Original Research Article

The Effect of Vitamin E Supplement on Creatine kinase MB, C-Reactive Protein, Creatinine and Urea Levels of Albino Rats Fed with High Fat Diet.

ABSTRACT

Aim: This study was designed to assess the effect of vitamin E supplement on Creatine Kinase (CK-MB) C - reactive protein, (C-RP), urea and creatinine of albino rats fed with high fat diet.
Methodology: A total of twenty (20) albino rats of both sexes were used in this study. They were grouped into four (4) groups comprising of five (5) rats each. Group 1 was fed with normal diet; group 2 with normal diet and treated with vitamin E supplement (70 mg/kg); group 3 was fed with high fat diet only, while group 4 was fed with high fat diet and treated with vitamin E supplement (70 mg/kg). The treatments were administered daily using oral gavage method for a period 21 days. After the experimental period, the animals were sacrificed and blood samples collected for the analysis of CK-MB, CRP, urea and creatinine respectively using ELISA and colorimetric method with a spectrophotometer. Data generated were analyzed statistically using SPSS version 20 and P = 0.05 was considered statistically significant.
Results: The results revealed a significance difference in C-RP (mg/l) levels ($P < 0.001$) amongst the groups with a decreased C-RP levels in group 2 (1.43 ± 0.39) compared to group 1 (1.94 ± 0.53), then in group 4 (2.27 ± 0.53) which is also decreased compared to group 3 (3.31 ± 0.63). For CK-MB there was also a significant difference among the different groups. In group 2, it was 1.94 ± 0.65 ng/ml which was decreased compared to group 1 (2.22 ± 0.55), and group 4 (2.74 ± 0.68) compared to group 3 (3.31 ± 0.62). For the urea and creatinine results, there was no significant difference ($p > 0.05$) among the groups when the mean levels were compared.
Conclusion: This shows that administration of vitamin E to rats fed normal or high fat diet could significantly reduce the plasma levels of CRP and CK-MB hence having some ameliorative effects on high fat induced inflammatory and cardiovascular risks or conditions.

Key Words: *Vitamin E Supplement, Creatine Kinase-MB.C - Reactive protein, Urea, Creatinine, High fat diet, albino rats.*

1. INTRODUCTION

"High fat diets are responsible for high global prevalence of chronic non communicable diseases such as cardio-vascular diseases and renal diseases" [1,2]. This is a serious health problem all over the world especially in industrialized countries. High fat diets especially those of trans fats and saturated fats such as margarine, butter, fats cut of beef and pork, high fat dairy products and poultry skin has been implicated in obesity which has a high-risk factor for the development of cardio-vascular disease, fatty liver disease, pulmonary dysfunction, colon cancer, diabetes, chronic renal failure and metabolic syndrome [3,4]. Some studies have shown that high fat diet increase low density lipoprotein-cholesterol (LDL-C) which is the major cause of atherosclerosis, a buildup of fats and other substances in and on artery wall [5]. Some studies have also shown that lipid-rich diets are capable of generating reactive oxygen species (ROS) because they can alter oxygen metabolism hence contributing to renal and heart diseases [6]. Having in mind that most of our Nigerian diets are heavily loaded with fats and our eating pattern also favours the accumulation of fats in the system, therefore it is necessary to undertake studies which will help to a larger extent to ameliorating the resultant effects that might arise as a result of consumption of high fat diets on the cardiac and renal organs.

"Non-communicable diseases kill 41 million people each year, which is about 71% of the world's total deaths" [7]. "It is reported that fats account for 20-35% of total energy intake [8], but daily total fat consumption accounts for 50% of total energy intake in some countries" [9]. "High-fat diets, in general, are associated with metabolic disorders, and the type of dietary fat involved is a determinant risk factor since saturated fats are more linked to a positive fat balance and visceral adipose tissue accumulation than to other types of fat" [10, 11]

The CPK-MB test (creatine phosphokinase-MB), also known as CK-MB test, is a cardiac marker used to assist diagnoses of an acute myocardial infarction, myocardial ischemia, or myocarditis. C-Reactive protein is one of the common test parameters used in clinical practice, for assessment and diagnosis of inflammation.

"Vitamin E is the major lipid-soluble component in the cell antioxidant defense system" [12]. "Vitamin E has been known to possess both antioxidant and anti- inflammatory properties which prevents or reduces oxidative stress, which is known to be an important contributor of renal and cardiac diseases. Vitamin E functions as a chain-breaking antioxidant by preventing chain initiation and propagation of free radical reaction and lipid peroxidation in cellular membrane. In addition to its antioxidant function, vitamin E supplementation influences the cellular response to oxidative stress through modulation of signal-transduction pathway" [13]. Therefore, it is necessary to assess the effect of vitamin E supplementation on the renal and cardiac functioning state of rats fed with high fat diet.

2. MATERIALS AND METHODS

2.1 Experimental Animals

A total of twenty (20) albino rats weighing between 140 – 160 g of different sexes were procured from the animal house unit of the Department of Human Physiology, Faculty of Basic Medical Science, University of Port Harcourt.The animals were kept in a well-ventilated clean plastic cage wounded round with metallic wire gauze with 12 hours normal light/ dark cycle. Sawdust was used as bedding. They were fed with commercially prepared grower mash feed (manufactured by Grand Cereal Limited) and water. Grower mash ingredients comprise of cereals/Grains (Maize), Vegetable Protein (SBC, SBM and GNC), Premix (Vitamins/Minerals), Essential Amino Acids (Lysine, Methionine), salt, Antioxidant (vitamin E), Probiotics (Fermentative) and Enzymes (NSP). The nutritional information/ Average composition: Crude Protein 16%, Fat 5%, Crude Fibre 5%, Calcium 1.0%, Phosphorus 0.46%, Metabolizable Energy 3,150Kcal/Kg (Min). The animals were allowed to acclimatize for 2 weeks before the commencement of treatment for 21days. The conditions of the rats were in conformity with standards as outlined by the National academy of Science [14. 15].

2.2 Preparation of High Fat Diet

"The design of the test meal was a modification of the Matos *et al*.; Waribo *et al*. [16.17] dietary models for inducing hyperlipidemia in rats. The diet is composed of 68% grower (chicken) mash, 30% soybean oil and 2% cholesterol. It was compounded by dissolving 20g of margarine in 300g of soybean oil, stirring thoroughly and mixing it evenly with 680g of grower chicken marsh to make up 1000 grams of the high cholesterol diets. The crude fat content of both the normal and high fat diets fed to the rats were determined using Soxhlet Extraction method".

2.3 Pilot Study of Rats to check Hyperlipidemic State

A total of 10 albino rats were divided into two groups: Group A (n = 5; control group received normal diet for 21 days) and Group B (n = 5; Test group received HFD prepared according to modification of Matos *et. al.* [16] for 21 days to induce hyperlipidemia). They were fasted overnight, then sacrificed on the 22nd day under chloroform anesthesia [18]. Blood samples were collected by puncture of the jugular vein into lithium heparin sample bottles which was centrifuged to obtained the plasma that was used to determine total cholesterol levels using Allan *et al.* Method [19]This is to verify the hyperlipidemic status of the rats as a result of the high fat diet given.

2.4 Drug / Reagents

Reagents for the analysis of total cholesterol, urea and creatinine were purchased from Randox Laboratories Ltd, while C - reactive protein and Creatine kinase-MB (a solid phase direct sandwich kits) were purchased from Calbiotech California USA. Vitamin E supplement (dl- alpha tocopherol) Puritan's Pride was purchased from Remaplus pharmacy Oyigbo, Rivers State, Nigeria. (lot number; 1781).

2.5 Calculation of Vitamin E Supplement Treatment Doses

Vitamin E used is in the form of DL-α-tocopherol acetate and 1.0 ml of the vitamin E contains 500 mg of DL-α-tocopherol acetate (water soluble form of vitamin E). In the preparation, the dose for the rats (AED) was extrapolated from the human dose (HED) of 11.2 mg/kg/day for 60 kg weight (i.e 670 mg/day) to give 70 mg/kg for 0.15 kg rat weight. The 70 mg/kg was obtained by administering 0.02 ml (10.5 mg) in 0.15 kg rats. The conversion of human equivalent dose (HED) to animal equivalent dose (AED) as seen was obtained using the equation: HED (mg/kg) = AED (mg/kg) × 0.16 as described by Nair & Jacob [20] and Umezulike *et al,* [21].

2.6 Experimental Design

1.After confirming hyperlipidemic status.A total of twenty (20) albino rats divided into four (4) groups comprising of five (5) animals were used.

2.Group 1 rats were fed with normal diet daily without any form of treatment for 21 days.

3.Group 2 rats were fed with normal diet and orally administered 70mg/kg of Vitamin E supplement daily for 21 days.

4. Group 3 rats were fed with high fat diet daily without any form of treatment for 21 days.

5.Group 4 rats were fed with high fat diet and orally administered 70mg/kg of Vitamin E supplement daily for 21 days.

6.The administration of vitamin E was done using oro-gastric gavage tube daily for a period of 21 days. The rats were fasted overnight, then sacrificed on the 22nd day under chloroform anesthesia. Blood samples were collected by puncture of the jugular vein into lithium heparin sample bottles. They were centrifuged to obtained the plasma which was stored at a temperature of - 20^{o}C in a freezer and later used for the estimation of Creatine kinase MB (CK-MB), C-Reactive protein (CRP), Urea and Creatinine.

2.7 Methods

1.Estimation of Creatine kinase MB isoenzyme (CK-MB) was carried out using Enzyme-Linked Immunosorbent Assay [22].

2.Estimation of C-Reactive Protein (CRP) was carried out using Enzyme-Linked Immunosorbent Assay [23].

3. Estimation of Urea was carried out using the Urease-Berthelot method (Weatherburn [24].

4.Estimation of Creatinine was carried out using the Jaffe's reaction method [25].

2.8. Data Collection and Analysis Method

Results were expressed as mean ± standard deviation (SD). Data obtained were analysed using one-way analysis of Variance (ANOVA) followed by Tukey's Multiple Comparison post hoc test using Statistical Package for Social Sciences (SPSS) version 23. For all statistical analysis, results were considered significant at $p<0.05$.

3.RESULTS

Table 1: Mean ± SD levels of Total Cholesterol for Control and Test Rats in the preliminary study (n-10)

Groups/Parameters	Total Cholesterol (mg/dl)
Control (ND)	158. 00 ± 25.88
Test (HFD)	341.00 ± 58.14
t-value	6.430
P – value	0.003
Remark	S

KEY: n = Number of Observations, ND = Normal Diet, HFD = High Fat Diet, S = Significant.

Table 2: Mean ± SD of CK-MB and C-RP in Rats Fed with High Fat Diet and Treated with Vitamin E over a period of 21 days

Groups /Parameter	CK-MB (ng/ml) Mean ± SD	C-RP (mg/L) Mean ± SD

Group 1 (ND)	2.22 ± 0.55^{a}	1.94 ± 0.53^{a}
Group 2 (ND/E)	1.94 ± 0.65^{b}	1.43 ± 0.39^{b}
Group 3 (HFD)	3.31 ± 0.62^{c}	3.31 ± 0.63^{c}
Group 4 (HFD/E)	2.74 ± 0.68^{d}	2.27 ± 0.53^{d}
F- Value	3.143	11. 312
P – value	0.004	0.0003
Remark	S	S

KEY: *Mean values with different superscripts are significantly different from each other, P = 0.05, S - Significant. CK-MB - Creatine kinase MB, C-RP - C-Reactive Protein, Group 1 ND - Normal Diet, Group 2 ND/E - Normal Diet with Vitamin E, Group 3 HFD - High Fat Diet, Group 4- HFD/E - High Fat Diet with Vitamin E*

Table 3: Mean ± SD of Urea and Creatinine in Rats Fed with High Fat Diet and Treatedwith Vitamin E

Groups	**Urea (mg/dl) Mean ± SD**	**Creatinine mg/dl Mean ± SD**
Group 1 (ND)	13.94 ± 0.65	0.95 ± 0.11
Group 2 (ND/E)	13.54 ± 0.53	0.79 ± 0.16
Group 3 (HFD)	13.73 ± 0.60	0.92 ± 0.18
Group 4 (HFD/E)	13.62 ± 0.62	1.06 ± 0.31
F- Value	2.137	1.369
P – value	0.14	0.29
Remark	NS	NS

KEY: *P = 0.05, NS -Non-Significant. Group 1 ND - Normal Diet, Group 2 ND/E - Normal Diet with Vitamin E, Group 3 HFD - High Fat Diet, Group 4- HFD/E - High Fat Diet with Vitamin E*

4.DISCUSSION

4.1 Effect of Vitamin E supplementation on CK-MB and C-RP levels

From the study, the significant increases observed in CK-MB and C-RP levels in rats fed with high fat diet compared to rats fed with normal diet (Table 2) corroborates the work of Muller *et al*.[26], who reported that consumption of saturated fat is associated with increased total cholesterol than consumption of other types of fatty acid. This might be attributed to the fact that high fat is able to create a disparity between fat balance and its visceral tissue accumulation hence increases the total cholesterol level [10,11].

Vitamin E in this study significantly reduced the C-RP levels in both rats fed with normal diet and especially high fat diet. This is evident in this study as a significant decrease (P= 0.0003) was observed in the plasma level of C-RP among the group fed with high fat diet and treated with vitamin E supplement compared with the group fed with high fat diet alone as reflected in Table 2. The anti-inflammatory effect of vitamin E as revealed in this study is in agreement with the works done by Asbaghi *et al. and* Nazrun *et al* [27,28] who both reported that high doses of vitamin E was found to exhibit anti-inflammatory effect by decreasing C-RP and inhibiting the release of pro inflammatory cytokines [29].

The result also reveals a significant difference in the plasma levels of CK-MB in both rats fed with normal diet and high fat diet as observed in the levels of CK-MB among the group fed with high fat and treated with vitamin E supplement compared to Group 1 fed with normal diet alone. This corroborates with the study by [30, 31] which states that individuals who consume vitamin E may have a reduced rate of developing chronic cardiovascular disease. This result is also in consonance with the work of Birsen [32] which reveals that the administration of vitamin E in rats fed with high fat diet reduces CK-MB significantly. However, according to a recommendation [33], there is no net benefit in using Vitamin E supplementation for the prevention of cardiovascular diseases.

4.2 Effect of Vitamin E Supplementation on Creatinine and Urea levels

There was no significant difference ($P > 0.05$) in the levels of urea and creatinine among the group fed with high fat and treated with vitamin E. This mean that there was no significant variation in the levels of urea and creatinine in those animals fed with high fat and treated with vitamin E and those fed with high fat without treatment. This is in contrast to the study by Kume *et al* [34] and Muller *et al.* [26] that reported kidney damages to be as a result of the consumption of high fat diet.

This result also disagrees with the work of Birsen [32] reported that the administration of vitamin E to a high fat diet reduces the high value of urea and creatinine to normal. However, this study agrees with the report of Jayawardena [35] that reported no beneficial effect of vitamin E supplementation on renal function.

4. CONCLUSION

High fat diet adversely alters some of the biochemical parameters especially CK-MB and C-RP values in the test rats however, Vitamin E significantly reduced the C-RP values, an inflammatory protein and CK-MB,a cardiovascular biomarker, while there were no significant changes in the levels of urea and creatinine. Vitamin E exhibits an ameliorating property to prevent fat induced inflammatory and cardiovascular risk in the rats.

A supplemented diet rich in vitamin E could ameliorate any fat induced cardio or inflammatory conditions based on wrong eating habits which could aggravate the accumulation of fats in the systemic circulation hence leading to other complications of health,

ETHICAL APPROVAL

All authors hereby declare that "Principles of laboratory animal care" (NIH publication No. 85-23, revised 1985) were followed, as well as specific national laws where applicable. All experiments have been examined and approved by the appropriate ethics committee.

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