Physalis angulata. This increase of NO release is thought to occur through [12]. The previous study proved that Ciplukan (the management of endothelial dysfunction associated kidney diseases. vascular rarefaction, oxidative stress, and inflammation is important in capillary rarefaction is associated with aging and renal fibrosis and is an rarefaction indicated by the decrease in vascular density [9,10]. Kidney leads to excessive inflammation and cell damage [8]. The damaged inflammatory mediators, and vice versa [7]. Excessive NF-κB activation factors, such as nuclear factor kappa-light-chain-enhancer of activated B cells, causes kidney damage [3,4].

Endothelial dysfunction creates an imbalance between NO and reactive oxygen species (ROS) which causes kidney damage [3,4]. This compound could increase the release of NO from endothelial cell in vitro. This increase of NO release is thought to occur through genomic effects by increasing the expression of endothelial NO synthase (eNOS) and inducible NO synthase and non-genomic effects by increasing cytosolic calcium [13]. Another study proved the target of P. angulata leaf water extract in NO synthesis pathway through increased levels of vascular endothelial growth factor (VEGF) and eNOS [14].

The objective of this study is to investigate the effects of P. angulata leaf water extract, which contains physalin, withanolides [13,15,16], and flavonoid [17] on vascular rarefaction, oxidative stress, and inflammation in the kidney of NOS-inhibited Wistar rats by L-NAME.

METHODS
Animal preparation
A total of 25 male Wistar rats, weighing 250–300 g, were placed in a quiet room with cage temperature 21±2°C, in which a 12–12 h light-dark cycle was maintained. They were fed and watered by ad libitum. After 7 days of acclimatization, Wistar rats were divided into five groups [control negative: normal saline 0.9%, i.p; L-NAME group: 40 mg/kg/day L-NAME i.p; and L-NAME 40 mg/kg/day, i.p. + P. angulata leaf water extract doses 500 mg/kg/day, 1500 mg/kg/day, and 2500 mg/kg/day, respectively]. The treatment lasts for 15 days [3,18,19].

INTRODUCTION
Endothelial dysfunction is indicated by the decreased bioavailability of vasodilators, primarily nitric oxide (NO) [1,2]. In the kidney, NO has various important functions, such as regulation of renal hemodynamics, regulation of glomerular microcirculation and salt balance, blunting of tubuloglomerular feedback, and modulation of renal sympathetic nerve activity [3,4]. The inhibition of NO synthesis by Nω-nitro-L-arginine methyl ester (L-NAME) in rats leads to endothelial vasoconstriction and activation indicated by pro-inflammatory, proliferative, and procoagulant conditions [4,5]. It results in severe hypertension and causes kidney damage [3,4].

Endothelial dysfunction creates an imbalance between NO and reactive oxygen species (ROS) which leads to oxidative stress [6]. ROS can attack various biomolecular components of the cells, such as lipid, which causes lipid peroxidation (LPO). This reaction released LPO products, such as malondialdehyde (MDA) [3]. ROS also activates one of the transcription factors, such as nuclear factor kappa-light-chain-enhancer of activated B cells (NF-kB), which will subsequently induce the synthesis of inflammatory mediators, and vice versa [7]. Excessive NF-kB activation leads to excessive inflammation and cell damage [8]. The damaged kidney vascular cells lead to structural changes called renal vascular rarefaction indicated by the decrease in vascular density [9,10]. Kidney capillary rarefaction is associated with aging and renal fibrosis and is an indicator of impaired renal function [11]. Therefore, preventing kidney vascular rarefaction, oxidative stress, and inflammation is important in the management of endothelial dysfunction associated kidney diseases.

Ciplukan (Physalis angulata L.) leaf is known to have high antioxidant effects in vitro [12]. The previous study proved that P. angulata leaf extract contains Physalin, a class of secosteroids. This compound could increase the release of NO from endothelial cell in vitro. This increase of NO release is thought to occur through genomic effects by increasing the expression of endothelial NO synthase (eNOS) and inducible NO synthase and non-genomic effects by increasing cytosolic calcium [13]. Another study proved the target of P. angulata leaf water extract in NO synthesis pathway through increased levels of vascular endothelial growth factor (VEGF) and eNOS [14].

The objective of this study is to investigate the effects of P. angulata leaf water extract, which contains physalin, withanolides [13,15,16], and flavonoid [17] on vascular rarefaction, oxidative stress, and inflammation in the kidney of NOS-inhibited Wistar rats by L-NAME.
Extract preparation

P. angulata leaves were obtained from Balai Penelitian Tanaman Obat dan Rempah, Lembang, Indonesia. The sample of the herbs was determined and confirmed as P. angulata species by the Laboratory of School of Life Sciences and Technology, Bandung Institute of Technology, Indonesia. As much as 10 g of P. angulata leaves dry powder were soaked in 100 ml of boiled water for an hour. The solution was filtered from the precipitated product by cotton clot to obtain 80 ml thick extract. The same process was repeated for the remaining product. It was soaked again in 30 ml boiled water for an hour and then filtered to obtain 20 ml extract. From this procedure, we got 100 ml P. angulata leaf extract with a concentration of 10% (w/v).

Determination of MDA level

Kidney LPO was used as an indicator of oxidative stress [21]. It was measured according to the concentration of thiobarbituric acid (TBA) reactive substances. The amount of produced MDA was used as an index of LPO [22]. MDA and TBA react and produce pink pigment with a maximum absorption at 535 nm [23]. Tissue homogenate (0.1 ml) was mixed with 2 ml reagent consisting of 0.37% TBA, 0.25 N HCL and 15% trichloroacetic acid with 1:1:1 ratio. The mixture was then placed in a boiling water bath for 15 min. After cooling, the mixture was centrifuged at room temperature for 10 min and the absorbance of the clear supernatant was read at 535 nm [24]. Data were expressed as nm/300 mg tissue.

Immunohistochemical preparation and evaluation

At the end of the experimental period, the rat kidneys were removed, fixed in 10% buffered formalin solution, and then embedded in paraffin. Each kidney was cut in a sagittal section into two halves. Paraffin kidney sections (5 mm) were prepared for immunohistochemical examination, and another was stained with hematoxylin and eosin. For immunohistochemical analysis of NF-κB, antigen unmasking was performed by heating the sections in citrate buffer, pH 6.0, using a water bath 95°C for 20 min. Sections were incubated overnight with primary antibodies (1:100 polyclonal p65, Santa Cruz Biotechnology) at 4°C. The detection of immunopositive cells used the avidin-biotin-peroxidase complex method. Immunoreactivity was visualized with diaminobenzidine. Hematoxylin was used as the counterstain. Negative controls consisted of each case in which the primary antibody was omitted.

Renal sections were scored for the presence of p65 in renal cells, whether in the cytoplasm or in the nucleus. Renal cells were quantitatively measured by counting at least 20 randomly selected high-power fields (×400) in the cortex and outer medulla area. The final score obtained was expressed as the number of positive cells per high-power field [25].

Histopathological evaluation

The vascular density of the kidneys was evaluated by counting the vascular in 20 randomly selected field sites in the renal cortex and outer medulla with a magnification of ×400. It will be considered as vascular if there is a lumen containing erythrocytes and coated by endothelial cell(s) [26]. Data were expressed as the number of vascular per high-power field.

Statistical analysis

The data were analyzed with SPSS 23.0 for Windows using independent t-test to compare the control and L-NAME group. One-way analysis of variance was used to compare the L-NAME group and the treatment groups. Differences between group were determined using post hoc analysis in which the significance level was described as p<0.05.

RESULTS

The L-NAME effect on vascular density, MDA levels, and NF-κB expression in rat kidney

The effects of L-NAME on vascular density, MDA levels, and NF-κB expression in rat kidney are shown in Fig. 1a-c, respectively. L-NAME tended to decrease the vascular density in rat kidney compared to the control group, but it was not statistically significant, while the MDA level and NF-κB expression tended to increase in the L-NAME group compared to the control group, but it was not significantly different.

The effect of P. angulata leaf water extract on kidney vascular density

Administration of P. angulata leaf water extract at dose 1 (500 mg/kg/day) and dose 2 (1500 mg/kg/day) could increase vascular number significantly to L-NAME group. Increasing the dose of P. angulata leaf water extract tended to decrease the vascular number in renal cortex compared to dose 1 (500 mg/kg/day). At dose 3 (2500 mg/kg/day), there was significant decrease on vascular number compared to dose 1 (500 mg/kg/day) but not significant to dose 2 (1500 mg/kg/day) (Figs. 2 and 3).

The effect of P. angulata leaf water extract on kidney MDA level

The effect of P. angulata leaf water extract on MDA level is an important indicator of oxidant status. The data of MDA level in L-NAME plus P. angulata leaf water extract with various dose groups are indicated in Fig. 4. There was a decrease in MDA level of P. angulata leaf water extract at dose 1 of 500 mg/kg/day compared to L-NAME group. Increasing the dose of the P. angulata leaf extract at dose 2 (1500 mg/kg/day) and 2500 mg/kg/day tended to increase the MDA level compared to the lowest dose (Fig. 4).

The effect of P. angulata leaf water extract on NF-κB p65 expression

The effect of L-NAME and P. angulata leaf water extract on NF-κB expression is shown in Fig. 5. It was shown that the administration of 500 mg/kg/day (dose 1) P. angulata leaf water extract tends to decrease p65 expression on rat kidney, but it was not significant. Administration of P. angulata leaf water extract at higher doses (doses 2 and 3) tends to increase p65 NF-κB expression compared to dose 1 (Figs. 5 and 6).

DISCUSSION

This study showed that the vascular density in the renal cortex was decreased after 15 days of L-NAME induction followed by the increasing level of MDA and NF-κB expression (Fig. 1). It was in accordance with the previous studies, showing that L-NAME may cause endothelial dysfunction by lowering NO which promotes thrombosis, vasospasm, vascular inflammation, and proliferation of vascular smooth muscle cells [1]. Oxidative stress also contributes to the mechanisms of endothelial dysfunction. L-NAME may increase oxidative stress by enhancing nicotinamide adenine dinucleotide phosphate oxidase expression [27,28]. Cell membranes composed of poly-unsaturated fatty acids are particularly susceptible to oxidative attack, which resulted in the changes of permeability, membrane fluidity, and cellular metabolic functions. MDA, one of the LPO products, was found to increase in oxidative stress state [21]. L-NAME can induce inflammation and kidney damage by increasing angiotensin (AT) 2 stimulation to AT1-receptor [29] so that it activates the transcription factor NF-κB [30]. These processes finally will lead to microvascular rarefaction [9,31], which was indicated by a decreased in vascular density [10].

The insignificant result of all three variables in the L-NAME group compared to the control group can be caused by a relatively short duration of treatment and/or less L-NAME dose being used. A study by Cipolla et al. [32] showed that it took 5 weeks of L-NAME administration at a dose of 0.5 g/L drinking water to cause loss of vascular (rarefaction) structures in the Sprague-Dawley rat brain capillaries. Meanwhile, the other studies were performed for 7–8 weeks to make significant renal damage and inflammation [4,33-36]. The other needed 28 days to significantly increase rat kidney MDA level [37-39]. These studies indicate that the kidney needs a longer duration than 15 days of L-NAME administration to significantly decrease the vascular density and increase the MDA level as well as the NF-κB expression. Kidney endurance may contribute to this phenomenon. The kidney can protect itself from L-NAME-induced hypertension by its autoregulation mechanism. This autoregulation mechanism causes kidney blood
pressure to be maintained normally despite an increase in systemic blood pressure [40].

Moreover, kidney cells can protect themselves from oxidative stress by synthesizing antioxidant enzymes. A study mentioned that renal ischemia would increase renal antioxidant enzymes [41]. This study showed that MDA levels in the L-NAME group did not increase significantly at the end of the treatment (Fig. 1b). It indicates that the antioxidant enzymes can still compensate for the free radical generated by L-NAME induction. Furthermore, AT2 also plays a role in kidney endurance [42]. The balance of AT2 effects on AT1 and AT2 receptors has an important impact on inducing kidney injury due to L-NAME administration. AT2 stimulation to AT1-receptor induces pro-oxidant and pro-inflammatory effects [43]. Meanwhile, AT2 stimulation to AT2-receptor counteracts those effects [44]. AT2-receptor stimulation can increase NO levels [45], possibly through direct stimulation of NOS.
and bradykinin pathway [46]. The AT2-receptor stimulation reduces inflammatory response through JAK/STAT inhibition, NF-κB inhibition, and COX2 synthesis inhibition [47]. Therefore, in this study, we assumed that the reduction of NO levels induced by L-NAME still can be compensated by AT2 stimulation to AT2-receptor: Thus, the vascular density in the L-NAME group decreases insignificantly, following MDA level and NF-κB expression which increase insignificantly when compared to the control group (Fig. 1.a-c).

Ciplukan (P. angulata) was reported as an important herbal medicine in the Indian Traditional System of Medicine [15,16]. Qualitative analysis of the content of P. angulata leaf water extract and ethanol extract found the presence of flavonoids, saponins, terpenoids, polyphenols, tannins, alkaloids, and steroids [48,49]. Our result showed that the treatment groups receiving 500 mg/kg/day and 1500 mg/kg/day P. angulata leaf water extract had significantly higher vascular density compared to the L-NAME group (Figs. 2 and 3). Therefore, it can be assumed that supplementation of P. angulata leaf water extract can prevent L-NAME-induced vascular rarefaction. This result is in accordance with a previous study, showing that P. angulata could increase NO level of endothelial cell culture [13]. NO has a vasoprotective effect by preventing endothelial cell apoptosis [50] and by inhibiting caspase through S-nitrosylation of cysteine residues [51]. Adequate NO level promotes neovascularization, one of which through the VEGF pathway and fibroblast growth factor [52]. NO stimulates endothelial migration by inducing endothelial cell podokinesis, increasing the expression of αβ3, and enhancing dissolution of the extracellular matrix through the fibroblast growth factor-induced upregulation of urokinase-type plasminogen activator [53]. Furthermore, NO may suppress the production of angiotatin, an endogenous antagonist of angiogenesis [54]. Sulistyowati also proved that the effect of Ciplukan (P. angulata L.) leaf water extract on NO synthesis pathway was by increasing VEGF levels [4]. VEGF was known as a substance needs to promote vasculogenesis [52,55].

P. angulata leaf water extract has a high antioxidant effect in vitro [12, 16]. This might be due to the flavonoid content of P. angulata which are 5-Methoxy-6,7-methylenedioxyflavone and 5,6,7-trimethoxyflavone [56]. Flavonoids prevent tissue damage from free radicals through various mechanisms. First, it reacts with free radical molecules directly because the flavonoids have hydroxyl groups with high reactivity. Second, it inhibits xanthine oxidase to prevent superoxide formation when reoxygenation occurs [57]. In accordance with those previous studies, the addition of 500 mg/kg/day and 1500 mg/kg/day P. angulata leaf water extract was able to lower the MDA level of the L-NAME-induced Wistar rat (Fig. 4). Therefore, it can be assumed that supplementation of P. angulata leaf water extract can prevent oxidative stress in the kidney of endothelial dysfunction Wistar rat model.

Treatment with P. angulata leaf water extract at a dose of 500 mg/kg/day and 1500 mg/kg/day also decreased p65 NF-κB expression (Figs. 5 and 6). The previous study reported that P. angulata leaves have anti-inflammatory effects through tumor necrosis factor-α (TNF-α) inhibition [16,20]. It was known that TNF-α can trigger the classic pathway of NF-κB activation, resulting in an inflammatory response. This is reinforced by the research of Grumbach et al. [58], stating that NO can inhibit NF-κB activation in vitro. NF-κB activation will be manifested as an increase in the expression of p65 proteins of the NF-κB complex [59]. The interesting result from the immunohistochemistry analysis above is that the p65 expression is never found in glomerular cells (Fig. 6). It indicates the involvement of glomerular autoregulation mechanism which protects the glomerulus from L-NAME-induced inflammation. Despite the changes in renal perfusion pressure, the autoregulatory mechanism keeps the renal blood flow and glomerular filtration rate constant [40].

Ciplukan (P. angulata) leaves have a unique characteristic. At lower dose (500 and 1500 mg/kg/day), it serves as proangiogenic, antioxidant, and anti-inflammatory substance. Conversely, at high dose (2500 mg/kg/day), it showed to decrease vascular density (Fig. 2), increase MDA level (Fig. 4), and increase NF-κB expression (Fig. 5). It indicates that there was an excessive NO formation induced by high-dose administration of P. angulata leaf water extract. This finding was in accordance with the previous study, showing that high NO concentration could promote endothelial cell damage [60,61]. NO cytotoxic effect is related to the chemical reactivity of peroxynitrite (ONOO−) formed from NO [62]. ONOO− caused persistent activation of NF-κB [63]. Conversely, the cytoprotective action of NO is attributed to the inhibition of NF-κB-mediated gene expression which produces ubiquitous anti-inflammatory activity [62]. The molecular mechanisms underlying the proapoptotic effect of high-dose NO remain speculative. The factors determining whether endothelial cells undergo apoptosis when exposed to NO include the amounts of NO, the different redox states of NO, and the local environment that may promote the further production of cytotoxic moieties such as ONOO−. The previous study showed that NO and ONOO− can damage DNA directly. The damaged DNA triggers the p53-dependent or p53-independent apoptotic cell death pathways which further activate caspases [60]. The damaged cells are not able to synthesize VEGF again [31]. Finally, the decrease in VEGF led to vascular rarefaction [64].

Moreover, exogenous antioxidants can act as a double-edged sword, becoming antioxidant at low doses and prooxidant at high doses [65,66]. Therefore, increasing P. angulata leaf water extract dose will probably increase the antioxidant which reacts as a prooxidant. Another study by Nnami et al. [67] reported that P. angulata leaves contain cyanide. Cyanide can cause oxidative stress in cells resulting in cell death [68,69]. Therefore, we hypothesized that a high concentration of P. angulata leaf water extract has a contrary effect, which is antiangiogenic, pro-oxidant, and pro-inflammatory. We also hypothesized that the high MDA level of the treatment group receiving 2500 mg/kg/day P. angulata leaf water extract might be caused by the effect of cyanide which becomes more dominant.

CONCLUSION

Based on those results, we concluded that the administration of P. angulata L. leaf water extract in particular concentrations has a vasoprotective effect by preventing kidney vascular rarefaction, oxidative stress, and inflammation on L-NAME-induced male Wistar rat. This study implies the importance of the optimal dose of P. angulata leaf water extract supplementation for the prevention of endothelial dysfunction-induced kidney injury. However, vascular rarefaction pathway is not only triggered by NF-κB and oxidative stress but also triggered by several pathways such as VEGF and pro-apoptotic signaling for vascular rarefaction. To investigate this pathway, further studies are needed.

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AUTHOR CONTRIBUTION

All the authors have the same contribution in this research (carried out the research, collected the data, analyzed the data, and formatted the manuscript).

CONFLICTS OF INTEREST

The authors declare no conflicts of interest.

REFERENCES

1. Endemann DH, Schiffrin EL. Endothelial dysfunction. J Am Soc Nephrol 2004;15:1983-92.
2. Föstermann U. Nitric oxide and oxidative stress in vascular disease. Pflugers Arch 2010;459:923-39.
3. Hadi HA, Carr CS, Al Awadhi J. Endothelial dysfunction: Cardiovascular risk factors, therapy, and outcome. Vasc Health Risk Manag 2005;1:183-98.

4. Higashi Y, Itoh M, Toma N, Kihara Y. Oxidative stress and endothelial dysfunction: Clinical evidence and therapeutic implications. Trends Cardiovasc Med 2014;24:165-9.

5. Talas ZS, Ozdemir I, Ciftci O, Cakir O, Gulhan MF, Pasaoğlu OM, et al. Role of propolis on biochemical parameters in kidney and heart tissues against L-NNAME-induced oxidative injury in rats.Clin Exp Hypertens 2014;36:492-6.

6. Tsuchiya K, Tomita S, Ishizawa K, Abe S, Ikeda Y, Kihara Y, et al. Dietary nitrite ameliorates renal injury in L-NNAME-induced hypertensive rats. Nitric Oxide 2010;22:98-103.

7. Oecckinghaus A, Ghelichian L. The NF-kappaB family of transcription factors and its regulation. Cold Spring Harb Perspect Biol 2009;1:a000034.

8. Guzik TJ, Harrison DG. Endothelial NF-kappaB as a mediator of kidney damage: The missing link between systemic vascular and renal disease? Circ Res 2007;101:227-39.

9. Goligorsky MS. Pathogenesis of endothelial cell dysfunction in chronic kidney disease: A retrospective and what the future may hold. Kidney Res Clin Pract 2015;34:76-82.

10. Olufsen MS, Hill NA, Vaughan GD, Sainsbury C, Johnson M. Rarefaction and 50% pressure loss in systemic and pulmonary arteries. J Fluid Mech 2012;705:280-305.

11. Schmitt R, Melk A. Molecular mechanisms of renal aging. Kidney Int 2017;92:569-79.

12. Kusumaningtyas RW, Lailya N, Limandha P. Potential of Cuclplanum (Physalis angulata L.) as source of functional ingredient. Procedia Chem 2015;14:367-72.

13. Nurdiana PN, Karyono S. Efek non genomik dan genomik ekstrak Physalis minima (L.) pada kultur sel endotel manusia. Cilacap Agrilife J 2010;2017;9:183-71.

14. Nuraeni PN, Karyono S. Efek non genomik dan genomik ekstrak daun ceplukan (Physalis minima L.) pada kultur sel endotel manusia (HUV-EC-C1). J Ilmu Ilmu Hati 2010;22:14-9.

15. Susiutyowati W, Setiawan Aksi Eksktr Air Herba Ceplok (Physalis angulata L.) Terstandar Fisalin pada Jalur Sintesis Nitric Oxide Titus Sprague Dawley Dinduksi Nicotinamide Dan Stretzpotocokin. Disertas. Universitas Gadjah Mada; 2015.

16. Chothani DL, Vaghaisya HU. A phyto-pharmacological overview of Physalis angulata leaves extract by subcritical water extraction. Mod Appl Sci 2015;9:190-8.

17. Talas ZS, Ozdemir I, Ciftci O, Cakir O. Malondialdehyde (MDA) in brown adipose tissue of Sprague-Dawley rats treated with propolis. Acta Biologica Hungarica 2008;59:19-29.

18. Sharma N, Bano A, Dhalwai HS, Sharma V. A pharmacological comprehensive review on ‘rassbhary’ Physalis angulata (L.). Int J Pharm Pharm Sci 2018; 10:73-40.

19. Susanti RF, Kurnia K, Vania A, Reynaldo IJ. Total phenol, flavonoid and antioxidant activity of Physalis minima. J Chem Pharm Sci 1987;7:30-4.

20. Sharma N, Bano A, Dhalwai HS, Sharma V. A pharmacological comprehensive review on ‘rassbhary’ Physalis angulata (L.). Int J Pharm Pharm Sci 2018;10:73-40.

21. Gaweł S, Wardas M, Niedworok E, Wardas P. Malondialdehyde (MDA) as marker of oxidative stress. Atheroscler Suppl 2001;2:S45-9.

22. Ayala A, Munoz MF, Arguelles S. Lipid peroxidation: Production, 4-hydroxy-2-nonenal. Oxid Med Cell Longev 2014;2014:31.

23. Sahibzada S, Shahid M, Kaur R, Arshad M. Oxidative stress and oxygen free radicals as potential risk factors for diabetic nephropathy. J Diabetes Metab 2019;10:67.

24. Ganie SA, Haq E, Hamid A, Qurishi Y, Mahmood Z, Zargar BA, et al. Proinflammatory signals. Hypertension 2000;36:103-9.

25. Spandou E, Tsouchnikas I, Karkavelas G, Dounousi E, Simeonidou C, Galiatsou E, et al. Differential role of angiotensin II receptor subtypes on endothelial superoxide formation. Br J Pharmacol 2000;131:667-72.

26. Santos D, Meneses V, Mendez J, Risco P, Barriuso E. Role of AT1 receptors in the kidney damage: The missing link between systemic vascular and renal disease? Circ Res 2000;87:434-9.

27. Kaschina E, Namsolleck P, Unger T. AT2 receptors in cardiovascular enhancing of activated B cells-induced cardiovascular damage. Bad CARMACS. Hypertension 2016;64:933-4.

28. Chade AR. Renal vascular structure and rarefaction. Compr Physiol 2013;3:187-31.

29. Carlström M, Wiholm CS, Arendshorst WJ. Renal autoregulation in renal hypertension. Hypertension 2006;47:719-22.

30. Chade AR. Renal vascular structure and rarefaction. Compr Physiol 2013;3:187-31.

31. Chade AR. Renal vascular structure and rarefaction. Compr Physiol 2013;3:187-31.

32. Chade AR. Renal vascular structure and rarefaction. Compr Physiol 2013;3:187-31.

33. Chade AR. Renal vascular structure and rarefaction. Compr Physiol 2013;3:187-31.

34. Chade AR. Renal vascular structure and rarefaction. Compr Physiol 2013;3:187-31.

35. Chade AR. Renal vascular structure and rarefaction. Compr Physiol 2013;3:187-31.

36. Chade AR. Renal vascular structure and rarefaction. Compr Physiol 2013;3:187-31.

37. Chade AR. Renal vascular structure and rarefaction. Compr Physiol 2013;3:187-31.

38. Chade AR. Renal vascular structure and rarefaction. Compr Physiol 2013;3:187-31.

39. Chade AR. Renal vascular structure and rarefaction. Compr Physiol 2013;3:187-31.

40. Chade AR. Renal vascular structure and rarefaction. Compr Physiol 2013;3:187-31.

41. Chade AR. Renal vascular structure and rarefaction. Compr Physiol 2013;3:187-31.

42. Chade AR. Renal vascular structure and rarefaction. Compr Physiol 2013;3:187-31.

43. Chade AR. Renal vascular structure and rarefaction. Compr Physiol 2013;3:187-31.

44. Chade AR. Renal vascular structure and rarefaction. Compr Physiol 2013;3:187-31.

45. Chade AR. Renal vascular structure and rarefaction. Compr Physiol 2013;3:187-31.

46. Chade AR. Renal vascular structure and rarefaction. Compr Physiol 2013;3:187-31.

47. Chade AR. Renal vascular structure and rarefaction. Compr Physiol 2013;3:187-31.

48. Chade AR. Renal vascular structure and rarefaction. Compr Physiol 2013;3:187-31.

49. Chade AR. Renal vascular structure and rarefaction. Compr Physiol 2013;3:187-31.

50. Chade AR. Renal vascular structure and rarefaction. Compr Physiol 2013;3:187-31.
vascular endothelial growth factor by rat vascular smooth muscle cells. 

53. Cooke JP, Losordo DW. Nitric oxide and angiogenesis. Circulation 2002;105:2133-5.

54. Matsunaga T, Weihrauch DW, Moniz MC, Tessmer J, Warltier DC, Chilian WM, et al. Angiostatin inhibits coronary angiogenesis during impaired production of nitric oxide. Circulation 2002;105:2185-91.

55. Nerkar D, Mukherjee A, Mehta BK, Banerjee S. Metabolic syndrome associated complications. Int J Pharm Sci 2015;7:22-5.

56. Ser NA. Flavonoids from Physalis minima. Phytochemistry 1988;27:3708-9.

57. Nijveldt RJ, van Nood E, van Hoorn DE, Boelens PG, van Norren K, van Leeuwen PA, et al. Flavonoids: A review of probable mechanisms of action and potential applications. Am J Nutr 2001;74:418-25.

58. Grumbach IM, Chen W, Mertens SA, Harrison DG. A negative feedback mechanism involving nitric oxide and nuclear factor kappa-B modulates endothelial nitric oxide synthase transcription. J Mol Cell Cardiol 2005;39:595-603.

59. Giani JF, Muñoz MC, Pons RA, Cao G, Toblli JE, Turyn D, et al. Angiogenesis and suppression of proteinuria and diminishment of structural damage in renal tissue of stroke-prone spontaneously hypertensive rats. Am J Physiol Renal Physiol 2011;300:F272-82.

60. Shen YH, Wang XL, Wilcken DE. Nitric oxide induces and inhibits apoptosis through different pathways. FEBS Lett 1998;433:125-31.

61. Stefanec T. Endothelial apoptosis. Chest 2000;117:841-54.

62. Hattori Y, Kasai K, Gross SS. NO suppresses while peroxynitrite sustains NF-kappaB: A paradigm to rationalize cytoprotective and cytotoxic actions attributed to NO. Cardiovasc Res 2004;63:31-40.

63. Clancy RM, Gomez PF, Abramson SB. Nitric oxide sustains nuclear factor kappaB activation in cytokine-stimulated chondrocytes. Osteoarthritis Cartilage 2004;12:552-8.

64. Lindenmeyer MT, Kretzler M, Boucherot A, Berra S, Yasuda Y, Henger A, et al. Interstitial vascular rarefaction and reduced VEGF-A expression in human diabetic nephropathy. J Am Soc Nephrol 2007;18:1765-76.

65. Rahal A, Kumar A, Singh V, Yadav B, Tiwari R, Chakraborty S, et al. Oxidative stress, prooxidants, and antioxidants: The interplay. Biomed Res Int 2014;2014:19.

66. Bouayed J, Bohn T. Exogenous antioxidants-double-edged swords in cellular redox state: Health beneficial effects at physiologic doses versus deleterious effects at high doses. Oxid Med Cell Longev 2010;3:228-37.

67. Nnamani C, Ani O, Belunwu G. Larvicidal effects of ethanol extracts on leaves and fruits of Physalis angulata L. on the larvae of anopheles mosquitoes from Ebonyi state, Nigeria, Anim Res Int 2010;6:1059-62.

68. Shou Y, Gunasekar PG, Borowicz JL, Isom GE. Cyanide-induced apoptosis involves oxidative-stress-activated NF-kappaB in cortical neurons. Toxicol Appl Pharmacol 2000;164:196-205.

69. Prabhakaran K, Li L, Borowicz JL, Isom GE. Cyanide induces different modes of death in cortical and mesencephalon cells. J Pharmacol Exp Ther 2002;303:510-9.