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Original Article

Antioxidative Activity of the Leaf of *Nelumbo nucifera* Gaertn. on Oxidative Stress-Induced Erythrocyte Hemolysis in Hypertensive and Normotensive Rats

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ABSTRACT **Hypertension is considered as a state of oxidative stress which contribute to several organ damages. The crude leaf of *Nelumbo nucifera* Gaertn. (NN extract) is traditionally used as a folk medicine, and it was reported to have antioxidant activity. The present study aimed to test our hypothesis that if there is an increase in erythrocyte lipid peroxidation in hypertensive rats, then there should be increased in erythrocyte hemolysis as well. In addition, the NN extract would protect erythrocyte membrane from free radical-induced oxidation.**

The constriction of left renal artery was used to induce hypertension in male Sprague-Dawley rats. The oxidative hemolysis of erythrocytes was performed by using the azo-compound 2,2'-Azobis (2-amidinoporpane) dihydrochloride (AAPH) as a peroxyl radical initiator. The free radical scavenging activity of NN extract was measured by 1,1-diphenyl-2-picryl-hydrazil (DPPH). The total of NN extract phenolic contents was determined by Folin-Ciocalteu reagent.

The time-dependent hemolysis curve of erythrocyte from hypertensive rats was similar to that of normotensive ones. The 50 percent inhibitory concentration hemolytic (IC_{50}) NN extract was 48.2 ± 0.2 and 46.8 ± 1.1 μg/ml in hypertensive and normotensive rats respectively. The concentration of the NN extract required to scavenge 50 percent DPPH radical (IC_{50}) was 18.14 ± 3.13. The NN extract was rich in phenolic compounds in that 20 - 160 μg of NN extract, and was corresponded to the amount of phenolic compounds 3.98 - 20.38 μg gallic acid equivalents.

The susceptibility of rat erythrocytes to peroxidation in hypertensive rats was similar to that of normotensive ones. NN extract have the ability to protect rat erythrocytes against AAPH-induced hemolysis in hypertensive as well as in normotensive rats. It can be concluded that NN extract possesses antioxidant properties, and may have health promotion activities. The antioxidant activity of NN extract may be due to the presence of phenolic compounds.

Key words: **Antioxidant, Oxidative Stress, Leaf of *Nelumbo nucifera* Gaertn., Hypertension, Erythrocyte Hemolysis**

INTRODUCTION

Reactive oxygen species (ROS) are a major cause of tissue injury in human diseases, and share common mechanisms of molecular and cellular damage. The imbalance of ROS in hypertension is also associated with increased level of lipid peroxides and decreased of antioxidant concentrations in plasma of hypertensive patients[1, 2]. ROS play a dual role in hypertension. They inactivate nitric oxide in peroxynitrite by causing vasoconstriction. On the other hand, ROS serve as trigger mechanism for oxidative damage of numerous macromolecules for example DNA, lipid and protein[3]. The initiation or propagation of lipid and protein peroxidation

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will lead to destruction of cell membranes and cellular lysis. Termination of this chain reaction can only be achieved by chain-breaking antioxidants that can destroy the ROS production[4].

Several reports have documented that erythrocyte Na^+ transport systems are abnormal in human with essential hypertension, and in rats with spontaneous hypertension[5,6]. The erythrocyte membrane contains abundant of polyunsaturated fatty acids, which are very susceptible to ROS-induced peroxidation. The increased generation of ROS, which occurs in the condition of hypertension, may be responsible for oxidative injury to erythrocyte membranes. Moreover, ROS could lead to induce the chains oxidations of lipids and proteins and eventually cause oxidative hemolysis. In our knowledge, the properties of the erythrocyte membrane in hypertension have not yet been thoroughly investigated. In order to protect the membrane lipids against free radical chain reaction, the antioxidative nutrition from natural sources has become an attractive strategy[4].

Lotus (*Nelumbo nucifera* Gaertn.) is one of the oldest plants consumed throughout Asia. All parts of lotus are used in various medicinal purposes and support many therapeutic effects. According to ancient medicine, lotus leaf was served as healthy beverages to treat hypertension, diarrhea, fever, weakness, infection and body heat imbalance. The seed and seedpod are reported to have hepatoprotective[7], and antioxidative effects[8]. The plumule inhibited the production of pro-inflammatory cytokine TNF-α and increased that of anti-inflammation cytokine IL-6[9].

The leaf extract is useful for treatment of hyperlipidemia that affected on increased activity of amylase and lipase, accelerated lipid metabolism and up-regulated energy expenditure[10]. The several alkaloids and flavonoids isolated from the leaf exhibited significant anti-HIV activity[11]. The methanolic extract from the leaf showed protective effect against ROS-induced cytotoxicity, and also exhibited antioxidative activities[12]. Recently, the leaf extract showed the hypotensive effects that mediated by direct vasodilatation via nitric oxide[13, 14].

The major constituents present in the leaf are flavonoids and alkaloids. The flavonoids such as (+)-1(R)-coclaurine, (-)-1(S)-norcoclaurine, together with quercetin 3-O-b-D-glucuronide, which isolated from the leaf, show the potency of anti-HIV activity. The alkaloids, including liensinine, neferine and isoliensinine which isolated from the leaf and embryo can serve as new leads for further development of anti-AIDS agents[11].

However, little is available known the antioxidative activities of the leaf extract in hypertensive model. Antioxidative effects motivate us to study the effect of the leaf extract on ROS-induced erythrocyte hemolysis in hypertensive and normotensive rats. We hypothesized that if there is an increase in erythrocyte lipid peroxidation in hypertensive rats, there should be increased in erythrocyte hemolysis as well. On the other hand, absence of erythrocyte hemolysis changes has suggested the absence of abnormal lipid peroxidation. The present study is being undertaken to quantitatively evaluate antioxidant potential of the crude leaf of Nelumbo nucifera Gaertn. (NN extract) in vitro.

MATERIALS AND METHODS

Materials

The following reagents were obtained from either Sigma-Aldrich or Cayman: sodium chloride (NaCl), sodium phosphate dibasic (anhydrous) (Na2HPO4), ascorbic acid and (-tocopheral, 2,2'-Azobis (2-amidinoporpane) dihydrochloride (AAPH), 1,1-diphenyl-2-picryl-hydrazyl (DPPH), Folin-Ciocalteu's phenol reagent.

Preparation of the NN extract

Fresh leaves of Nelumbo nucifera Gaertn. were obtained from reservoir situated in Chonburi City during December 2002 to January 2003, and the specimen was identified by comparison with an authentic sample at department of biology, Burapha University. Fresh leaves were dried at room temperature for 3 days and kept away of sunlight. Dried leaves were ground and extracted in 5% ethanol solution. The water-ethanol extract was prepared with 50 g of Nelumbo nucifera (dry powder) in 2,000 ml, under 100 °C for 15 min. Afterwards, the extract was evaporated at 50 °C and lyophilized to form crude material. The NN extract were dissolved in PBS and filtered through Whatman paper number 2, and then the filtrates were collected. The appropriate stock solutions prepared on the day of the experiment.

Experimental animals and renal artery constriction procedure

Male Sprague Dawley rats were purchased from National Laboratory Animal Care, Mahidol University and were approximately 14 to16 weeks at time of experimentation. The animals were allowed to acclimate for at least one week before experimentation. All animals were housed in the animal facilities at department of medical science with a cycle of 12 hours light and dark, and allowed free choice access to standard rat chow and water. Animal procedures in this study were approved by Ethic on Animal Committee, Burapha University.

Hypertension was induced by left renal artery constriction adapted to the rat. All the rats were randomly divided into 2 groups (n = 8 per group): normotensive and hypertensive group. Rats were anesthetized with pentobarbital sodium (60 mg/kg, intraperitoneal), an incision was made in the mid abdomen, and the left renal artery was carefully dissected from the renal vein under aseptic conditions. A stainless wire (0.3-mm diameter) was parallel placed on the left renal artery of 8 weeks old rats and constricted the left renal artery with rope. After retrieve the stainless wire, the abdomen was closed. Rats were placed in a heating cage until they awoke. After operation, all rats were allowed an ordinary rat chow diet and tap water as desired and kept on a cycle of 12-hours light and dark. The systolic blood pressure (SBP) was measured by an indirect tail-cuff sphygmomanometer in preheated (37°C, 15 min) conscious rats before and at weekly intervals after renal artery constriction. Rats were considered to be hypertensive when SBP was significantly increased compare to control.

Preparation of erythrocyte suspensions

Whole blood was obtained from male Sprague Dawley rats via heart puncture, and collected in a heparinized tube. Erythrocytes were centrifuged and washed three times with 10 volumes of 10 mmol/L phosphate buffered saline (PBS; 125 mM NaCl and 10 mM sodium phosphate buffer, pH 7.4). The plasma and buffy coat were carefully removed by aspiration after each wash. The washed erythrocytes were finally resuspended in PBS and adjusted to a hematocrit of 4 percent.

Hemolysis Assay

Erythrocyte hemolysis mediated by AAPH, a peroxyl radical initiator, was measured according to the method of Niki et al.[15] with minor modification. Preliminary experiments were performed to choose the dose of AAPH to be used and it was concluded that 50 mM was the AAPH concentration giving the best resolution.

Time-dependent hemolysis assay

Erythrocyte suspension at 4 percent hematocrit was incubated with 50 mM AAPH (in PBS at pH 7.4). This reaction mixture was shaken gently while being incubated for indicated time at 37°C. At indicated time, the reaction mixture (0.5 ml) was diluted with PBS (1.5 ml) and control was diluted with distilled water (1.5 ml) to induce 100% hemolysis. The hemoglobin contents of the supernatant after centrifugation were determined by measuring the absorbance (Abs) at 540 nm. The percent hemolysis was calculated by using the following equation:

$$\%\text{Hemolysis} = \frac{\text{Abs at 540 nm of sample}}{\text{Abs at 540 nm of control}} \times 100$$

Dose-dependent hemolysis assay

Erythrocyte suspension at 4 percent hematocrit was incubated with PBS (control), and preincubated with the NN extract at the concentration 0-90 μg/ml for 30 min. Then, they were incubated with 50 mM AAPH (in PBS, pH 7.4) at 37°C for 3 hours with gently shaken. The hemoglobin contents of the supernatant after centrifugation were determined by measuring the absorbance at 540 nm. Ascorbic acid was used as a positive control in this experiment. The percent inhibition of hemolysis was calculated by using the following equation:

DPPH radical scavenging activity

DPPH radical scavenging activity was deterimined according to the method of Blios with a minor modification[16]. The odd electron in DPPH radical gives a strong absorption maximum at 517 nm and radical color is purple. DPPH radical solution in ethanol at 1 mM for 1 ml was mixed with 3 ml of NN extract at 0-90 μg/ml. Reaction mixture was vortexed vigorously and left for 30 min at room temperature in the dark. Disappearance of DPPH radical color upon radical reduction was monitored by measuring the absorbance at 517 nm. An α-tocopherol was used as a positive control. Percent DPPH scavening was calculated

according to the following equation:

$$\%DPPHscavening = \frac{\text{Abs at 517 nm of (control - sample)}}{\text{Abs at 517 nm of control}} \times 100$$

Determination of Total Phenolic Compounds

The total phenolic compounds in NN extract were determined with Folin-Ciocalteu reagent according to the method of Slinkard & Singleton[17] using gallic acid as a standard phenolic compound. Briefly, 0.5 ml of extract solution diluted with distilled water (9.5 ml) to obtain final solution of NN extract at 0-160 μg/ml. Folin-Ciocalteu reagent (0.5 ml) was added and the reaction mixtures were mixed thoroughly. After 5 min, 2 ml of Na_2CO_3 (10%) was added, and then the mixture was allowed to stand for 20 min with intermittent shaking. The absorbance was measured at 760 nm. The total phenolic compounds of extract were determined in micrograms of gallic acid equivalents, using the equation obtained from the standard curve of gallic acid graph:

$$Y = 0.00036X + 0.000058, r^2 = 0.997$$

where Y is gallic acid equivalents (μg) and X is the absorbance.

Statistics and data processing

All results are expressed as mean ± standard error of mean (S.E.M) of 8 different trials, and analyzed with the software Microcal™ Origin 6. The difference between erythrocyte hemolysis curves was performed using analysis of variance (ANOVA) and the Student's t-test were used for paired data. Significance was accepted at the $P < 0.05$ level.

RESULTS

Blood Pressure

The mean of SBP in male Sprague-Dawley rats was 155 ± 10 mmHg before renal artery constriction, and rose progressively to a peak mean value of 180 ± 8 mmHg at 8th weeks postoperation in hypertensive groups. In this regard, SBP was increased up to 16%. SBP in normotensive groups was found to be 158 ± 11 and 168 ± 6 at 1st and 8th weeks respectively, which increased only 6%. The percent increased blood pressure in hypertensive groups was significantly higher than that of normotensive ones ($P<0.05$).

Time-dependent curve of AAPH-induced erythrocyte hemolysis

When erythrocytes were incubated in PBS or PBS plus NN extract (without AAPH), they were stable and no significant hemolysis within 4 h, thus to exclude any membrane-perturbing effect of the compounds (data not shown). When a water-soluble radical initiator, AAPH (final concentration 50 mM) was added to the erythrocyte suspension, it induced hemolysis in a time-dependent manner. The kinetic profile of the hemolysis curve was sigmoid from which one can calculate several quantitative parameters. The lag time, which is defined as the absence of hemolysis, reflects the endogenous antioxidant of the cell to inhibit peroxyl radicals. After depletion of all endogenous antioxidants, hemolysis occurred rapidly. The other parameters were time required to achieve 50% hemolysis and maximum hemolysis.

In erythrocyte exposed to AAPH, hemolysis started after 100 min incubation and plateau (98-100% hemolysis) at approximately 170 min incubation. In this experiment condition, the time-dependent hemolysis curves of erythrocytes from hypertensive and normotensive groups were not significantly different in all 3 parameters as shown in Figure 1. The addition of NN extract

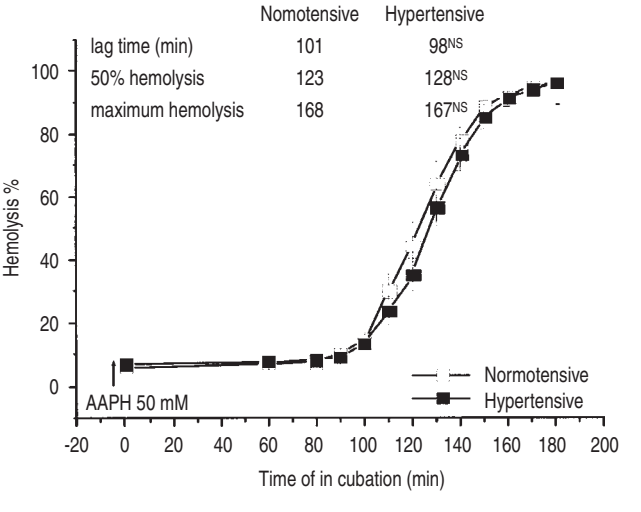

Fig. 1 Time-dependent hemolysis curve of erythrocytes in PBS (pH 7.4) at 37°C in the presence of 50 mM AAPH. The inset shows the parameters measured from the hemolysis curves of erythrocytes. Data are expressed as mean ± S.E.M of n = 8 samples, respectively. NS = statistically not different from normotensive rats, $P>0.05$.

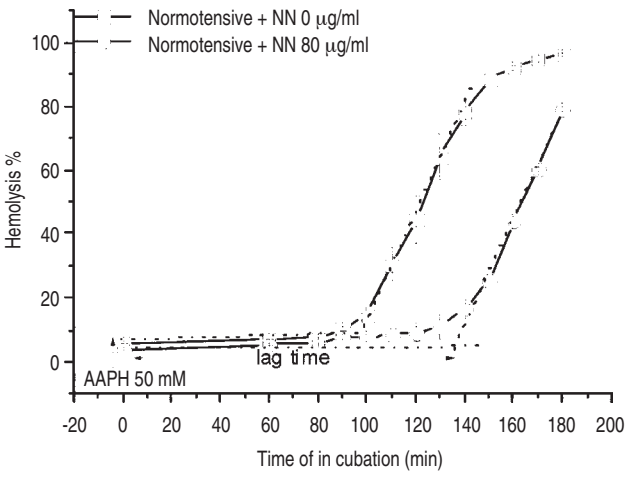

Fig. 2 Time-dependent hemolysis curve of erythrocytes in PBS (pH 7.4) at 37°C in the presence of 50 mM AAPH. The lag time is defined as the absence of hemolysis. Data are expressed as mean ± S.E.M of n = 8 samples, respectively.

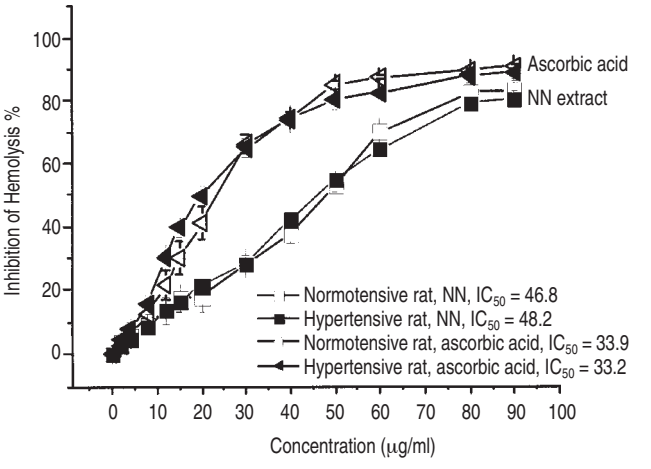

Fig. 3 The relationship between concentration and percent inhibition of AAPH-induced hemolysis. The incubation period is 3 hours in PBS (pH 7.4), at 37°C. Ascorbic acid was used as a positive control. IC_{50} is inhibitory concentration of 50% hemolysis. Data are expressed as mean ± S.E.M of n = 8 samples, respectively.

showed a tendency to prolong the lag time when compared to one from normotensive groups (Figure 2). The other parameters were not analyzed due to scarcity of erythrocytes.

Inhibitory effect of NN extract on AAPH-induced erythrocyte hemolysis

In order to compare the antioxidative activity of NN extract quantitatively, it is essential that the relationship between concentration of NN extract and percent inhibition is established, in which the appropriated time of AAPH incubation is 3 hours in this case. NN extract inhibited AAPH-induced hemolysis at concentrations ranging from 10 to 90 μg/ml with varying effectiveness (Figure 3). Concentration for 50% inhibition of hemolysis (IC_{50}) of NN extract was 48.2 ± 0.2 and 46.8 ± 1.1 μg/ml in hypertensive and normotensive rats respectively. The IC_{50} of ascorbic acid in hypertensive rats (33.2 ± 3.22 μg/ml) was similar to that of normotensive ones (33.9 ± 1.8 μg/ml). However, the IC_{50} of ascorbic acid was stronger than that of NN extract in both groups. The IC_{50} of hypertensive groups did not show any significant difference to that of normotensive ones.

Free Radical Scavenging Potential Measured by DPPH Method

To confirm the antioxidant property, NN extract

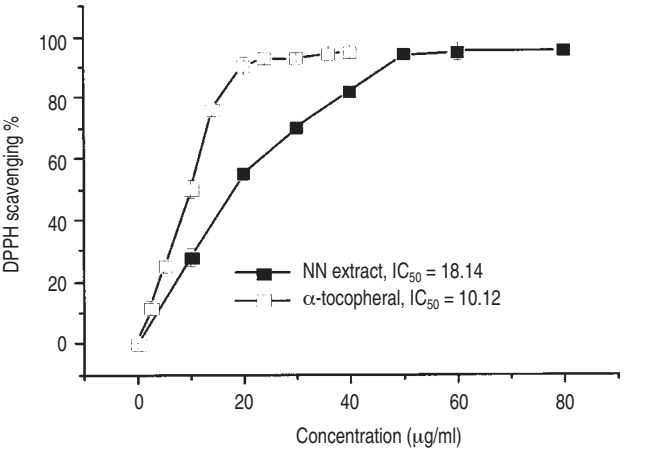

Fig. 4 Percent DPPH scavenging activities of NN extract. α-tocopheral was used as a positive control. IC_{50} is the concentration required to scavenge 50% DPPH radical. Data are expressed as mean ± S.E.M of n = 6 samples.

was tested *in vitro* free radical scavenging potential by DPPH method. The relationship between concentrations of NN extract and percentage of DPPH radical scavenging activity showed in Figure 4. The scavenging rate increased in a concentration-dependent manner for NN extract and α-tocopheral, which showed the highest activity (94% inhibition) at a concentration of 50 ± 2.13

Table 1 Amount of total phenolic compounds in NN extract.

NN Extract (μg)	Absorbance (760 nm)	Gallic Acid Equivalents (μg)
20	0.095	3.98
40	0.159	6.22
60	0.252	9.54
80	0.315	11.76
120	0.441	16.26
160	0.557	20.38

and 36 ± 2.01 μg/ml respectively. Concentration of NN extract and α-tocopheral required to scavenge 50% DPPH radical (IC_{50}) was 18.14 ± 3.13 and 10.1 ± 2.15 (g/ml respectively. This showed a comparable value to that of α-tocopheral, which was used as a positive control.

Total Phenol Contents of NN extract

Phenolic compounds are a class of antioxidant agents which act as free radical terminators. As shown in Table 1, the contents of total phenols that were measured by Folin Ciocalteu reagent in terms of gallic acid equivalent (standard curve equation: y = 0.00036x + 0.000058, r^2 = 0.997). NN extract was rich in phenolic compounds in that 20 - 160 μg of NN extract corresponded to the amount of phenolic compounds 3.98 - 20.38 μg gallic acid equivalents.

DISCUSSION

Oxidative damage of erythrocyte membrane is implicated in hemolysis, which associated with pathogenic role in several diseases including hypertension. Erythrocyte membrane from hypertensive rats showed the same parameters of hemolysis curves in vitro as compared to normotensive rats. Above observation reflects the absence of abnormal lipid peroxidation in erythrocytes of hypertensive rats. NN extract inhibited the hemolysis of erythrocyte mediated by AAPH in a dose-dependent manner, thus protecting against oxidative damage. In addition NN extract showed a significant DPPH scavenging activity. The phenolic compounds in NN extract may contribute directly to the antioxidative action.

AAPH generates peroxyl radicals by thermal decomposition of an azo compound in oxygen. This method has advantages because azo compound decomposes thermally to give radicals without biotransformation or enzyme and that rate and site of radical generation are easily controlled and measured. In order to get comparable results, it is essential to generate free radicals at a constant rate. Thermal decomposition of AAPH met this requirement in that the generation of radicals at constant temperature is virtually constant within the time used in this study[18]. Peroxyl radicals generated from AAPH induce the chain oxidations of lipids and proteins in erythrocytes membrane components and eventually cause changes in the structure and function of membranes. This is evidenced by the significant accumulation of malonyldialdehyde and decrease in an activity of free radical scavenging enzymes such as cytosolic glutathione[19].

As a result, a time-dependent erythrocyte hemolysis was observed during cell incubation with AAPH. Erythrocytes membranes are more vulnerable to lipid peroxidation due to high oxygentension and richness in polyunsaturated fatty acid. On the other hand erythrocytes contain multiple concentration defense mechanisms against free-radical-induce lipid and protein peroxidation, which include both enzymatic and non-enzymatic antioxidants[20]. The oxidation of erythrocytes membranes serves as a model for oxidative damage of biomembranes. We therefore studied erythrocytes to determine the end organ effect of oxidative stress related hypertension. On the basis of above-mentioned findings, we hypothesized that if there is an increase in erythrocyte lipid peroxidation in hypertensive rats, and then there should be decreased in lag time or increased in rate of hemolysis (leftward shift curve) as well. On the other hand, absence of oxidative hemolysis change has suggested the absence of abnormal lipid peroxidation.

From the kinetic profile of time-dependent hemolysis curve, the same pattern was observed both in hypertensive and normotensive rats. The data presented here demonstrated that susceptibility of rat erythrocytes to peroxidation was the same degree in both groups. The endogenous antioxidant activity (lag time) in hypertensive groups may not different than those of normotensive ones. We acknowledge that the present study did not assess all parameters of oxidative stress and antioxidant system. These include superoxide radical, superoxide dismutase, catalase and glutathione peroxidase. Adding these parameters would increase the validity of

our study. The results of this preliminary study did not support the hypothesis that erythrocytes from renovascular hypertensive rats may be linked to abnormal lipid peroxidation, and suggested that erythrocytes of hypertensive rats were not prone to haemolysis as evidenced by AAPH-induced hemolysis test. This study would tend to confirm the work of Garay et al.[21] which reported abnormalities in erythrocyte membrane sodium transport in patients with essential hypertension but not in patients with renovascular hypertension. Erythrocyte membrane in essential hypertension reflects a polygenic disorder that is different from renovascular hypertension.

In the case of erythrocytes pre-incubated with NN extract (0-90 μg/ml), our findings clearly indicated that NN extract significantly reduced the extent of lipid peroxidation and hemolysis of erythrocyte treated with AAPH in a dose-dependent manner. In this regard, NN extract possess an inhibitory effect on peroxyl radical, which induced erythrocyte hemolysis *in vitro*. The maximum effect was at concentration of 80 μg/ml. Resistance of biomembrane to oxidation is positively associated with the content of antioxidants in or surrounding the membrane[22]. Like an antioxidative nutraceuticals, NN extract may protect erythrocyte membrane from peroxyl radical attack by trap the initiating and/or propagating radicals. This process inhibit the peroxidation of polyunsaturated fatty acid in erythrocyte membrane[23]. When the *in vitro* quantitative antioxidant activity of NN extract and ascorbic acid were compared, ascorbic acid was more effective against AAPH-induced hemolysis than NN extract 1.5 times.

The present study was in agreement with that of Wu et al[12] which showed that the methanolic extract from leaf of *Nelumbo nucifera* Gaertn has a dose-dependent protective effect against H_2O_2-mediateded Caco-2 cytotoxicity. The seeds showed a potent free radical scavenging and hepatoprotective effects by restorating antioxidant enzymes activities in carbon tetracholide-induced lipid peroxidation of biomembrane[7]. Higashi et al[24] reported that patients with unilateral renovascular hypertension had impaired endothelium-dependent vasodilatation of the brachial artery and that this impairment was corrected by administration of an antioxidant ascorbic acid. A number of drugs for treatment hypertension such as β-blockers, ACE (angiotensin-converting enzyme) inhibitors, calcium antagonists have been reported to scavenge free radical and inhibit lipid peroxidation[25]. The hypotensive effect of NN extract may be explained by its capability as therapeutic agent against oxidative stress in blood vessel[13, 14].

It is noteworthy that NN extract has in vitro antioxidant activity. However, its mechanism especially by donating hydrogen to free radical is not guarantee. To evaluate free radical scavenging activity, DPPH method is based on the measurement of a scavenging effect of antioxidants on stable radical DPPH. The odd electron of DPPH gives a strong absorption at 517 nm (purple color). As the odd electron of DPPH becomes paired in the presence of a hydrogen donor, that is an antioxidant molecule, and then the absorption at 517 nm is decreased. The resulting decolorization is stoichiometric with respect to number of electron captured. DPPH assay is considered a valid and easy assay to evaluate the ability of compounds to act as free-radical scavengers or hydrogen donors[26]. NN extract was confirmed by the hydrogen-transferring ability to the stable free radical DPPH, using α-tocopheral as reference compounds. The potent antioxidant of NN extract to donate a hydrogen atom to DPPH radical under these experimental conditions was comparable with α-tocopheral.

Since it has been proved that antioxidant activity of NN extract is medically important, analysis of various chemical methods have been purposed to evaluate antioxidant compounds such as polyphenols. The polyphenol content of NN extract is usually evaluated by the Folin-Ciocalteu reagent, which provides an appropriate response to phenolic structures. According to our study, the high contents of these polyphenols in NN extract may be in a part responsible for its in vitro free radical scavenging activity, which was evaluated by the significant dose dependent protect erythrocyte membrane from AAPH-induced oxidation and scavenging DPPH radical. Gallic acid is widely present in plants and has two functional groups in the same molecule, hydroxyl groups and a carboxylic acid group which is a strong natural antioxidant[27]. The high scavenging property of NN extract may be due to hydroxyl groups in the phenolic compounds that can provide the necessary component as a radical scavenger.

Results of other studies have indicated that a various part of lotus is found to contain phenolics, which are suggested to be responsible for the antioxidant activ-

ity. The seeds contain alkaloids, saponins, phenolics and carbohydrates that were responsible for the antioxidant and hepatoprotective activities in vitro and in vivo models[7]. The procyanidins of lotus seedpod have a scavenging on superoxide anion, inhibit lipooxygenase activity[8]. Although there are many works that deals with the effect of lotus on antioxidant activity, the constituent of extracts differ from the extraction method, even the season of its collection. The present study proves the significant antioxidant potential of the leaf of NN extract which is beneficial in the treatment and prevention in a variety of diseases. However, further investigation of its possible compounds which may be explained the antioxidant activity need to be further characterized.

CONCLUSION

Erythrocytes suspensions were treated with AAPH induce membrane lipid peroxidation and hemolysis. There were no significant differences in time-dependent hemolysis curve of erythrocytes between the hypertensive and normotensive rats. These results suggested that the susceptibility of rat erythrocytes to peroxidation was not changed according to hypertensive disease. NN extract have the ability to protect rat erythrocytes against AAPH-induced hemolysis in hypertensive as well as in normotensive rats. It can be concluded that NN extract possess antioxidant properties as evidenced by the significant dose dependent scavenge DPPH radical. Antioxidant activity of NN extract may be due to the presence of phenolics compounds. These results supported that NN extract could be a potential therapeutic agent in oxidative stress-induced diseases. Further investigation of components and the effects on other active oxygen species is necessary to understand the significance of oxidative stress in hypertension.

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