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EFFECT OF ETHANOL EXTRACT OF FONIO MILLET ON THE HEPATIC HEALTH AND BODY WEIGHT OF DIABETIC RATS

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Abstract

Diabetes mellitus (DM) affects liver health and appetite. Natural therapies are often preferred over synthetic medications for treating DM. Therefore, the aim of this study was to evaluate the effect of *Digitaria exilis* (Fonio millet) extract on hepatic health and body weight of diabetic rats. A total of 200 g of *Digitaria exilis* powder was extracted. Twenty-five adult male rats were divided into five groups of five rats each. Group I served as the normal control and received 2 ml of distilled water only. Diabetes mellitus was induced in rats by a single intraperitoneal injection of 60 mg/kg body weight of streptozotocin. Group II was negative control and was induced with diabetes without any treatment. Groups III and IV were induced with diabetes and subsequently administered 200 mg/kg and 400 mg/kg of the extract, respectively. Group V was induced with diabetes and then treated with a standard drug (2.5mg/kg body weight metformin). The results of the study showed that the activities of AST, ALT, and ALP in diabetic untreated rats (Group II) were significantly elevated ($p < 0.05$). However, the administration of the extract significantly reduced the activities of AST and ALP, while there was no significant difference ($p > 0.05$) in the activity of ALT between the treated groups and the normal control. The body weight of diabetic rats treated with the extract progressively increased during the treatment period. In conclusion, this study suggests that Fonio millet extract has therapeutic potential to enhance hepatic health in diabetic conditions.

Keywords: Fonio Millet, Diabetes, Alkaline phosphatase, Hyperglycaemia

Introduction

Diabetes mellitus (DM) refers to a group of metabolic disorders characterized by elevated blood glucose levels and abnormalities in insulin production and function. It encompasses various types, such

as Type 1 diabetes (T1DM), Type 2 diabetes (T2DM), gestational diabetes (GDM), and other subtypes like maturity-onset diabetes of young and latent autoimmune diabetes in adults. DM poses a significant health risk and is one of the leading causes of morbidity worldwide (Zeng *et al.*, 2018).

The liver plays a crucial role in maintaining glucose balance, clearing insulin, and producing inflammatory cytokines (Loffing and Verrey, 2022). Key liver enzymes, including gamma-glutamyl transferase, aspartate aminotransferase (AST), and alanine transaminase (ALT), are involved in these processes and can indicate liver damage when present at abnormal serum levels (Kunutor *et al.*, 2014). These enzymes have also been identified as reliable markers for evaluating liver health in the context of hepatic insulin resistance (Hanley *et al.*, 2004).

The presence of liver disease in individuals with diabetes mellitus can exacerbate with treatment strategies that involve synthetic medications, putting additional strain on the liver. This underscores the need for alternative therapies that can improve liver health with minimal or no extra burden on the liver (Forlani *et al.*, 2008).

Fonio millet, an annual herbaceous plant that grows to heights of 30 to 80 cm, features ears consisting of two to five narrow parts, reaching lengths of up to 15 cm. The spikelets contain both a sterile and a fertile flower, with the latter producing the fonio grain (Kanlindogbe *et al.*, 2020). Fonio millet is endowed with phytochemicals of immense health significance (Izadi *et al.*, 2012).

Research efforts have shown that fonio millet can be used as a functional food, but there is limited information on the specific health benefits of its consumption (Bello *et al.*, 2022). Therefore, the aim of this study was to evaluate the effect of ethanol extract of fonio millet on the hepatic health and body weight of diabetic rats.

Materials and Methods

Collection of Seed Samples

A total of 350g of dried seeds of *Digitaria exilis* were obtained from the Afor Ngugo market in the Ikeduru Local Government Area of Imo State, Nigeria. The seeds were then identified at the herbarium unit of the Department of Forestry at Michael Okpara University of Agriculture, Umudike (MOUAU) in Abia State, Nigeria.

Seed Processing

The seeds were carefully inspected, and any unsuitable ones were discarded. An industrial blender was used to grind the dried seeds into a fine powder, which was then sieved. The resulting powder was subjected to extraction, with 200 g of *Digitaria exilis* powder mixed with 2 L of 50% ethanol for 48 hours. The remaining marc was further extracted using 1.2 L of 50% ethanol for an additional 48 hours, followed by filtration. The combined filtrate was concentrated under reduced pressure using a rotary evaporator, resulting in a crude extract weighing 75 g, which was stored in an airtight container for future use.

Animals

Twenty-five adult male Wistar rats were obtained from the animal facility of the Department of Science Laboratory Technology at Akanu Ibiam Federal Polytechnic, Uwana Afikpo, Ebonyi State. The rats were housed under controlled conditions, including regulated humidity, temperature, and a 12-hour light/dark cycle. Prior to the study, the animals underwent a two-week acclimatization period. All animal procedures followed established guidelines for the care and handling of laboratory animals as outlined by the National Research Council.

LD_{50} Determination

The median lethal dose 50% (LD50) was determined using a two-phase experimental

approach. In the initial phase, nine adult male wistar rats were divided into three groups, each consisting of three rats. These groups were administered oral doses of 10, 100, and 1000 mg/kg of the extract and were monitored for 24 hours for signs of toxicity. Since no mortality occurred, the second phase involved three additional groups, each with one rat, receiving doses of 1600, 2900, and 5000 mg/kg of the extract. These animals were observed for 48 hours for any signs of toxicity (Lorke, 1983).

Induction of Diabetes

Diabetes mellitus was induced in rats by a single intraperitoneal injection of 60 mg/kg body weight of streptozotocin, prepared in a 0.1 mol/L fresh cold citrate buffer at pH 4.5. This procedure was performed on rats that had been fasted for 24 hours (Burcelin *et al.*, 1995). Blood glucose levels were measured three days later using a glucometer (Accu-Chek Advantage, Roche Diagnostics GmbH, Germany), and rats with fasting blood glucose levels exceeding 126 mg/dl were classified as diabetic and selected for further experimentation.

Experimental Design

Group I: (Normal Control) rats were fed rat chow and water.

Group II: Diabetic rats without treatment.

Group III: Diabetic rats were administered 200 mg/kg of the extract of *D. exilis* orally.

Group IV: Diabetic rats were administered 400 mg/kg of the extract of *D. exilis* orally.

Group V: Diabetic rats were administered 2.5 mg/kg body weight metformin daily

Determination of Animals' Body Weight

The measurement of body weight for the animals was conducted at the start of the study, designated as day 0, and continued on a weekly basis for two weeks before the conclusion of the experiment.

Biochemical Assessment

Sample Preparation: The rats were treated for two weeks, after which they were humanely sacrificed, and blood samples were collected for liver function test. 2 mL of blood collected in an EDTA tube was centrifuged at 4,000 rpm for 15 minutes. The resulting plasma was then preserved for biochemical assessment.

Liver Function Test

Determination of Alkaline Phosphatase Activity

The method described by Bassey *et al.* (1946) and modified by Wright *et al.* (1972) using Randox kits were used to determine ALP activity. 500 µL of the reagent was added to a cuvette containing 10 µL of the sample before mixing. The absorbance was initially measured at 405 nm. The ALP activity was calculated using the formula:

ALP activity (IU/l) = 2742 × ΔA 405 nm/min 1

Where: 2742 = Extinction coefficient; ΔA 405 nm/min = change in absorbance per minute for the sample.

Determination of Alanine Amino Transferase Activity

Exactly 50 µl of the sample was mixed with ALT reagent in a test tube. The initial absorbance was read at 340 nm after 1 minute following the method described by IFCC (1980) using Randox kits. The absorbance readings were taken at 1, 2, and 3 minutes.

ALT activity (nm/min) = 1746 × ΔA 340 nm/min.....2

Where ΔA 340 nm/min = change in absorbance per minute for the sample.

1746 = Extinction coefficient

Determination of Aspartate Transaminase Activity

The same procedure as for ALT was used to determine AST activity, but with the AST reagent.

AST activity (nm/min) = 1746 × ΔA 340 nm/min 3

Where ΔA 340 nm/min = change in absorbance per minute for the sample

1746 = Extinction coefficient.

Histopathological Examination

Histopathological examinations were conducted on harvested liver that were preserved in a 4% formaldehyde solution, subsequently embedded in paraffin wax, and sectioned. The resulting slices were mounted on slides and subjected to H&E staining. Microscopic analysis (×400) was performed to assess any cellular alterations (Justin *et al.*, 2015).

Data Analysis

The data collected were presented as mean ± standard deviation utilizing SPSS (Version 23). A one-way Analysis of Variance (ANOVA) was employed for data analysis. The means were compared using the Turkey test, with p-values below 0.05 considered statistically significant.

Results

Figure 1 shows the activity of the liver enzymes (alkaline phosphatase, aspartate aminotransferase, and alanine aminotransferase) in diabetic rats administered ethanol seed extract of *Digitaria exilis* (Fonio millet), indicating that the activities of the enzymes in diabetic untreated rats (Group II) were significantly ($p < 0.05$) elevated. However, following oral administration of the extract, there was a significant ($p < 0.05$) reduction in the activities of AST and ALP, while there was no significant ($p > 0.05$) difference in the activity recorded for ALT between the treated groups and the normal control (Group I). It is worth noting that there was no significant ($p > 0.05$) difference in the activity recorded in the group administered the standard drug and the

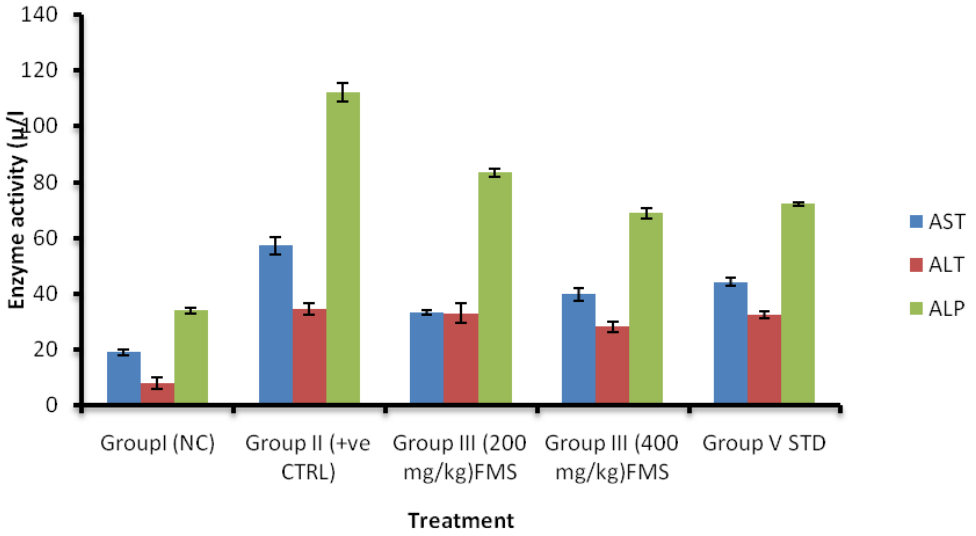

Figure 1: Liver enzyme activity in diabetic wistar rats administered ethanol seed extract of Fonio Millet

Key: Bars with similar alphabets are not significantly ($p<0.05$) different

groups administered the extract.

Figure 2 shows the body weight of diabetic Wistar rats treated with ethanol seed extract of fonio millet, indicating that the body weight of diabetic rats treated with the extract progressively increased within the treatment period. However, a contrary observation was made in Group II (diabetic untreated rats) and Group V (diabetic rats treated with the standard anti-diabetic group) as the body weight of rats declined progressively across the treatment period. Although an increase in body weight was observed in Group I (normal control), the body

weight recorded in week 1 was not significantly different ($p > 0.05$) from that

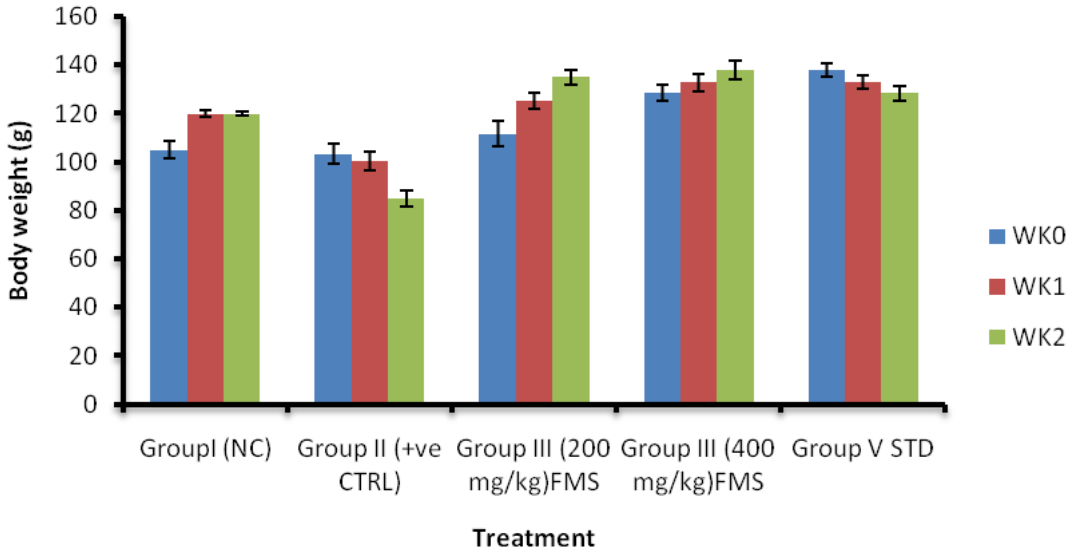

Figure 2: Body weight of diabetic Wistar rats treated with ethanol seed extract of Fonio millet

Key: Bars with similar alphabets are not significantly ($p<0.05$) different

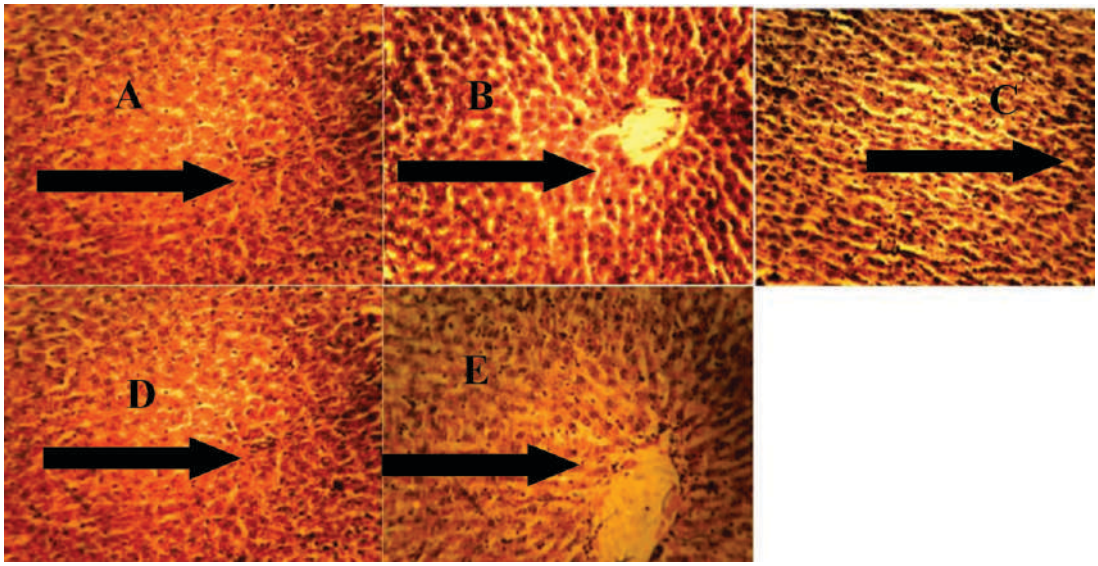

Plate 1: Photomicrograph of the liver of diabetic rats administered Fonio millet extract (Magnification: ×400; H&E)

NC, (b) Negative control (c) Diabetes without treatment (d) Diabetes+200 mg/kg extract (e) Diabetes+400 mg/kg extract
a, c,d, and e show relatively normal histoarchitectural features of the liver
b. shows histoarchitectural distortion

recorded in week 2.

Discussions

Diabetes mellitus is a metabolic disorder that affects carbohydrate processing, marked by the body's reduced capacity to produce or respond to insulin, which is essential for regulating blood glucose levels effectively. The role of Type 2 diabetes in the pathogenesis of hepatic dysfunction is known (Rogha *et al.*, 2011). The activity of the liver

enzymes was significantly raised following the induction of diabetes. This is substantiated by the outcome of a study conducted by Eltayeb *et al.* (2012), which reported a notable elevation in the levels of liver enzymes ALT, AST, GGT, and ALP in patients with type 2 diabetes, implying that these liver enzymes may serve as effective biomarkers for evaluating type 2 diabetes. The decreased enzyme activity observed in the diabetic rats treated with the extract could be attributed to the presence of certain bioactive compounds, some of which have established antioxidant and anti-diabetic potentials, including polyphenols, flavonoids, phytic acid, and dietary fiber. This is in line with the findings of a study by Singh *et al.* (2022) that highlighted the anti-diabetic potential of dietary bioactives from millets. Bello et al. (2022) found that polyphenol extracts from finger millet, Fonio millet, and Pearl millet have therapeutic potential in the prevention and management of Type-2 diabetes. They specifically highlighted the impressive antioxidant potential of fonio millet, which contributes to its health benefits. The study also revealed a decrease in body weight after diabetes induction, possibly due to metabolic dysfunctions leading to increased satiety and reduced hunger, characteristic of diabetes (Fedder *et al.*, 2013). This suggests that the observed increase in body weight after treatment could indicate the anti-diabetic properties of the extract, correcting metabolic imbalances that contribute to increased food intake and subsequent weight gain.

Conclusion

The study focused on the ability of fonio millet to preserve hepatic function in diabetic condition. The extract lowered the activities of alkaline phosphatase, aspartate aminotransferase and alanine transaminase implying that fonio millet extract has the potential to preserve hepatic health in diabetic conditions.

Conflict of interest

Authors declare that no conflict of interest exists

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