ORIGINAL ARTICLE

INTERNATIONAL JOURNAL OF RESEARCH IN PHARMACEUTICAL SCIENCES

Published by JK Welfare & Pharmascope Foundation

Journal Home Page: www.ijrps.com

Effect of nori, a combination of turmeric (*Curcuma longa*) and gotu kola (*Centella Asiatica*) on blood pressure, modulation of ACE, eNOS and iNOS gene expression in hypertensive rats

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Article History:

Received on: 01 Oct 2021
Revised on: 05 Nov 2021
Accepted on: 08 Nov 2021

Keywords:

Centella asiatica,
Curcuma longa,
Hypertensive,
Gene expression,
Nori

ABSTRACT

Hypertension is a major risk factor for causing life-threatening cardiovascular diseases such as myocardial infarction, coronary heart failure, kidney failure, and stroke. Its cases continue to increase worldwide and it is estimated that 1.56 billion adults would live with the condition in 2025. Therefore, this study aims to examine the antihypertensive effect of nori supplement prepared with a combination of turmeric (*Curcuma longa*) and gotu kola (*Centella Asiatica*) on L-NAME-induced and non-induced rats. It was conducted for 28 days on 25 wistar rats that were randomly assigned to the negative, positive, comparison, supplement, and test control groups. CODA was then used in measuring the blood pressure of the rats, while ECG and PPG sensors were utilized for arterial stiffness assessment, as well as for spatial QRS-T and heart rate analysis. Additionally, serum NO levels were measured using griess reagents by spectrophotometric λ540 nm. At the same time, the gel-based PCR semi-quantitative method was used in assessing the activity of ACE, including eNOS and iNOS gene expression. The results showed that nori preparations which contained a combination of 5% turmeric and gotu kola in a feed mixture, had an antihypertensive effect. The effect was characterized by a decrease in systolic, diastolic, and mean arterial blood pressure, as well as heart rate, arterial stiffness, and spatial QRS-T. Additionally, it occurred due to increased NO availability, which resulted from eNOS expression as well as a decrease in iNOS and ACE expression.

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ISSN: 0975-7538

DOI: https://doi.org/10.26452/ijrps.v12i4.4908

Production and Hosted by

IJRPS | www.ijrps.com

INTRODUCTION

High blood pressure or hypertension is a condition of systolic blood pressure of 140 mmHg and diastolic blood pressure of 90 mmHg. It is a major risk factor for life-threatening cardiovascular diseases such as myocardial infarction, coronary heart failure, kidney failure, and stroke (Ntchapda *et al.*, 2020). Its cases continue to increase worldwide, and it is estimated that 1.56 billion adults would live with the condition by 2025 (Bell *et al.*, 2015).

When the cause of hypertension is unknown, the condition is called essential hypertension. It occurs from the combined action of genetic, environmental, and behavioral factors (Bolivar, 2013). However, when hypertension is caused by specific causes such

as drugs or medical conditions, it is known as secondary hypertension.

The renin-angiotensin system (RAS) is involved in the pathogenesis of cardiovascular disease, of which the angiotensin-converting enzyme (ACE) is the most important component. This is because it converts angiotensin I to angiotensin II, which is the main vasoactive peptide (Ferreira *et al.*, 2011) in blood pressure regulation, and a potent vasoconstrictor (Ngo *et al.*, 2014).

Another variable involved in the development of hypertension is oxidative stress, and it occurs due to nitric oxide deficiency (Ntchapda *et al.*, 2020), which is associated with endothelial dysfunction (Sinha and Dabla, 2015). NO is synthesized from its precursor, L-arginine, by the NO synthases (NOS) family.

Furthermore, decreased NOS endothelial activity is primarily responsible for an increase in blood pressure because NO from eNOS plays a role in vasodilation (Lee *et al.*, 2016).

Many studies have shown that Nω-nitro-L-arginine methyl ester (L-NAME) is a chronic inhibitor of NO synthesis, causing endothelial dysfunction and vasoconstriction. This leads to a significant increase in blood pressure in rats (Alwi, 2018; Pantan *et al.*, 2019)

Based on previous research, the combination of turmeric (*Curcuma longa*) and gotu kola (*Centella asiatica*) has a beneficial effect on blood pressure and arterial stiffness (P Hasimun et al., 2019).

The antihypertensive effect of turmeric is mainly due to its active compound curcumin (diferuloylmethane), which is a polyphenolic compound from the *Curcuma longa Linn* (Zingiberaceae) plant. Meanwhile, the antihypertensive effect of gotu kola is due to its active component, asiatic acid, which is a triterpenoid compound.

The antihypertensive effect of asiatic acid is related to the upregulation of eNOS, which causes an increase in NO bioavailability (Bunbupha *et al.*, 2014).

Additionally, this asiatic acid could directly reduce ACE activity, Angiotensin II levels, oxidative stress, and inflammation in hypertensive rats (Maneesai *et al.*, 2016).

In this study, the combination of turmeric and gotu kola was used in the preparation of nori because it is a processed food that is familiar and favoured by many people.

Additionally, the study aims to determine the effectiveness of nori on blood pressure, arterial stiffness, heart rate, spatial QRS-T angle, NO levels, and modulation of ACE, eNOS and iNOS gene expression in L-NAME induced and non-induced hypertensive animal models.

MATERIALS AND METHODS

Non-invasive measurement of blood pressure, arterial stiffness, heart rate, and spatial QRS-T angle was carried out in vivo to ascertain the antihypertensive activity of the nori preparations. The measurement was also carried out in vitro to analyze the expression of ACE, eNOS and iNOS genes, as well as to ascertain the serum NO levels.

Nori Preparation

Fresh turmeric rhizome and gotu kola leaves were cleaned of dirt with running water. A total of 2 g of the turmeric rhizome, 3 g of gotu kola leaves, and 95 g of euchema cottoni seaweed were then processed into a slurry mixture with water in the ratio of 2:1, using a blender. The slurry was then mixed and boiled at 70^{o}C for 3-5 minutes and added with 1% glycerol. Finally, the mixture was printed and dried in an oven at 50^{o}C for 7 hours.

Experimental Design

A total of 25 male Wistar rats of 3-4 months old and weighing between 190-230 g were randomly divided into 5 groups (N=5 each group). The rats were then adapted for 7 days with a 12:12 dark: light cycle at the animal facility of the Faculty of Pharmacy, Bhakti Kencana University. During the experimental period of 28 days, the rats were given treatment according to the following groups. Na CMC at 0.5% was administered in the negative control group, while L-NAME at 40mg/kg in the positive control group. Additionally, Kaptopril at 2.5mg/kg and L- NAME at 40 mg/kg were administered in the comparison group, 5% nori in the supplement group, and 5% nori and L-NAME at 40 mg/kg in the test group. The measurements of non-invasive parameters, namely, blood pressure, arterial stiffness, heart rate, and spatial QRS-T angle, were then performed on days 0, 14, and 28. Finally, in vitro tests on NO levels, ACE, eNOS, and iNOS gene expression were conducted after in vivo testing.

The experimental protocol was approved by the Ethics Commission of Padjadjaran University, Sumedang, Indonesia, and an ethical license (Number: 090/UN6.KEP/EC/2021) was also obtained.

Blood Pressure Measurement

Systolic and diastolic blood pressure measurements were carried out through a non-invasive blood pressure method using the "CODA® Kent Scientific Corporation" device. This method uses a tail-cuff

attached to the rat's tail to monitor blood pressure. Furthermore, in the CODA device, there is a VPR (Volume Pressure Recording) sensor that utilizes a differential pressure transducer specially designed to measure the blood volume in rat tails.

Evaluation of Arterial Stiffness, Heart Rate, and Spatial QRS-T Angle

Arterial stiffness (PWV) was measured through the previously published method (Zakaria and Hasimun, 2017), using an ECG device that utilizes an electrocardiogram (ECG) and photoplethysmogram (PPG) sensor. The ECG sensors were mounted on the right palm, left palm, right foot, and left foot. While the PPG sensor was placed at the base of the tail as a second reference time point to mark the arrival time of blood pumped from the heart (Zakaria and Hasimun, 2017). The QRS-T angle was then measured by adding two channels to the ECG device to obtain the frontal leads. The frontal QRS-T angle was then derived from those lead (Zakaria and Hasimun, 2019). Finally, heart rate was determined by measuring the R-R distance in the ECG PQRST waves.

Determination of Serum NO Levels

The Griess reaction was used to detect nitric oxide levels by measuring the nitrite levels in the blood serum sample. The sample was obtained from the orbital sinus of the eye. The measurement procedure involved first reacting the serum with cadmium for 15 minutes to reduce the nitrate. Then Griess reagent of the same amount was added and incubated for 30 minutes at room temperature. Subsequently, absorbance was measured using a spectrophotometer at λ540 nm, while NO concentration was determined from the standard curve of sodium nitrite ($NaNO_2$).

Gene Expression

Gene expression was carried out using the gel base PCR semi-quantitative method. The method involved first isolating the aortic vessels to determine the eNOS and iNOS gene expression. Furthermore, the kidney organs were also isolated to determine the ACE gene expression.

RNA was isolated using the SV Total RNA kit, while cDNA synthesis was carried out as stated in the GoScript™ Reverse Transcription System (Promega) kit protocol. Finally, cDNA amplification was according to the Specific primers as listed in Table 1, using a Thermal Cycler (Thermo Scientific®) for 35 cycles, including initial denaturation stage at 95°C for 2 minutes, annealing stage at 57°C for 30 seconds, extension stage at 72°C for 30 seconds, and final extension at 72°C for 5 minutes. The amplified product (amplicon) was then stored at 4°C.

Subsequently, amplicons were detected using the agarose gel electrophoresis process, which was run at 110 volts for 35 minutes. The results of the electrophoresis were visualized under Blue Light (Mupid) and then documented.

The area under the curve (AUC) of the image was determined using the ImageJ software. This value interprets the expression of the total gene and DNA in intact condition because it is directly proportional to the band thickness.

Statistic Analysis

The data obtained were processed by statistical one way ANOVA and the results showed that there was a significant difference between the groups indicated by $p < 0.05$. The supplement group was compared with the normal group, while the test group was compared with the comparison group.

RESULTS AND DISCUSSION

Blood Pressure and Heart Rate

Table 2 presents the mean systolic blood pressure (SBP) and diastolic blood pressure (DBP) in all treated groups. The positive control group induced with L-NAME 40 mg/kg showed a significant increase in SBP and DBP compared to the normal control group ($p<0.05$). Furthermore, the group which was given treatment with the nori preparation and the comparison group that received captopril showed a significant decrease in SBP and DBP compared to the positive control group ($p<0.05$). While the normal group that was given the nori preparation showed a significant decrease in SBP and DBP compared to the negative control group ($p<0.05$).

Table 3 shows values of mean arterial pressure (MAP) and heart rate (HR) in all treated groups. The positive control group showed a significant increase in MAP and HR compared to the negative control group on days 14 and 28 of measurement. Furthermore, on day 14, the group that received treatment with the nori preparation did not show a significant decrease in MAP and HR compared to the positive control group ($p>0.05$), while at day 28, it showed a significant decrease ($p<0.05$). This decrease was also significantly different from the negative control group ($p>0.05$) and the comparison group ($p>0.05$). The group receiving treatment with captopril at 2.5 mg/Kg showed a significant decrease in MAP and HR compared to the positive control group ($p<0.05$) at 14 days and a significant decrease compared to the

Table 1: Specific Primer

Gene	Primary Sequence	Product Length
ACE	Forward: TCCTGCTAGACATGGAGACGA Reverse: CAGCTCTTCCACACCCAAAG	142 bp
eNOS	Forward: AAGTGGGCAGCATCACCTAC Reverse: GCCTGGGAACCACTCCTTTT	207 bp
iNOS	Forward: AGCATCACCCCTGTGTTCCACCC Reverse: TGGGGCAGTCTCCATTGCCA	388 bp

Table 2: Effect of nori combination of turmeric and gotu kola on systolic and diastolic blood pressure

Group	SBP (mmHg)			DBP (mmHg)		
	T0	T14	T28	T0	T14	T28
Negative	122 ± 1.53	$123 \pm 4.04^{\beta}$	$126 \pm 2.65^{\beta}$	93 ± 1.00	$88 \pm 2.00^{\beta}$	$96 \pm 2.31^{\beta *}$
Positive	123 ± 0.58	$142 \pm 2.08^{\alpha *}$	$164 \pm 2.08^{\alpha *}$	91 ± 2.52	$96 \pm 2.52^{\alpha *}$	$106 \pm 2.65^{\alpha *}$
Comparison	123 ± 1.53	$129 \pm 1.53^{\beta}$	$122 \pm 8.18^{\beta}$	93 ± 2.08	$90 \pm 1.73^{\beta}$	$82 \pm 1.53^{\alpha\beta}$
Nori Supplements	122 ± 0.58	$114 \pm 6.51^{\alpha}$	$110 \pm 7.21^{\alpha}$	92 ± 2.52	84 ± 5.13	$78 \pm 2.08^{\alpha}$
Nori test	124 ± 1.00	$132 \pm 4.04^{\alpha\beta}$	$131 \pm 3.22^{\beta}$	92 ± 1.00	$94 \pm 1.53^{\alpha}$	$89 \pm 4.16^{\alpha\beta *}$

Negative = normal control (Na-CMC 0.5%), Positive = hypertension control (L-NAME 40 mg/kg), Comparison =Captopril 2.5 mg/kg+L-NAME, Nori supplement = normal + nori 5% in feed, Nori test = 5% nori in feed+L-NAME.
α : There is a significant difference with the negative control group ($p<0.05$)
β : There is a significant difference with the positive control group ($p<0.05$)
* : There is a significant difference with the comparison group ($p<0.05$)

negative control group ($p<0.05$) at 28 days of treatment. Finally, the normal group that received the nori preparation for 28 days showed a significant decrease in MAP and HR compared to the negative control group ($p<0.05$).

Evaluation of Arterial Stiffness and Spatial QRS-T Angle

Table 4 shows the parameters of arterial stiffness characterized by PWV values and the spatial QRS-T angle after 28 days of treatment. The table also showed that the positive control group experienced a significant increase in PWV values and widening of the spatial QRS-T angle compared to the negative control group ($p<0.05$).

Furthermore, it showed that the administration of the nori preparation in the induced test group caused a decrease in PWV and spatial QRS-T angle compared to the positive control group ($p<0.05$), but significantly different from the comparison group ($p>0.05$). The preparation, when administered to the normal rats, also showed a significant decrease in PWV and spatial QRS-T angle compared to the negative control group ($p<0.05$) at 28 days. Finally, the group receiving captopril treatment showed a decrease in PWV and spatial QRS-T angle compared to the positive control group ($p<0.05$), as well as a significant decrease compared to the negative control group ($p<0.05$).

Evaluation of Serum NO Levels

Figure 1 shows that in the L-NAME-treated group, the serum NO concentration was significantly reduced compared to the negative control group.

Furthermore, the normal group that received the nori preparation had the highest serum NO levels compared to the negative control group, and the group treated with the preparation had significantly increased serum NO levels compared to the positive control group and the captopril-treated group.

Evaluation of ACE, eNOS, and iNOS Gene Expression

Table 5 and Figure 2 show the gene expression activity. Herein, the L-NAME-treated group showed a significant increase in ACE, iNOS gene expression and a significant decrease in eNOS gene expression com-

Table 3: Effect of nori combination of turmeric and gotu kola on mean arterial pressure and heart rate

Group	MAP (mmHg)			HR (denyut/menit)		
	T0	T14	T28	T0	T14	T28
Negative	103 ± 1.00	99 ± 2.52$^{\beta}$	106 ± 3.51$^{\beta*}$	372.4 ± 18.58	373.5 ± 10.12$^{\beta}$	376.7 ± 5.3$^{\beta*}$
Positive	102 ± 1.53	112 ± 1.53$^{\alpha*}$	125 ± 0.58$^{\alpha*}$	341.6 ± 23.82	499.0 ± 4.28$^{\alpha*}$	677.2 ± 6.19$^{\alpha*}$
Comparison	103 ± 1.16	103 ± 1.73$^{\beta}$	96 ± 3.51$^{\alpha\beta}$	349.8 ± 13.46	420.4 ± 17.61$^{\alpha\beta}$	356.8 ± 5.43$^{\alpha\beta}$
Nori Supplements	102 ± 1.53	94 ± 5.57	89 ± 4.04$^{\alpha}$	379.6 ± 30.03	315.6 ± 17.17$^{\alpha}$	319.4 ± 16.69$^{\alpha}$
Nori test	103 ± 1.00	107 ± 1.53$^{\alpha}$	102 ± 3.51$^{\beta*}$	371.6 ± 73.46	432.7 ± 22.29$^{\alpha\beta}$	394.9 ± 3.51$^{\alpha\beta*}$

Negative = normal control (Na-CMC 0.5%), Positive = hypertension control (L-NAME 40 mg/kg), Comparison =Captopril 2.5 mg/kg+L-NAME, Nori supplement = normal + nori 5% in feed, Nori test = 5% nori in feed+L-NAME, MAP = mean arterial pressure, HR = heart rate

α : There is a significant difference with the negative control group (p<0.05)

β : There is a significant difference with the positive control group (p<0.05)

* : There is a significant difference with the comparison group (p<0.05)

Table 4: Effect of nori combination of turmeric and gotu kola on PWV and spatial QRS-T angle

Group	PWV (cm/s)			Sudut QRS-T spasial (^{o})		
	T0	T14	T28	T0	T14	T28
Negative	436.7 ± 0.88	435.6 ± 4.99$^{\beta*}$	428.8 ± 6.57$^{\beta*}$	75±5.0	75 ± 5.0$^{\beta}$	80 ± 0.0$^{\beta*}$
Positive	441.6 ± 4.46	525.7 ± 9.61$^{\alpha*}$	674.7±31.79$^{\alpha*}$	75 ± 5.0	95 ± 5.0$^{\alpha*}$	122 ± 2.9$^{\alpha*}$
Comparison	437.2 ± 1.76	390.6±27.37$^{\alpha\beta}$	333.7±14.31$^{\alpha\beta}$	70±10.0	70 ± 10.0$^{\beta}$	70 ± 0.0$^{\alpha\beta}$
Nori Supplements	438.5 ± 2.30	423.1 ± 0.79	362.6±5.35$^{\alpha}$	73 ± 5.8	70± 10.0	68 ± 2.9$^{\alpha}$
Nori test	439.8 ± 1.82	468.8 ± 4.69$^{\alpha\beta*}$	450.1±27.61$^{\beta*}$	76 ± 5.8	77 ± 5.8$^{\beta}$	95 ± 5.0$^{\alpha\beta*}$

Negative= normal control (Na-CMC 0.5%), Positive = hypertension control (L-NAME 40mg/kg), Comparison = Captopril 2.5 mg/kg+L-NAME, Nori supplement = normal +nori 5% in feed, Nori test = 5% nori in feed+L-NAME, PWV = Pulse Wave Velocity.

α : There is a significant difference with the negative control group (p<0.05)

β : There is a significant difference with the positive control group (p<0.05)

* : There is a significant difference with the comparison group (p<0.05)

Table 5: Effect of nori combination of turmeric and gotu kola on gene expression of ACE, eNOS, and iNOS

Group	Area Under Curve (AUC)		
	ACE	eNOS	iNOS
Negative	$14903.69 \pm 222.3^{\beta *}$	$8256.92 \pm 448.3^{\beta *}$	$4692.35 \pm 191.6^{\beta}$
Positive	$18375.33 \pm 809.5^{\alpha *}$	$5368.84 \pm 206.5^{\alpha *}$	$10857.49 \pm 92.9^{\alpha *}$
Comparison	$13589.13 \pm 365.1^{\alpha\beta}$	$7271.13 \pm 573.8^{\alpha\beta}$	$4952.20 \pm 73.3^{\beta}$
Nori Supplements	$12280.35 \pm 703.5^{\alpha}$	$10104.83 \pm 224.9^{\alpha}$	$4128.71 \pm 172.5^{\alpha}$
Nori test	$17526.47 \pm 339.2^{\alpha *}$	$8542.34 \pm 337.8^{\alpha\beta *}$	$8580.37 \pm 189.6^{\alpha\beta *}$

Negative= normal control (Na-CMC 0.5%), Positive = hypertension control (L-NAME 40mg/kg), Comparison = Captopril 2.5 mg/kg+L-NAME, Nori supplement = normal +nori 5% in feed, Nori test = 5% nori in feed+L-NAME, ACE=Angiotensin ConvertingEnzym, eNOS= endotel Nitric Oxide Syntase, iNOS= inducible Nitric Oxide Syntase.
α : There is a significant difference with the negative control group ($p<0.05$)
β : There is a significant difference with the positive control group ($p<0.05$)
* : There is a significant difference with the comparison group ($p<0.05$)

pared to the negative control group.

Subsequently, the captopril-treated group showed a significant decrease in the regulation of ACE gene expression ($p<0.05$) compared to the positive control group.

While the group treated with the nori preparation showed a significant increase in eNOS gene expression compared to the positive control group and the comparison group ($p<0.05$). The iNOS gene expression showed a significant decrease in the captopril and nori-treated group compared to the positive control group ($p<0.05$).

Finally, the normal group receiving the nori preparation showed a significant decrease in ACE and iNOS gene expression compared to the negative control group ($p<0.05$). Also, it showed a significant increase in eNOS expression compared to the negative control group ($p<0.05$).

Oral administration of L-NAME caused a significant increase in systolic, diastolic, and arterial mean blood pressure, including heart rate, arterial stiffness, and spatial QRS-T angle. This occurred through the decreased availability of NO from endothelial cells and increased expression of ACE and iNOS genes. Nitric oxide (NO), synthesized and released by endothelial nitric oxide synthase (eNOS), was the main source of circulating NO and played an important role in modulating vascular tone (Kumar *et al.*, 2010).

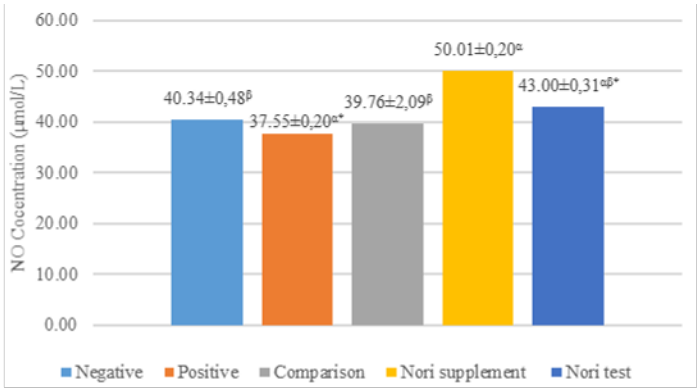
L-NAME inhibits eNOS activity during NO synthesis, resulting in decreased NO availability and vasodilating effects (Alwi, 2018). Therefore, increased peripheral resistance is characterized by an increase in systolic blood pressure, diastolic blood pressure, MAP, heart rate, and spatial QRS-T angle. NOS endothelial expression was observed in aortic vessels because eNOS expression mainly occurs in renal tubular epithelial cells, capillary bundle endothelial cells, and aortic, kidney, and heart blood vessel endothelial cells (Abdel-Rahman, 2017).

Increased heart rate is a strong predictor of cardiovascular morbidity and mortality. This is because a higher heart rate has a direct impact on artery walls, resulting in atherosclerotic plaque (Hasimun *et al.*, 2020). Shifts and changes in the abnormal QRS-T angle also describe abnormalities in the heart (Oehler *et al.*, 2014).

This is because when the QRS-T angle reaches 120° or more, there would be abnormalities in the left ventricle, which are generally identified as ischemia (Hasimun *et al.*, 2020). Finally, the QRS-T angle is defined as the peak or average direction of the T wave relative to the QRS.

The inhibition of NO production by L-NAME caused an increase in the activity of ACE expression in the kidney (Abdel-Rahman, 2017) or the activity of the renin-angiotensin system (Kumar *et al.*, 2010). ACE is a carboxypeptidase that converts angiotensin I to angiotensin II. Expression of iNOS in the L-NAME-treated group caused an increase in the aortic vasculature. This is because iNOS is a cytoplasmic enzyme that increases during inflammation in response to proinflammatory cytokines, such as tumor necrosis factor-alpha (TNF-α), interleukin-1 beta (IL-1β), interleukins 2 and 10 (IL-2, IL-10), interferon-gamma (IFN-γ), or lipopolysaccharide (Antošsová *et al.*, 2015).

Increased iNOS activity contributes to the development of endothelial dysfunction (Lee *et al.*, 2016), where NO would react with superoxide radicals to form peroxynitrite, which triggers nitrosative stress and endothelial damage. Additionally, the increase is associated with the upregulation of arginase activity that competes with eNOS during NO formation in



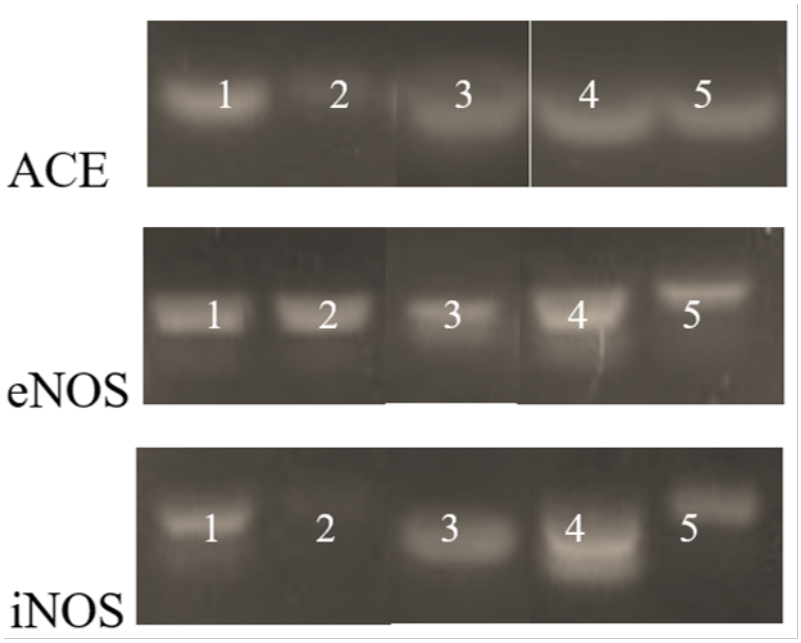
Negative = normal control (Na-CMC 0.5%), Positive = hypertension control (L-NAME 40 mg/kg), Comparison = Captopril 2.5 mg/kg+L-NAME, Nori supplement = normal + nori 5% in feed, Nori test = 5% nori in feed+L-NAME, NO = Nitric Oxide

α : There is a significant difference with the negative control group ($p<0.05$)

β : There is a significant difference with the positive control group ($p<0.05$)

* : There is a significant difference with the comparison group ($p<0.05$)

Figure 1: Effect of nori combination of turmeric and gotu kola on serum NO levels.



1=negative control, 2=comparison, 3=positive control, 4=nori supplement, 5=nori test group, ACE=Angiotensin Converting Enzym, eNOS= endotel Nitric Oxide Syntase, iNOS=inducible Nitric Oxide Syntase

Figure 2: Band of gel electrophoresis result

L-arginine (Abdel-Rahman, 2017).

The development of endothelial dysfunction is related to arterial stiffness due to NO deficiency or the long-term effects of hemodynamic overload on arterial wall remodeling (Paulis, 2012).

In this study, the administration of L-NAME showed an increase in arterial stiffness, which was indicated by an increase in the PWV value compared to the negative control group. Meanwhile, Pulse Wave Velocity (PWV) is a direct sign of arterial stiffness that indicates a risk factor for cardiovascular disease (Paulis, 2012).

Nori containing a combination of turmeric and gotu kola has been proven to lower blood pressure and reduce the development of hypertension in L-NAME-induced rats, as well as provide preventive and hemodynamic improvement effects in normal rats.

The effect of the nori preparation in reducing blood pressure occurs through the mechanism of increas-

ing the activity of eNOS expression. Previous findings have shown that the active ingredient of curcumin (diferuloylmethane) from turmeric has an antihypertensive effect by increasing the availability of NO, thereby reducing the abundance of ROS (Greish, 2020). Moreover, the asiatic acid content of gotu kola as an antihypertensive is associated with the upregulation of eNOS, leading to an increase in NO bioavailability (Bunbupha *et al.*, 2014).

The restoration of NO availability from eNOS causes an increased vasodilation effect resulting in a decrease in systolic, diastolic, and mean arterial blood pressure. This positively correlated with improvements in heart rate, vascular elasticity (PWV), and spatial QRS-T angle.

The combination of turmeric and gotu kola could also reduce the activity of iNOS expression in order that the restriction of NO production by eNOS is lower. However, the effect on decreasing ACE activity was not significant compared to the positive control group and captopril-treated rats. Captopril showed the best antihypertensive effect through the mechanism of decreasing the activity of ACE expression the best.

CONCLUSION

The combination of turmeric (*Curcuma longa*) and gotu kola (*Centella asiatica*) in processed nori has an antihypertensive activity which was characterized by improvements in blood pressure parameters and eNOS expression. However, the ACE inhibitory mechanism was not very significant in induced rats. In addition, it provides a preventive effect and improves hemodynamics in normal rats.

ACKNOWLEDGEMENT

The authors are grateful to LPPM Bhakti Kencana University and the Ministry of Research, Technology, and Higher Education of the Republic of Indonesia for their support and funding for research.

Conflict of Interest

The authors declare that they have no conflict of interest.

Funding Support

The authors declare that they have no funding support for this study.

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