of\ standard\ - Absorbance\ of\ sample}{Absorbance\ of\ sample} \times 100$$

2.4.2.5 Determination of Fe^{2+} Chelation potential

The ability of the extract to chelate Fe^{2+} was determined using a modified method of Minotti&Aust (1987) by Puntel *et al* (2005). Briefly, 150mM $FeSO_4$ was added to a reaction mixture containing 168ul of 0.1M Tris- HCl pH 7.4, 218ul saline and extract and the volume is made up 1ml with distilled water. The reaction mixture was incubated for 5min, before the additional of 13ul of 1, 10-phenantroline the absorbance will be read at 510nm.

2.4.3 In Vivo Antioxidant Markers

In Vivo Antioxidant Markers: Glutathione levels and catalase enzyme activity and were analyzed in serum to assess oxidative stress response.

2.4.3.1 Determination of reduced glutathione content (GSH)

The plasma Reduced Glutathione (GSH) concentration was determined using the methodology of Othman *et al*. (2021) Aliquots of 0.5 ml from the tissue homogenates were mixed with 0.2M phosphate buffer, pH 8.0, and 0.1 ml of 0.01MEllman'sreagent (DTNB). Following that, the mixture was centrifuged at room temperature at 3000 ×g for 15 minutes. The absorbance of the resultant cleared supernatants was measured using a spectrophotometer at 420 nm.

2.4.3.2 Determination of catalase (CAT) Activity

Catalase activity in homogenized tissue was determined using the procedures outlined by

Sinha *et al.*, (2011). A solution of 50 µl tissue homogenate, 0.27M H_2O_2, and 250µl 10 µM phosphate buffer (pH 7.0) was made. Potassium dichromate in acetic acid was then added, and the absorbance was measured at 620nm. Catalase activity was measured as mol H_2O_2 consumed per mg protein.

2.5 Statistical Analysis

The results were presented as mean±standard deviation (SD) and the test for the Statistical difference was performed using one –way analysis of variance (ANOVA). The statistical package used to determine significant difference was Statistical Package for Social Science (SPSS,Version 20). Significant means were separated using Duncan's New Multiple Range Test (DNMRT) and differences were considered significant at $p<0.05$.

3. Results

3.1 Blood Glucose Lowering Potential of the Experimental Samples

The results of blood glucose level of diabetic treated with Fig and Cake tea were significantly reduced compared to the diabetic control ($p<0.05$).

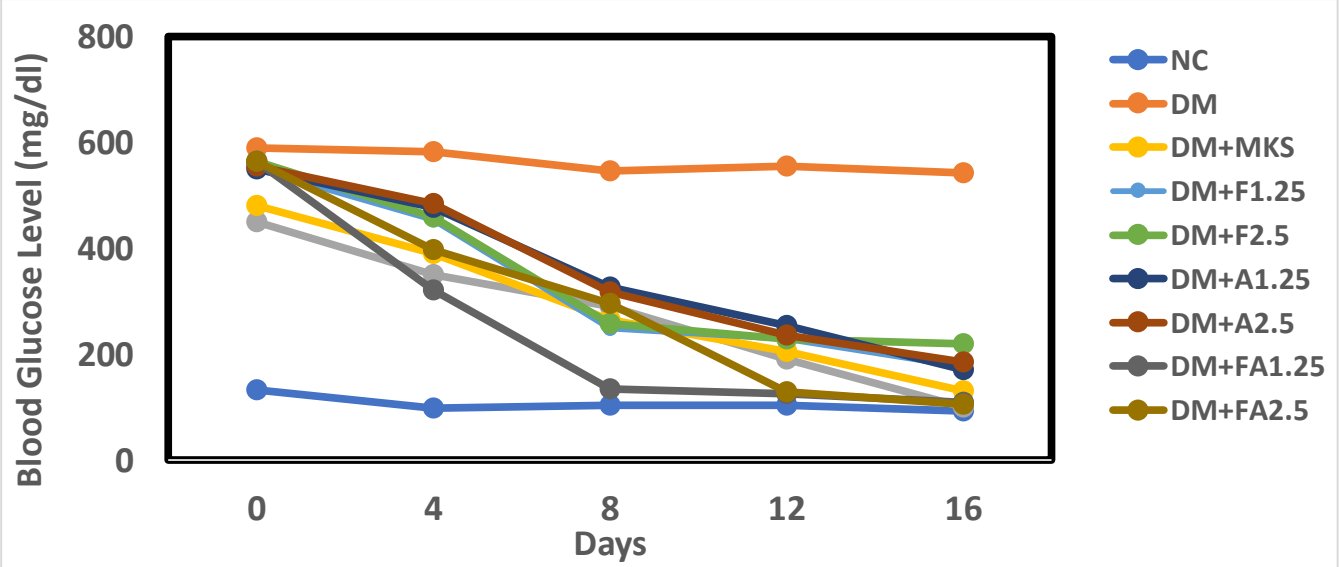

Figure 1: Blood Glucose Lowering Potential.

NC: Normal Control, DM: Untreated STZ induced diabetic rats; DM+Met: Induced diabetic rat treated with Metformin (25mg/kg); DM+MKS: Induced diabetic rat treated with market sample; DM+F1.25: STZ induced diabetic rats treated with 1.25g fig leaf extract; DM+F2.5: STZ induced diabetic rats treated with 2.5g fig leaf extract; DM+A1.25: STZ induced diabetic rats treated with 1.25g ackee leaf extract; DM+A2.5: STZ induced diabetic rats treated with 2.5g ackee leaf extract

DM+FA1.25: STZ induced diabetic rats treated with 1.25g fig and ackee leaf extract

DM+FA2.5: STZ induced diabetic rats treated with 2.5g fig and ackee leaf extract

Figure 1: Effect of fig and ackee leaf extract on fasting blood glucose level of treated diabetic rats. Data is mean ± standard deviation (n=3) ($p<0.05$). X and Y rep mean values are significantly different ($p<0.05$) compared to control group and induced group.

The effect of fig and ackee leaf extract on the glucose level of the diabetic treated rats is shown in figure 1. The normal control shows a consistent low blood glucose level throughout the 16 days, as expected for nondiabetic subjects. The untreated diabetic animals, with persistently elevated blood glucose levels (around 600 mg/dl), showing minimal reduction throughout the 16 days. This shows hyperglycemia in untreated diabetes. The metformin treated group shows a significant reduction in blood glucose almost approaching normal levels by day 8 and stabilizing thereafter, demonstrating the effectiveness of metformin, a standard antidiabetic drug (Rena *et al*., 2017). The market sample group also shows a sharp decrease in glucose levels, comparable to metformin, suggesting that the MKS treatment is similarly effective in controlling blood glucose. Both fig and ackee at different doses (1.25 mg/kg and 2.5 mg/kg) show a gradual reduction in blood glucose levels. The combination treatments of fig and ackee at (2.5 mg/kg) show synergistic effects, with blood glucose levels decreasing more rapidly than with fig or ackee alone. This could suggest a potential synergistic action between the phytochemicals in fig and ackee leaves. Figure.1 shows fig and ackee extracts, either individually or combined, can significantly lower blood glucose levels in diabetic animals. The effectiveness seems dose-dependent, with the 2.5mg/kg doses being more potent than 1.25 mg/kg. The DM+FA groups (combination therapy) show enhanced glucose-lowering effects compared to single treatments, possibly due to complementary actions of the bioactive compounds in fig and ackee (Asgary *et al*., 2018) These results support the potential of fig and ackee as sources of natural antidiabetic agents, which could complimentary serve as alternatives to conventional treatments like metformin (Yin *et al*., 2014).

3.2 In Vitro Antioxidant Activity: DPPH and FRAP, F^{2+}, ABTS and OH^{+} content assays showed increased antioxidant capacity in treated groups (Figure 2).

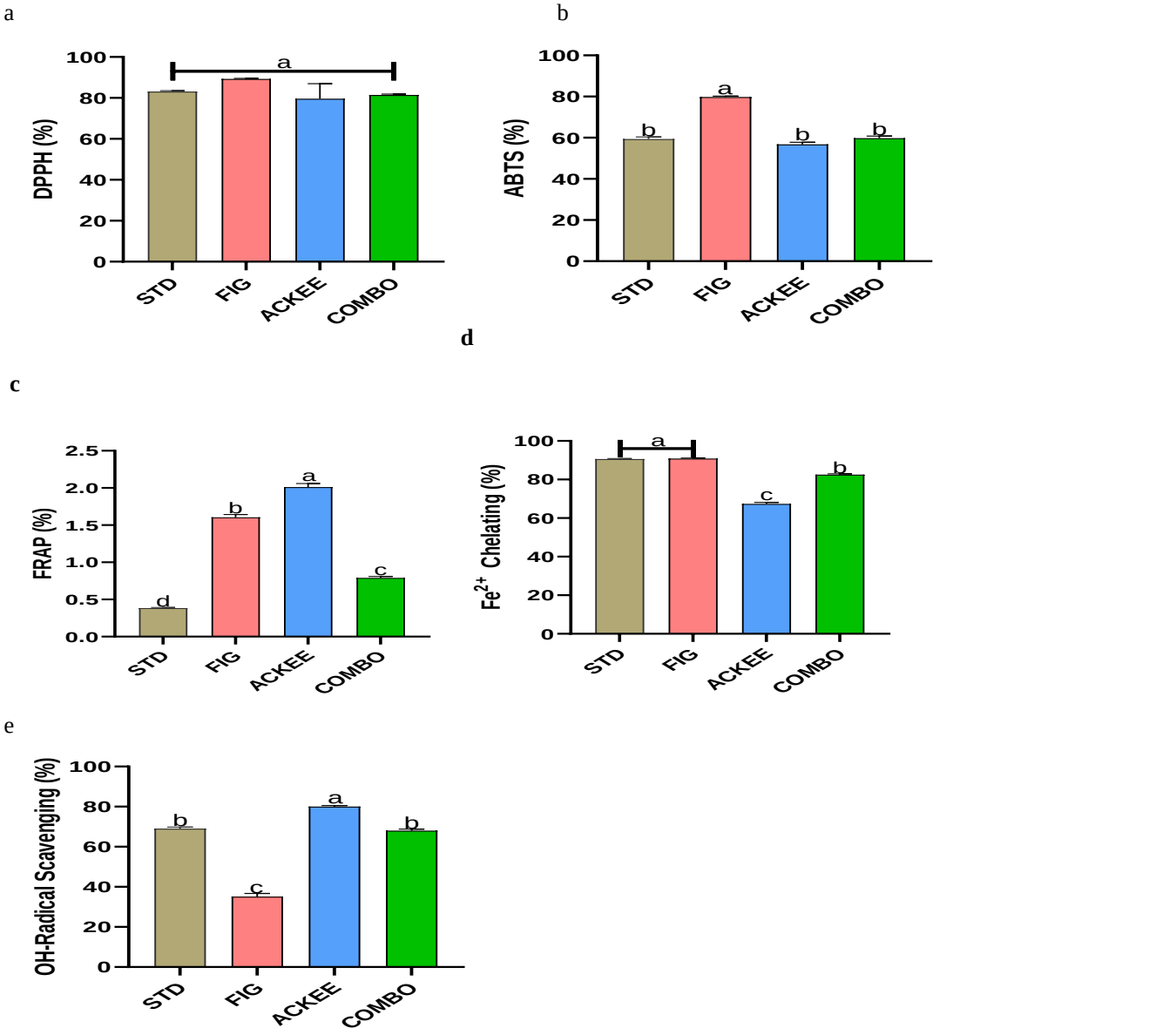

Figure 2: In-vitro antioxidant properties of the Standard Tea Samples, Fig, Ackee, and Fig-Ackee leaves Powder. COMBO = FIG: ACKEE 50:50

The antioxidant activities of the extracts were evaluated by five assays which are DPPH, ABTS, FRAP, Fe^{2+} Chelation, and OH^- radical scavenging ability. It has been reported that Ficus species are rich source of antioxidants that help in prevention of oxidative stress related diseases (Onyeto, 2015). A decolonization from purple to yellow by DDPH when reacted with a food substance indicates that the substance sample has a proton radical scavenger. The DPPH radical scavenging activity of the leaves extract is about 80%, with no significant difference between the samples. Even compared to a standard antidiabetic market sample as shown in (figure 2b). The ABTS assay is based on the generation of a bluish-green radical cation that can react with both hydrophilic and hydrophobic antioxidant systems to produce its colorless form (Gupta, 2015). The ABTS radical scavenging activity of the beverages ranged from 60 to 80% with Fig extract showing significantly higher scavenging activity. Ackee, Combo and Market sample showed lower ABTS scavenging activity with no significant difference between the three samples (Figure 2a).

The FRAP assay was used to measure the reducing potential of the antioxidant in the extract that is reacting with ferric tripyridyltriazine (Fe^{3+}–TPTZ) complex to produce a blue colored ferrous tripyridyltriazine (Fe^{2+}–TPTZ). There is a highly significant difference in the ferric reducing antioxidant activities of the samples. Fig exhibited the highest FRAP activity, followed by Ackee and then the Combo. However, the three samples exhibited higher Ferric reducing antioxidant activities than the standard market sample (Figure 2e).

Fe^{2+} chelation assay was used to measures the ability of the samples to chelate ferrous ions (Fe^{2+}). Chelation involves binding metal ions to a molecule, preventing their oxidation (Gulcin and Alwasel, 2022). All samples showed high Fe^{2+} chelating activities, but Fig showed the highest potential with no significant difference with the market sample as shown in figure 2c. Fig leaf extract showed the lowest OH^- radical scavenging activities, while Ackee extract showed the highest activities. With no significant difference to the standard market sample, the equal ratio combination also showed relatively high OH^- radical scavenging activities (Figure 2d).

3.3 In Vivo Antioxidant Markers: Catalase activity and glutathione levels were significantly higher in tea-treated groups (Figure 3), suggesting improved oxidative stress resistance.

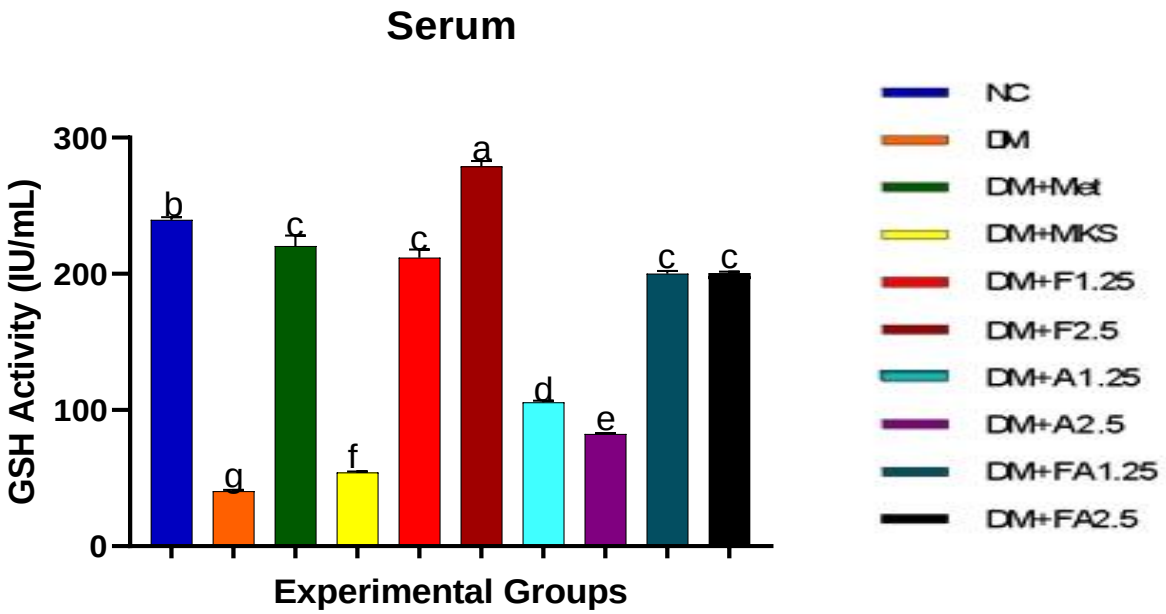

Figure 3: Effect of fig and ackee leaf extract on Glutathione (GSH) Levels of Treated Diabetic Rats. Data is mean ± standard deviation (n=6) ($p<0.05$). X and Y rep mean values are significantly different ($p<0.05$) compared to control group and induced group

Keys: NC- Normal control
DM- Untreated induced diabetic rats
DM+ Met- Treated induced diabetic rats
DM+MKS- Treated with market sample
DM+F1.25-Treated with Fig leaf extract (1.25g)
DM+F2.5- Treated with Fig leaf extract (2.5g)
DM+A1.25- Treated with Ackee leaf extract (1.25g)
DM+ A2.5- Treated with Ackee leaf extract (2.5g)
DM+FA1.25- Treated with Fig and ackee leaf extract (1.25g)
DM+FA2.5- Treated with Fig and ackee extract (2.5g)

Figure 3 shows that the levels of endogenous antioxidant enzymes were significantly reduced in the serum of untreated diabetic rats compared to the group fed with fig and ackee leaf extract. The groups receiving the extract exhibited significantly higher levels of GSH activity which is an antioxidant enzyme.

Moreover, the decrease is naturally occurring in GSH in untreated diabetic rats emphasizes the weakened antioxidant defense system caused by diabetes. GSH plays a crucial role in neutralizing damaging free radicals and limiting oxidative stress. Diabetic circumstances cause a persistent increase in blood sugar levels, leading to the excessive creation of ROS. These ROS exceed the body's natural defenses against oxidation and cause damage to tissues, including the liver (Hanley *et al.*, 2005; Ibrahim *et al*., 2020; *et al*). This study results indicate that the extract administered to the treated groups was effective in enhancing the levels of these crucial antioxidant enzymes.

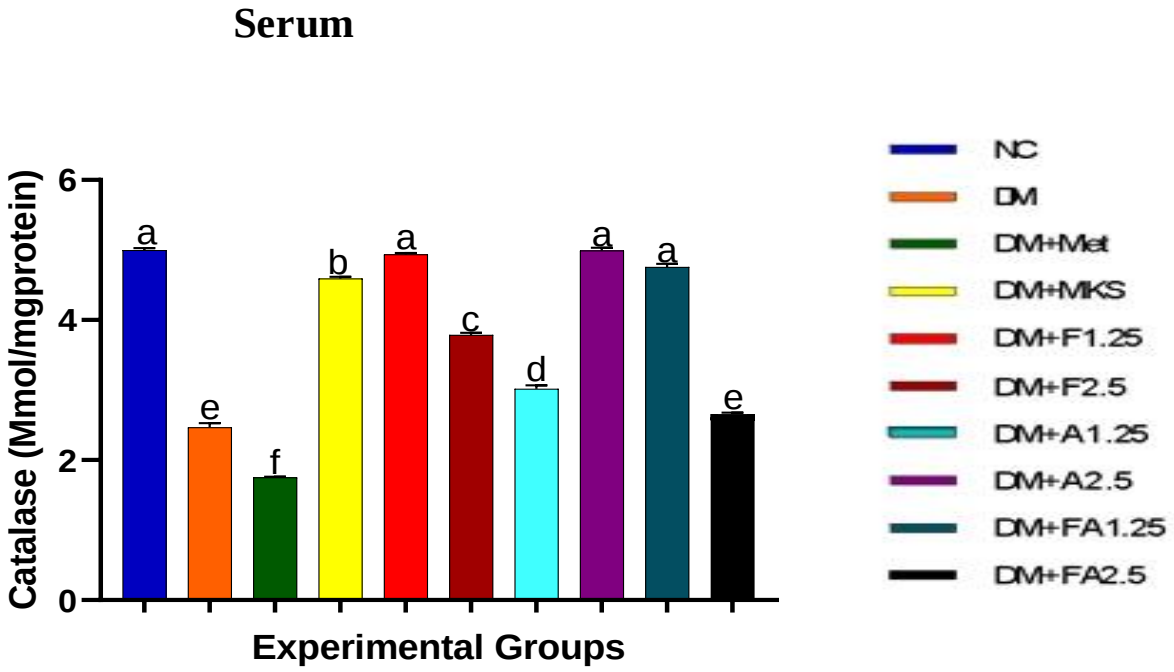

Figure 4: Effect of fig and ackee leaf extract on α-amylase Levels of Treated Diabetic Rats. Data is mean ± standard deviation (n=6) ($p<0.05$). X and Y rep mean values are significantly different ($p<0.05$) compared to control group and induced group.
Keys: NC- Normal control
DM- Untreated induced diabetic rats
DM+ Met- Treated induced diabetic rats
DM+MKS- Treated with market sample
DM+F1.25-Treated with Fig leaf extract (1.25g)
DM+F2.5- Treated with Fig leaf extract (2.5g)
DM+A1.25- Treated with Ackee leaf extract (1.25g)
DM+ A2.5- Treated with Ackee leaf extract (2.5g)
DM+FA1.25- Treated with Fig and ackee leaf extract (1.25g)
DM+FA2.5- Treated with Fig and ackee extract (2.5g)

Figure 4 shows that the levels of endogenous antioxidant enzymes were significantly reduced in the serum of untreated diabetic rats compared to the group fed with fig and ackee leaf extract. The groups receiving the extract exhibited significantly higher levels of Catalase activity which is an antioxidant enzyme.

Moreover, the decrease is naturally occurring in Catalase in untreated diabetic rats emphasizes the weakened antioxidant defense system caused by diabetes. Catalase plays a crucial role in neutralizing damaging free radicals and limiting oxidative stress. Diabetic circumstances cause a persistent increase in blood sugar levels, leading to the excessive creation of ROS. These ROS exceed the body's natural defenses against oxidation and cause damage to tissues, including the liver (Hanley *et al*., 2005; Ibrahim *et al.*, 2020).

This study results indicate that the extract administered to the treated groups was effective in enhancing the levels of these crucial antioxidant enzymes. The glucose-lowering effect of Fig and Ackee tea may be attributed to their polyphenols and flavonoids, which have been shown to improve insulin sensitivity and reduce oxidative stress. The enhancement of in vitro antioxidant activity supports their free radical scavenging potential. Furthermore, increased catalase activity and glutathione levels suggest systemic antioxidant defense improvement. These findings align with previous studies on plant-based interventions for diabetes management.

4. Conclusion

Fig and Ackee tea demonstrated significant glucose-lowering and antioxidant effects in STZ-induced diabetic rats. Their bioactive compounds may contribute to oxidative stress reduction and improved glucose homeostasis. These findings suggest that incorporating such plant-based extracts into treatments could provide a natural and effective approach to enhancing health outcomes in diabetes management. Further studies are needed to explore their molecular mechanisms and potential clinical applications.

Conflict of Interest

The author declares no conflict of interest.

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