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ORIGINAL RESEARCH article

Effect of resveratrol on total protein and albumin in type 2 diabetes wound healing in rats

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Abstract: Resveratrol, a polyphenol predominantly present in red grapes, has attracted interest due to its possible health advantages. The anti-inflammatory, antioxidant, and vasodilatory properties of this substance indicate that it may have a beneficial effect on wound healing in individuals with diabetes mellitus. This study aims to determine the role of resveratrol on type 2 diabetic wound healing on total protein and albumin levels in rats. 20 male adult Albino Wistar rats were rendered diabetic using a high-fat diet and an alloxan injection (120 mg/kg). The rats were grouped into four: non-diabetic control group (negative control), diabetic control group (positive control), diabetic treatment group 1 (resveratrol: 10 mg/kg) and diabetic treatment group 2 (resveratrol: 20 mg/kg). Excisional wounds were created and monitored for wound closure over a defined treatment period of 14 days. Studies were conducted and expressed using physical and biochemical indices. The data demonstrated wound healing activities *via* biochemical indices, and histological and macroscopic methods. There was a difference in fasting blood glucose between the diabetic control group with the treatment groups. There was a difference between the diabetic control group compared to the group treated with resveratrol 10 mg/kg and 20 mg/kg in the weight of the rats. There was no significant acceleration in total albumin and globulin levels in the diabetic wounded group treated with resveratrol (10 mg and 20 mg). The results suggest that resveratrol treatment does not affect on total albumin and globulin levels in diabetic rats.

Introduction

Diabetes mellitus (DM) is become a frequent chronic condition. The newest research shows that about 500 million people globally have DM or one in 10 adults. The global DM prevalence rate for 20 - 79-year-olds was 10.5% (536.6 million) in 2021 and will climb to 12.2% in 2045 [1]. Chronic DM skin ulcers are one of many diabetes consequences that depend on type, onset time, and metabolism regulation. Diabetic foot ulcers (DFUs) are associated with high mortality in DM, according to epidemiological research. Death rates reach 39.0%-68.0% within five years of amputation [2]. Diabetic patients experience a reduced capacity to metabolize glucose, leading to hyperglycemic circumstances that further impede the process of wound healing. This can lead to the

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development of persistent chronic wounds [3, 4]. Diabetic wounds are characterized by delayed healing, increased risk of infections, and poor tissue regeneration due to factors such as hyperglycemia-induced oxidative stress and inflammation [5]. The majority of diabetes-related deaths, over 80.0%, take place in nations with low- and intermediate-income levels, with diabetes wounds accounting for 50.0%-70.0% of all limb amputations [6]. It has been stated that one leg is amputated every 30 seconds worldwide owing to diabetes wounds. Age-related physiological changes such as poor blood circulation, obesity, DM, and environmental stress are the main concerns. Chronic and acute wounds are classified by healing capability. Chronic wounds require more than 12 weeks to heal and do not heal in stages [7]. Nutrition therapy is crucial for DM management and complications. Poor nutrition before or during healing can slow and weaken wounds [8]. Protein, an essential macronutrient, is crucial for wound healing as it is required for all stages of the healing process [9]. This includes the development of fibroblasts, the synthesis of collagen, the creation of new blood vessels, and the proper functioning of the immune system [10, 11]. Resveratrol is a polyphenolic substance that is naturally found in plants. It is also present in other plants and food items, including peanuts, grapes, and red wine. Resveratrol exhibits several pharmacological effects, principally functioning as an anti-inflammatory, antioxidant, anti-apoptotic, and overall cytoprotective agent [12]. The abovementioned effects have mostly been reported in preclinical research. However, other studies have evaluated the antidiabetic effects of resveratrol and the associated problems [13], and many aspects of its impact on blood indices, and biochemical, and molecular impacts are still under investigation. Conventional treatment approaches often fall short of effectively addressing the complications of diabetes type 2, especially diabetic wound healing while mitigating the adverse effects [14, 15]. This study aims to investigate the effect of resveratrol on the total protein and albumin in type 2 diabetes mellitus wound healing in rats.

Materials and methods

Experimental animals: 20 adult male Wistar rats with body weights of 150 gm to 200 gm were purchased from the Animal House of the Department of Human Physiology, Faculty of Basic Medical Sciences, Ahmadu Belo University, Zaria, Nigeria. Before the experiment, the rats were acclimated in a well-lit room for one week on a 12/12 light-dark cycle. Before the experiment, the rats had access to commercial pellet feed and water (ad libitum).

Ethical Approval: The study followed Ahmadu Bello University's guidelines for using laboratory animals, as accepted internationally by the National Institute of Health. Ethical approval on guidelines for the care and use of laboratory animals in scientific research was sought from the Ahmadu Bello University Committee on Animal Use and Care (ABUCAUC) with the approval number ABUCAUC/2023/105.

Induction of diabetes mellitus: We modified Sen et al.'s method for high-fat diet (HFD) preparation and type 2 diabetes induction [16]. NDF: 18.0% fats, 28.0% proteins, 54.0% carbohydrates; Simas margarine: 99.9% fats. Mixing 20 gm NDF and one-gram Simas margarine yielded HFD. After six weeks of HFD, the rats were fasted overnight and injected intraperitoneally with 120 mg/kg alloxan diluted in 0.1 M citrate-buffered saline (pH 4.5) [16]. Right after alloxan administration, rats were given 5.0% glucose water. Rats with fasting blood glucose levels ≥ 200 mg/dL were diagnosed with diabetes 72 hours after alloxan administration and validated one week later the establishment of the diabetic wound model. After rat diabetes was confirmed, the upper back was shaved using a small animal clipper and examined for skin abnormalities. After disinfecting with methylated spirit, rats were sedated with intraperitoneal ketamine injection (75.0 mg/kg and diazepam 5.0 mg/kg). A full-thickness circular excisional wound was excised from the back of all rats by surgical blade -10 mm x10 mm [17].

Experimental design: The rats were randomly divided into four groups as shown in **Table 1**.

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Table 1: Experimental groups

Group, n = 5	Induction	Treatment
I	Experimental control group (wound)	No treatment (negative control group)
II	Experimental control group (diabetic & wound)	No treatment (positive control group)
III	Experimental group (diabetic & wound)	Resveratrol 10 mg/kg body weight (i.p.) [18]
IV	Experimental group (diabetic & wound)	Resveratrol 20 mg/kg body weight (i.p.) [18]

Measurement of wound healing area: Digital venire calipers marked the wound healing area reduction. In wound healing, wound contraction was measured as a percentage of the healed area: wound healing ability = (F0 - F7, F14) per F0 by 100%, where F0 is the primary wound area and F7, 14 are the wound areas on days 7 and 14 [19].

Sample collection: The rats were starved overnight and anesthetized with 75.0 mg/kg ketamine hydrochloride and 5.0 mg/kg diazepam after two weeks of therapy. The serum used for biochemical indices was collected by cardiac puncture with 5.0 ml syringes into plain bottles and centrifuged for 15 min at 3500 rpm.

Determination of body weight: Changes in the body weight of rats were recorded using digital balance, before and after the experiment, and were recorded as initial body weight (IBW) and final body weight (FBW).

Determination of blood glucose level: A glucose level test was conducted to verify the induction of diabetes. Blood samples were collected from the tail vein of all rats and analyzed for blood glucose levels using a glucometer (ACCU-CHEK Active®). The results were reported as mg/dL.

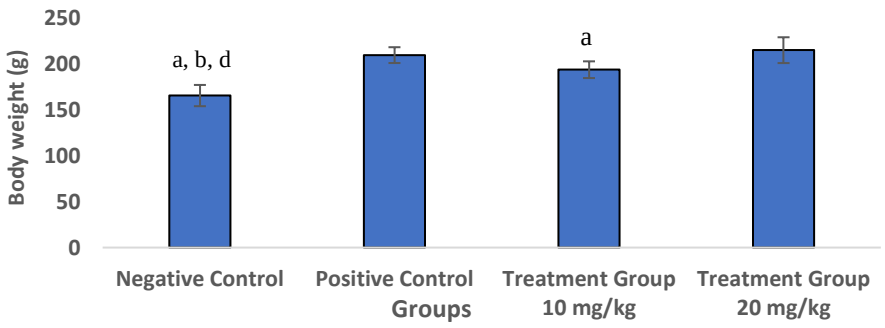
Determination of total protein and albumin: Blood samples from the caudal vena cava were centrifuged to obtain hemolysis-free clear serum. The activities of total protein (TP) and albumin (ALB) were assayed using an autoanalyzer (Dir- chem 4000i, Fujifilm, Tokyo, Japan) by standard methods as described previously [20].

Statistical analysis: Data was analyzed by one-way analysis of variance (ANOVA). Individual groups were then compared by Turkey's post-hoc test. Significant difference considered at probability value less than 0.05.

Results

Figure 1 shows the weight of each group of type 2 diabetic wounds in rats treated with resveratrol. The results obtained showed that after treating type 2 diabetic wound rats with 10 mg/kg resveratrol, there was no significant difference in the body weight when compared to the diabetic control group. There was also no significant difference in the body weight of rats treated with 20 mg/kg resveratrol when compared to the control group.

Figure 1: Total body weight of rats



a = significant compared to the negative control group, b = significant compared to the diabetic control group & d = significant compared to the group treated with resveratrol 20 mg/kg.

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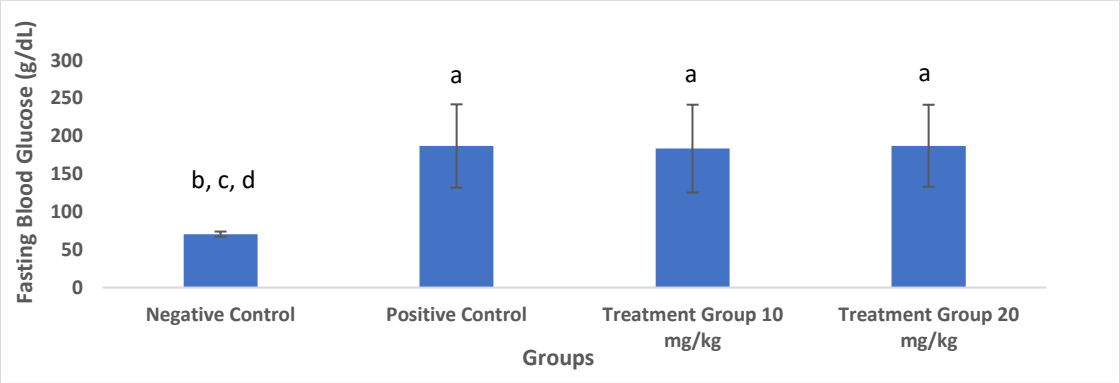
Figure 2 shows the blood glucose level of each group of type 2 diabetic wound in rats treated with resveratrol. There was no significant difference in the glucose level of rats treated with 10 mg resveratrol when compared with the diabetic control group. 20 mg of resveratrol administration also showed no significant difference in blood glucose level when compared to the diabetic control group.

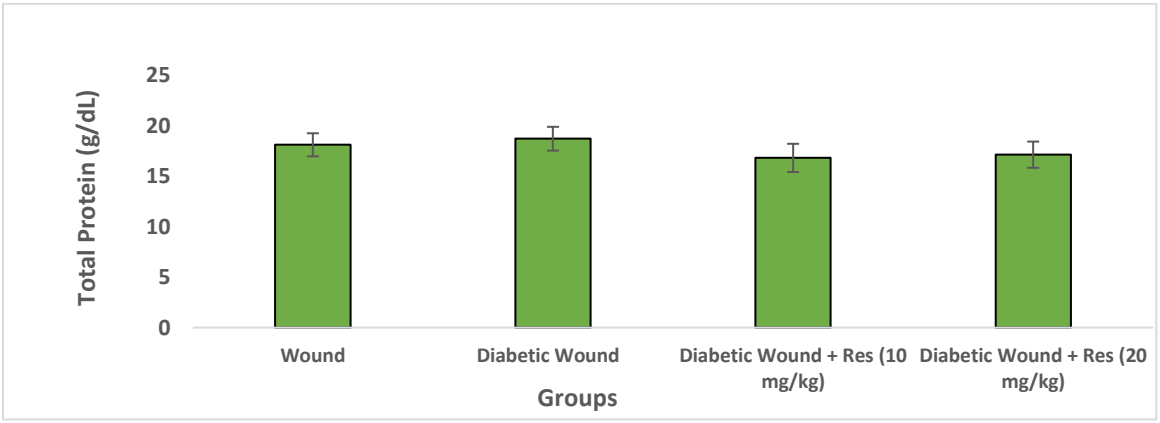
Figure 2: Fasting blood glucose levels in rats treated with resveratrol



a = significant when compared to the normal control group, b = significant when compared to the diabetic control group, c = significant when compared to the group treated with resveratrol 10 mg/kg, & d = significant when compared to the group treated with resveratrol 20 mg/kg.

In **Figure 3**, after the oral administration of resveratrol to the different groups of diabetic wound-healing rats, there were no significant changes observed in the total protein concentration of the control (wound) group rats when compared to the other groups. There were also no significant changes observed in the total protein concentration in rats treated with resveratrol as compared to the diabetic control (wound) group rats.

Figure 3: Total protein level of diabetic wound healing rats across all treatment



Data presented as Mean ± SEM.

In **Figure 4**, after the oral administration of resveratrol to the different groups of diabetic wound-healing rats, there were no significant changes observed in the total albumin concentration of the control (wound) group rats when compared to the other groups. 10 mg and 20 mg resveratrol administration in type 2 diabetic wound healing show no significant difference in total albumin concentration when compared to the diabetic control (wound) group.

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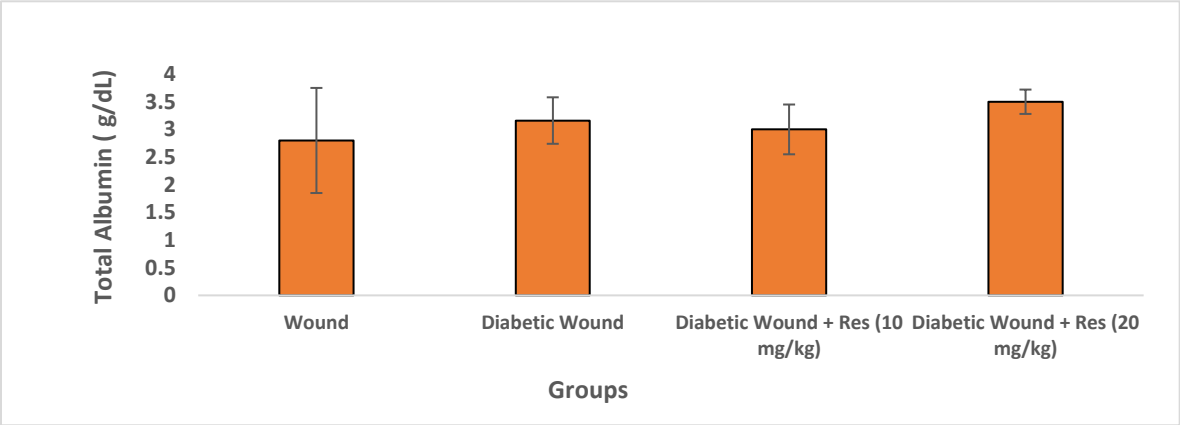
Figure 4: Total albumin concentration in diabetic wound healing rats with resveratrol



a = significant when compared to wound group & d = significant when composed to diabetic wound + res. (20 mg/kg) group.

Discussion

In this study, no change in the body weight of the rats which suggests that resveratrol does not affect diabetic rats' body weight as previously reported in diabetic animal models [21]. These data support the notion that the main therapeutic effect of resveratrol may not be associated with the management of body weight in the context of diabetes mellitus [22]. The result could be a result of the antidiabetic effect of resveratrol via hypoglycemic, antioxidant and anti-inflammatory roles of resveratrol [23]. The blood glucose data indicated no disparity between the diabetic rats treated with resveratrol and the diabetic rats that were not treated. The findings align with Yonamine et al. [18], which demonstrated that the use of resveratrol as an additional therapy improved glycemic control and returned plasma fructosamine concentration to levels comparable to those of non-diabetic rats. However, it did not have any effect on the expression of glucose transporters GLUT2 and SGLT1 in the intestine, GLUT2 and SGLT2 in the kidney, and GLUT4 in the soleus. This suggests that the movement of glucose remained unchanged. The findings were consistent with Zhu et al. [24], who showed that resveratrol decreased the homeostasis model assessment of insulin resistance (HOMA-IR) index and blood pressure in individuals with T2 DM. However, the effect of resveratrol on glucose homeostasis and insulin resistance reversal in T2DM is largely inconclusive based on reported results. However, a high dose of resveratrol can lower fasting blood glucose levels in individuals with T2 DM [25, 26]. The effect of resveratrol on the total protein level of wound healing type 2 diabetic rats showed no difference in the control (wound) group compared to the diabetic control and the two resveratrol-treated groups, also, no difference between resveratrol-treated groups and diabetic control. It can be said that resveratrol has no effect on total protein concentration in T2 DM, contrary to a reported result where resveratrol attenuated protein and albumin concentrated in rats [27]. The effect of resveratrol on the albumin level of wound healing type 2 diabetic rats showed no difference in the diabetic control when compared to diabetic resveratrol-treated groups, it also showed no difference between the control (wound) and all the other groups. It can be said that resveratrol has no significant effect on total albumin concentration in type 2 diabetic wound healing in rats. Resveratrol was shown to have properties antidiabetic that are attributed to its antioxidant, anti-inflammatory, proapoptotic, and antiproliferative features thereby restoring homeostasis but not to affect metabolic biomarkers [28]. This is not farfetched when it comes to causing a decrease in total protein and albumin levels in some research [29] while having an increase in total protein and albumin levels [30]. The absence of inherent negative regulation of gluconeogenesis in the liver leads to heightened catabolism of lipids and proteins,

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together with the transformation of glucogenic amino acids into glucose, resulting in elevated glucose levels. Hemodilution can contribute to this decrease, whereas the increase may be attributed to hyperglycemia and renal filtration, resulting in the buildup of harmful waste products in individuals with diabetes [31].

Conclusion: Resveratrol did not have a significant effect on total protein and albumin levels in rats with diabetes, despite its known antidiabetic effects.

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Author contribution: NOY, AJ, HAI & FAD designed the study, NOY & PJF performed experiments and collected data, and AI & PJF contributed to data analysis. AI & AJ performed the analysis and interpreted the data. AI, AJ, HAI & FAD drafted the manuscript. All the authors approved the final version of the manuscript and agreed to be accountable for its contents.
Conflict of interest: The authors declare the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.
Ethical issues: Including plagiarism, informed consent, data fabrication or falsification, and double publication or submission have completely been observed by authors.
Data availability statement: The raw data that support the findings of this article are available from the corresponding author upon reasonable request.
Author declarations: The authors confirm that all relevant ethical guidelines have been followed and any necessary IRB and/or ethics committee approvals have been obtained.