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Protective Effect of Resveratrol Co-Administration with Cholesterol Diet on Erythrocyte Osmotic Fragility and Malondialdehyde Concentration in Rabbits

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Authors' contributions

This work was carried out in collaboration between all authors. Author AJ designed the study, wrote the protocol, and wrote the first draft of the manuscript. Author YT managed the literature searches, analyses of the study performed the spectroscopy analysis and author JOA managed the experimental process and author AA identified the species of plant. All authors read and approved the final manuscript.

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ABSTRACT

The aim of the experiments was to investigate the protective effect of resveratrol co-administration with cholesterol diet on erythrocyte osmotic fragility (EOF) and malondialdehyde (MDA) concentration in rabbits. Thirty rabbits divided into six group of five animals (n = 5) each were used for the experiment: Group 1 = normal control (C), group 2 = cholesterol diet (CD) only, group 3 = resveratrol 200 mg/kg (R200), group 4 = resveratrol 400 mg/kg (R400), group 5 = CD + R200 and group 6 = CD + R400. Eight weeks after the treatment period, blood sample of about 5 ml (3 ml in EDTA bottle for erythrocyte osmotic fragility (EOF) test and 2 ml in plane tube for extraction of serum for malondialdehyde concentration (MDA) were drawn from the heart of each sacrificed

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animal from all group by cardiac puncture. At 0.45% of NaCl concentration, the percentage haemolysis of 100% obtained in the CD group was considerably higher than the value of 59% recorded in the CD + R400 mg/kg and 30% haemolysis recorded for normal control group. Increases in haemolysis were indicated on the point in the graph presented. The MDA concentration obtained in the CD group rabbits (2.64±0.18 nmol/ml) was higher compared to those of CD + R200 and CD + R400 with a value of 1.70±0.14 nmol/ml and 1.64±0.12 nmol/ml respectively. It is concluded that CD increased haemolysis and MDA concentration in rabbits, ameliorated by resveratrol administration.

Keywords: Cholesterol diet; erythrocyte osmotic fragility; rabbit; malondialdehyde.

1. INTRODUCTION

The excessive consumption of high cholesterol diet has been associated with an increased incidence of obesity, enhanced by formation of oxidative stress and lipid peroxidation. Consequently, the high levels of circulating free fatty acids (FFA) and glucose are potent inducers of reactive oxygen species (ROS) in cells [1-3]. Lipotoxicity impairs cell function and viability due to chronic exposure to FFA, leading to the induction of β-cell endoplasmic reticulum stress [4], and glucose-induced β-cell dysfunction and apoptosis [5]. The ROS are involved in the regulation of multiple processes in the body, including ageing, cell membrane integrity and obesity [6]. Visceral adipose tissue may be increased in the body by over-consumption of nutrients, which is involved in the increased generation of ROS that enhance the expression and secretion of inflammatory adipokines [1-3]. Adipokines have been shown to cause imbalance in the activities of erythrocyte redox enzymes, including glutathione reductase and peroxidase [7,8], glucose -6- phosphate dehydrogenase and damage structural integrity of plasma membrane [9]. The erythrocyte membrane is inertly connected with membrane stability and functions due to relative composition of fatty acids, phospholipids and cholesterol [10]. Recently, [11] reported that exposure of rabbits to oxidative stress increased haemolysis, which was ameliorated by ascorbic acid. Thus measures aimed at preventing or reducing ROS formation in the cells especially involving the administration of antioxidants may be an efficient means of reducing the damage induced in cell membrane [12-14].

Resveratrol is a potent antioxidant found in the skin of red grapes and in other fruits as well as in the roots of Japanese knotweed [15]. Chemically, resveratrol (3, 5, 4`-trihydroxy-trans-stilbene) is a naturally-occurring, non-flavonoid; a phytoalexin belonging to a class of polyphenolic compounds called stilbenes [16]. It is produced in plants with the help of the enzyme stilbene synthase, and the production is in response to infection by the pathogen, environmental stressors that include water deprivation, ultraviolet and radiation [17]. Attenuation of ROS by resveratrol has been demonstrated to prevent, tread, or cure some of the diseases using laboratory model [18,19]. There is paucity of information on the protective effect of resveratrol co- administered with cholesterol diet on MDA concentration and EOF in rabbits. The EOF and MDA are very important biomarkers of oxidative stress [20-23].

The aim of the present study was to evaluate the effect of co-administration of resveratrol with cholesterol diet on EOF and malondialdehyde concentration in rabbits.

2. MATERIALS AND METHODS

2.1 Chemicals

All chemicals were obtained commercially and were of analytical grade: Cholesterol Mumbai India, M. W 386.67, CAS No. 57-88-5, LoT No. 100413) and Mega resveratrol (99% pure trans-resveratrol Batch Number: MR 131120, Average particle size: 2.5 µm Sigma, USA).

2.2 Experimental Animals and Management

Seven-week-old male rabbits of different crossbreeds (New Zealand and local breed) weighing between 300-350 g were used for the study. They were bred in the Animal house of the Department of Human Physiology Ahmadu Bello University Zaria Nigeria. The animals were kept in well-aerated laboratory cages and were allowed to adjust to the laboratory conditions for a period of three weeks before the commencement of the experiment. They were given free access to grower and starter mash (Vital Feeds Company, Kaduna, Nigeria, and water *ad libitum* during the stabilizing period.

2.3 Induction of Oxidative Stress

The animals were fasted from feeds for 16-18 hours before the commencement of the experiment. The normal groups were fed with standard animal feeds only, while the cholesterol diet groups were fed with standard animal feeds + cholesterol diet (10% groundnut oil, 20% groundnut mill and 2% cholesterol) in order to induce induction oxidative. The feedings last for eight weeks which was the experimental period.

2.4 Resveratrol Preparation and Administration

Trans-resveratrol, due to its low solubility in water, was suspended in 10 g/L of carboxymethylcellulose (CMC), and administered orally according to the method of [24].

2.5 Ethical Approval

The rabbits were handled in accordance with the principles guiding the use and handling of experimental animals Ahmadu Bello University Zaria, Nigeria.

2.6 Experimental Design

2.6.1 Groupings

In the study, thirty (30) rabbits weighing between 300-350 g were used, each group comprising five rabbits (n = 5) as follows:

Group 1: Received 10 g/L CMC each orally (C).
Group 2: Receive cholesterol diet as feed only (CD).
Group 3: Received 200 mg/kg body weight of resveratrol orally (R200 mg/kg).
Group 4: Received 400 mg/kg body weight of resveratrol orally (R400 mg/kg).
Group 5: Received 200 mg/kg body weight of resveratrol and cholesterol diet (R 200 mg/kg + CD).
Group 6: Received 400 mg/kg body weight of resveratrol and cholesterol diet (R400 mg/kg + CD).

2.6.2 Collection and Preparation of Serum Samples for Analysis

Eight weeks after the treatment period, all rabbits were subjected to light anaesthesia by exposing them to chloroform, soaked in cotton wool placed in anaesthetic box covered with lid. Blood samples of about 5 mL were drawn from the heart of each sacrificed animal from all groups by cardiac puncture. The blood sample was divided into two (2 ml for EOF and 3 ml for MDA): the EOF part was put in EDTA bottle to prevent clotting, and the other, without anticoagulant was used to extract serum sample for the determination of MDA concentration.

2.7 Erythrocyte Osmotic Fragility Determination

Sodium chloride (NaCl) solution was prepared according to [25] with slight modification by me in volume of 500 mL for each of the samples; and in concentrations, ranging from 0.0 to 0.9% at pH 7.4. A set of six test tubes, each containing 10 ml of NaCl solution of concentrations, ranging from 0.0 to 0.9% was arranged serially in a test tube rack. One set was used to analyse each sample. The test tubes were labeled with corresponding NaCl concentration. One mL pipette was used to transfer 0.02 mL of blood sample into each of the six test tubes. Mixing was done by gently inverting the test tubes for about 5 times. The test tubes were allowed to stand at room temperature (27°C) for 30 minutes. The contents of the tubes were maintained at pH 7.4. Thereafter, they were re-mixed and centrifuged at 1,500 × g for 15 min. the supernatant of each test tube was transferred into a cuvette. The concentration of haemoglobin in the supernatant solution was measured using a spectrophotometer (Shimadzu UV160 UV-VIS, Shimadzu corp, Kyoto, Japan) at 540 nm by reading the absorbance. The same procedure was repeated for every blood sample of each rabbit used for the study. The percent haemolysis was calculated using the formula [25]:

2.8 Determination of serum Malondialdehyde Concentration

The level of thiobarbituric-acid reactive substance, MDA, as an index of lipid peroxidation was evaluated. Quantitative measurement of lipid peroxidation of MDA was determined using NWLSSTM MDA assay kit (Northwest Life Science Specialities, Product NWK-MDA01, Vancouver, WA, and specificity: Malondialdehyde, sensitivity: 0.08 μM). The principle was based on the reaction of MDA with thiobarbituric acid (TBA); forming an MDA-TBA2 adduct that absorbs strongly at 532 nm [26].

2.9 Statistical Analysis

Samples were expressed in mg/dL as mean ± SEM. The data were analyzed using ANOVA, followed by Dunett's post-hoc test to show multiple comparisons versus control group using SPSS version 17.0 software and Microsoft Excel (2007). Values of P ≤ 0.05 were considered as significant [27].

3. RESULTS

3.1 Malondialdehyde Concentration Assay

Fig. 1 shows the result of MDA concentration in the resveratrol co-administered with CD, C group, resveratrol treated groups alone, and CD group only. Resveratrol co-administered with CD showed significant (P < 0.05) decrease in MDA concentration (CD + R200 = 1.70±0.14 nmol/ml), (CD + R400 = 1.64±0.12 nmol/ml) when compared to CD only, with a value of 2.64±0.18 nmol/ml. Rabbits administered with R200 mg/kg and R400 mg/kg also showed a significant (P < 0.05) decrease in MDA level with a value of 0.76±0.10 nmol/ml and 0.84±0.09 nmol/ml when compared with that of CD only with a value of 2.64±0.18 nmol/ml. The result also show that R200 mg/kg and R400 mg/kg showed a significant (P < 0.05) decrease in MDA level from 0.76±0.01 nmol/ml and 0.84±0.09 nmol/ml, when compared to that obtained in rabbits given CD + R200 mg/kg or CD + R400 mg/kg with a value of 1.70±0.14 nmol/ml and 0.84±1.64 nmol/ml.

3.2 Erythrocyte Osmotic Fragility Test

The result of EOF is shown in Fig. 2. Mean percentage of haemolysis at varying NaCl concentration of 0.25%, 0.30%, 0.35%, 0.40%, 0.45%, 0.50%, 0.55%, and 0.60% are shown on the curve. Complete haemolysis occur for C group at 0.25% NaCl concentration, R200 mg/kg at 0.35% NaCl concentration, R400 mg/kg at 0.40% NaCl concentration, CD at 0.45% NaCl concentration, R200 mg/kg + CD at 0.45% Nacl concentration and R400 mg/kg + CD at 0.45% NaCl concentration. At 0.45% of NaCl concentration, the percentage haemolysis of 100% obtained in the CD group was considerably higher than the value of 30%, recorded in the C group. At 0.45% of NaCl concentration, the percentage haemolysis 100% obtained in the R200 mg/kg + CD was higher than the value of 55%, obtained for R200 mg/kg only. However, the percentage haemolysis in the R400 mg/kg + CD rabbits was not different from that, obtained for R400 mg/kg alone. Finally, the complete haemolysis occurred for all the groups at 0.35% NaCl, except for C group which withstood haemolysis up to 0.25% NaCl concentration.

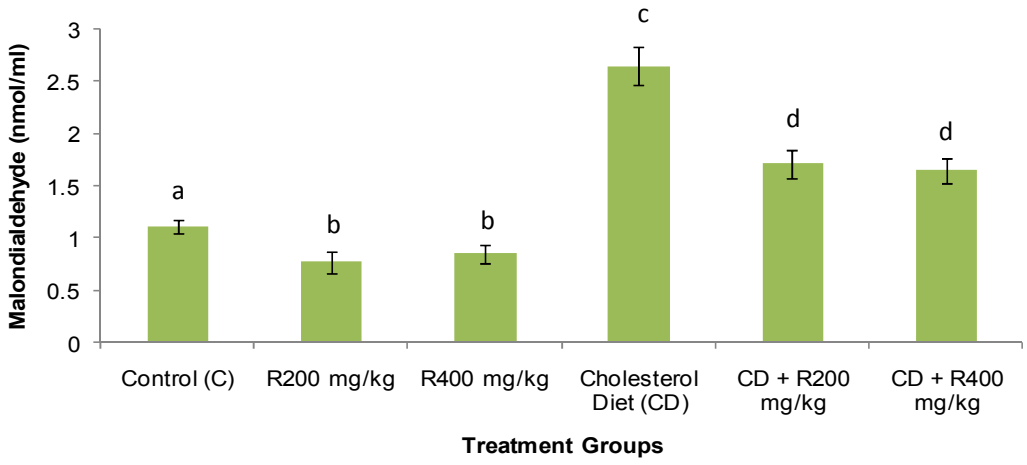

Fig. 1. Effect of co-administration of resveratrol and high-fat diet on serum MDA concentration level in rabbits fed with high fat diet. Values are expressed as mean ± SEM; n = 5;

Values with error bars having different superscripts letters are significant ^{a,b,c,d} = p < 0.05 significant. Resveratrol 200 mg/kg (R200), resveratrol 400 mg/kg (R400)

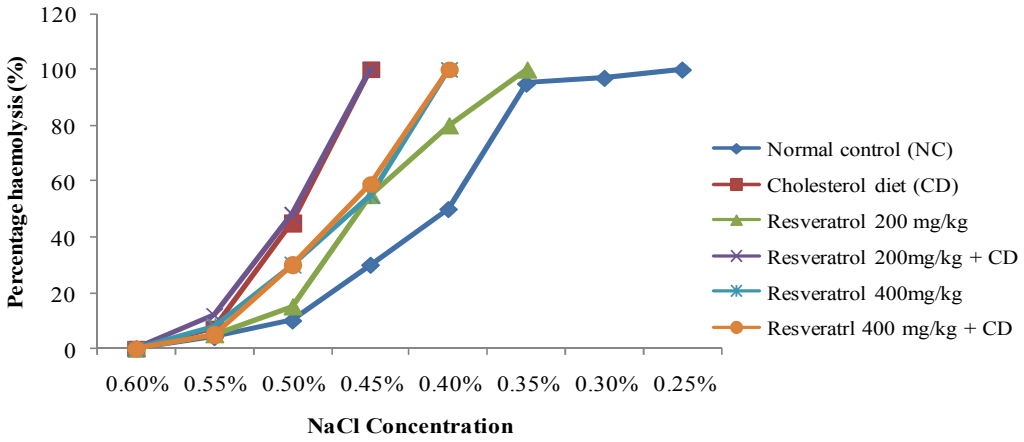

Fig. 2. Percentage haemolysis of effect of co-administration of resveratrol and cholesterol diet on erythrocyte osmotic fragility in rabbits fed with cholesterol diet

4. DISCUSSION

The result of (EOF) showed that CD increased haemolysis in rabbits, and the effect was more evident in CD-fed group only than CD groups, treated with resveratrol. The result agrees with the finding of [11], which showed that exposure of rabbits to oxidative stress increased haemolysis. Continuous exposure to ROS renders the erythrocyte to be sensitive to redox imbalance that alters its mechanical properties and resulting into oxidative stress [28,29]. The alteration in EOF obtained in the present study shown increased membrane fragility of the erythrocytes in CD group, apparently due to ROS induced changes in the molecular properties of the erythrocyte. Over-consumptions of nutrients have been implicated to play a central role in visceral fat stores. As visceral fat expand, adipocytes generate increasing levels of ROS [1,2,3]. The decrease in EOF observed resveratrol treated with CD group as compared to CD only indicated that resveratrol may serve as a protective agent against CD-induced damage to the red blood cell membrane. The increase ROS may further result into disruption of structural integrity of the red blood cell membrane leading to increased haemolysis [21]. The accumulation of ROS in adipose tissue is one of the early events in the development of metabolic syndrome [30,31].

The result obtained in the MDA levels in CD-treated groups with resveratrol showed a significant ($P < 0.05$) decrease, when compared to that of the CD group only. The decrease in plasma MDA concentration observed in the present study suggests that the entire process of lipid peroxidation may be reduced by daily consumption of resveratrol. The result of the present study agreed with the finding of [32], which showed the effectiveness of spice phenolic antioxidant in reduction of oxidative associated with metabolic complications. Circulating MDA levels are higher in diabetic and obese subjects and cholesterol diet has been known to induced obesity and generation of vascular ROS, [33]. Studies have shown that elevating lipid peroxide in erythrocyte cell membrane decreases antioxidant enzymes activities in hyperglycaemia and hypercholesterolaemia-associated with obesity and membrane damage [34]. Although the mechanism of action of resveratrol was not investigated in the present study, resveratrol has been shown to exert antioxidant activity via ROS scavenging, up regulation of natural antioxidant defence of cells [35-38]. More than one phenol groups (polyphenol) of chemical structure of resveratrol have been associated with antioxidant properties. The formation of ROS by the phenol group gives a stable molecule that is less toxic than the original radical [39]. Resveratrol has also been demonstrated to prevent oxidative stress-induced DNA damages [40] and also prevent ROS generated by high fat diet and fructose diet fed rats associated with cardiovascular complication [41,42].

5. CONCLUSION

Cholesterol diet-induced increased MDA concentration level and EOF which was ameliorated by administration of resveratrol.

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CONSENT

It is not applicable.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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