Journal of Infertility and Reproductive Biology

J Infertil Reprod Biol, 2020, Volume 8, Issue 4, Pages: 90-98

L-Arginine, Quercetin and Their Combination on Testicular Function and Sexual Behaviors in Rats

Johnson Olawumi Feyisike[1]*, Oyewopo Adeoye Oyetunji[1], Adeleke Opeyemi Samson[2], Akingbade Olabanji[3]

[1]Department of Anatomy, University of Ilorin, Ilorin, Nigeria
[2]Department of Anatomy, Osun state University, Osogbo, Nigeria
[3]Department of Anatomy, Ekiti State University, Ado Ekiti, Nigeria

Received: 14/09/2020 Accepted: 19/11/2020 Published: 20/12/2020

Abstract

L-arginine is the substrate for nitric oxide (NO) synthesis in biologic system. NO, being a free radical may induce some cytotoxic cascades related to impaired spermatogenesis. This study was undertaken to determine the effects of L-arginine, quercetin and their combination on the histology of the testes, oxidative stress biomarkers, hormones, sperm parameters and sexual behaviors in rats. 35 adult male rats divided into seven groups were used. A (Control), B (Sildenafil treated, 1.4mg/kg/day), C (L-arginine treated, 30 mg/kg/day), D (L-arginine treated, 100 mg/kg/day), E (Quercetin treated, 10 mg/kg /day), F (L-arginine, 30 mg/kg/day and Quercetin, 10 mg/kg/day) co-administrated, G (L-arginine, 100 mg/kg/day and Quercetin, 10 mg/kg/day) co-administrated. Animals were sacrificed after 8 weeks and the histology of the testes, oxidative stress biomarkers, hormonal assay, sperm parameters and some sexual behaviors were done. The results obtained from the study showed that L-arginine has a relatively adverse effect on spermatogenesis which was improved by co-administration with Quercetin. The findings from this study suggests that synergism between L arginine and Quercetin can improve spermatogenesis as well as sexual behaviors in rats.

Keywords: Antioxidants, Arginine, Nitric oxide, Quercetin, Spermatogenesis

Introduction

Infertility has become a global concern affecting up to 186 million people worldwide (1) and male factors put up to about 30%-55% of all cases, which are mainly related to decreased spermatogenesis (2). Infertility is the inability to achieve successful pregnancy after 12 months of consistent unprotected sex. It is estimated that approximately 15% of reproductive-age couples suffer from infertility (3). Male infertility refers to the incapability of a man to impregnate a woman after 12 months of regular and unprotected sexual intercourse. Factors including drug treatment, chemotherapy, toxins, air pollutions and inadequate vitamins intake have harmful effects on spermatogenesis and normal sperm output (4, 5).

To help improve male sexual performance, men consume L arginine, a semi essential amino acid as dietary supplement. L-arginine is one of the most commonly used food supplements for the management of erectile dysfunction. It is an amino acid used in the body to manufacture nitric oxide (6). It has various metabolic and immunologic effects and has been considered to be conditionally essential. L-Arginine is a notable precursor to nitric oxide (NO), a key component of endothelial-derived relaxing factor, an endogenous messenger molecule connected to diverse endothelium-dependent physiological effects in the cardiovascular system (7). Nitric oxide (NO) is produced from L-arginine by the activity of NO synthase (NOS), an enzyme that occurs in three isoforms (8).

Quercetin, a dietary flavonoid ubiquitously present in a broad range of foods has received considerable attention because of its overwhelming presence in foods (9). Quercetin has a wide range of reported biologic effects, including antioxidant, antihypertensive, antimicrobial and antiprotozoan activities (10-13). Most studies conducted on it had focused on the antioxidant properties of quercetin, its effects on several enzyme systems and effects on biological pathways involved in carcinogenesis, inflammation and cardiovascular diseases (14-17) as it is believed to prevent lipid peroxidation. It is also attracting intense scientific interest for its unique anti-aging and immune boosting activities (18) and several recent studies show that organisms exposed to high levels of quercetin live longer, healthier lives and have good pregnancy outcome (19-22).

Investigations in infertility has confirmed that excessive production of reactive oxygen and nitrogen species (ROS and RNS), in other words, oxidative stress, is connected to the aetiology of infertility, especially male infertility (23, 24). All of the reports have linked ROS/RNS, generated both exogenously and endogenously, with some aspects of male infertility, such as decreased sperm motility, abnormal morphology and reduced sperm–egg penetration. There are conflicting arguments on the role or effects of nitric oxide, the product of L-arginine on male fertility (25-32). Despite the important physiological benefits, NO as a free radical can induce cytotoxic cascades in the biological

***Corresponding author:** Johnson Olawumi Feyisike, Department of Anatomy, University of Ilorin, Ilorin, Nigeria. Email: olawumi_2005@yahoo.co.uk

system. Quercetin is a naturally occurring flavonoid with antioxidant properties (8). It has been found to prevent testicular toxicities (33).

This study would provide useful insights into the interactions between compounds used in the management of erectile dysfunction and testicular function and the role of quercetin on these interactions. The aim of the study therefore was to evaluate the effect of L-arginine and quercetin oral administration and their combination on histology of the testes, oxidative stress biomarkers, hormones (FSH, LH & TT), sperm parameters and sexual behaviors.

Materials and methods

Animal materials

35 adult male and 5 female Wistar rats (180g-200g) were procured and used for the experiment. The rats were randomly assigned into seven (7) groups with 5 male rats in each group. Thereafter, the rats were allowed to acclimatize to the animal house condition for two weeks with free access to feed and water and the grouping was maintained throughout the period of the experiment. Ethical approval for the study was obtained from the University of Ilorin Ethical Committee on the Use of Animals for Experiments. The studies were conducted in accordance with the laws and regulations guiding the use of animals. The seven (7) groups were as follows: A (Negative Control - Normal healthy animals), B (Sildenafil citrate treated rats, 1.4mg/kg/day) positive control, C (L-arginine treated rats, 30 mg/kg/day), D (L-arginine treated rats, 100 mg/kg/day), E (Quercetin treated rats, 10 mg/kg /day), F (L-arginine, 30 mg/kg/day and Quercetin, 10 mg/kg/day) co-administrated rats, G (L-arginine , 100 mg/kg/day and Quercetin , 10 mg/kg/day) co-administrated rats.

L-Arginine and quercetin preparation

The pure L-arginine and Quercetin were purchased in powder form (Sigma Chemical). The powder forms were dissolved in distilled water (34) and administered to rats as suspension in distilled water by oral gavage for 8 weeks.

Sildenafil citrate (Viagra) preparation

Viagra was purchased from Pfizer pharmaceuticals. Each 100 mg tablet of sildenafil citrate was dissolved in 100 ml of distilled water so each 1ml contained 1mg of the drug and administered 1.4 mg/kg/day (35) to rats in group B by oral gavage for the 8 weeks of experiments.

Animal sacrifice, Sample collection and Histology

Animals were euthanized with ketamine (100 mg/kg bodyweight) at the end of the period of treatment. Blood was collected through cardiac puncture. For histological studies, the testis was excised and fixed in Bouin's fluid and processed using Haematoxylin and Eosin (H&E)(36) staining. Blood samples were centrifuged at 3500rpm for 10 min and then sera were stored at -80ºC until analysis.

Oxidative stress biomarkers

Superoxide dismutase (SOD) was assayed according to the method of (37). Reduced glutathione (GSH) level was determined according to the method of (38).

Glutathione peroxidase was assayed according to the method of (39). Nitric oxide is an unstable molecule with a short half-life that reacts with molecular oxygen and accumulates in serum or plasma as nitrate (NO_3^-) and nitrite (NO^-) ions; thus, the quantification of NOx in biological samples provides essential information about NO production. NO level was assayed by assessing the nitrites content (i.e. the stable end product of NO according to the method of (40)

Measurement of serum LH, FSH and testosterone levels

The serum levels of LH, FSH and testosterone were analyzed using an enzyme-linked immuno-absorbent assay (ELISA) kit (Shanghaicrystat Day Biotech Co. LTD, Shanghai, China for LH & FSH and IBL International GMBH Flughafenstrasse Hamburg, Germany for testosterone) according to the manufacturer's guidelines.

Sperm parameters

The left epididymis of each rat was dissected and put in 1 mL of pre-warmed Ham's F10, then minced into small sections and incubated for a few minutes (37 °C, 5% CO_2) to allow the spermatozoa to swim out of the epididymal tubules.

Sperm parameters (count, motility, viability, and morphology) were studied in all groups. An aliquot of sperm suspension was diluted 1: 20 with Ham's F10 medium and transferred into a Neubar's hemocytometer under a coverslip to estimate the sperm count. Spermatozoa were counted under a light microscope at ×400 (Olympus Light Microscope; Olympus Corp., Tokyo, Japan) and expressed as million per ml of suspension. A drop of sperm sample was placed on clean and 37 °C pre-warmed slides, and a minimum of 200 spermatozoa motility was accessed under a light microscope. Motility was shown as the percentage of motile sperm according to the World Health Organization manual criteria. Sperm viability was analyzed by eosin-nigrosin staining in 500 spermatozoa. A drop of stained sperm suspension was put on a clean slide, and a thin smear was made and allowed for drying. This slide was analyzed under a light microscope at ×1000 and spermatozoa with white and pink heads were considered as alive and dead, respectively (41).

Sexual behaviors

For behaviorsal studies, female rats were introduced in the cages of the male rats 3 weeks after the start of administration and using a camcorder the following parameters were monitored: Mounting frequency, intromission frequency and sexual motivation (42).

Data Analysis

Data obtained was analyzed using GraphPad prism 5 software. All results were expressed as mean± standard error of mean (SEM), and one way ANOVA analysis, followed by Tukey's post hoc test was performed. $P<0.05$ was considered statistically significant.

Results

Histology

Figure 1 shows the representative histological sections of the testes of rats after the administration of L-arginine, Quercetin and

their combination. Testicular sections of control rats showing normal seminiferous tubules, exhibiting spermatogenesis in different stages and Leydig cells in interstitial. Reduced diameter of the germinal epithelium (blue arrows) in the treated groups except for the Quercetin and the co-administrated groups was observed. Black arrows demonstrate Sertoli cells.

Histological analysis showed that the structure of the testis and seminiferous tubule is entirely typical in control and Quercetin groups. Destruction of the germinal epithelium of the seminiferous tubule was observed and formation of vacuoles in the L-arginine treated groups. Shrinking and irregular shape of the basement membrane of the seminiferous tubules and disorders of cellular arrangement and organization were revealed in the L-arginine treated groups. Moreover, tissue structural improvement was evident in the co-administrated groups as against what was obtained in the L-arginine treated groups.

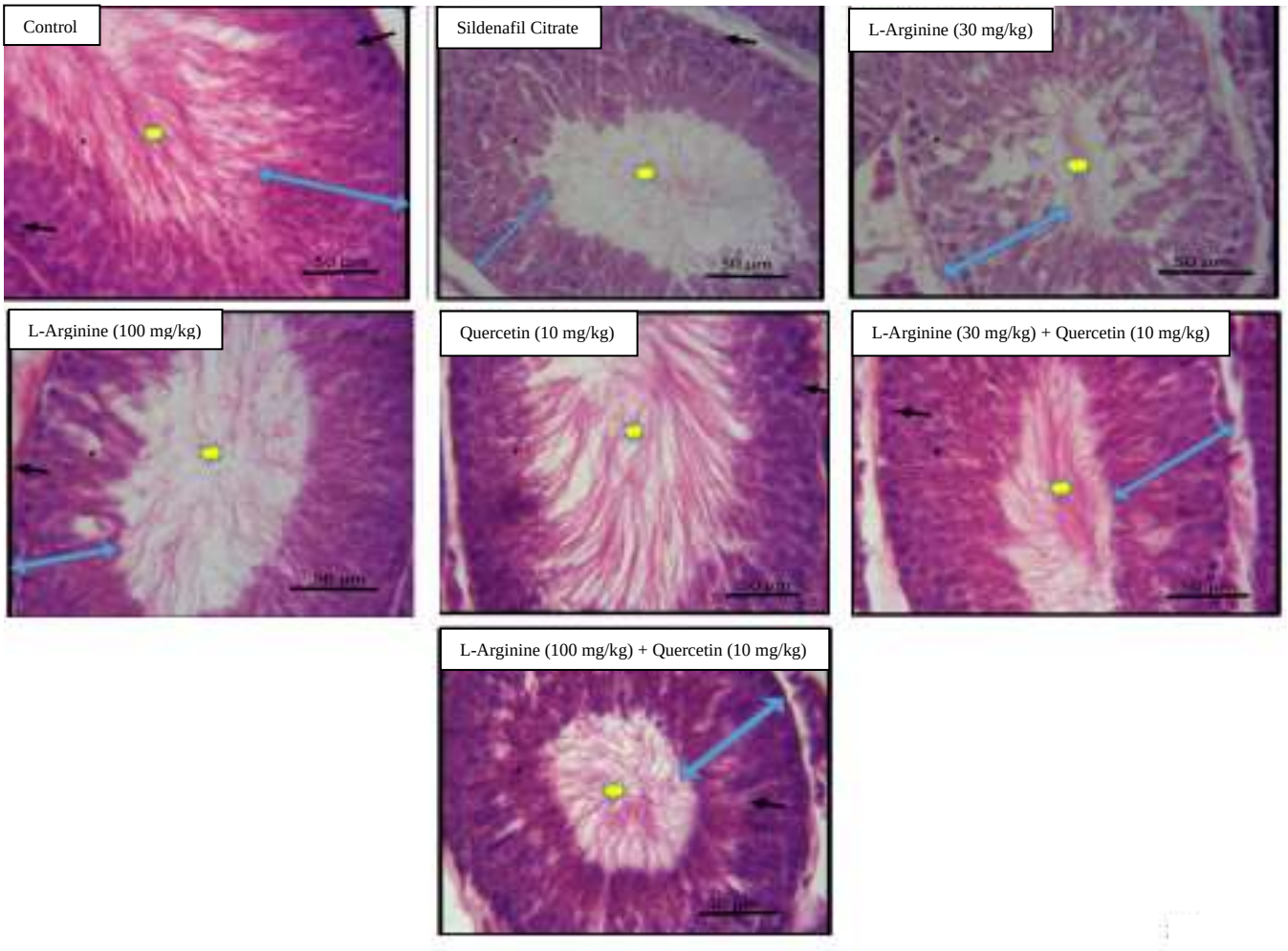

Figure 1. Representative micrographs of H&E showing the general histology of the testes of the Wistar rats administered with L-arginine, Quercetin and their combination and Sildenafil citrate and positive control ×400. . Reduced diameter of the germinal epithelium (blue arrows) in the treated groups except for the Quercetin and the co-administrated groups was observed. Black arrows demonstrate Sertoli cells.

Oxidative stress biomarkers

The effects of L-arginine, Quercetin and their combination on oxidative stress biomarkers are presented in Table 1. It was observed that L-arginine (100 mg/kg) decreased significantly ($P<0.05$) the level of serum GPx & SOD as compared to the control group and the Quercetin treated group.

The level of this biomarkers were increased in the co-administrated groups (L-arginine, 30 mg/g + Quercetin, L-arginine, 100 mg/kg + Quercetin). The serum levels of level of nitric oxide was significantly increased in the L-arginine groups (100 mg/kg being the highest) as compared to the control group, the sildenafil citrate group and especially the Quercetin group. (Table 2).

Hormonal assay

The effect of L- Arginine, Quercetin and their combination on serum levels of FSH, LH and testosterone (TT) were determined and presented in Table 2. The levels of LH, FSH and TT were significantly decreased in the L-arginine treated groups when compared with the control group and Quercetin group but was significantly improved 0.05) in the co-administrated groups.

Sperm parameters

The effects of L-arginine, Quercetin and their combination on sperm parameters in rats are showed in Table 3.

Sperm Count

Sperm count was decreased in the L-arginine treated rats as compared to the control group and the Quercetin treated group.

Sperm Motility

There was significant decrease (P< 0.05) in sperm motility in L-Arginine treated groups as compared with the Control and Quercetin groups. There was significant improvement in sperm motility of the co-administrated groups as compared to what was obtained in the L-arginine treated groups. There was no significant difference between the control and Quercetin, 10 mg/kg group (P>0.05). The difference between Sildenafil citrate group and control group was not statistically significant (P< 0.05) (Table 3).

Sperm Normal Morphology

There was significant decrease (P<0.05) in the percentage of sperm with normal morphology in the L-Arginine groups irrespective of dose when compared with the Control group and the Quercetin group. There was no significant difference (P>0.05) in the percentage of sperm with normal morphology in the Control group and the Quercetin group (Table 3).

Sexual behaviors

At the end of the first four weeks of treatments, frequency of mounting was significantly increased in the sildenafil citrate group as compared with the other groups, but at 8 weeks, there was significant increase in the L-arginine and sildenafil citrate group as compared to the other groups (Table 4). For sexual motivation there was significant increase at the end of the 8 weeks in the L-arginine treated groups compared to the control and sildenafil citrate groups as against what was obtained after 4 weeks (Table 4). Frequency of intromission was significantly increased in the L-arginine group (100 mg/kg) and co-administrated group (L-arginine, 100 mg/kg and Quercetin, 10 mg/kg) as compared to the control group at the end of the 8 weeks of administration compared to after four weeks of administration (Table 4).

Table 1: Effect of L- Arginine, Quercetin and their combination on oxidative stress biomarkers

		Treatments					
Parameters	**Control**	**Sildenafil Citrate**	**L-Arginine, 30 mg/kg**	**L-Arginine, 100 mg/kg**	**Quercetin, 10 mg/kg**	**L-Arginine, 30 mg/kg + Quercetin, 10 mg/kg**	**L-Arginine, 100 mg/kg + Quercetin, 10 mg/kg**
NO	5.15±0.16	9.55±0.37***	10.91±0.66****	15.64±0.65****	4.12±0.12^{ns}	7.64±0.46*	10.94±0.45****
GSH	10.0±0.3	21.0±5.0*	10.0±0.2^{ns}	6.0±0.1^{ns}	23.0±3.0*	26.0±3.0**	12.0±2.0^{ns}
GPx	99.72± 3.30	54.51±16.48^{ns}	33.56±5.94	45.1±5.73*	164.3±7.92*	103.46±2.15*	74.73±19.70^{ns}
SOD	57.56±14.57	83.69±4.49^{ns}	45.1±5.73^{ns}	33.56±5.94^{ns}	164.3±7.93****	103.46±2.15*	119.51± 6.28***

Values are expressed as mean ± SEM, n=5 (numbers of animals in each group). One-way analysis of variance (ANOVA) was used followed by Tukey's post hoc test. Statistical differences between control and different groups: *; P<0.05,**; P<0.005, ***; P<0.0005, ns – not significant. P value<0.05 was considered statistically significant compared to the control group.

Table 2: Effect of L- Arginine, Quercetin and their combination on FSH, LH & TT

		Treatments					
Parameters	**Control**	**Sildenafil Citrate**	**L-Arginine, 30 mg/kg**	**L-Arginine, 100 mg/kg**	**Quercetin, 10 mg/kg**	**L-Arginine, 30 mg/kg + Quercetin, 10 mg/kg**	**L-Arginine, 100 mg/kg + Quercetin, 10 mg/kg**
LH	55.30±0.32	20.39±1.86****	40.46±1.91**	35.23±4.34***	39.38±0.29**	55.35±1.0^{ns}	45.85±2.46^{ns}
FSH	80.13±2.22	33.56±5.94****	28.74±3.68****	27.18±3.52****	64.3±7.93^{ns}	77.02±1.45^{ns}	48.44±2.84**
TT	24.33±2.0	22.31±1.10^{ns}	17.74±0.13*	11.61±0.24***	23.23±1.22^{ns}	20.86±0.2^{ns}	18.31±1.44*

Values are expressed as mean ± SEM, n=5 (numbers of animals in each group). One-way analysis of variance (ANOVA) was used followed by Tukey's post hoc test. Statistical differences between control and different groups: *; P<0.05,**; P<0.005, ***; P<0.0005, ns – not significant. P value<0.05 was considered statistically significant compared to the control group.

Table 3: Effect of L- Arginine, Quercetin and their combination on Sperm parameters

	Treatments						
Parameters	**Control**	**Sildenafil Citrate**	**L-Arginine, 30 mg/kg**	**L-Arginine, 100 mg/kg**	**Quercetin, 10 mg/kg**	**L-Arginine, 30 mg/kg + Quercetin, 10 mg/kg**	**L-Arginine, 100 mg/kg + Quercetin, 10 mg/kg**
Sperm count	50 ± 5.45	54 ± 2.45^{ns}	36 ± 6.96^{ns}	35.6 ± 4.23^{ns}	44 ± 4.00^{ns}	32 ± 7.35*	26 ± 6.78^{ns}
Sperm motility	76.2 ± 2.11	74.2 ± 3.23^{ns}	51 ± 6.60**	51.7 ± 2.76**	73.4 ± 2.96^{ns}	58.8 ± 4.87**	75.63 ± 4.44^{ns}
Sperm morphology	71 ± 4.58	71 ± 3.32^{ns}	35 ± 4.47***	38 ± 3.55***	65 ± 4.47^{ns}	50 ± 8.37^{ns}	59 ± 4.00^{ns}

Values are expressed as mean ± SEM, n=5 (numbers of animals in each group). One-way analysis of variance (ANOVA) was used followed by Tukey's post hoc test. Statistical differences between control and different groups: *; P<0.05,**; P<0.005, ***; P<0.0005, ns- not significant. P value<0.05 was considered statistically significant compared to the control group.

Table 4: Effect of L-arginine, Quercetin and their combination on sexual behaviors in rats after 8 weeks

	Treatments						
Parameters	**Control**	**Sildenafil Citrate**	**L-Arginine, 30 mg/kg**	**L-Arginine, 100 mg/kg**	**Quercetin, 10 mg/kg**	**L-Arginine, 30 mg/kg + Quercetin, 10 mg/kg**	**L-Arginine, 100 mg/kg + Quercetin, 10 mg/kg**
Frequency of mounting	2.5±0.5	11.0± 1.0*	6.67±0.9^{ns}	13.5±0.5**	7.0±1.0^{ns}	7.67±1.5^{ns}	9.67±1.7*
Frequency of intromission	13.0±2.0	14.25±2.7^{ns}	17.0±2.1^{ns}	26.75±3.4**	9.5±1.2^{ns}	10.0±0.4^{ns}	17.5±2.1^{ns}
Sexual motivation	24.25±1.5	33.33±2.4^{ns}	43.35±5.3*	54.28±6.2***	39.13±3.1^{ns}	35.4±3.3^{ns}	46.2±2.5*

Values are expressed as mean ± SEM, n=5 (numbers of animals in each group). One-way analysis of variance (ANOVA) was used followed by Tukey's post hoc test. Statistical differences between control and different groups: *; P<0.05,**; P<0.005, ***; P<0.0005, ns – not significant. P value<0.05 was considered statistically significant compared to the control group.

Discussion

In the present study, there were some histological changes in the sildenafil citrate and the slightly in the L-arginine treated group (Figure 1) which may lead to impaired spermatogenesis, in the form of decreased cells of the spermatogenic layers reduction in the diameter of the seminiferous tubules. Also, congested and dilated blood vessels with increased number of Leydig cells were also realized in the sildenafil citrate group. This finding is in agreement with study (43) which investigated the effect administration of Sildenafil Citrate on sperm count, sperm malformations and testicular histological changes of male rats. The histological changes seen in the L-arginine treated group (Figure 1) could be as a result of the increase. These effects may be according to the changes in the expression of various receptors associated cGMP or responsive effect of these receptors in the brain and this will cause damage of testicular tissues and failure in spermatogenesis (44, 45). The results from this study showed the protective role of Quercetin in the co-administrated groups. This is in correlation with a recent study that Quercetin protects testicular structure and histology in case of testicular toxicity (13). This study showed that oral administration of L-arginine had a significant (P<0.05) increase in NO concentration in seminal plasma in rats (Table 1); indicating that the dosage and duration of L-arginine administration may produce a significant effect in increasing NO concentration in this study. L-arginine is the sole substrate for NO synthesis with the presence of enzyme nitric oxide synthase (NOS), oxygen and nicotinamide nitric oxide synthase (NOS), oxygen and nicotinamide adenine dinucleotide phosphate (NAPDH). Thus, higher oral intake of L-arginine may increase NO concentration in the plasma where there was a significant (P<0.05) positive association between L-arginine consumption and NO concentration. However, the increment of NO concentration was higher in the L-Arginine, 100 mg/kg group suggesting that higher dose of L-Arginine may produce higher NO concentration in seminal plasma. These findings support previous studies done which demonstrated that certain concentrations of L-arginine used have ability of increasing the NO levels in the brain and the reproductive organs (42, 46). A recent study also supports this finding that L-arginine can significantly increase serum NO level (47). NO reacts with superoxide anion to form peroxynitrite that react directly with lipid, protein and DNA results in cellular deterioration and tissue damage. In the testes, antioxidant defense system protects the cells from oxidative damages (48). The rate of NO production may be critically dependent on the availability of arginine. Decreased arginine availability may result to decreased NO synthesis (49). NO has a double function, being both a cytotoxic and necessary molecule for normal spermatogenesis. Under physiological conditions, NO plays a key role in normal spermatogenesis. Low NO concentrations have been shown to enhance sperm motility (50), whereas high NO concentrations reduce it (51). Hence confirming the role of Quercetin in this present study.

However, in this study, SOD and GPx were markedly decreased in the L-arginine treated groups when compared to the control and Quercetin groups. This finding indicates the inability or dysfunction of antioxidants to act as NO and ROS scavenging machinery in the testes due the high level of NO in the L-arginine treated groups. The levels of SOD, GSH & GPx was improved in the co-administrated groups. The rise in these cellular antioxidants in the co-administrated rats signifies defense against the depletion in antioxidant status via its antioxidant activity. This is in consonant to several studies that Quercetin enhances expression levels of endogeneous antioxidant enzymes showing a strong antioxidant activity by maintaining oxidative balance (52, 53

The findings from this study showed that the level of gonadotropins were reduced in the L-arginine treated and the sildenafil citrate treated group when compared to the control groups and the co-administrated groups. This findings correlates with a previous study which reported that NO donors suppresses GnRH-stimulated LH release from pituitaries in male rats (54). Another study demonstrated that NOS inhibitor p-nitro-L-arginine methyl ester (L-NAME) improved GnRH-induced LH release from pituitaries in rats (55). The decrease in the levels of FSH and LH in the L-arginine treated group could have been as a result of increase in NO level in these groups which were reduced in co-administrated groups. The improved levels of FSH and LH in the co-administrated groups could have been the anti-oxidative effect of Quercetin which mopped up the excess NO produced from L-arginine in the blood. It was previously reported that low level of NO stimulates the release of LH from the pituitaries of male rats (56). Leydig cells produce and release testosterone under the influence of LH and acts as autocrine and/or paracrine hormones in the gonads under modulation of NO (57). The reduced level of testosterone in the L-arginine treated groups when compared to the control group and co-administrated groups could have still pointed to the level of NO. It was reported that NO seemingly exerted a transient stimulatory effect on testosterone secretion (58). At low concentrations, NO exerted a transitory stimulatory effect on testosterone secretions mediated by cyclic GMP, whereas at high concentrations, it inhibited steroidogenesis by Leydig cells (59). This point to the fact that coadministration of L-arginine with Quercetin lowered the level of nitric oxide which improved the level of testosterone.

The results obtained from sperm count showed non-significant results except in L-Arginine (100 mg/kg) + Quercetin (10 mg/kg), which showed a significant ($P<0.05$) increase in sperm count (Table 3). This may be due to the fact that NO is a key regulator of gonadotrophin releasing hormone (GnRH), which is involved in the stimulation of luteinizing hormone (LH) that regulates the function of the Leydig cells in testes to produce testosterone. Elevated testosterone may cause an increase in sperm count. These findings supported previous studies done where the anatomical localization of NO neurons in close proximity to GnRH neurons in hypothalamus has been demonstrated and suggested that NO may be an important indicator of GnRH secretion (60).

Sperm motility is a pivotal parameter of semen quality (61). Accordingly, a principal cause of male infertility is decreased sperm motility, and men with poorly motile or immotile sperm are usually sterile (62).The finding that free radicals can affect male fertility has received substantial scientific evidence (24). The proposed mechanism for loss of sperm function upon oxidative stress has been shown to involve excessive generation of reactive oxygen species (ROS) (63). The nitrogen derived free radical nitric oxide (NO) and peroxynitrite anion (ONOO-) also appear to play a key role in reproduction and fertilization. This study showed that there was a significant ($P<0.05$) drop in percentage of sperm motility in the L-Arginine, 30 mg/kg and L-Arginine, 100 mg/kg, L-arginine treatment groups as compared to the control groups (Table 3). This showed that sperm motility is inversely proportional to the NO production. The results obtained here are closely related to the previous studies on human sperm, where NO supplied by a variety of donors inhibits human sperm motility, possibly by impeding cellular respiration (46, 64). Studies carried out by Weinberg and colleagues had that high concentration of NO (at milimolar to micromolar concentrations of sodium nitroprusside) distrupted sperm motility and viability (65). The results are consistent with other reports showing that high concentration of NO have a cytotoxic effect on spermatozoa (42).

Sperm need high levels of adenosine triphosphate (ATP) to sustain their motility. ATP is generated by the glycolytic pathway and the mitochondrial electron transport system. Based on a previous study, NO can reduce ATP levels in ability of enzymes in these pathways (e.g., iron-containing proteins of the electron transport system and the tricarboxylic acid cycle and glyceraldehydes phosphate dehydrogenase of the glycolytic pathway) (66). NO may also hinder heme containing the mitochondrial enzymes, aconitase, NAPH:ubiquinone, and NADH:succinate oxidoreductase, leading to the reduction in sperm motility by inhibition of ATP production (65). Thus, excessive amounts of NO may cause sperm dysfunction and damage (46). The significant increase in the percentage of sperm motility in the L-Arginine (100 mg/kg) + Que (10 mg/kg group as compared to the L-Arginine (100 mg/kg) group suggests the protective effect of quercetin against the cytotoxic effect of excessive production of NO on sperm motility. This finding support previous study on the antioxidative effect of quercetin in manganese induced testicular toxicity in rats (67).

Sperm morphology is a vital parameter that reflects the degree of normalcy and maturity of the sperm population in the ejaculate and corresponds with fertility (68). Deformity of the head and mid-piece have been classified as primary defects of spermatogenesis, and occur during testicular degeneration. Main defects of spermatogenesis are more likely to be associated with decreased fertility (69). Oxidative stress due to excessive production of NO gives rise a range of pathologies that are believed to negatively affect the male reproductive function. Oxidative stress-induced damage to sperm mediated by lipid peroxidation can cause damage to DNA in sperm nucleus (70, 71). The significant decrease ($P<0.05$) in the percentage of sperm with normal morphology in the L-Arginine, 30 mg/kg and L-Arginine, 100 mg/kg groups when compared with the Control group and the Quercetin, 10 mg/kg group (Table 1) possibly suggests a relationship between the production of NO and abnormal sperm morphology. Suggesting that increased production of NO may exert cytotoxic effect on sperm morphology through oxidative stress. This finding is supported by a study which showed that high concentrations NO induces atypical sperm morphology (72). It was also observed from the study that there was improvement in normal sperm morphology

as compared with the L-arginine treated groups and the sildenafil citrate group. This improvement could be as a result of the antioxidative properties of quercetin in the co-administrated groups.

This study also shows that L-arginine and sildenafil citrate significantly increased sexual motivation and the frequency of mounting and intromission (Table 4) at the end of the 8 weeks of experiment compared to the control group. It was also observed from the study that there was significant increase in frequency of mounting and sexual motivation in the co-administrated groups when compared to the control groups but there was a decrease when compared with the L-arginine only groups. In a study, L-arginine and yohimbine were compared; results demonstrated that arginine supplementation improved sexual behaviors, while pure yohimbine supplementation did not have the same effect (73). NO is a vasodilator that increases blood flow in penis arteries (74). Arginine increases synthesis and release of NO, thus it can be the mechanism of arginine effect on sexual behaviors. Male sexual behaviors of rat is impeded by L-NAME and this effect has been credited to reduction of NO at brain centres controlling reproduction (75). Thus, the ability of L-arginine to affect sexual behaviors suggests that NO exists in brain for the regulation of male sexual function. This finding is however with agreement with some studies that have investigated the effects of exogenous l-arginine on restoring NOS activity and improving erectile function. Chen et al. reported that oral administration of l-arginine in high doses (5 g/d) caused significant subjective improvement in sexual function in men with organic erectile dysfunction only if they had decreased NO_x excretion or production prior to arginine supplementation (76). It was showed that long-term oral administration of supraphysiological doses of l-arginine improves erectile function in the aging rat (77). It was also shown that that administration of oral l-arginine to diabetic rabbits increases endothelium-dependent relaxation of rabbit corpus cavernosum but had no effect on neurogenic relaxation in diabetic animals (78). Angulo et al. also investigated the effects of phentolamine and l-arginine on neurogenic relaxation of healthy rabbit corpus cavernosum in organ bath studies (79).

Conclusion

The results obtained from the study showed that prolonged use of L-arginine has a relatively adverse effects on spermatogenesis because of the buildup of nitric oxide in the system. This however was improved by co-administration with Quercetin. The findings from this study suggests that synergism between L arginine and Quercetin can improve spermatogenesis as well as sexual behaviors in rats (as the role of L-arginine cannot be ruled out in fertility).

Authors' contribution

OF conceived the study and its design, participated in experimental procedures, interpretation of data and drafted the manuscript. AO participated in the experimental procedures and helped in interpretation of data and revised the manuscript. OS contributed to the study design, participated in interpretation of data. O assisted with the acquisition of data and participated in experimental procedures. All authors approved the final manuscript.

Declaration of interest

The author declares that there is no conflict of interest.

References

1. Inhorn MC, Patrizio P. Infertility around the globe: new thinking on gender, reproductive technologies and global movements in the 21st century. Human reproduction update. 2015;21(4):411-26.
2. Wu H, Sun L, Wen Y, et al. Major spliceosome defects cause male infertility and are associated with nonobstructive azoospermia in humans. Proceedings of the National Academy of Sciences. 2016;113(15):4134-9.
3. Sharlip ID, Jarow JP, Belker AM, et al. Best practice policies for male infertility. Fertility and sterility. 2002;77(5):873-82.
4. Sule J, Erigbali P, Eruom L. Prevalence of infertility in women in a southwestern Nigerian community. African Journal of Biomedical Research. 2008;11(2).
5. Alaee S, Talaiekhozani A, Ziaei GR, Lohrasbi P. Evaluation of Iranian college students' awareness about infertility risk factors. Jundishapur Journal of Health Sciences. 2016; 8 (2):
6. Ivanova S, Pankova S, Petkova V, Dimitrov M. Food additives with beneficial effects in the treatment of erectile dysfunction, containing L-Arginine, Pycnogenol and Ginseng extract. World journal pharmacy and pharmaceutical science. 2014;3(11):234-45.
7. Blum A, Hathaway L, Mincemoyer R, et al. Oral L-arginine in patients with coronary artery disease on medical management. Circulation. 2000;101(18):2160-4.
8. Rosselli M, Keller R, Dubey RK. Role of nitric oxide in the biology, physiology and pathophysiology of reproduction. Human reproduction update. 1998;4(1):3-24.
9. Bentz AB. A review of Quercetin: chemistry, Antioxident properties, and bioavailability. Journal of young investigators. 2017.
10. Liu J-J, Song C-W, Yue Y, et al. Quercetin inhibits LPS-induced delay in spontaneous apoptosis and activation of neutrophils. Inflammation Research. 2005;54(12):500-7.
11. Bischoff SC. Quercetin: potentials in the prevention and therapy of disease. Current Opinion in Clinical Nutrition & Metabolic Care. 2008;11(6):733-40.
12. Chan S-T, Chuang C-H, Yeh C-L, et al. Quercetin supplementation suppresses the secretion of pro-inflammatory cytokines in the lungs of Mongolian gerbils and in A549 cells exposed to benzo [a] pyrene alone or in combination with β-carotene: in vivo and ex vivo studies. The Journal of nutritional biochemistry. 2012;23(2):179-85.
13. Khodabandeh Z, Dolati P, Zamiri MJ, et al. Protective Effect of Quercetin on Testis Structure and Apoptosis Against Lead Acetate Toxicity: an Stereological Study. Biological Trace Element Research. 2020:1-11.
14. Formica J, Regelson W. Review of the biology of quercetin and related bioflavonoids. Food and chemical toxicology. 1995;33(12):1061-80.
15. Pérez-Vizcaíno F, Ibarra M, Cogolludo AL, et al. Endothelium-independent vasodilator effects of the flavonoid quercetin and its methylated metabolites in rat conductance and resistance arteries. Journal of Pharmacology and Experimental Therapeutics. 2002;302(1):66-72.
16. Kris-Etherton PM, Lefevre M, Beecher G, et al. Bioactive compounds in nutrition and health-research methodologies for establishing biological function: the antioxidant and anti-

inflammatory effects of flavonoids on atherosclerosis. Annu Rev Nutr. 2004;24:511-38.
17. Williams RJ, Spencer JP, Rice-Evans C. Flavonoids: antioxidants or signalling molecules? Free radical biology and medicine. 2004;36(7):838-49.
18. Li Y, Yao J, Han C, et al. Quercetin, inflammation and immunity. Nutrients. 2016;8(3):167.
19. Proshkina E, Lashmanova E, Dobrovolskaya E, et al. Geroprotective and radioprotective activity of quercetin,(-)-epicatechin, and ibuprofen in Drosophila melanogaster. Frontiers in pharmacology. 2016;7:505.
20. Jung H-G, Kim H-H, Dey DK, et al. The Anti-inflammatory and Immune-Boosting Potential of Quercetin-3-O-β-D-glucopyranosyl-(1→6)-β-D-glucopyranoside in LPS–Stimulated RAW264. 7 Macrophages. Revista Brasileira de Farmacognosia. 2020:1-7.
21. Mrityunjaya M, Pavithra V, Neelam R, et al. Immune-Boosting, Antioxidant and Anti-inflammatory Food Supplements Targeting Pathogenesis of COVID-19. Frontiers in Immunology. 2020;11:570122.
22. Bolouki A, Zal F, Alaee S. Ameliorative effects of quercetin on the preimplantation embryos development in diabetic pregnant mice. Journal of Obstetrics and Gynaecology Research. 2020;46(5):736-44.
23. Aitken RJ, Baker MA. Oxidative stress, sperm survival and fertility control. Molecular and cellular endocrinology. 2006;250(1-2):66-9.
24. Agarwal A, Makker K, Sharma R. Clinical relevance of oxidative stress in male factor infertility: an update. American journal of reproductive immunology. 2008;59(1):2-11.
25. Di Fiore MM, Lamanna C, Assisi L, Botte V. Opposing effects of D-aspartic acid and nitric oxide on tuning of testosterone production in mallard testis during the reproductive cycle. Reproductive Biology and Endocrinology. 2008;6(1):1-9.
26. Ducsay CA, Myers DA. eNOS activation and NO function: differential control of steroidogenesis by nitric oxide and its adaptation with hypoxia. Journal of Endocrinology. 2011;210(3):259-69.
27. Doshi SB, Khullar K, Sharma RK, Agarwal A. Role of reactive nitrogen species in male infertility. Reproductive Biology and Endocrinology. 2012;10(1):1-11.
28. Wang J, He Q, Yan X, et al. Effect of exogenous nitric oxide on sperm motility in vitro. Biological research. 2014;47(1):44.
29. Chaturvedi CM, Kumar P. Nitric oxide modulates gonadal and adrenal function in Japanese quail Coturnix coturnix japonica. General and comparative endocrinology. 2007;151(3):285-99.
30. Kim K, Kim S-H, Kim J, et al. Glutathione s-transferase omega 1 activity is sufficient to suppress neurodegeneration in a Drosophila model of Parkinson disease. Journal of Biological Chemistry. 2012;287(9):6628-41.
31. Shan L, Wang B, Gao G, et al. L-Arginine supplementation improves antioxidant defenses through L-arginine/nitric oxide pathways in exercised rats. Journal of applied physiology. 2013;115(8):1146-55.
32. Singh VK, Lal B. Nitric oxide (NO) stimulates steroidogenesis and folliculogenesis in fish. Reproduction. 2017;153(2):133-46.
33. Ebokaiwe AP, Mathur PP, Farombi EO. Quercetin and vitamin E attenuate Bonny Light crude oil-induced alterations in testicular apoptosis, stress proteins and steroidogenic acute regulatory protein in Wistar rats. Drug and chemical toxicology. 2016;39(4):424-31.
34. Jarad AS, AL-Samawy ER, ALBadran ASH. Effct of L-Arginine on Spermatogenesis of the Diabetic Rat. Basrah Journal of Veterinary Research. 2011;10(2):19-32.
35. Abdalla E-EE, Gebaly ZM, Moustafa A-EA, Amr IM. Evaluation the Effect of Sildenafil Citrate (sc or Viagra) on Senile Albino Rat Testis: Histological and Biochemical Study. The Egyptian Journal of Hospital Medicine. 2012;31(760):1-44.
36. Drury R, Wallington E. Carelton's Histology Technique, 5th edit. Oxford University Press, Oxford, New York, Toronto; 1980.
37. Misra HP, Fridovich I. The role of superoxide anion in the autoxidation of epinephrine and a simple assay for superoxide dismutase. Journal of Biological chemistry. 1972;247(10):3170-5.
38. Beutler E, duron O, kelly B. Improved method for the determination of blood glutathione. The Journal of laboratory and clinical medicine. 1963;61:882.
39. Wendel A. Enzymatic Basis of Detoxication.-Vol. 1, 333 S. Academic Press, New York; 1980.
40. Green LC, Wagner DA, Glogowski J, et al. Analysis of nitrate, nitrite, and [15N] nitrate in biological fluids. Analytical biochemistry. 1982;126(1):131-8.
41. Kolahian S, Sadri H, Larijani A, et al. Supplementation of diabetic rats with leucine, zinc, and chromium: effects on function and histological structure of testes. Int J Vitam Nutr Res. 2015;85:311-21.
42. Ratnasooriya W, Dharmasiri M. L-arginine, the substrate of nitric oxide synthase, inhibits fertility of male rats. Asian journal of andrology. 2001;3(2):97-104.
43. Al-Fartosi KG. Effect of long-term administration of sildenafil citrate (Viagra) on some sperm characteristics and testis architecture of male rats. Basrah Journal of Veterinary Research. 2009;8(2):91-103.
44. Canteros G, Rettori V, Genaro A, et al. Nitric oxide synthase content of hypothalamic explants: increase by norepinephrine and inactivated by NO and cGMP. Proceedings of the National Academy of Sciences. 1996;93(9):4246-50.
45. Tocharus C, Jeenapongsa R, Teakthong T, Smitasiri Y. Effects of Long-term Treatment of Butea superba on Sperm Motility. Naresuan University Journal: Science and Technology (NUJST). 2013;13(2):11-7.
46. Sukardi S, Yaakub H, Ganabadi S. Effects of L-Arginine on the reproductive system of male rabbits. 2006.
47. Ragy MM, Ali FF, Toni ND. Comparing the preventive effect of sodium hydrosulfide, leptin, and curcumin against L-arginine induced acute pancreatitis in rats: role of corticosterone and inducible nitric oxide synthase. Endocrine regulations. 2019;53(4):221-30.
48. Adedara IA, Abolaji AO, Awogbindin IO, Farombi EO. Suppression of the brain-pituitary-testicular axis function following acute arsenic and manganese co-exposure and withdrawal in rats. Journal of Trace Elements in Medicine and Biology. 2017;39:21-9.

49. Wu G, Morris Jr SM. Arginine metabolism: nitric oxide and beyond. Biochemical Journal. 1998;336(1):1-17.
50. Herrero MB, de Lamirande E, Gagnon C. Tyrosine nitration in human spermatozoa: a physiological function of peroxynitrite, the reaction product of nitric oxide and superoxide. Molecular human reproduction. 2001;7(10):913-21.
51. Rosselli M, Dubey RK, Imthurn B, et al. Andrology: Effects of nitric oxide on human spermatozoa: Evidence that nitric oxide decreases sperm motility and induces sperm toxicity. Human Reproduction. 1995;10(7):1786-90.
52. Chen BH, Park JH, Ahn JH, et al. Pretreated quercetin protects gerbil hippocampal CA1 pyramidal neurons from transient cerebral ischemic injury by increasing the expression of antioxidant enzymes. Neural Regeneration Research. 2017;12(2):220.
53. Xu D, Hu M-J, Wang Y-Q, Cui Y-L. Antioxidant activities of quercetin and its complexes for medicinal application. Molecules. 2019;24(6):1123.
54. Ceccatelli S, Hulting A-L, Zhang X, et al. Nitric oxide synthase in the rat anterior pituitary gland and the role of nitric oxide in regulation of luteinizing hormone secretion. Proceedings of the National Academy of Sciences. 1993;90(23):11292-6.
55. Chatterjee S, Collins T, Yallampalli C. Inhibition of nitric oxide facilitates LH release from rat pituitaries. Life sciences. 1997;61(1):45-50.
56. Barnes MJ, Lapanowski K, Rafols JA, et al. GnRH and gonadotropin release is decreased in chronic nitric oxide deficiency. Experimental Biology and Medicine. 2001;226(7):701-6.
57. Peers C, LaManna JC. Hypoxia in the central nervous system. Essays in biochemistry. 2007;43:138-52.
58. Valenti S, Cuttica CM, Fazzuoli L, et al. Biphasic effect of nitric oxide on testosterone and cyclic GMP production by purified rat Leydig cells cultured in vitro. International journal of andrology. 1999;22(5):336-41.
59. Sokanovic SJ, Baburski AZ, Janjic MM, et al. The opposing roles of nitric oxide and cGMP in the age-associated decline in rat testicular steroidogenesis. Endocrinology. 2013;154(10):3914-24.
60. Bhat GK, Mahesh VB, Lamar CA, et al. Histochemical localization of nitric oxide neurons in the hypothalamus: association with gonadotropin-releasing hormone neurons and co-localization with N-methyl-D-aspartate receptors. Neuroendocrinology. 1995;62(2):187-97.
61. Wang M-J, Ou J-X, Chen G-W, et al. Does prohibitin expression regulate sperm mitochondrial membrane potential, sperm motility, and male fertility? : Mary Ann Liebert, Inc. 140 Huguenot Street, 3rd Floor New Rochelle, NY 10801 USA; 2012.
62. Turner RM. Moving to the beat: a review of mammalian sperm motility regulation. Reproduction, Fertility and Development. 2005;18(2):25-38.
63. Aitken RJ, Krausz C. Oxidative stress, DNA damage and the Y chromosome. Reproduction Cambridge. 2001;122(4):497-506.
64. Husein RH, Ahmed MO, Muhammed SM. Effects of L-Arginine, vitamin E and their combinations on sperms morphology in albino male mice. Al-Nahrain Journal of Science. 2011;14(2):137-43.
65. Weinberg JB, Doty E, Bonaventura J, Haney A. Nitric oxide inhibition of human sperm motility. Fertility and sterility. 1995;64(2):408-13.
66. Dimmeler S, Lottspeich F, Brüne B. Nitric oxide causes ADP-ribosylation and inhibition of glyceraldehyde-3-phosphate dehydrogenase. Journal of Biological Chemistry. 1992;267(24):16771-4.
67. Adedara IA, Subair TI, Ego VC, et al. Chemoprotective role of quercetin in manganese-induced toxicity along the brain-pituitary-testicular axis in rats. Chemico-biological interactions. 2017;263:88-98.
68. Memon M, Bretzlaff K, Ott R. Comparison of semen collection techniques in goats. Theriogenology. 1986;26(6):823-7.
69. Schumacher J, Moll HD. manual of equine diagnostic procedures: Teton NewMedia; 2006.
70. Garg V, Garg S. Role of nitric oxide in male infertility. Journal of Indian Academy of Forensic Medicine. 2011;33(1):65-8.
71. Bakhtari A, Nazari S, Alaee S, Kargar-Abarghouei E, Mesbah F, Mirzaei E, Molaei MJ. Effects of dextran-coated superparamagnetic iron oxide nanoparticles on mouse embryo development, antioxidant enzymes and apoptosis genes expression, and ultrastructure of sperm, oocytes and granulosa cells. International Journal of Fertility & Sterility. 2020; 14(3): 161.
72. Wu TP, Huang BM, Tsai HC, et al. Effects of nitric oxide on human spermatozoa activity, fertilization and mouse embryonic development. Archives of andrology. 2004;50(3):173-9.
73. Lebret T, Hervé J-M, Gorny P, et al. Efficacy and safety of a novel combination of L-arginine glutamate and yohimbine hydrochloride: a new oral therapy for erectile dysfunction. European urology. 2002;41(6):608-13.
74. Padma-Nathan H, McCullough A, Forest C. Erectile dysfunction secondary to nerve-sparing radical retropubic prostatectomy: comparative phosphodiesterase-5 inhibitor efficacy for therapy and novel prevention strategies. Current urology reports. 2004;5(6):467-71.
75. Ratnasooriya W, Dharmasiri M, Wadsworth R. Reduction in libido and fertility of male rats by administration of the nitric oxide (NO) synthase inhibitor N-nitro-l-arginine methyl ester. International journal of andrology. 2000;23(3):187-91.
76. Chen J, Wollman Y, Chernichovsky T, et al. Effect of oral administration of high-dose nitric oxide donor L-arginine in men with organic erectile dysfunction: results of a double-blind, randomized, placebo-controlled study. BJU int. 1999;83(3):269-73.
77. Moody J, Vernet D, Laidlaw S, et al. Effects of long-term oral administration of L-arginine on the rat erectile response. The Journal of urology. 1997;158(3):942-7.
78. Yildirim S, Ayan S, Sarioglu Y, et al. The effects of long-term oral administration of L-arginine on the erectile response of rabbits with alloxan-induced diabetes. BJU international. 1999;83:679-85.
79. Angulo J, Cuevas P, Gabancho S, de Tejada IS. Combination of phentolamine and L-arginine or sildenafil synergistically

improves neurogenic relaxation of rabbit corpus cavernosum smooth muscle. Urology. 2001;57(3):585-9.