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Research Article

Effect of High-Cholesterol Feed and Antioxidant Vitamins Supplement on Tear Production of Rabbits

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Abstract

Cholesterol is a waxy, shiny, white substance, important for sustenance of life. It consists of 50% of all lipid components in cellular membranes. This alcohol sterol is significant for many metabolic activities in all cellular milieu. Cholesterol thus is necessary for survival. It is therefore not a question of whether cholesterol is required but how it is to be utilized and the amount and type necessary for homeostasis..

The purpose of this study was to investigate the effect of high-cholesterol feed on tear production and assess the impact of antioxidant intervention. Dry Eye Diseases (DEDs) are on the increase. Factors such as dietary patterns, environment, genetics, etc are suspected to be implicated in the pathophysiology of DEDs.

Twenty New Zealand Rabbits were grouped into 4 groups, each with five (5) rabbits. Group 1 (CTRL) was the control which received normal feed daily. Group 2 (CHOL) was fed daily with 200 mg/kg body weight of cholesterol for 28 days without any interventional agent. Group 3 (ANTX) was fed daily with the normal diet and supplemented with vitamins A, B, C and E, while Group 4 (CHOL+ANTX) was fed daily with the normal diet, high cholesterol (200mg/kg body weight) and supplemented with a combination of vitamins A, B, C and E. Tear production was inspected with Schirmer's test strips on day 0, and day 28. The results showed that after four (4) weeks of treatment, group 2 (CHOL) had significantly reduced tear production ($p = 0.004$). Group 3 (ANTX) indicated normal outcome of tear production. Group 4 (CHOL+ANTX) which had high cholesterol but an intervention with antioxidant vitamin, showed no significant difference in tear flow rate ($p = 0.343$). The results show that high-cholesterol feed militates against normal tear production in rabbits. However, the intake of antioxidant vitamins mitigates this effect.

Keywords: *Tear Production; High-Cholesterol Feed; Antioxidant Vitamins*

Abbreviations

CTRL: Group 1 which was the control and received normal feed daily; CHOL: Group 2 which was fed daily with 200mg/kg body weight of cholesterol for 28 days without any interventional agent; ANTX: Group 3 which was fed daily with the normal diet and supplemented with vitamins A, B, C and E; CHOL+ANTX: Group 4 which was fed daily with the normal diet, high cholesterol (200 mg/kg body weight) and supplemented with a combination of vitamins A, B, C and E.

Introduction

Generally, cholesterol described as a white, waxy, fat-like substance, is ubiquitous in cells of the body. It is an organic compound, belonging to the group of compounds known as sterols and in the family of alcohols. This means that cholesterol is very important for

homeostasis. It is the building block of cellular membranes, a rich source of energy for basal metabolism, acts as cushion for all skeletal systems of organisms and is the precursor for production of vitamin D and essential hormones. This same substance insulates nerve cells and assists in maintenance of skin cell moisture. Thus cholesterol is a steroid that modulates eukaryotic cell membrane fluidity [1].

Cholesterol, triacylglycerols and other lipids are transported in body fluids by a series of lipoproteins classified according to increasing density which include chylomicrons, very low density lipoproteins (VLDL), low density lipoprotein (LDL) and high density lipoproteins (HDL). The lipoproteins possess core hydrophilic lipids surrounded by polar lipids and then by a shell of apoproteins [2]. Though *de novo* biosynthesis of cholesterol occurs in virtually all cells, this is more profound in the liver, intestine, adrenal cortex and reproductive tissues such as ovaries, testes and placenta. There is therefore unambiguous ubiquity of cholesterol in all cells especially in the cell membranes. Becker and colleagues explain that typical animal cell contains large amounts of cholesterol, up to 50% of total membrane lipid on a molar basis [3].

Therefore, it is important to know the influence of excess or limited cholesterol in cells of tissues of the body. When the quantity of dietary cholesterol increases, cholesterol synthesis in the liver and intestine is almost totally inhibited. Thus rate of de novo cholesterol synthesis is inversely related to the amount of dietary cholesterol taken up by the body. The correlation between high levels of blood cholesterol and debilitating disease is a major cause of concern.

Hypercholesterolemia is the manifestation of high levels of cholesterol in the vascular system. This implies elevated levels of low density lipoprotein in the blood which is associated with increased risk of atherosclerosis and Coronary Heart Disease (CHD)

Worldwide, coronary heart disease is on the increase. It is still the major cause of death in advanced countries while in developing countries the incidence is on a mad rush. According to United States of America's Centre for Disease Control and Prevention, about 32% of Americans above 20 years are hypertensive, implying that about 28% of them suffer from hyperlipidemia which is high cholesterol in the plasma [4]. Presence of hyperlipidemia may result to dyslipidemia. The eye is of great importance for survival. Visual impairment and blindness are important issues in public health systems around the world. Though there are 285 million visually impaired individuals, about 40 million of them are blind [5]. Dyslipidemia in the eye is now of great concern. A Hollenhorst plaque (due to cholesterol emboli) is suspected to be associated with long term elevation of serum cholesterol. Other hyperlipidemic issues of the eye include arcus senilis and juvenillis, xanthelasma palpebrarum, cataract [6], choroidal irregularities [7], retinal abnormalities [8] and optic nerve anomalies [9].

Objectives of the Study

The objectives of this study therefore were to investigate the ocular manifestation of short-term intake of high cholesterol feed on mechanism of tearing and the effect of antioxidants on high cholesterol feeds and on tear production.

Materials and Methods

Twenty (20) New Zealand rabbits, weighing between 1.40 - 2.15 kg, of mean weight 1.67 ± 0.05 kg were used for this study. The rabbits were purchased from Aduwawa Animal market, Benin City, of the same colony and were acclimatized for two (2) weeks before the experiment began. The rabbits were divided into four groups, and each group consisted of five (5) rabbits. Group 1 (CTRL) was the control. Group 2 (CHOL) was fed daily with 200mg/kg body weight cholesterol plus the normal diet. Group 3 (ANTX) was fed daily with the normal diet in combination with antioxidant supplements which included vitamins A, B, C and E. Group 4 (CHOL+ANTX) was daily given 200mg/kg of body weight of cholesterol in combination with supplement of antioxidant vitamins - A, B, C and E.

Approval for consent for the animal research was gotten from the departmental research committee.

Materials

Pricon Schirmers Ophthalmic strips [U.S.P]. Registered trademark of Iscon Surgicals Ltd. Lot No: PRS11071 and PRS11051. Manufacture Date: 07-2011. Expiry: 06-2016. Each strip contained Fluorescein Sodium I.P. 1 mg. Made in India.

Cholesterol Powder Sigma Life Science - Cholesterol. Sigma Aldrich St Louis USA 314 771 5765.

Vitamin A (Retinol) 200,000IU (USP). Banner Pharmacaps Ltd, Canada. Batch No 30120262. Manufacture date: 11-2010. Expiry: 11-2013.

Vitamin C (Nature Vit C 500mg) Mason Vitamins Inc. Miami lake, USA. Lot/Expiry date 9524E/2-15.

Dr Meyer's Vit B-complex (High Potency). Dietary Health Supplements Formula. Vitamin B1 5mg (BP) Batch No T1931 Manufacture date 3-2013. Expiry 2-2015. Vitabiotics Nigeria Ltd, Lagos, Nigeria. NAFDAC No 04-0076.

Dr Meyer's Folic acid. Vitamin B12. 5mcg (BP). Folic acid 5mg (BP). Vitabiotics Nig Ltd, Lagos, Nigeria. Batch No T101212 Manufacture date 12-2012. Expiry 11-2014

Vitamin E (dl - Alpha Tocopherol Acetate) 1000IU (USP). Future biotics LLC, New York, USA.

Method

Schirmer's tear test 1 describes the quantitative production of tears. This test was used to estimate tear production by measuring the amount of wetting on a special filter paper measuring 5 mm by 35 mm. It was performed with minimal restraint, and without the prior application of ocular medications or stains. Surrounding light was dimmed in the laboratory. A strip within a plastic sleeve was first bent at a notch 5 mm below a rounded tip. This step was done within the packaging to avoid contamination of the strip by oil from the hands. Once released from its packaging, the rounded bent tip of the strip was then placed within the lower conjunctival fornix (in contact with the cornea) near the junction of the middle and temporal third of the lower eyelid. The strip remained in place for 5 minutes. Tear production was visualized by tear migration down the strip (distance in mm) and recorded as millimetres (mm)/ 5 minutes.

Statistical analyses of all parameters were carried out using SPSS 16.0 version for windows. The values were represented as mean ± standard error mean (SEM). The paired *t*-test was used to compare tear production before and after treatments. The level of statistical significance was set at $p < 0.05$.

Results and Discussion

Results

After four (4) weeks of treatment Group 2 (CHOL) showed a significant reduction tear production, $p = 0.004$ (Table 1). In spite of that, Group 4 (CHOL+ANTX), which had high cholesterol plus antioxidants, showed no significant difference in tear production ($p = 0.343$). Also, Groups 3 (ANTX) and 1 (CTRL) showed no significant difference in tear production before and after treatments ($p > 0.05$).

	Group 1 (CTRL)	Group 2 (CHOL)	Group 3 (ANTX)	Group 4 (CHOL+ANTX)
Day 0	10.10 ± 1.41	10.85 ± 1.14	11.35 ± 0.95	9.30 ± 1.00
Day 28	11.30 ± 1.40	6.85 ± 1.33	10.75 ± 1.0	8.85 ± 1.55

Table 1: *Mean Schirmer's test values (mm/5minutes) ± Standard error mean in all groups on Days 0 and 28.*

Discussion

Our results show that after 28 days of maintenance on high cholesterol diet, a significant reduction in tear production was observed in the CHOL group. This group was loaded with cholesterol continuously without any intervention, for 28 days. Two possible explanations for the reduction in tear production are oxidative stress and depletion of body antioxidant quantities (such as vitamin C) generated by the high-cholesterol feed [10]. Oxidative stress has been defined as a disruption in the equilibrium between the production of reactive oxygen types (especially free radicals) and antioxidant defence which may precede tissue injury [11]. It is generated by a disproportion

between the production of reactive oxygen species and the biological system's fortification mechanisms needed to remove the stress. Oxidative stress is associated with many acute and chronic diseases and is a basic cause of dry eye disease, eventually leading to ocular surface inflammation by disorganized osmolarity of the tear film. It strongly tempers intracellular calcium (Ca^{2+}) routes, which are dire for the actions exocrine glands. The lacrimal glands encounter serious oxidative stress as a consequence of these disease operations, and compromised vitamins and minerals except individuals distressed with them are supplementing or always feeding exceedingly well. A compromise of antioxidant quantities and the existence of oxidative stress by loose radical injury on the lacrimal gland and tear film can stir dry eye [12,13]. This accounts for the reduced mean tear production observed in the CHOL group. Furthermore, the mechanism by which high-cholesterol feed decreased the mean tear production may have been from an over-exhibition of unchained radical reactive oxygen species production thereby putting oxidative strain on the lacrimal and accessory lacrimal glands and deranging their secreting roles [11]. It may have also resulted from an interference in the equilibrium between reactive oxygen species (chiefly free radicals) and antioxidant resistance footing in the tear film [12]. According to Dogru., *et al*. (2007) oxidative stress is involved in a significant task in the spread of cellular injury that results in dry eye [12]. It is known to change the lacrimal glands and foster diminished tear production with age, systemic diseases like diabetes, or numerous micronutrient shortage in which oxidative stress occurs.

In this study, the Schirmer's test was used to inspect tear production. Although this test is considered a crude screening test for the observation of tear production, when executed as a standardized procedure with the same researcher, as in our study, it furnishes useful data [11]. The test, also known as a dry eye test, is fundamentally used to detect dry eye disorders. Schirmer's test deduces if the eye generates sufficient tears to retain its moistness - it reveals if the eye produces too few or too many tears to support stellar eye health. Exclusively, dry eye syndrome is a class of disorders of the tear film due to inadequate tear production or uncontrolled evaporation. It can trigger irritation and mutilate the ocular surface, predominantly the cornea, conjunctiva and eyelids. The symptoms are unflagging painful, stinging, burning or itching eyes. The feeling is frequently referred to as gritty or scratchy sensation and it usually intensifies as the day goes on. There may be stringy mucus within or around the eyes, eye irritation from smoke or wind, and distress wearing contact lenses. Sufferers with the most extreme diseases are at a heightened chance of developing corneal scarring or ulcerations or infections. All these can engender lots of hardship, poor psychological well-being and depreciated calibre of living.

In this experiment, the incorporation of antioxidant vitamins in the chow of rabbits on high cholesterol diet (as in the ANTX+CHOL group) significantly prevented the reduction in tear production. The metabolic stress caused by the high cholesterol diet in the CHOL group was prevented by the consumption of antioxidant vitamins. Antioxidants are known to ebb the actions of oxidation, minimize oxidative vandalism, and enhance lacrimal gland function [11,14]. It is not inconceivable that the combined therapy of antioxidant vitamins A, C, E, B1, B3, B6, B9, and B12 had an important role in reducing the oxidative damage, separately impeding free radical generation from hypercholesterolemia and ameliorating the ocular surface environment [11,14]. Antioxidants are defined as any compound that can give at the minimum one hydrogen atom to a free radical, developing in the cessation of radical chain reactions. Healthy foods copious in antioxidants aid in decelerating the process of oxidation. Normally vitamins are required for cell differentiation, development and maintenance. For example vitamin A, an antioxidant which lessens oxidative stress, is useful in neutralizing free radical damage [15] and is vital in the function of both the outer and inner parts of the eye; Vitamin E, a free radical scavenger, acts as an effective sequence-shattering antioxidant, averting lipid peroxidation [16,17]; Vitamin C, a powerful water-soluble antioxidant vitamin, safeguards crucial ocular areas against oxidative damage [18]; and the B group vitamins - BI, B2, B3, B4, B6 and B12 - show outstanding antioxidant activity and have radical-hunting capacity [19,20]. The antioxidant features of all these vitamins fortified the ocular surface of the rabbits from free radical incursion and safeguarded the integrity of the surface epithelium. They moved to interrupt the series of cell death of the lacrimal glands from oxidative stress induced by the high-cholesterol feed [11]. They sustained lacrimal gland function by arresting oxidative stress-kindled dysfunction of intracellular Ca^{2+}-signalling to keep lacrimal gland functioning. This finding suggests that antioxidant treatment may militate against dry eye induced by high-cholesterol feed. This management of oxidative stress may proffer a novel way for the prevention and remedial care for dry eye syndromes [12].

Conclusion

High-cholesterol feed militates against tear production in rabbits. However, the intake of antioxidant vitamins mitigates this effect.

Conflicts of Interest

The authors declare no conflicts of interest.

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