Protective effects of *Nymphaea lotus* Linn. (*Nymphaeaceae*) on N (ω)-nitro-L-arginine methyl ester-induced tissular oxidative damages and erectile dysfunction in hypertensive male rat

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**ABSTRACT**

**Introduction:** Hypertension represents one of the major risk factors for the development of erectile dysfunction (ED). Protective effects of *Nymphaea lotus* Linn. on ED and tissular oxidative stress in the N (ω)-nitro-L-arginine methyl ester (L-NAME) hypertensive male rats were investigated in this study. **Materials and Methods:** Fifty adult male Wistar rats were randomly classified into five groups; control, L-NAME (10 mg/kg), L-NAME + losartan (10 mg/kg), L-NAME + *N. lotus* at the dose of 75 mg/kg and 200 mg/kg. L-NAME was administered during 8 weeks, but others treatment started from the 4th week and was administered continuously with L-NAME (10 mg/kg) during 4 additional weeks. **Results:** L-NAME administration caused marked hit of sexual behavior, specifically failure of penile insertion or difficulty to ejaculate during the test interval. Further L-NAME causes a significant decrease in antioxidant products as reduced glutathione (GSH) and nitric oxide (NO) in aorta and penile tissues, as compared to control group. *N. lotus* co-treatment for 4 weeks increased markedly the erectile index and the ejaculation rates contrarily to losartan in comparison to negative control receiving only L-NAME. *N. lotus*, but not the positive drug losartan, induced a significant increase in the antioxidant enzymes activities and GSH and NO levels, as well as, a significant decrease in malondialdehyde levels compared to L-NAME group. These results suggest *N. lotus* may provide significant protection against L-NAME-induced tissular oxidative damages by upregulation of antioxidant systems and promotion of the vasodilator factors in chronic NO-deficient rats. These alleviating effects of *N. lotus* denote a prosexual, antioxidant, and vasodilatory properties that were more appreciated after histological examination where remodeling of aorta was visibly reduced. **Conclusion:** *N. lotus* may be considered as a potentially useful strategy to limit ED and toxicity associated with hypertension.

**KEY WORDS:** Erectile index, lipid peroxidation, nitric oxide-deficiency, *Nymphaea lotus*, prosexual

**INTRODUCTION**

Hypertension (HT) is an upgrading challenge throughout the world, which holds the third place as a life-threatening cardiovascular disease [1]. HT is characterized by the incrementing of the arterial blood pressure and involves complex structural and functional alterations of the cardiovascular system. Sexual dysfunction is a frequent problem in hypertensive patients [2,3]. Among patients with HT, 30% have been reported to have erectile dysfunction (ED) [4], as erectile function is mainly a vascular phenomenon. ED is a discomforting condition that considerably reduces the men life quality. A deficiency of nitric oxide (NO) has been reported to be the primary cause of erectile disorder in patients suffering from HT [5]. NO is the key actor involved in the relaxation mechanism of the penile Corpus cavernosum (CC). It mediates male sexual behavior [6]. In the most essential respects, NO is a ubiquitous molecule derived from L-arginine along with different NO synthase (NOS) isoforms acting as catalysts. NO modulates blood pressure variations and it is released controlled by nitricergic nerves fibers and vascular endothelial cells in the CC commands erectile function [7]. Atherosclerotic vascular alterations have been often mentioned as the principal responsible for the setting of ED in HT [8], however, the exact pathogenesis of ED in HT has not been fully clarified.
Chronic administration of N (ω)-nitro-L-arginine methyl ester (L-NAME) is known to produce a persistent increase in arterial blood pressure [9,10]. This animal model is considerably used to investigate the mechanism of vasculogenic diseases caused by NO deficiency. In order to reduce blood pressure and improving erectile function in hypertension-related ED patients between whiles, a large number of antihypertensive drugs are prescribed. However, HT remains inadequately managed everywhere [11]. Effectively, populations of developing countries are mainly of the belief that natural products have better efficacy and/or have more difficulties to access modern health system. Therefore, population showed greater liking for medicinal plants as complementary or alternative medicine over modern antihypertensive therapies. The ethnomedical treatment methods come into sight as noticeable ways to improve health and treat sexual disorders and could greatly benefit people with middle or low economic incomes.

*Nymphaea lotus* Linn. is well known for it is a beautiful flower which is traditionally used in Cameroon West region to treat sexual disorders [12]. Furthermore, called “white waterlily,” *N. lotus* belongs to the water plants family of Nymphaeaceae. In this study, we investigated if chronic administration of L-NAME induced-HT in rats resulted in ED and tissular oxidative stress and whether concomitant treatment with *N. lotus* Linn. prevent both impairments.

**MATERIALS AND METHODS**

**Plant Materials**

Flowers of *N. lotus* freshly collected in Nkol-bisson (Yaounde, Cameroon) were cleaned, and the whole plant was identified at the National Herbarium of Cameroon compared to the voucher specimen number 8647/HNC. Shade dried flowers of *N. lotus* were powdered using an electric grinder and 50 g (equivalent of 20 flowers) of this powder were infused with 1 L of freshly boiled tap water (100°C) and infused until the mixture became cool (method used by population). The resulting mixture was filtered with Whatman number 3 papers and dried in an oven at 45°C until obtention of brown powder.

**Animals**

Healthy Wistar albino rats were provided by the animal house of the Laboratory of Animal Physiology, University of Yaounde 1, Cameroon. Female rats were 12 weeks old weighing between 180 and 200 g. Male rats were 12 weeks old and weighing between 200 and 220 g. The animals were housed in plastic cages (5/cages) cleaned twice a week. They were allowed free access to a fed solid laboratory rat food and tap water. Their solid food consumption was recorded once a week. Rats were treated in respect to the guidelines of the Cameroonian Bioethics Committee (reg number FWA-IRB00001954) and in accordance with NIH-care and use of laboratory animals manual (8th Edition). Efforts were also made to reduced animal pain as well as the number of animal used in the experiment.

**Chemicals**

L-NAME and estradiol benzoate were obtained from Sigma-Aldrich (St. Louis, MO, USA). Progesterone caproate was procured from Bayer Schering Pharma (Berlin, Germany) and losartan (Losar-Denk 50) was obtained from Denk-Pharma (München, Germany).

**L-NAME-induced Arterial HT Model**

Many studies already proved that chronic administration of L-NAME causes hypertension in rats [1,13,14]. Thus hypertension was induced by daily L-NAME administration at a concentration of 10 mg/kg in the form of oral solution for 8 weeks.

**Estrus Induction**

A total of 30 female Wistar rats were ovariectomised under ketamine and valium anesthesia (10 and 50 mg/kg ip, respectively). 14 days after ovariectomy, estrus was induced by subcutaneous injection of 12 μg of estradiol benzoate and progesterone 0.5 mg injected in olive oil, respectively, at 56 h and 8 h before matching.

**Animal Treatments**

The animals were randomly divided into five groups: (1) Control group, receiving only distilled water; (2) negative group treated only with L-NAME at the dose of 10 mg/kg as oral gavage solution, everyday for 8 weeks, in the aim to induce hypertension; [13,14] (3) L-NAME-treated group receiving a daily dose of losartan, an antagonist of angiotensin receptors at the dose of 10 mg/kg; (4) and (5) two L-NAME-treated groups receiving the aqueous flowers extract of *N. lotus*, respectively, at the level of 75 and 200 mg/kg. The aforementioned dose of the plant extract was selected following a screening study and previously published reports regarding *N. lotus* biological effects [12]. The animals were weighed weekly. Losartan was freshly prepared by dissolving one pill of 50 mg in 50 ml of distilled water and administered daily by gavage (1 ml/100 g). The treatment with losartan and *N. lotus* began after 4 weeks L-NAME administration period and lasted for 4 additional weeks concomitantly with L-NAME administration.

**Copulatory Behavior Evaluation**

The evaluation of male rat’s sexual behavior was recorded in all groups in the evening of the last day of treatment. Each male rat was treated 1 h prior evaluation with vehicle or drugs and placed individually in a cage for a 10 min adaptation period. Thereafter, a female rat chemically brought into estrus was introduced in the cage. The male rat was observed for 30 min, and the percentage of animals that exhibit mount, intromission, and ejaculation (among those that achieve intromission) in each group was noted. Penile erection was determined as the number of penile licking occurred during the observation period. Penile erectile index was determined by dividing the sum of erection
in a group with the total number of penile erection observed in all groups performing the test multiplied by 100.

**Organs Collection and Preparation of Homogenate**

After 8 weeks, the rats were killed between 09:00 and 11:00 h by exsanguinations. Aortas and penis were weighed using 4-digital electronic balance (Mettler PL301) and crushed respectively in McEwen solution and Tris buffer to obtain homogenates at 10%. After centrifugation (3000 g for 30 min), the supernatant was collected and kept at the storage temperature of −20°C for biochemical analyses.

**Estimation of Oxidative Stress Parameters**

**Determination of NO**

The Griess method was used to perform the determination of NO levels [15]. The magenta absorbance was determined at 546 nm. The construction of a standard curve from the absorbance of standards was used to determine the concentration of NO in the tissues. The results of NO levels were expressed in µmol/L.

**Determination of superoxide dismutase (SOD)**

The assessment of SOD was made by the method of Misra and Fridovich [16]. The unit activity (U) of the enzyme was defined as the enzyme needed for 50% inhibition of adrenaline auto-oxidation. The results were expressed in U/mg protein.

**Determination of glutathione**

The reduced tissular glutathione (GSH) was measured in respect to the Ellman method [17]. An equivalent quantity of 100 µL of tissue homogenate or tris-HCl buffer for the blank sample was mixed with 1500 µL of Ellman reagent. The mixture was mixed and the absorbance was read at 412 nm against the blank within 1 h at room temperature. The GSH concentration was calculated according to the following equation:

\[
\text{GSH concentration} = \text{Absorbance}/(1.36 \times 10^5/\text{mol/cm})
\]

The concentration of reduced glutathione was expressed as mg/g tissue.

**Determination of catalase (CAT)**

The assessment of CAT activity was made by the following method of Aebi [18]. The reagent mixture consisted of 187.5 µL of phosphate buffer (0.1 M, pH 7.5) and 100 µL of supernatant. The reaction started by adding 50 µL of H₂O₂ 50 mM, incubated at 37°C for 1 min. The addition of 500 µL of dichromate acetic acid reagent served to stop the reaction. The melange was kept in a water bath at 100°C during 15 min. Thereafter, the green color developed during the reaction was read at 570 nm with a spectrophotometer. Tubes used as control were entirely lacking of enzyme and read in parallel. The measure of CAT activity was determined by the difference in absorbances per unit.

**Determination of malondialdehyde (MDA)**

The concentration of MDA, which is proportional to lipid peroxidation, was measured colorimetrically using the thiobarbituric acid [19]. The MDA concentration was calculated according to the following equation:

\[
\text{MDA concentration} = \text{Absorbance}/(1.56 \times 10^5/\text{mmol/cm})
\]

The MDA levels results are expressed per mg/g of tissue.

**Histomorphological Analysis**

The paraffin-embedded tissues fixation for the aortas tissues (n = 5) was performed to evidence damages on tissue. The tissues (3 sampling from the upper to the lower part of the thoracic aorta of each animal) were mounted on slides and then stained by immersing them in Mallory trichrome solutions. Following this staining, the aorta was photographed at ×10 magnification using a light microscope Olympus. The complete equipment consisted of a digital camera (DCM35 350K pixels) related to a computer (Compaq nx9010) with Minisee software for image transfer and analysis.

**Statistical Analysis**

SPSS Statistics Software (San Diego, California, USA) was used to perform data analysis. One-way analysis of variance and the Tukey post-test (to correct for multiple comparisons) were used. 5% was referred as the level of significance to indicate the difference between group means. Unless specified, data are presented as mean ± standard error.

**RESULTS**

**Effect of N. lotus Aqueous Extract on Body Weight Variation**

The slight increment of body weight observed in all hypertensive animals was lower than in control group, whereas, only the variation in losartan treated group was significantly (P < 0.01) lower in comparison to control at the days 35 of the treatment period [Figure 1].

**Effect of N. lotus Aqueous Extract on Food Consumption**

The effects of chronic coadministration of N. lotus on food consumption are showed in Table 1. From the 5th week to the end of treatment L-NAME induced significant decrease of food intake while cotreatment with losartan and plant extract considerably improved the fewer food intake observed in negative group. This effect was significantly marked with the plant aqueous extract at the dose of 200 mg/kg during the treatment so that no significant difference was observed.
compared to control group receiving only distilled water.

Effect of *N. lotus* Aqueous Extract on Copulatory Behavior

L-NAME induced marked decrease of precoital sexual behavior, and a failure of the majority of rats to penile insertion (% rats intromitting) or to ejaculate during the test interval [Table 2]. These hindering effects of L-NAME on percentage of male that ejaculated were prevented by concomitant administration of *N. lotus* at both doses, particularly at the level of 75 mg/kg, but not with the angiotensin receptor antagonist losartan.

Effect of *N. lotus* Aqueous Extract on Some Oxidative Stress Markers in Tissue

The effects of L-NAME and losartan and *N. lotus* cotreatments on some tissular oxidative stress parameters of hypertensive rats are shown in Figure 2. Administration of L-NAME by oral route decreased significantly MDA concentrations in aorta (P < 0.05) and penile tissue (P < 0.001) versus baseline whereas additional treatment with losartan or *N. lotus* at both doses used conserved the MDA levels in normal range. In penile tissue, MDA concentrations were higher in losartan and *N. lotus* treated rats than in negative control group. However, these levels were significantly lower in penis (P < 0.001) of the cotreated with *N. lotus* (75 mg/kg) animals compared to those cotreated with losartan [Figure 2a]. The supplementation with *N. lotus* and losartan for 4 weeks significantly reversed (P < 0.001) the decreased release of nitrates by L-NAME-treatment in penile tissue, but also in aorta compared to L-NAME non cotreated animals [Figure 2b].

L-NAME did not significantly affect the activities of the intrinsic antioxidant enzymes, CAT and SOD [Figure 2c and d]. Cotreatment with *N. lotus*, but not with losartan, induced a significant elevation of the activities of these enzymes particularly in aortas. Further, L-NAME treatment for 60 days reduced markedly the levels of antioxidant compounds (nitrites, GSH) in aorta and penile tissues. Effectively, treatment of rats with L-NAME dropped the glutathione level in aorta and penis. Hence, the declined percentages of GSH levels in aorta were by 15.85%, by 37.71% and by 32.32%, respectively, for L-NAME, losartan, extract at the dose of 200 mg/kg compared to control. Only the cotreatment with *N. lotus* at the dose of 75 mg/kg increased slightly the glutathione level in both aorta (by 5.95%) and penile (by 40.3%) tissues when compared to control [Figure 2e].

Histology

The histopathological analysis of the aortas cross-sections of the chronically NO-blocked animals exhibited marked morphological changes including intimal hypertrophy, increased leukocytic cells adjacent to endothelium and subendothelial (intimal) deposition of lipids in the artery wall forming atherosclerotic plaque. The coadministration of *N. lotus* aqueous flowers extract as well as losartan to L-NAME rats visibly alleviated the remodeling of aorta [Figure 3].

DISCUSSION

Previous studies mentioned that chronic administration of L-NAME induced arterial hypertension associated with NO deficiency [9,10,20]. This study analyzed the protective effects of 4 weeks of *N. lotus* treatment on erectile function and tissular oxidant/antioxidant markers in hypertensive male rats. The results of this study showed that oral administration of L-NAME (10 mg/kg) significantly hindered sexual behavior in male rats. The lower percentage of animals achieving intromission in comparison to the number of those achieving mounts (decreased copulatory ratio) was the most deleterious effect of hypertension on copulatory behavior. The number of males succeeding in ejaculations in nontreated hypertensive group was also significantly reduced. This L-NAME inhibitory action was worsened by the 30 days cotreatment with losartan at the level of 10 mg/kg that drastically reduced ejaculatory rate. The most likely justification of these results is the dysfunction or abolition of the erectile process. This agreed with the findings of Hull et al. [21] who showed that chronic administration of

![Figure 1: Percentage of body weight variation. Each points represents the mean ± standard error of the mean of group; *P* < 0.01 significantly different compared to control n = 10](image)

<table>
<thead>
<tr>
<th>Groups</th>
<th>Food consumption (g/100 g of body weight)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Week 1</td>
</tr>
<tr>
<td>Control</td>
<td>91±0.67</td>
</tr>
<tr>
<td>L-NAME</td>
<td>100±0.97</td>
</tr>
<tr>
<td>L-NAME-LSRT</td>
<td>89±1.34</td>
</tr>
<tr>
<td>L-NAME-NL75</td>
<td>107±1.24</td>
</tr>
<tr>
<td>L-NAME-NL200</td>
<td>100±1.01</td>
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</tbody>
</table>

Each value represents the mean ± SEM of group. n=10; *a,b,c* P<0.05; *P* <0.01 and *P* <0.001 significantly different compared to control; *a,b,c* P<0.01 and *P* <0.001 significantly different compared to L-NAME; *P* <0.01 significantly different compared to losartan. L-NAME: L-nitroarginine methyl ester, *N. lotus*: *Nymphaea lotus*
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L-NAME lowered the frequency of erections during copulatory behavior in rats. It should be emphasized that the reduced intromission rate is related to ED due to the L-NAME inhibition of NO synthesis at the endothelium level or at the level of the parasympathetic nervous system innervating the corpora cavernosa of penile tissue [22].

The mechanism of penile erection remains on a synchronized extra activity of sacral parasympathetic-nitrergic innervations and decreased activity of the sympathetic to increase blood flow to the corpora cavernosa [23]. The complete inhibition of ejaculatory potential observed in losartan cotreated animals may counteract as specific side effect of angiotensin receptors blockers and suggest an inhibitory role on the NO potential to activate the expression of sexual reflexes such as ejaculation at the spinal cord level [24]. This is in good agreement with earlier report documented that the possible mechanism involved in this type of ED is the decreased NO-synthase activity with consequent reduced NO production associated with increased renin angiotensin system activation [25,26].

Our results showed that losartan and N. lotus at both doses used, improved the decrease of body weight observed in L-NAME rat all along the treatment period. This could be related to food intake that is proportional to NO level as described by several studies [27,28]. In addition, the evaluation of food consumption was in agreement with the standard acceptable dietary intake of 15 g/day per rat for maintenance of growing or adult rats [29]. The slightly increased consumption of food in control rats, compared to the mean consumption in hypertensive rats, may be related to the ability of NO regulating appetite [30].

In addition, long-term inhibition of NO synthesis induced vascular inflammation (presence of leukocytic cells) and

Table 2: Effects of N. lotus on the percentage of male rats that achieved mount, intromission and ejaculation after 60 days oral treatment with L-NAME (n=10)

<table>
<thead>
<tr>
<th>Groups</th>
<th>Rats mounting (%)</th>
<th>Rats intromitting (%)</th>
<th>Rats ejaculating (%)</th>
<th>Erectile index (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>100</td>
<td>100</td>
<td>100</td>
<td>27.34</td>
</tr>
<tr>
<td>L-NAME</td>
<td>100</td>
<td>51.34</td>
<td>40</td>
<td>13.94</td>
</tr>
<tr>
<td>L-NAME-LSRT</td>
<td>100</td>
<td>71.34</td>
<td>0</td>
<td>18.99</td>
</tr>
<tr>
<td>L-NAME-NL75</td>
<td>100</td>
<td>72.82</td>
<td>100</td>
<td>19.27</td>
</tr>
<tr>
<td>L-NAME-NL200</td>
<td>100</td>
<td>75.50</td>
<td>80</td>
<td>20.46</td>
</tr>
</tbody>
</table>

L-NAME: L-nitroarginine methyl ester, N. lotus: Nymphaea lotus

Figure 2: Effect of the coadministration of Nymphaea lotus and losartan on aorta and penile (a) malondialdehyde levels, (b) nitrites concentrations, (c) catalase activity, (d) superoxide dismutase activity, and (e) reduced glutathione concentrations of L-nitroarginine methyl ester (L-NAME) treated rats. Each bar represents the mean ± standard error of the mean of group, n = 10; a,b,c,d,e,f P < 0.05; P < 0.01 and P < 0.001 for comparisons among control and experimental groups; a,b,c,d,e,f P < 0.05; P < 0.01 and P < 0.001 for comparisons between L-NAME untreated and L-NAME cotreated animals; P < 0.001 significantly different compared to losartan; ***P < 0.001 dose-dependence between two extract doses.
subsequent atherosclerosis. The aqueous extract of N. lotus coadministered during 4 last weeks of treatment at the both doses used, as well as, losartan were able to delete inflammatory process in this model of L-NAME-induced atherosclerosis. Noticeably, animals treated simultaneously either by L-NAME and losartan or by L-NAME and N. lotus had lowered, also qualitatively alleviated, intimal remodeling. We assumed that substantial morphological changes on aorta exhibited in this model such as intimal hypertrophy or vascular remodeling may also appears on penile small arteries leading thus to diminished blood flow to the erectile tissue and finally to ED observed in L-NAME treated animals. Studies have showed that the incidence of atherosclerosis among patients with ED is approximately 40% [31] and that atherosclerotic vascular damage might be involved in the pathogenesis of hypertensive ED in animals [8].

Oxidative stress, defined by the increased bioavailability of reactive oxygen species (ROS), plays a principal role in the progression of vascular dysfunction related to hypertensive diseases [32]. Our results revealed decreased levels of reduced GSH in hypertensive animals in comparison to control. Glutathione is an endogenous antioxidant, which gets converted to its oxidized form (glutathione disulfide). This oxidized form of GSH reacts with free radicals and prevents the generation of most toxic hydroxyl radicals [33]. An explanation to the decrease of GSH levels after L-NAME treatment is the enhanced consumption of GSH in nomenzymatic biological removal of ROS. The decrease in GSH levels in aorta and penile tissue of N. lotus cotreated animals compared to untreated animals of the negative control indicated that L-NAME treatment induced an excessive generation of ROS, and as a compensatory mechanisms glutathione levels were reduced while fighting oxidative stress [34,35]. However, N. lotus particularly at the dose of 75 mg/kg hindered this decrease and alleviates the GSH level in the tissues of hypertensive rats.

The protective effect of N. lotus may be associated secondly to NO pathways. Our findings revealed significant reduction of nitrites and MDA levels in the aorta and penile tissues of the L-NAME untreated animals compared to those cotreated with the plant extract at the dose of 75 mg/kg or to those receiving losartan as cotreatment. Further, nitrites are considered as the stable end product of NO in living organisms and MDA as stable end product of lipid peroxidation. Treatment with N. lotus, at both doses used supplemented the inhibited NO production through enhancing the nitrites concentrations in aorta and penile tissues. Nevertheless, Joshi et al. [36] have mentioned two opposite effects of NO; low-level of NO may act as an antioxidant while higher level as proantioxidant. They proposed that the mechanism of low concentration of NO’s protection may involve diminished metal-catalyzed lipid peroxidation and the high concentration of NO’s potentiation of oxidative stress may involve mitochondrial dysfunction. The large increase in nitrites concentration that was obtained in this study may justify the high amount of lipid peroxidation marker in animals treated with losartan and N. lotus aqueous flowers extract at the dose of 200 mg/kg. Previously, it has been evidenced that N. lotus possesses lipid peroxidation inhibitory effect [12]. Considering the prosexual properties exhibited by our plant extract, the latter implies that adequate antioxidant potential can improve ED.

Further, under normal conditions, ROSs are removed by endogenous antioxidant enzymes as SOD, CAT or glutathione peroxidase [37,38]. In our study, the activities of SOD and CAT enzymes were not significantly influenced by L-NAME treatment whereas cotreatment with the plant extract reacted dose dependently by enhancing SOD and CAT activities in aorta and penile tissues. This suggests that chronic administration of L-NAME at the level of 10 mg/kg do not influence these two intrinsic antioxidant enzymes. However, the depletion of GSH and nitrites levels in tissue of L-NAME rats confirmed that oxidative stress is one of the causes of the ED observed in hypertensive rats. Interestingly, the combined administrations of N. lotus plus L-NAME contrarily to losartan to rats, significantly enhanced dose dependently the activity of these enzymes underlying the antioxidant properties of N. lotus. Therefore, it can be speculate that N. lotus induces its prosexual effect by its combined effects on vascular system through anti-inflammatory and antioxidant properties. Others authors also mentioned that the aphrodisiac properties of the extract of Cyperus esculentus may be related to its antioxidant potential [39]. Indeed, these observations are interesting, since the prime site for control of blood pressure is at the level of the small arteries/arterioles. Our study scientifically evidenced that long-term treatment with N. lotus suppress ED and oxidative stress induced by chronic NO blockage.

CONCLUSION
In conclusion, our results showed that N. lotus hindered these functional and morphological changes induced by NO blockage in a dose-dependent mode. These suppressive effects are most likely due to the ability of N. lotus to attenuate oxidative stress and/or to increase NO release in penile vessels, improving thus erectile function.
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REFERENCES