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Research Article

Aloe vera Gel Ameliorates Fat-Rich and High Fructose (FRHF) Diet-Induced Pancreatic and Splenic Damage in Mice

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Abstract

High-fat diet alone or in combination with high fructose has been known to induce diabetes, obesity, hypertension, and immune dysfunction. The study evaluates the role of *Aloe vera* in fat-rich and high fructose diet-induced (FRHFD) hyperglycemias in addition to testicular and splenic morphology in mice. Twenty BALB/c Mice were randomly distributed into four groups (n=5). The groups were fed on a normal diet, FRHFD, FRHFD + 10 g *A. vera*, and FRHFD + 20 g *A. vera* for 10 weeks. All the mice were sacrificed a day after the 10 weeks of treatment. The result showed that mice fed on FRHFD plus *A. vera* had a significantly lower ($p<0.05$) blood glucose level relative to the FRHFD-fed mice. The mice fed on FRHFD plus *A. vera* had a significantly lower ($p<0.05$) blood glucose level relative to the FRHFD-fed mice. *Aloe vera* was found to ameliorate FRHFD-induced pancreatic islet and acini damage. It also prevented distorted lymphoid cells and testicular damage induced by FRHFD. *Aloe vera* prevents hyperglycemia and protects pancreatic islets in FRHFD-fed mice. It further prevents immune dysfunction and protects against testicular damage. Hence, *A. vera* supplementation could be an alternative and/or complementary therapy for hyperglycemia-related disorders.

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INTRODUCTION

Obesity is one of the common non-communicable diseases with an alarming global health concern[1]. It is caused by an imbalance between energy intake and expenditure, while fat-rich and high-calorie diets plus a sedentary lifestyle are the main driving forces[2]. Obesity is associated with many comorbidities, such as diabetes, immune dysfunction, infertility, dyslipidemia, and hypertension[2-4]. Excessive fat accumulation has been reported to induce insulin resistance, hyperglycemia, and hyperlipidemia[5,6]. Adipocytes reduce glucose uptake in peripheral tissues by realizing free fatty acids. Hence, obesity is a significant cause of diabetes-related insulin resistance and hyperglycemia[7].

Obesity is the primary cause of type 2 diabetes, as individuals with BMI ≥ 35 kg/m^2 are more than 90 times at risk of diabetes[8]. Previous studies[9,10] have connected obesity to menstrual dysfunction and ovulatory disorders in females with decreased sperm count and motility and defective Leydig cells in males. Obesity is also associated with lower plasma testosterone[11]. An earlier report has shown an altered immune response in obesity, leading to metabolic complications[12]. Another study[13] also reported increased susceptibility to infection in obese humans and animals. The changes in immune cells contribute to the progress of obesity-related complications and further affect innate and adaptive immune responses[14]. High-fat diet alone or in combination with high fructose has been known to induce diabetes, obesity, hypertension, and liver diseases by promoting fat uptake principal to weight gain and insulin resistance[15,16].

Aloe vera is an evergreen perennial succulent plant widely used in conventional and orthodox medicine to treat skin ailments, constipation, digestive disorders, and poor appetite[17,18]. *Aloe vera* has numerous bioactive compounds and is used in Africa and Asia as an antiseptic, antioxidant, and antibacterial in treating and preventing diabetes and obesity-related complications[19,20]. A previous study[21] suggests that *A. vera* has anti-hyperglycemic potentials and antioxidant properties and prevents dyslipidemia. The present study evaluates the role of *A. vera* in fat-rich and high fructose (FRHF) diet-induced hyperglycemia in addition to testicular and splenic morphology in mice.

MATERIALS AND METHODS

Materials

Aloe vera was collected from a garden at the University of Maiduguri. It was authenticated and deposited at the Faculty of Pharmacy herbarium (UMM/FPH/ASH/001). Other materials include formalin, ketamine injection, hematoxylin, and eosin stains (H & E), glucometer (AccuChek, Roche, Switzerland), paraffin wax, dibutyl phthalate polystyrene xylene (DPX), rotary microtome (Leica RM2125), microscope (Olympus BH2), and microscope camera (AmScope M500).

Methods

Diet formulation

Fresh *A. vera* gel was collected from the plant daily and used to formulate the diet. The normal diet contains 4% fat, 15% protein, and 6% fiber. Fat-rich and high fructose diet (FRHFD) contains a normal diet and margarine in a 7 : 3 ratio and 15% fructose in drinking water. The *A. vera* supplemented diet was constituted in two forms; the first contains 100 g of FRHFD plus 10 g *A. vera* gel (FRHFD 1AV), while the second contains 100 g of FRHFD plus 20 g *A. vera* gel (FRHFD + 2AV).

Ethics statement

The study was approved by the Human Anatomy Ethical Committee, University of Maiduguri (UM/HA/PGR20.21-08800). It was carried out according to the ARRIVE guidelines and the National Institute of Health Guide for the Care and Use of Laboratory Animals. All the dissections were carried out under ketamine anesthesia to minimize suffering.

Experimental design

Twenty BALB/c strains of mice (18–21 g) were randomly distributed into four groups, each consisting of five mice. The groups were fed a normal diet (Chikun Feed, Nigeria), FRHFD, FRHFD +1AV, and FRHFD +2AV for 10 weeks. All the mice were marked for identification and sacrificed a day after 10 weeks of treatment. The blood glucose level of all the mice was measured using a glucometer (AccuChek, Roche, Switzerland). Also, the pancreas, spleen, and testicles were dissected,

fixed in 10% formalin, processed for light microscopic observation, and stained with hematoxylin and eosin. The micrographs were observed at x100 and x200 magnifications.

Data analysis

The data were analyzed with GraphPad Prism 9.0 (San Diego, USA). One-way analysis of variance (ANOVA) followed by Sidak multiple comparisons was conducted, and statistical significance was considered at $p < 0.05$. The results were expressed as mean ± SE (standard error).

RESULTS AND DISCUSSION

Blood glucose level

The blood glucose level of all the mice was measured using a glucometer. One-way analysis of variance followed by Sidak multiple comparisons was conducted, and the results were presented in **Figure 1**. A significant increase ($p < 0.05$) in blood glucose levels was observed in FRHFD-fed mice compared to the control. The mice fed on FRHFD plus *A. vera* had a significantly lower ($p < 0.05$) blood glucose level relative to the FRHFD-fed mice. No significant change ($p > 0.05$) was observed in the blood glucose level when FRHFD plus *A. vera*-fed mice were compared with the control.

While a high-fructose diet alone was believed to induce hyperglycemia due to insulin resistance, a combination of fructose and high fat was reported to induce diabetes[22]. The present study also shows that a fat-rich and high-fructose diet induces pancreatic islet damage and hyperglycemia. Another previous report[23] highlights the ability of fructose to impair insulin action in both humans and animals by impairing fatty acid oxidation, triggering inflammation, and promoting *de novo* lipogenesis. *Aloe vera* prevents pancreatic islet cell damage and hyperglycemia in FRHFD-fed mice. The previous report has shown that *A. vera* could prevent metabolic disorders by enhancing insulin sensitivity and preventing oxidative stress[21,24]. We suggest that *A. vera* prevents hyperglycemia by preventing pancreatic islet damage and improving insulin sensitivity.

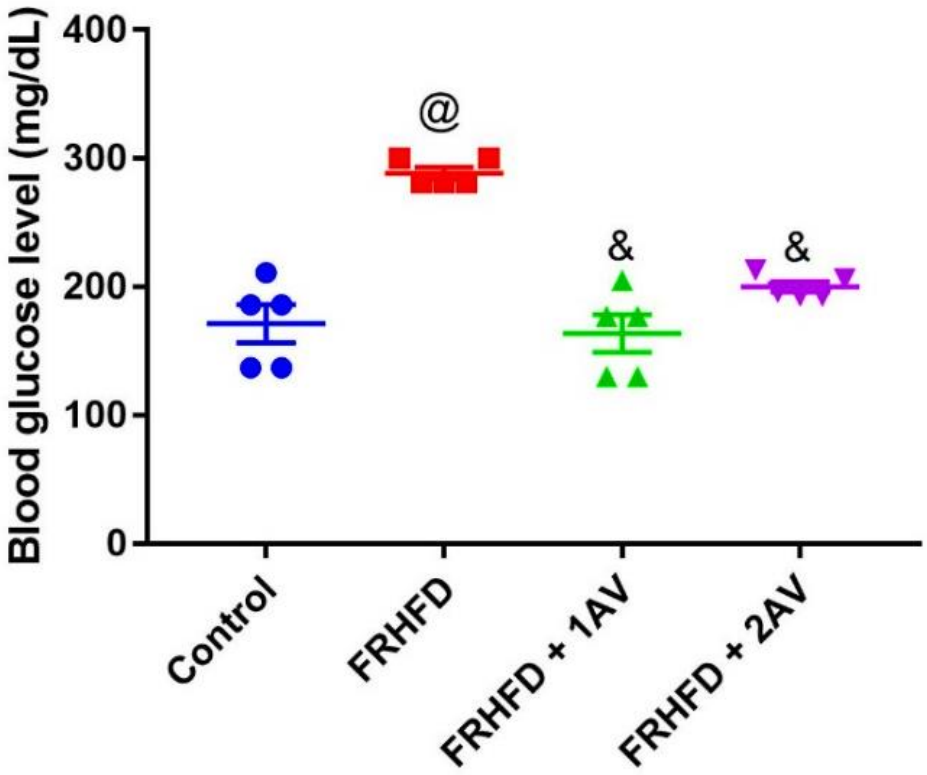

Figure 1. The blood glucose level of fat-rich and high fructose diet plus *A. vera*-fed mice. Data presented as Mean±SEM, n=5. SEM= standard error of the mean, FRHFD= fat rich and high fructose diet, FRHFD +1AV= fat rich and high fructose diet plus *A. vera* (10 : 1), FRHFD +2AV= fat rich and high fructose diet plus *A. vera* (10:2).

Histological study

The pancreas, spleen, and testicles were dissected, fixed in 10% formalin, processed for light microscopic study, and stained with H & E. The micrographs were observed at x100 and x200 magnifications and presented in **Figure 2**. The pancreas of control mice revealed normal pancreatic islets and acinar cells (**Figure 2a1**). The pancreas of FRHFD-fed mice revealed degenerating acini and islets (**Figure 2b1**). Feed supplementation with *A. vera* ameliorates FRHFD-induced pancreatic islet and acini damage. This was observed by the normal islet and acini found in the pancreas of FRHFD plus *A. vera*-fed mice (**Figure 2c1** & **2d1**).

The spleen of control mice showed normal lymphoid cells with visible white and red pulps (**Figure 2a2**). The spleen of FRHFD-fed mice revealed irregular aggregation of lymphoid cells with no clear demarcation between the white and red pulps, as the whole spleen is densely filled with lymphoid cells (**Figure 2b2**). The spleen of mice fed on FRHFD +1AV showed normal lymphoid cells comparable to the control with clear white and red pulps, while those of mice fed on FRHFD +2AV showed densely packed lymphoid cells with no demarcation between white and red pulps (**Figure 2c2** & **2d2**). The testicles of control mice showed normal seminiferous tubules with spermatozoa within the lumen (**Figure 2a3**). The testicles of mice fed on FRHFD showed spermatogonia instead of spermatozoa within the lumen (**Figure 2b3**). *Aloe vera* supplementation was shown to protect the testicles from FRHFD-induced damage by the presence of spermatozoa within the seminiferous tubular lumen (**Figure 2c3** & **2d3**).

The high-fat diet was also reported to induce immune dysregulation and inflammation[25]. Excessive fructose intake was reported to disrupt the immune system by altering the gut microbial structure and intestinal permeability[26]. Free fatty acids, lactate, and uric acid are the fructose metabolites believed to induce oxidative stress and alter the immune response[27-29]. The present study showed that *A. vera* prevented the irregular arrangement and distribution of lymphoid cells in the spleen of mice. The possible mechanism through which *A. vera* prevents immune dysfunction in FRHFD-fed mice is by promoting the breakdown of fructose into less harmful substances for excretion.

A diet rich in fat was previously reported to alter carbohydrate metabolism and reduce cell proliferation, eventually affecting spermatogenesis[30]. It was also reported to induce mitochondrial and testicular damage[31,32]. Hyperinsulinemia and decreased insulin sensitivity due to high-fat diet consumption were reported to be the probable cause of Leydig cell dysfunction and testicular damage[33]. The current study found *A. vera* to prevent spermatogonial cell damage in FRHFD-fed mice. We hypothesized that *A. vera* prevents testicular by promoting insulin sensitivity, enhancing Leydig cell function, and stimulating carbohydrate metabolism.

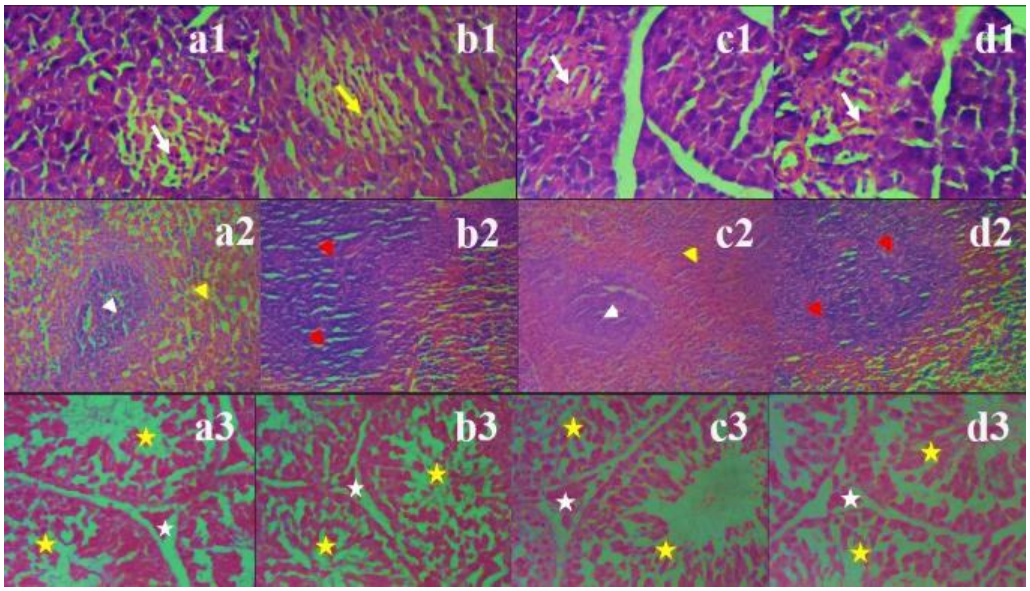

Figure 2. Photomicrographs of the pancreas, spleen, and testicles of mice showing normal pancreatic islets (white arrows), degenerating pancreatic islets (yellow arrow), white pulp of spleen (white arrowheads), red pulp of spleen (yellow arrowheads), irregular dense lymphocytes aggregation (red arrowheads), seminiferous tubules (yellow stars), and interstices (white stars). a=control, b=mice fed on FRHFD, c=mice fed on FRHFD +1AV, d=mice fed on FRHFD +2AV. H&E stain x200 magnification. FRHFD=fat rich and high fructose diet, 1AV A. vera (10:1), 2AV=A. vera (10:2).

CONCLUSION

The supplementation of a diet with *A. vera* was found to prevent hyperglycemia and protect pancreatic islets in FRHFD-fed mice. It further prevents immune dysfunction and protects against testicular damage. Hence, *A. vera* supplementation could be an alternative and complementary therapy for hyperglycemia-related disorders.

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AUTHORS' CONTRIBUTION

Conceptualization and design: All authors. Experimental procedure: UI, MMB, MGA, UAA, SJM, FMA, & MMS. Photomicrographs: SJM, NID, & SMC. Data analysis and interpretation: All authors. Supervision: NID, ZMG, MOOA, Writing initial draft: SJM & MNG. Review: NID & SMC, Final approved: All authors.

DATA AVAILABILITY

None.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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