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(RESEARCH ARTICLE)

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Ameliorative effect of beetroot aqueous extract on the liver of diabetic Wistar rats

Mary kabole [1], Isabel Namfukwe Luambia [1], Albertina Ng'andu [2], Olawole Temitayo Priscilla [3] and Uthman Ademola Yusuf [1,*]

[1] *Department of Human Anatomy, School of Medicine and Health Sciences, Mulungushi University, Livingstone Campus, Zambia.*
[2] *Department of Anatomy, Institute of Basic and Biomedical Sciences, Levy Mwanawasa Medical University, Zambia.*
[3] *Department of Anatomy, College of Medicine, University of Lagos, Nigeria.*

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Abstract

This study investigated the effects of beetroot extract on the liver of diabetic Wistar rats. Thirty male Wistar rats (8-10 weeks old, weighing 160-200 g) were randomly assigned to five groups: A) normal control, B) diabetic only, C) diabetic + beetroot, D) diabetic + Metformin, and E) beetroot extract only. Diabetes was induced via a single intraperitoneal dose of streptozotocin (70 mg/kg body weight). After 72 hours of sustained diabetes (blood glucose ≥ 7 mmol), the rats received oral administrations of beetroot extract and Metformin (500 mg/kg and 100 mg/kg body weight daily respectively) for four weeks, while being fed a standard diet with water access. Blood glucose levels and body weight were measured weekly. At the experiment's conclusion, the rats were euthanised, and relative liver weights were recorded. Data analysis included one-way ANOVA and graphical representation using Excel. Results indicated significant differences in blood glucose levels and average body weight in the diabetic rats ($p<0.05$) compared to other groups. The relative liver weight in the diabetic group was notably lower ($p<0.05$). Histological examination revealed normal liver architecture in the control and beetroot groups, whereas the diabetic group exhibited disrupted histoarchitecture with necrotic hepatocytes. The diabetic + beetroot group showed normalised histoarchitecture similar to the control group, while the diabetic + Metformin group displayed minor disruptions. All groups except the diabetic group displayed normal reactions to PAS staining. In conclusion, beetroot extract demonstrated hepatoprotective effects, mitigating diabetes-related liver damage and weight loss in male Wistar rats.

Keywords: Streptozotocin; Liver; Diabetes; Wistar Rats; Beetroot

1. Introduction

Diabetes mellitus is a medical condition characterised by abnormal metabolism of proteins, carbohydrates, and fats, leading to high blood glucose and lipid levels. The most common subtypes are type 1 and type 2 diabetes mellitus, though other forms exist [1]. Type 1 diabetes affects 5-10% of individuals with diabetes and is caused by dysfunction of beta cells, resulting in decreased insulin production and low levels of circulating insulin [2]. Symptoms typically manifest during childhood or adolescence but can appear later in life as well. Conversely, type 2 diabetes mellitus accounts for about 90% to 95% of diabetes cases globally [2]. It is primarily marked by insulin resistance in peripheral tissues and an insufficient insulin response. Obesity, especially abdominal fat accumulation, is a common trait among patients with type 2 diabetes. Both genetic and environmental factors play a role in its epidemiology, with genetics becoming increasingly significant in the context of sedentary lifestyles and high-calorie dietary habits [3]. Key organs involved in developing type 2 diabetes include the liver, skeletal muscle, kidneys, brain, small intestine, adipose tissue, and pancreas. Less common forms of diabetes include gestational diabetes, maturity-onset diabetes of the young, latent autoimmune diabetes in adults, and secondary diabetes, which can result from other medical conditions, such as

* Corresponding author: Uthman Ademola Yusuf

pancreatitis, or as a side effect of medications like corticosteroids [4]. The liver, comprising about 2.5% of the body weight, is vital for nutrient metabolism and waste elimination [5]. It plays an essential role in producing bile for lipid digestion and absorption in the small intestine and regulating coagulation through the manufacture of vitamin K and clotting factors [6]. Recently, there has been increasing interest in herbal and complementary therapies, particularly those utilising medicinal plants rich in antioxidants, to help manage diabetes by reducing inflammation [7]. Beetroot has emerged as a popular functional food due to its various biological activities and potential therapeutic benefits against conditions such as hypertension, depression, inflammation, infections, and diabetes [8]. Some studies indicate that beetroot extract may possess hepatoprotective and anti-diabetic properties [9]. Consequently, this study aims to evaluate the effects of beetroot extract on the liver of Wistar rats with induced diabetes mellitus.

2. Material and methods

2.1. Plant materials

The Beetroot was harvested from the Livingstone district, Southern Province of Zambia. It was subjected to identification at the University of Zambia, School of Natural Sciences, under the Department of Biological Sciences before the study began. The Beetroot was air-dried and pounded. The dry pounded Beetroot was then ground and sieved to obtain a homogeneous powder. The extraction was done using methods described by Borjan et al [10].

2.2. Animals and animal management

Thirty adult, presumably healthy male Wistar rats (Rattus norvegicus) were used for this study. The animals were between 8 to 10 weeks old, with a body weight of 160-200 g. They were kept in five cages (6 rats per cage) and housed in the animal holdings of the Department of Anatomy, Mulungushi University School of Medicine and Health Sciences. The rats were maintained on standard animal feeds (Wealth-gate pelletized feeds) and allowed free access to clean water and feed (ad libitum).

2.3. Induction of diabetes.

Streptozotocin (STZ) was used to induce diabetes. Rats were weighed, and a baseline glucose level was established following an overnight fasting period. The animals were injected with streptozotocin, calculated at a dose of 70 mg/kg body weight, and then returned to their normal feeding cycle [11]. It took about 72 hours for diabetes to be established in the animals following the administration of streptozotocin. Subsequently, a fasting blood sample was collected to confirm the establishment of diabetes using a tail vein puncture. A glucometer was used to assess blood glucose levels, with animals considered diabetic if fasting blood glucose levels exceeded 7 mmol/L (≥ 250 mg/dL).

2.4. Experimental design

Thirty adult healthy male Wistar rats were divided into five groups of six (6) Wistar rats each. Control Group A consisted of normoglycemic animals that received neither STZ nor beetroot extract. Group B was diabetic without receiving beetroot extract or metformin. Group C was diabetic treated with beetroot extract only. Group D was diabetic treated with Metformin only, and Group E received beetroot extract only.

2.5. Beetroot mode of administration

The dose of the aqueous extracts of Beetroot used in these studies was adopted from a report by [12]. Beetroot was dissolved in physiological saline daily and administered orally using an oro-gastric cannula to Group C rats (n=6) at a dosage of 500 mg/kg body weight (bw) at 9:00 – 10:00 a.m. each day for a maximum period of four weeks. Group D (n=6) received 100 mg/kg bw of metformin, and Group E rats (n=6) were administered 500 mg/kg bw of beetroot extracts. Group A rats (n=6) received neither STZ nor beetroot aqueous extract.

2.6. Measurement of blood glucose

Blood glucose levels were evaluated after overnight fasting the rats at 9:00 – 10:00 hours, using the Glucose oxidase method with a One Touch Ultra 2 glucometers (Accu-Chek Compact Plus). Blood samples were obtained from the median caudal vein of the tail by snipping the tip. The blood glucose level was monitored weekly for one week (acclimatisation period) before the induction of diabetes and monitored for four weeks during treatment [13].

2.7. Measurement of body weight (g)

Body weight (g) of the rats was recorded for one week (acclimatisation period) before the induction of diabetes and every week during the experimental treatment for four weeks. Body weight was measured using a weighing scale (Venus VT 30 SL) [13].

2.8. The relative organ weight (%)

The relative weight of the liver of the rats was evaluated as the ratio of the liver's respective weight to the terminal body weight of the same rat, recorded as a percentage (%) using a sensitive weighing balance (SonyF3G brand) [13].

2.9. Histological process.

At the end of the study, animals were sacrificed by euthanasia. They were laid supine on the dissecting board and pinned through the fore and hind paws. The abdomen of the animals were dissected using scalpel and surgical blade, and each organ was carefully removed and weighed. Tissues were fixed in freshly prepared formo saline for 72 hours and processed for routine histological examinations, stained with Haematoxylin and Eosin (H&E) to observe changes in cellular morphology. Periodic acid-Schiff (PAS) stain was also used to checked for the presence of the glycogen.

2.10. Photomicrography

Photomicrographs of histological sections of the liver were taken with an Olympus Microscope (New York, United States of America) coupled with a camera at the Department of Human Anatomy, Mulungushi University School of Medicine and Health Sciences, Livingstone Campus, Zambia.

2.11. Data analysis.

Data were presented as mean ± standard error of the mean (mean ± SEM) and analysed using one-way ANOVA. All graphs were drawn using Excel (Microsoft Corporation, U.S.A.). P values less than 0.05 ($p < 0.05$) were considered statistically significant.

3. Results

3.1. Average blood glucose levels on weekly basis

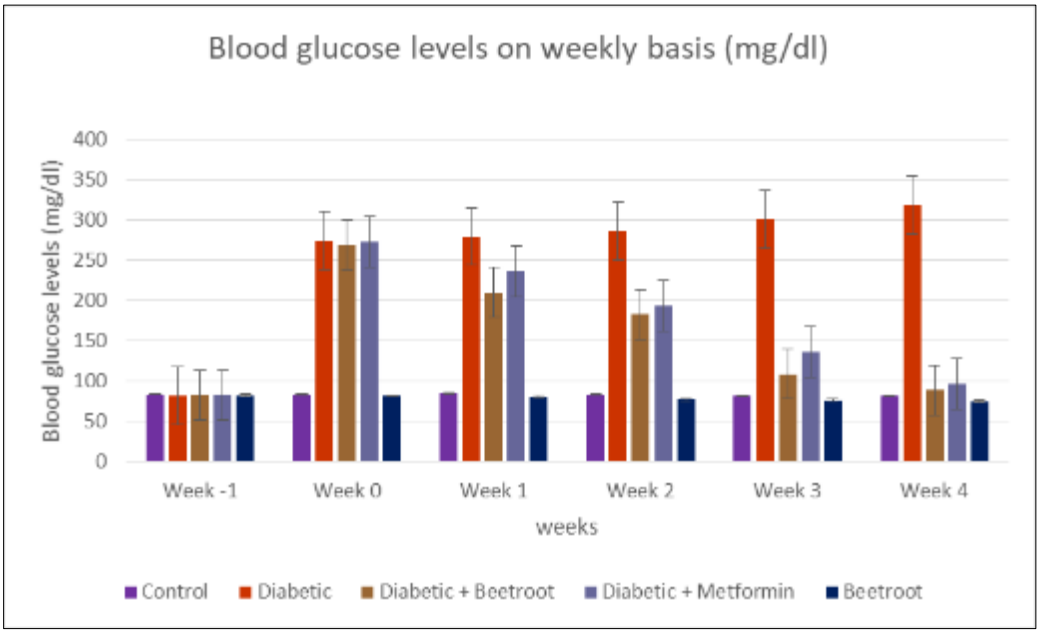

Figure 1 Blood glucose levels of Wistar rats on weekly basis. Data were expressed as mean ± SEM ($p<0.05$)

The average blood glucose levels were monitored weekly across different groups of Wistar rats. During the acclimatisation week (week-1), all rats were normoglycemic. The control and beetroot-only groups maintained stable blood glucose levels throughout the study. In week 0, which was the induction week, the diabetic group, as well as the

diabetic + beetroot and diabetic + metformin groups, showed elevated blood glucose levels. By week 1, the diabetic + beetroot group began to show a decrease in blood glucose levels, which was statistically significant ($p<0.05$) when compared to the control and beetroot-only groups. A similar decrease was observed in the diabetic + metformin group. In week 3, the diabetic + beetroot group's blood glucose levels were approaching the normal range, and when compared to the control and beetroot-only groups, the differences were no longer statistically significant ($p>0.05$). In contrast, the diabetic + metformin group still exhibited statistically significant differences ($p<0.05$). By week 4, all groups returned to normal blood glucose levels except the diabetic group.

3.2. Average body weight on weekly basis

At the start of the experiment, the acclimatisation week (week-1), the average body weight of all the Wistar rats from the various groups was nearly identical, with only a negligible difference observed. In week 0, during the induction phase, the control group showed the highest increase in weight, followed closely by the metformin groups, though no significant differences were noted across all groups. By week 2, a gradual decrease in weight was observed in the diabetic group, while the other groups continued to show weight increases. The comparison between the diabetic and control groups was statistically significant ($p<0.05$), but the comparison with the beetroot and metformin groups did not show significant differences ($p>0.05$). In week 3, the weight decrease in the diabetic group became statistically significant ($p<0.05$) compared to all other groups, and this trend continued up to week 4, indicating a consistent relationship throughout the experiment.

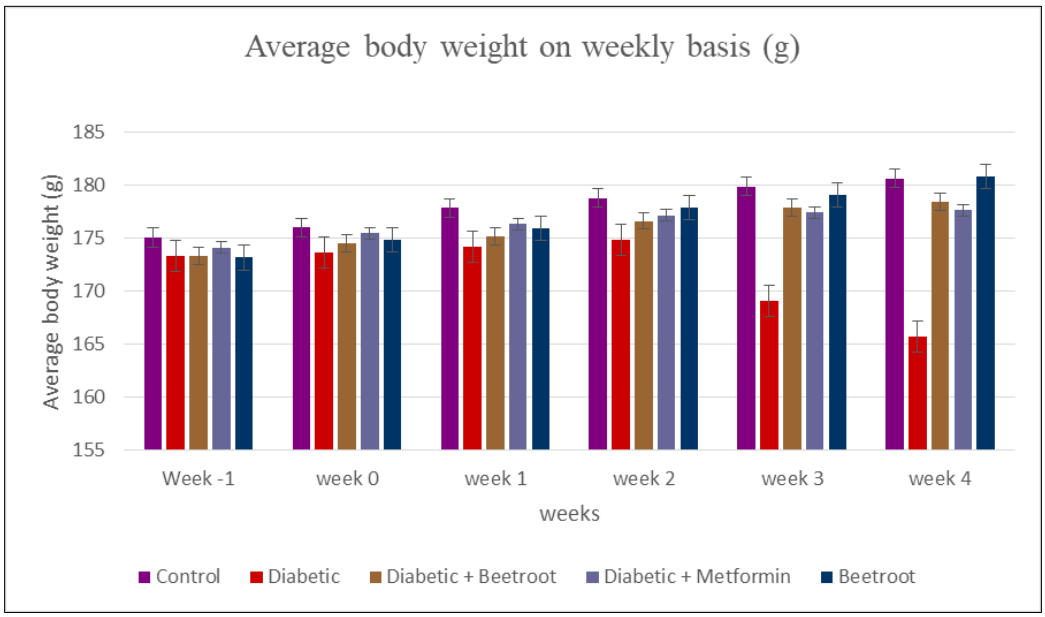

Figure 2 Average body weight on weekly basis. Data were expressed as mean ± SEM ($p<0.05$)

3.3. Relative weight of the liver (%)

Figure 3 presents the relative weight of the liver among different groups. The findings indicate that the diabetic group had the least liver weight, followed by the diabetic + metformin group, the diabetic + beetroot group, the beetroot-only group, and finally the control group. There was a statistically significant difference in the relative liver weight of the diabetic group compared to all other groups ($p<0.05$). However, the differences between the diabetic + beetroot and diabetic + metformin groups compared to the control and beetroot-only groups were not statistically significant ($p<0.05$).

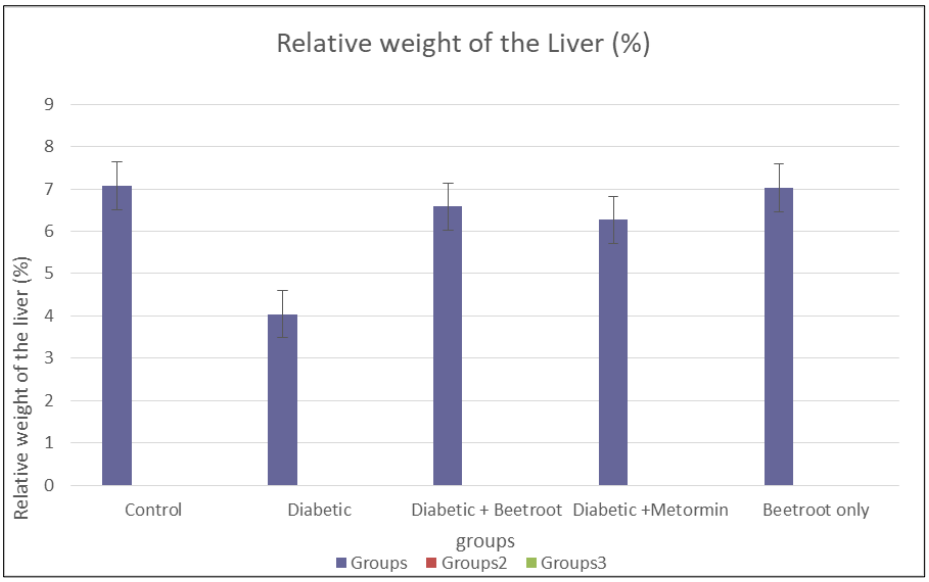

Figure 3 blood glucose levels of Wistar rats on weekly basis. Data were expressed as mean ± SEM ($p<0.05$)

3.4. Histology of the liver

The liver in the normal control and beetroot groups exhibited normal histoarchitecture with numerous healthy hepatocytes (Fig. 4 A and E). In contrast, the diabetic group displayed disrupted histoarchitecture characterised by many necrotic hepatocytes (Fig. 4 B). The diabetic+beetroot group demonstrated a histoarchitecture similar to that of the control group (Fig. 4 C), while the diabetic+metformin groups exhibited minimal disruption, containing both normal and necrotic hepatocytes (Fig.4 D). The normal control, beetroot only, diabetic+beetroot, and diabetic+metformin groups showed a normal reaction (Fig. 5 A, C, D, and E), while the diabetic group displayed a positive reaction to the PAS stain (Fig.5 B).

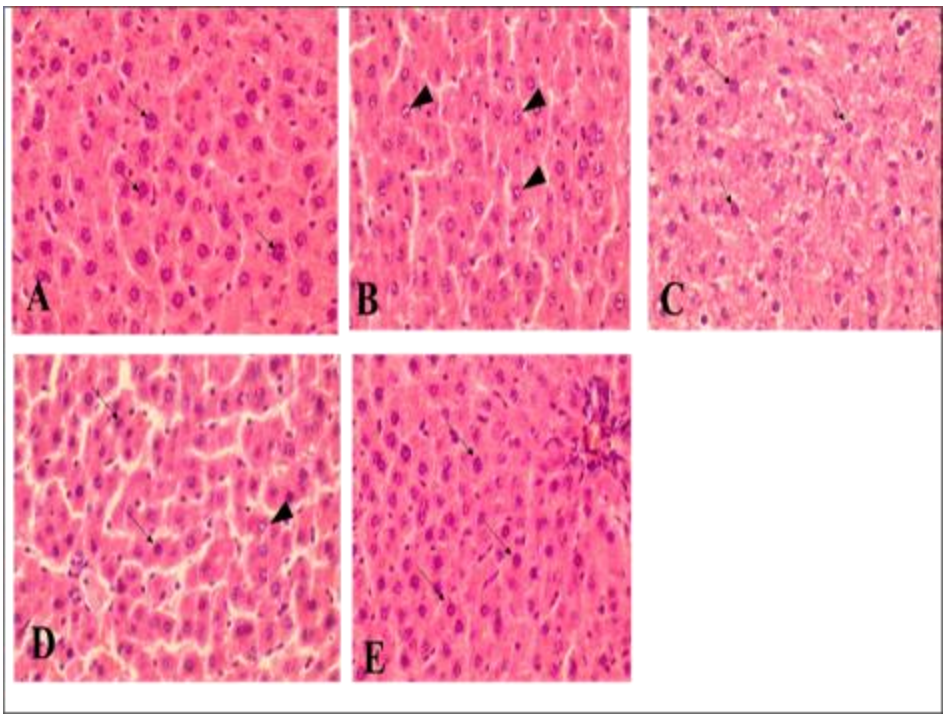

Figure 4 Photomicrograph showing the Liver at day 28. H&E stain X400. A- Normal control, B – Diabetic, C – Diabetic+beetroot, D – Diabetic+Metformin and E- Beetroot only. Arrow – hepatocyte, Arrow head – Necrotic hepatocyte

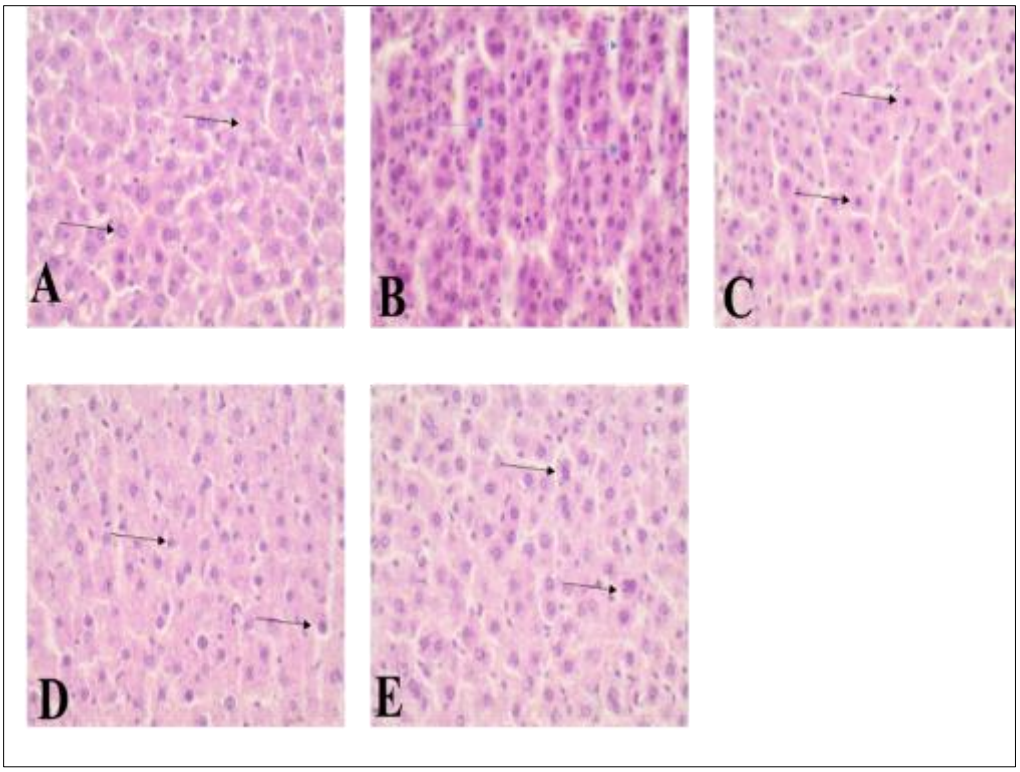

Figure 5 Photomicrograph showing the Liver at day 28. PAS stain X400. A- Normal control, B – Diabetic, C – Diabetic+Beetroot, D – Diabetic+Metformin and E- Beetroot only. Arrow – hepatocyte, deep coloration indicates positive PAS reaction

4. Discussion

Diabetes mellitus has been associated with multiple tissue damage and significantly contributes to the development of non-alcoholic fatty liver disease (NAFLD). In contrast, beetroot is gaining attention for its hepatoprotective and hypolipidemic properties in rats and rabbits subjected to high-cholesterol diets [12]. This study aimed to evaluate the effects of beetroot aqueous extract on the liver of Wistar rats with diabetes induced by streptozotocin.

As shown in Figure 1, Wistar rats in the diabetic group exhibited persistently elevated blood glucose levels throughout the experiment. This elevation is attributed to the damage inflicted by streptozotocin on pancreatic beta cells, resulting in inadequate insulin production [9]. Remarkably, in the diabetic + beetroot group, blood glucose levels began to normalise by week 3 and achieved normoglycemia by week 4. This improvement can be linked to the insulin sensitisation, antioxidant activity, and hypoglycemic effects of beetroot [12]. Conversely, the metformin-treated group remained diabetic until week 3 and only reached normoglycemia in week 4, indicating that beetroot's efficacy may stem from its higher concentration of beneficial biochemical constituents [14]. Numerous studies have underscored beetroot's ability to enhance nitric oxide production, a compound that is often deficient in insulin-resistant diabetes [15]. Refaat and El-Nassag [16] also highlighted that nitric oxide improves insulin signalling and promotes glucose transporter 4, suggesting that increasing nitric oxide could effectively improve insulin sensitivity and reduce blood glucose levels. Furthermore, Yusuf et al. [17] reported beetroot's capacity to stimulate insulin production and secretion through beta cell activation by betanin, aligning with the findings of the present study.

In Figure 2, the diabetic group showed a progressive weight loss from week 3 until the end of the study. This phenomenon is due to diabetes impairing glucose utilisation by cells, prompting a catabolic response involving fat and protein degradation, ultimately leading to muscle wasting [9]. Conversely, the diabetic + beetroot group maintained their body weight throughout the experiment, likely due to beetroot's ability to enhance insulin sensitivity in cells, thus preventing reliance on adipose tissue and muscle protein for energy [18]. Although the diabetic + metformin group displayed a diminished rate of weight gain after week 3, their overall weight increased, highlighting that metformin has a limited impact on peripheral insulin sensitivity [19]. These findings corroborate the observations of Pang et al. [20], who noted that inadequate insulin secretion from pancreatic beta cells reduced glucose uptake in cells, causing energy sourcing from tissue proteins and, consequently, weight loss. Additionally, as reported by Pu et al. [21], metformin's

action does not inhibit body weight reduction and is being explored as a potential weight loss agent. Beetroot's content of betalains, which prevent glycogenolysis and improve blood glucose levels, also plays a role in inhibiting adipose tissue breakdown [17]. Figure 3 illustrates that the diabetic group had the lowest relative liver weight, indicating liver damage caused by diabetes [4]. However, the diabetic + beetroot group did not show a statistically significant difference in liver weight compared to the control group ($p<0.05$). This may be attributed to beetroot's protective effects against oxidative stress and its role in reducing lipid accumulation, thereby preserving liver integrity [12]. These findings contrast with those reported by Eluehike and Onoagbe [22], who found an increase in liver weight among their diabetic subjects. However, our results align with Abd El-Ghffar et al. [7], who observed that beetroot positively influenced anabolic processes and improved both glucose and lipid metabolism, contributing to its hepatoprotective properties.

Histological assessment of the liver utilising H&E staining revealed normal histoarchitecture in both the control and beetroot groups, characterised by abundant hepatocytes. The diabetic + beetroot group also exhibited a similar histological profile, suggesting that beetroot effectively reconstructed the liver and prevented further damage [23]. In contrast, the diabetic + metformin group displayed minor disruptions in their liver architecture, with both normal and necrotic hepatocytes. This discrepancy indicates that metformin may not adequately repair liver damage resulting from diabetes mellitus [19]. The diabetic group demonstrated significant architectural disruption with numerous necrotic hepatocytes, attributable to the toxic effects of diabetes on the liver [18]. These results are consistent with prior studies documenting the hepatoprotective effects of beetroot, which has been shown to reduce liver enzyme levels and lipid accumulation. Elevated blood glucose levels lead to the generation of reactive oxygen species (ROS), causing oxidative stress that triggers inflammation and, ultimately, hepatocyte damage [24]. Similarly, Mahmoud Abbas [9] confirmed that beetroot protects the liver by its high concentrations of polyphenols and flavonoids, which impart potent antiradical properties. Findings parallel to those from Sui et al. [25] revealed that metformin has minimal effects on lipid levels and hepatocyte degeneration, consistent with our observations. In the PAS reaction assessment, the control, beetroot-only, diabetic + beetroot, and diabetic + metformin groups exhibited normal responses, while the diabetic group demonstrated a positive PAS reaction. This response indicates increased glycogen accumulation due to diabetes mellitus [18], aligning with findings by Ashcroft et al. [26] and [27] which attributed elevated glycogen levels to impaired glycogenolysis, glycosylation of proteins, and tissue damage resulting from hyperglycemia.

5. Conclusion

This study established the hepatoprotective effects of beetroot, evidenced by reductions in blood glucose levels and the preservation of normal histoarchitecture in liver tissue. Additionally, beetroot played a significant role in preventing body weight loss in diabetic Wistar rats. These findings suggest that beetroot has the potential to mitigate diabetes-related mortality and morbidity.

Compliance with ethical standards

Acknowledgments

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Disclosure of conflict of interest

No conflict of interest to be disclosed.

Statement of ethical approval

The ethical approval and permission for the study was obtained from Mulungushi University School of Medicine and Health Sciences Research ethic committee

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