Potential Benefits of Omega 3.5.7 on Cardiovascular and Reproductive Functions of Male Rats Administered L-N^G-Nitro Arginine Methyl Ester (*L-NAME*)

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ABSTRACT

Background: Dietary interventions and lifestyle changes are some of the ways to manage hypertension and its complications. Males with hypertension report infertility and erectile dysfunction.

Objective: This study determined the role of Omega 3.5.7 enriched diet on cardiovascular and reproductive functions in male rats administered L-N^G-Nitro arginine methyl ester (L-NAME; a nitric oxide synthase inhibitor).

Materials and Methods: Twenty-four male rats were divided into four groups of six rats each: Group 1 (control), Group 2 received L-NAME (50 mg/kg b.w. intraperitoneally), Group 3 received L-NAME + Omega 3.5.7, and Group 4 received 1.5% omega 3.5.7 enriched diet. Rats were fed for two weeks and blood pressure parameters were obtained under anaesthesia using a Power Lab system. Blood was withdrawn from the carotid arteries for biochemical and hormonal assays and the caudal epididymis was collected for the assessment of sperm parameters.

Results: Systolic, diastolic, mean arterial blood pressure, and rate pressure products were increased in the L-NAME and L-NAME + Omega 3.5.7 groups when compared with control ($p<0.05$). MDA level was significantly increased in the L-NAME group while levels of antioxidants (GSH, SOD, CAT) were decreased. Omega 3.5.7 supplementation with L-NAME increased GSH and CAT levels when compared with the L-NAME group. Plasma NO and sperm count, sperm motility, and testosterone levels were decreased by L-NAME. The Omega 3.5.7 + L-NAME group increased NO and testosterone levels.

Conclusions: Improved antioxidant status, testosterone, and NO levels are indicators that omega 3.5.7 enriched diet could reverse blood pressure and increase the decrease in sperm count and motility induced by L-NAME.

Keywords: L-NAME, Omega 3-5-7, Nitric oxide, Testosterone, Blood pressure, Antioxidant.

INTRODUCTION

Hypertension is the leading cause of cardiovascular disease and premature death worldwide. It also leads to many end-organ damages especially affecting the heart, kidney, brain, and eyes. The prevalence of hypertension has increased over the years due to various lifestyle risks, unhealthy diets, and lack of physical activity (1, 2). Hypertension affects around 700 million men of reproductive age with a great burden on low- and middle-income countries (3). About 61 million Nigerian adults are estimated to live with hypertension (4). Hypertension is also a risk factor for reproductive dysfunction (5-7). Males are responsible for 20-30% of infertility cases and contribute to 50% of cases overall (8). Numerous studies have evaluated the association between hypertension and impaired sexual health in men and women (2, 9, 10). Some of the detrimental effects of hypertension in men include erectile dysfunction, decreased sperm volume, sperm count and motility, abnormal sperm morphology, inflammation, and hormonal imbalance (9, 10).

Male reproductive disorders have become more prevalent during the last 25 years with a decrease in sperm concentrations (11-13). Studies have shown that circulatory concentrations of sex hormone-binding globulin and total testosterone level are decreased in male hypertensive patients, (14, 15). In hypertensive rats, hormonal imbalance, reduced spermatogenic functions, depressed antioxidant status in the testes and epididymis, and histological changes in the intratesticular arteries and seminiferous tubules have been reported (5, 12). Depletion of nitric oxide (NO) and the contribution of oxidative stress have been proposed to participate in these reproductive alterations in hypertension (16, 17). It has been reported that patients with hypertension have a high level of reactive oxygen species and a low level of plasma nitric oxide (18). Ngamma-nitro-L-arginine methyl ester hydrochloride (L-NAME), a nitric oxide synthase inhibitor, is widely used to induce hypertension in rats (19, 20). L-NAME causes nitric oxide deficiency, systemic vasoconstriction, and hypertension (21, 22).

Nature has been a source of medicinal agents for thousands of years and an impressive number of modern drugs have been isolated from natural sources (23). It has become increasingly apparent that long-chain polyunsaturated fatty acids (PUFA)

are involved in maintaining a variety of physiological processes and therefore are currently viewed to play key roles in human nutrition both in normal health and disease states (24). Omega 3.5.7, a product of Max International (Utah, USA), is purely derived naturally. The omega 3 content is derived from Norwegian cod, caught fresh in the icy arctic waters, omega 5 is derived from pomegranate seed oil, and omega 7 (palmitoleic acid) is derived from pollock fish. This proprietary blend is believed to offer health benefits by complementing each other to provide the body with essential nutrients to function adequately.

Studies demonstrated that dietary Omega-3 PUFA is important in mitigating the risk for cardiovascular diseases (25). Several organizations, such as the American Heart Association, recommend the consumption of 198 or 227 g of seafood (preferably, oily fish) per week to ensure ideal cardiovascular health (26). Evidence suggests that a reduction of the blood pressure by 5 mmHg can decrease the risk of stroke by 34%, ischemic heart disease by 21%, and reduce the likelihood of dementia, heart failure, and mortality from cardiovascular disease (27). Recently, the American Heart Association recommended the treatment of patients with prevalent coronary heart disease or heart failure with Omega-3 PUFA (28). Regarding the reproductive processes, Omega-3 PUFAs can modify the biosynthetic pathways involved in both prostaglandin (PG) synthesis and steroidogenesis (29). Studies in men (30) and boars (31) have demonstrated the benefits of Omega-3 PUFA consumption on male reproductive capacity, yet other studies in different species have reported no effects (32). Sperm cells contain very high proportions of polyunsaturated fatty acids (PUFA) (33) and normal spermatozoa possess a higher percentage of the most representative PUFA (C22:6 n-3) than those detected in blood serum phospholipids and cell membranes (34). The lipid composition, the degree of PUFA unsaturation, and the proportion of sperm PUFA have been shown to affect sperm quantity (30, 34). The omega 3 PUFA needs to be provided in the diet as these PUFAs are essential for numerous processes including growth, reproduction, vision, and brain development (35).

Therefore, this study investigated the influence of omega 3.5.7 oil on reproductive dysfunction in L-NAME-induced hypertensive rats.

MATERIALS AND METHODS

Experimental Animals

Twenty-four male Sprague-Dawley rats weighing between 180–200 g were used for this study (n=6). They were acclimatized for 2 weeks, kept at room temperature, and could feed and drink water *ad libitum*. All experiments were carried out following international best practices for the management and handling of laboratory animals approved by the College of Medicine, University of Lagos Animal Care and Use Research Ethics Committee (CMUL/ACUREC/06/21/866).

Experimental Groups

Group 1 (Control group): Received 10 mL/kg distilled water.
Group 2 (L-NAME): Injected daily with 50 mg/kg b.w. L-NAME subcutaneously for 2 weeks.

Group 3 (L-NAME + Omega 3.5.7): Received L-NAME (50 mg/kg b.w.) daily for 2 weeks and 1.5% Omega 3.5.7 enriched diet.

Group 4 (Omega 3.5.7): Received 1.5% Omega 3.5.7 enriched diet.

Chemicals

Ngamma-nitro-L-arginine methyl ester hydrochloride (L-NAME), was obtained from Apex Bio (Houston, USA) while Max 3.5.7 was procured from Max International, Utah, USA. Urethane and alpha chloralose are products of Sigma-Aldrich (Darmstadt, Germany).

Blood Pressure Measurement

The animals were anesthetized with a solution of 25% (w/v) urethane and 1% (w/v) alpha chlorase injected intraperitoneally at a dose of 5 mL/kg of body weight. Rats' invasive blood pressure measurement was carried out via arterial cannulation as earlier described using a pressure transducer (model SP 844, Physiological Pressure Transducer) that was attached through MLAC11 Grass adapter cable to a computerized data acquisition system with LabChart-7 pro software (Power Lab-4/24T, model MLT844/P; AD Instruments Pty Ltd., Castle Hill, Australia) (22).

Sperm Function Analysis

The reproductive tract of the rats was exposed, and the caudal epididymis was isolated and minced in 1 mL of physiological saline to release the sperm. Each chamber of the haemocytometer was loaded with 10 µL of diluted sperm and allowed to stand for 5 min. Counting was done under a light microscope (Olympus CX 21, Beijing, China) at 400× magnification. Sperm motility was done by placing 10 µL of sperm suspension on a slide for microscopic evaluation at a magnification of 400×. An average of 100 sperm cells per rat was examined and classified as either motile or immotile and expressed as a percentage (36). Sperm morphology was determined using the eosin and nigrosin stains. Briefly, 10 µL of eosin and nigrosin was mixed with 40 µL of sperm suspension. The sperm suspension was incubated at 40 °C for 5 min and then re-suspended with a micro-pipette. About 100 sperm cells per rat were morphologically examined under the microscope at 400× magnification. Morphological abnormalities were classified as headless sperm, banana head, bent neck, and bent tail (36).

Nitric Oxide and Testosterone Assay

Nitrite/nitrate (stable NO metabolites) in the serum samples were measured based on the Griess reaction. A colourimetric method using Enzo-life Science (Lausen, Switzerland) nitrate/nitrite assay kit was used to measure serum nitric oxide metabolite concentration (NOx). The reaction buffer, NADH reagent, and nitrate reductase enzyme provided were reconstituted as described by the manufacturer, while the standards for nitrate and nitrite were also prepared in various concentrations as described in the protocol booklet. Testosterone was assayed in serum samples collected from the rats using enzyme-linked immunosorbent assay (ELISA) kits from Elabscience (Wuhan, China).

Lipid Profile Analysis

Serum lipid profile assay for total cholesterol, triglycerides, and high-density lipoprotein (HDL) was carried out on a fully automated chemistry analyzer (Mindray BS-120, Shenzhen, China) based on spectrophotometric principle using kits obtained from ERBA diagnostics (Transasia Bio-Medicals Ltd, Mannheim, Germany). LDL-cholesterol was calculated from measured values of total cholesterol, triglycerides, and HDL-cholesterol according to the relationship:

[LDL-chol] = [total chol] - [HDL-chol] – [TG]/5

Where [TG]/5 is an estimate of VLDL-cholesterol, all values are expressed in mmol/L.

Atherogenic index = Log_{10} (Triglycerides / High density lipoprotein-cholesterol)

Atherogenic coefficient = (Total cholesterol – High density lipoprotein-cholesterol) / High density lipoprotein-cholesterol Cardiac Risk Ratio = Total cholesterol / High density lipoprotein-cholesterol.

Determination of Oxidative Stress Parameters

Malondialdehyde (MDA) was determined in the homogenized liver samples. This was determined based on its interaction with thiobarbituric acid to form a pink complex with an absorption maximum at 535 nm (37). Antioxidant enzyme activity of superoxide dismutase (SOD) was determined in the liver homogenate in the medium of 0.05 M sodium carbonate buffer pH 10.3 and was initiated by the addition of epinephrine in 0.005 N HCl (38). Catalase (CAT) activities in the liver homogenate were determined by measuring the exponential disappearance of H_2O_2 at 240 nm and expressed in units/mg of protein (39), while glutathione levels (GSH) were determined by adding 10% trichloroacetic acid (TCA) to the liver homogenate. Samples were then centrifuged and 1.0 mL of the supernatant was treated with 0.5 mL of Ellman's reagent (19.8 mg of 5,5-dithiol-bis-(2-nitrobenzoic acid) DNTB) in 100 mL of 0.1% sodium nitrate) and 3.0 mL of phosphate buffer (0.2 M, pH 8.0) and the absorbance read at 412 nm (40). Absorbance was recorded using PG Instruments T70 UV/VIS (Lutterworth, UK).

Statistical Analysis

All the values are expressed as mean ± standard error of the mean (SEM). The values were analysed by one-way ANOVA followed by Student's Newman-Keuls post-hoc test using the GraphPad Prism version 6 software. Differences were considered significant when $p<0.05$.

RESULTS

Effect of L-NAME and Omega 3.5.7 on Blood Pressure Parameters

Table 1 shows the effects of L-NAME and Omega 3.5.7 on blood pressure parameters. The results showed significant increase in systolic, diastolic and mean arterial blood pressure in the L-NAME and Omega 3.5.7 + L-NAME group compared to the control group ($p<0.05$). There was also a significant increase in the rate pressure product in the L-NAME and Omega 3.5.7 group compared to the control group ($p<0.05$). There was significant decrease in the diastolic, systolic, mean arterial blood pressure and rate pressure product in the Omega 3.5.7 group compared to L-NAME and L-NAME + Omega 3.5.7 group ($p<0.05$).

Effect of L-NAME and Omega 3.5.7 on Lipid Profile and Atherogenic Indices

Table 2 shows the effects of L-NAME and Omega 3.5.7 on lipid profile and atherogenic indices. Total cholesterol and LDL-cholesterol were significantly increased in the L-NAME administered rats ($p<0.05$). L-NAME + Omega 3.5.7 reduced the increases in the total cholesterol and LDL-cholesterol levels. A similar result was observed in the calculated atherogenic coefficient and cardiac risk ratio in which L-NAME increased these values and the values were significantly reduced by the supplementation of Omega 3.5.7 ($p<0.05$).

Effect of L-NAME and Omega 3.5.7 on Sperm Parameters

Table 3 shows the effects of L-NAME and Omega 3.5.7 on sperm parameters. The results showed that sperm count and motility were significantly reduced in L-NAME and L-NAME + Omega 3.5.7 groups when compared with control $p<0.05$. No difference was found in the sperm count, motility and morphology of rats administered Omega 3.5.7 when compared with the control. However, a significant increase in sperm count and sperm motility was observed in the Omega 3.5.7 group when compared with L-NAME and L-NAME + Omega 3.5.7 groups ($p<0.05$).

Effect of L-NAME and Omega 3.5.7 on Oxidative Stress Indices

Table 4 shows the effects of L-NAME and Omega 3.5.7 on oxidative stress indices. The results showed a significant increase in MDA level in the L-NAME group compared to the control group ($p<0.05$). There was also a significant decrease in the MDA level in the Omega 3.5.7 group compared to the

Table 1: Effect of L-N^G-Nitro Arginine Methyl Ester and Omega 3.5.7 on Blood Pressure Parameters

Parameters	Control	L-NAME	L-NAME +Omega 3.5.7	Omega 3.5.7
Systolic blood pressure (mmHg)	121.12 ± 7.81	209.45 ± 4.70*	187.65 ± 2.22*	127.45 ± 6.90#$
Diastolic blood pressure (mmHg)	96.82 ± 7.41	177.56 ± 1.41*	163.92 ± 3.22*	103.03 ± 7.94#$
Pulse pressure (mmHg)	24.29 ± 0.57	31.89 ± 3.86	23.73 ± 3.85	24.41 ± 1.56
Mean arterial blood pressure (mmHg)	104.92 ± 7.53	188.19 ± 2.32*	174.12 ± 3.91*	111.17 ± 7.58#$
Heart rate (beats/min)	341.33 ± 18.24	397.33 ± 4.64	409.33 ± 9.47	385.33 ± 28.04
Rate pressure product	42096.12 ± 4781.01	83333.23 ± 2734.98*	77753.43 ± 1652.02*	50090.56 ± 6047.76#$

**Signifies significant difference from control; #signifies significant difference from L-NAME; $signifies significant difference from L-NAME + Omega 3.5.7, p<0.05.*

Table 2: Lipid Profile of Rats Administered L-N^G-Nitro Arginine Methyl Ester and Omega 3.5.7

Parameters	Control	L-NAME	L-NAME + Omega 3.5.7	Omega 3.5.7
Total cholesterol (mmol/L)	1.32 ± 0.06	1.67 ± 0.03*	1.35 ± 0.09#	1.20 ± 0.11#
Triglycerides (mmol/L)	0.40 ± 0.05	0.55 ± 0.08	0.36 ± 0.05	0.30 ± 0.05#
HDL-Cholesterol (mmol/L)	0.76 ± 0.04	0.75 ± 0.03	0.8 ± 0.04	0.78 ± 0.04
LDL-Cholesterol (mmol/L)	0.47 + 0.10	0.80 ± 0.12*	0.37 ± 0.12#	0.35 ± 0.11#
VLDL (mmol/L)	0.08 ± 0.01	0.11 ± 0.01	0.07 ± 0.01	0.06 ± 0.01#
Atherogenic index	–0.30 ± 0.07	–0.15 ± 0.05	–0.36 ± 0.07	–0.46 ± 0.09#
Atherogenic coefficient	0.77 ± 0.08	1.26 ± 0.18*	0.70 ± 0.08#	0.56 ± 0.15#
Cardiac risk ratio	1.78 ± 0.17	2.25 ± 0.08*	1.70 ± 0.09#	1.56 ± 0.15#

**Signifies significant difference from control; #signifies significant difference from L-NAME, p<0.05.*

Table 3: Effect of L-N^G-Nitro Arginine Methyl Ester and Omega 3.5.7 on Sperm Parameters

Parameters	Control	L-NAME	L-NAME + Omega 3.5.7	Omega 3.5.7
Sperm count (sperm/million 10^6)	71.66 ± 1.03	53.36 ± 2.98*	56.71 ± 1.53*	66.45 ± 2.04#
Sperm progressive motility (%)	81.33 ± 3.66	52.16 ± 6.01*	61.33 ± 4.66*	74.83 ± 2.81#
Sperm normal morphology (%)	88.00 ± 2.67	78.16 ± 3.61	81.50 ± 2.25	84.00 ± 2.00

**Signifies significant difference from control; #signifies significant difference from L-NAME, p<0.05.*

L-NAME group. Glutathione (GSH) level, SOD and catalase activities were significantly reduced in the L-NAME group compared to the control group (p<0.05), while there was an increase in GSH level and catalase activity in the Omega 3.5.7 and Omega 3.5.7 + L-NAME groups compared to the L-NAME group (p<0.05).

Effect of L-NAME and Omega 3.5.7 on Serum Testosterone and Nitric Oxide Metabolites

Figure 1 shows the effects of L-NAME and Omega 3.5.7 on serum testosterone concentration. The results showed a significant decrease in the serum concentration of testosterone in the L-NAME group when compared to the control (p< 0.05). L-NAME + Omega 3.5.7 significantly increased the reduced testosterone levels when compared with L-NAME. A similar pattern also occurred in the nitric oxide level; Figure 2 shows the effects of L-NAME and Omega 3.5.7 on nitric oxide metabolites. There was a significant decrease in nitric oxide metabolites levels in the L-NAME group when compared to the control group (p<0.05), while the decrease was reversed by the supplementation of Omega 3.5.7 with L-NAME.

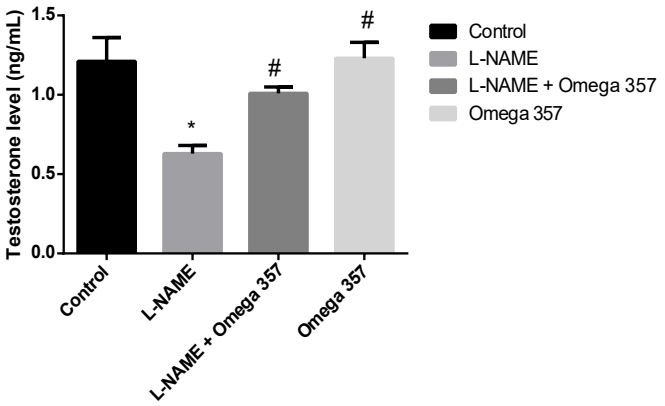

Fig. 1: Serum Testosterone Levels of Rats administered L-NAME and Omega 3.5.7. *Signifies significant difference from control; #signifies significant difference from L-NAME, p<0.05.

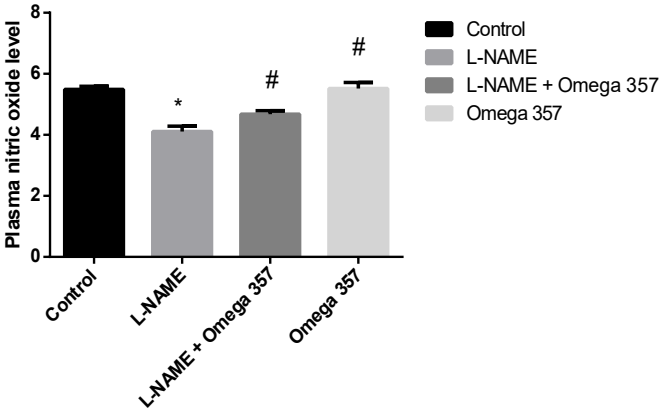

Fig. 2: Serum Nitric Oxide Level of Rats administered L-NAME and Omega 3.5.7. *Signifies significant difference from Control, # signifies significant difference from L-NAME, p<0.05.

DISCUSSION

Hypertension is one of the most common risk factors affecting cardiovascular health. It is often characterized by impaired vasodilation involving the dysfunction of multiple vasodilatory mechanisms. Several studies have shown that hypertension is associated with severe symptoms of target organ damage, including, but not limited to, complications of the heart, brain, kidney, and eye (41, 42). Recent studies have linked the testes as a target organ for hypertension due to drastic dysfunctional sperm parameters presented in a rat model

Table 4: Lipid Peroxidation and Oxidative Status of Rats Administered L-N^{G}-Nitro Arginine Methyl Ester and Omega 3.5.7

Parameters	Control	L-NAME	L-NAME + Omega 3.5.7	Omega 3.5.7
MDA level (µmol/ml)	2.11 ± 0.19	3.99 ± 0.39*	2.96 ± 0.51	2.19 ± 0.28$^{\#}$
GSH level (µmol/ml)	69.88 ± 5.50	45.97 ± 2.86*	65.30 ± 4.17$^{\#}$	74.08 ± 6.22$^{\#}$
SOD activity (µmol/ml/min/mg protein)	2.07 ± 0.06	1.43 ± 0.16*	1.80 ± 0.13	1.99 ± 0.14$^{\#}$
Catalase activity (µmol/ml/min/mg protein)	7.41 ± 0.30	4.10 ± 0.28*	6.29 ± 0.24$^{\#}$	6.34 ± 0.42$^{\#}$

**Signifies significant difference from control; $^{\#}$signifies significant difference from L-NAME; $^{\$}$signifies significant difference from L-NAME + Omega 3.5.7, $p<0.05$.*

of hypertension (7). The present study investigated the role of Omega 3.5.7, a proprietary blend of Max International on the cardiovascular and reproductive functions of male rats.

Attention was first drawn to the potential benefits of seafood and fish oils when several epidemiological studies reported a decreased incidence of cardiovascular disease, including hypertension, in regions of the world with high consumption of these foods (43, 44). Recent studies have shown that polyunsaturated fatty acids, docosahexaenoic acid (DHA and eicosapentaenoic acid (EPA) can reduce blood pressure and promote vasodilation by improving nitric oxide bioavailability (45, 46). In this study, we report that L-NAME increased systolic, diastolic, mean arterial blood pressures, and the rate pressure product. L-NAME, an established NO synthase inhibitor, is known to induce hypertension characterized by endothelial dysfunction along with marked deficiency of NO (47). Our studies confirm a reduction in NO levels in rats administered L-NAME. The endothelium-derived vasodilatory factor, NO, is a widespread biological mediator involved in many physiological and pathological processes and a crucial regulator of vascular tone (48, 49). The increased blood pressure parameters were not ameliorated significantly by the supplementation of Omega 3.5.7. This could be because of the short-term administration of the supplement or because the supplement response could be described as slow or long acting. The increased nitric oxide level by the supplementation of omega 3.5.7 enriched diet with L-NAME buttresses this point that Omega 3.5.7 could reduce blood pressure in L-NAME administered rats over time. Omega 3 has been suggested to possess vasodilatory capacity and decreases blood pressure by activating vascular eNOS which produces NO. This action was said to be mediated by cytochrome P450 1A1 enzyme (45, 46).

Oxidative stress is known to play a key role in the pathogenesis of hypertension (50, 51). The induced oxidative stress in hypertension might be one of the contributory factors to reproductive dysfunction in men living with hypertension. DNA damage, largely owing to oxidative stress, is a leading cause of defective sperm function, and increased levels of ROS result in damage to sperm DNA, RNA transcripts, and telomeres which results in male infertility and recurrent pregnancy loss (52, 53). Our study showed that L-NAME increased the index of lipid peroxidation and reduced the levels of antioxidant SOD, GSH, and CAT. This agrees with previous studies that showed that L-NAME elevates levels of oxidative stress markers such as vascular superoxide ($O_2^{\bullet-}$), plasma malondialdehyde (MDA), and plasma protein carbonyl (21, 22). Antioxidant supplementations are one of the therapeutic approaches to ease the burden of oxidative-stress-induced male factor infertility and could aid the improvement in the treatment of hypertension. Our study showed that Omega 3.5.7 + L-NAME reversed the effect of L-NAME on oxidative stress parameters. Though Omega 3 is said to possess antioxidant properties, omega-5 fatty acid (punicic acid), is a more potent antioxidant that is an agonist of PPAR gamma and capable of restoring endogenous antioxidant enzymes levels (54). It acts by inhibiting the expression of proinflammatory cytokines through PPAR and modulation delta (54). Thus, the combination of omega 5 with omega 3 might possess a synergistic effect which might alleviate oxidative stress, thus resulting in a potential decrease in blood pressure.

Another disturbing effect of hypertension is its link with a perturbed lipid profile which can contribute to atherosclerosis, the formation of fibrofatty lesions in the artery wall, which is responsible for most myocardial infarctions, strokes, as well as disabling peripheral artery disease. In our study, L-NAME increased total cholesterol, LDL-cholesterol, atherogenic coefficient, and the cardiac risk ratio. The reduction in these indices by the supplementation of omega 3.5.7 confirms its potent antilipidaemic activity. It has been suggested that endothelial nitric oxide plays a role in lipid metabolism through the activation of hepatic sterol regulatory element-binding protein (SREBP)-2, a transcriptional factor necessary for cholesterol metabolism and expression of LDL receptors (55, 56). Therefore, inhibition of nitric oxide synthase resulted in the dysregulation of lipid profile in the L-NAME administered groups.

High serum triglycerides, total cholesterol, and low-density lipoprotein in cholesterol are well-known risk factors for cardiovascular disease, conversely, high-density lipoprotein-cholesterol is considered as protective cholesterol. The atherogenic indices are calculated from the total cholesterol, triglycerides, and HDL-cholesterol. They help predict metabolic disturbances such as dyslipidaemia and atherosclerosis. The current study reports that L-NAME disrupts the atherogenic coefficient and cardiac risk ratio of the rats. This is in line with the study of Aluko (55). However, supplementation of L-NAME with Omega 3.5.7 resulted in a reversal of the atherogenic coefficient and the cardiac risk ratio back to normal. Studies have reported Omega 3 to possess anti-atherogenic activity,

by reducing the hepatic secretion of apoB-100 and SREBP-1c and by an increase of lipoprotein lipase activity (57).

Nitric oxide and nitric oxide synthase participate in the control of the levels of cytokines and hormones in the testes. They play a vital role in the homeostasis of the seminiferous epithelium microenvironment (58). Our study reported decreased sperm count, sperm motility, testosterone, and nitric oxide levels in rats administered L-NAME. This is in line with previous studies by Adedare *et al*. (5) in which L-NAME reduced sperm count, motility, and testosterone level. This might be due to the reduction in the levels of nitric oxide by the action of L-NAME on the nitric oxide synthase enzyme. We reported an increase in testosterone and nitric oxide level in the rats supplemented Omega 3.5.7 in their diet and administered L-NAME. This suggests the potential of Omega 3.5.7 to improve sperm function. However, the results of sperm count and sperm motility in L-NAME administered rats in which there was no full recovery of the sperm indices suggest that more time might be required for the administration before full recovery of sperm function could occur.

CONCLUSION

Taking together, we suggest that the improved antioxidant activity, antilipidaemic activity, improved nitric oxide, and testosterone levels are indicators that Omega 3.5.7 could help improve the activity of NOS inhibited by L-NAME. Thus, Omega 3.5.7 over time possesses a potential benefit for blood pressure reduction and improvement in sperm function in L-NAME administered rats.

Conflict of Interest

The authors declare no conflict of interest.

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