

**Betel Leaf Extract (*Piper betle* L.) Antihyperuricemia Effect  
Decreases Oxidative Stress by Reducing the Level of MDA and Increase Blood SOD Levels of  
Hyperuricemia Wistar Rats (*Rattus norvegicus*)**

<sup>1,2</sup>Sumarya, I M., <sup>3</sup>Adiputra, N., <sup>3</sup>Sukrama, I D.M, <sup>4</sup>Putra-Manuaba, I.B.

<sup>1</sup>Doctoral Program in Medical Science of Postgraduate Program of Udayana University, Bali, Indonesia

<sup>2</sup>Faculty of Mathematics and Natural Sciences of Hindu University of Indonesia, Bali-Indonesia

<sup>3</sup>Faculty of Medicine of Udayana University, Bali, Indonesia

<sup>4</sup>Faculty of Mathematics and Natural Sciences of Udayana University, Bali-Indonesia  
sumaryaimade@yahoo.com

**Background:** Betel leaf extracts (*Piper betle* L.) antioxidant activity and enzyme inhibitors of XO. Hyperuricemia cause oxidative stress by increasing the formation of reactive oxygen species (ROS) cause lipid peroxidation and oxygenation of low-density lipoprotein cholesterol (LDLc). **Objective:** The aim of this research was to determine the betel leaf extract as an anti hyperuricemia that can lower the blood uric acid levels and oxidative stress by lowering the levels of MDA and increase the SOD of hyperuricemia of the rat's blood. **Method:** Experimental research was conducted with the design of The Randomized Post Test Only Control Group Design, on normal Wistar rats (*Rattus norvegicus*), administered with oxonic potassium (hyperuricemia) and the hyperuricemia rats either given betel leaf extract and allopurinol. After the experiment of uric acid levels, MDA and SOD in rat blood determined. **Results:** The results showed that the betel leaf extract significantly ( $p < 0.05$ ) lower uric acid levels, MDA and increase levels of SOD in rat blood. There is a positive correlation between the levels of uric acid with MDA levels and a negative correlation, although not significantly with SOD ( $p > 0.05$ ). **Conclusion:** It can be concluded that the betel leaf extract as an anti-hyperuricemia can lower the uric acid levels and decreases oxidative stress by lowering the levels of MDA and increasing the SOD.

**Keywords:** Hyperuricemia, Betel Leaf Extract, Oxidative Stress, MDA and SOD.

**DOI:** 10.15562/bmj.v5i2.218

**Cite This Article:** Sumarya, I., Adiputra, N., Manuaba, P., Sukrama, D. 2016. Betel Leaf Extract (*Piper betle* L.) Antihyperuricemia Effect Decreases Oxidative Stress by Reducing the Level of MDA and Increase Blood SOD Levels of Hyperuricemia Wistar Rats (*Rattus norvegicus*). Bali Medical Journal 5(2). DOI:10.15562/bmj.v5i2.218

## INTRODUCTION

The use of betel leaves as traditional medicine has been known for centuries to prevent body odor, medicine of shortness of breath, throat problems and lung treatment. It is also used to prevent and cure coughs, to prevent itching caused by fungi and bacteria.<sup>1,2</sup> Due to the use of betel leaf is traditionally promising, the various chemical and biological research have been conducted on betel leaf extract.

### Corresponding author:

**I Made Sumarya**

**Address: Doctoral Program in Medical Science  
of Postgraduate Program of Udayana  
University, Bali, Indonesia**

**Email: sumaryaimade@yahoo.com**

The survey results revealed that, betel leaf extract has bioactivity that serves as anti-mutagenic, anti-carcinogenic, anti-diabetic, anti-inflammatory and anti-bacterial.<sup>3,4,5</sup>

Fractionation and pure compounds from betel leaf extract, has anti-diabetic activity, cardiovascular, anti-inflammatory, antioxidant, and anti-platelet aggregation. Besides, betel leaf extract also leads to increasing antioxidant enzymes such as superoxide dismutase (SOD) and catalase (CAT).<sup>2</sup> Research on the activity of antioxidant and anti-inflammatory properties of betel leaf extract showed that the betel leaf extract contains active compounds hydroxychavicol (HC) and eugenol (EU), through a test with the enzyme xanthine oxidase (XO) showed strong inhibition against the enzyme.<sup>5</sup>

Hyperuricemia can lead to oxidative stress by increasing the formation of reactive oxygen species (ROS) through enzymatic oxidation system with enzyme NADPH-oxidase, xanthine oxidase (XO) and the enzyme endothelial nitric oxide synthase (eNOS).<sup>6,7</sup>

ROS accumulation causes lipid peroxidation and oxygenation of low-density lipoprotein cholesterol (LDLc) which can further enhance the stickiness of platelets in the formation of thrombus.<sup>8,9</sup> Oxidation of LDL form malondialdehyde (MDA) as a result of acid hydrolysis of lipid peroxides.<sup>7</sup>

## MATERIALS AND METHODS

### Materials

The materials used were fresh betel leaves (*Piper betle* L.) collected from farmers in the village of Senganan, Tabanan, Bali. 10-12 week old experimental Wistar rats (*Rattus norvegicus*) with 200-250 gram weight obtained from the Laboratory of Animal Unit, Section of Pharmacology Faculty of Medicine of Udayana University. Materials/ chemicals used are all in analytical grade unless otherwise stated. Chemicals used include: 96% ethanol, oxonic potassium and allopurinol (from Sigma-Aldrich, USA), 0.5% CMC solution. Uric Acid Assay Kit (Sigma-Aldrich, USA). Rat Malondialdehyde (MDA) ELISA Kit (Cusabio, UK). Rat superoxide dismutase (SOD) ELISA Kit (Kamiya Biomedical Company, Seattle).

### Method

Betel leaf extracts. Fresh betel leaves were cleaned by washing them on the stream of tap water and air dried for 3-5 days and then finely ground. 1 kg of dry powder betel leaves were macerated with 96% ethanol for 72 hours at room temperature (28-30°C). The extract was filtered and the filtrate is collected and then the residue was macerated again with 96% ethanol for 72 hours at room temperature until the filtrate was colorless. The filtrate was collected and evaporated to dry with a rotary evaporator at 40 ° C (yield 86.96 g) and stored at -20 ° C until it was used.<sup>3,10</sup>

GCMS analysis of betel leaf extracts. GC 6890N from the Hewlett-Packard (Palo Alto, CA, USA) put together with mass detector (MS5973, Hewlett-Packard) was used for sample analysis of the betel leaf extracts. The experimental conditions were as follows: column: HP 5MS (30 mm x 0.25 mm and a film thickness of 0.25 mm) was used and the flow moving phase (He) was set at 0.7 ml / min. In the gas chromatography, temperature was kept at 50°C for 1 minute and then increased until 150°C with 10°C increase in heating / min. After this

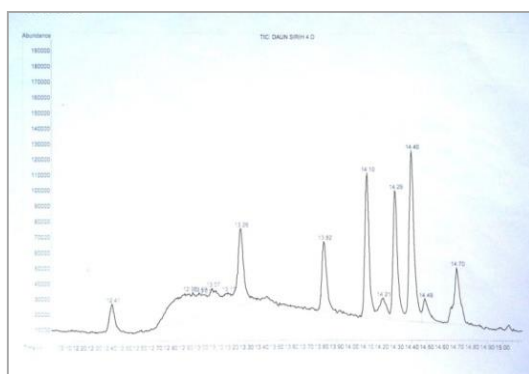
period, the temperature was maintained at 150° C for 2 minutes. Lastly the temperature was increased up to 280°C with the increase in heating of 20°C / minute and then maintained at 280°C for 30 minutes.<sup>9,11</sup> The percentage of each compound was calculated as the ratio of the peak areas on the total area of chromatography. The tops of the GCMS were identified by comparing some of the data reported by other researchers and the profile of the library of Nist98 and Wiley 7n.<sup>4</sup>

The research was conducted with true experimental method with The Randomized Post-test Only Control Group Design. The protocol followed the instructions and approval of the Animal Care Committee of the Faculty of Veterinary Medicine of Udayana University. Experiments were conducted using 24 Wistar rats with 4 treatments. Rats were divided into 4 groups each of 6 rats (n = 6). (1) the normal group/ without treatment were given standard feed/ pellet (50 g/ kg bw/ day) and CMC solution of 0.5% as Vehicle. (2) the negative control group (hyperuricemia/ oxonic potassium) were fed a standard / pellet (50 g/ kg bw/ day) and oxonic potassium 750 mg/ kg bw/ day. (3) the positive control group (oxonic potassium + allopurinol) were given a standard feed pellets (50 g/ kg bw/ day), oxonic potassium 750 mg/ kg bw/ day and allopurinol 5.0 mg/ kg bw/ day. (4) The treatment group with betel leaf extract (oxonic potassium + betel leaf extract) were given a standard feed pellets (50 g/ kg bw/ day), oxonic potassium 750 mg/ kg bw/ day and betel leaf extract 300 mg/ kg bw/ day.

At the end of the experiment (7 weeks) rat blood was collected through the retro-orbital plexus of rats using micro hematocrit tubes, for specifying the levels of uric acid, MDA and SOD by the method of spectrophotometry using Uric Acid Assay kit, Rat Malondialdehyde (MDA) ELISA Kit and Rat superoxide dismutase (SOD) ELISA Kit. The data of uric acid levels, MDA and SOD in blood shown as mean ± SE and statistically analyzed by One-way ANOVA followed by Post-hoc analyzes using LSD to compare between groups. Correlation analysis of Pearson-Product Moment was conducted between blood uric acid levels with the blood levels of MDA and SOD.

## RESULTS

From the extraction of 1 kg maceration of dried betel leaf powder, it was obtained results in the form of viscous greenish-black betel leaf extract as much as 86.96 g. Based on the results of spectroscopic analysis of GC-MS of betel leaf extract, it showed 11 detected peak compounds with retention time ( $t_R$ ), the peak area (%) as shown in Figure 1 and the details is presented in Table 1.



**Figure 1**  
Betel Leaf Ethanol Extract chromatograms

The results of chromatogram show that 1<sup>st</sup> and the 7<sup>th</sup> until the 11<sup>th</sup> peaks are classes of sesquiterpene compounds of terpenoids derivatives. The 2<sup>nd</sup> until the 6<sup>th</sup> peaks are derivatives of phenol namely chavibetol and eugenol.

The experimental results showed that means of uric acid levels, MDA and SOD in all 4 treatment groups are as shown in Table 2.

Administration of oxonic potassium (hyperuricemia) significantly increased the rats' blood uric acid levels compared to the normal rats ( $3.467 \pm 0.3018$  vs.  $1.983 \pm 0.1682$  mg/dL,  $p < 0.05$ ,

$n=6$ ). Significantly increased the rats' MDA blood levels compared to the normal rats ( $46.91350 \pm 9.546585$  vs.  $22.82016 \pm 4.605019$  pg/mL,  $p < 0.05$ ,  $n=6$ ). Not lowering blood SOD rats compared normal rats ( $48.23520 \pm 10.979368$  vs.  $47.05883 \pm 7.565573$  %,  $p > 0.05$ ,  $n = 6$ ).

When the administration of oxonic potassium jointly given with allopurinol or betel leaf extract the elevated uric acid levels totally prevented or reduced significantly by allopurinol ( $3.467 \pm 0.3018$  vs.  $1.833 \pm 0.1542$  mg/dL,  $p < 0.05$ ,  $n=6$ ) or by a betel leaf extract ( $3.467 \pm 0.3018$  vs.  $1.950 \pm 0.1384$  mg/dL,  $p < 0.05$ ,  $n=6$ ). Increased levels of MDA prevented or reduced, though not significantly reduced by allopurinol ( $46.91350 \pm 9.546585$  vs.  $34.16115 \pm 6.404795$  pg/mL,  $p > 0.05$ ,  $n=6$ ). But prevented or reduced significantly by betel leaf extract ( $46.91350 \pm 9.546585$  vs.  $23.43454 \pm 3.199519$  pg/mL,  $p < 0.05$ ,  $n=6$ ). Increases levels of SOD, although not significantly by allopurinol ( $48.23520 \pm 10.979368$  vs.  $64.46083 \pm 3.529713$  %,  $p > 0.05$ ,  $n = 6$ ). But increased significantly by betel leaf extract ( $48.23520 \pm 10.979368$  vs.  $77.45100 \pm 1.296985$  %,  $p < 0.05$ ,  $n = 6$ ).

**Table 1** Compounds Detected at Betel Leaf Extract

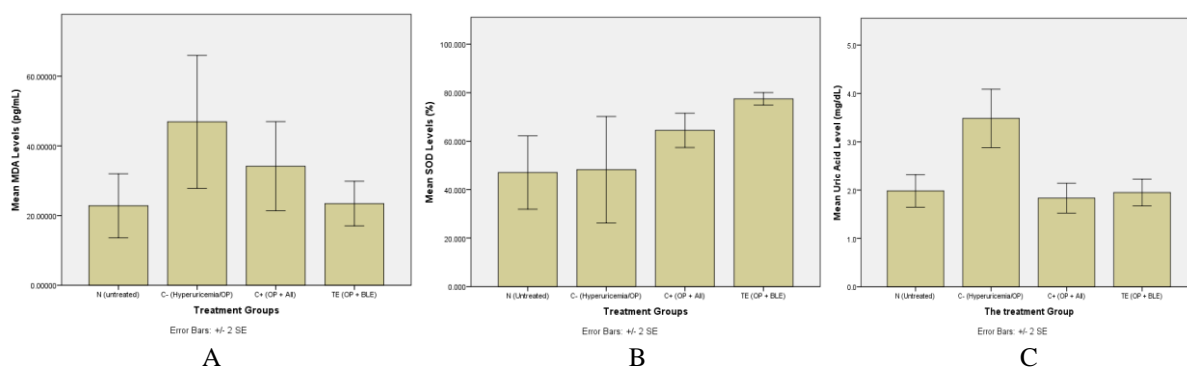
Peak Number	Retention Time (Minute)	Area Size (%)	Molecule Formula	Names of compounds
1	12,41	1,88	C <sub>15</sub> H <sub>24</sub>	α-Cubebene
2	12,92	1,94	C <sub>10</sub> H <sub>12</sub> O <sub>2</sub>	Isochavibetol
3	12,98	1,90	C <sub>10</sub> H <sub>12</sub> O <sub>2</sub>	Chavibetol
4	13,01	2,29	C <sub>10</sub> H <sub>12</sub> O <sub>2</sub>	Eugenol
5	13,07	6,08	C <sub>10</sub> H <sub>12</sub> O <sub>2</sub>	Isoeugenol
6	13,17	3,31	C <sub>10</sub> H <sub>12</sub> O <sub>2</sub>	Cis-Isoeugenol
7	13,26	14,66	C <sub>15</sub> H <sub>24</sub>	Caryophyllene
8	13,82	7,94	C <sub>15</sub> H <sub>24</sub>	α-Caryophyllene
9	14,10	9,88	C <sub>15</sub> H <sub>24</sub>	α-Cadinene
10	14,29	8,71	C <sub>15</sub> H <sub>24</sub>	β-Selinene
11	14,40	11,79	C <sub>15</sub> H <sub>24</sub>	α-Selinene

**Table 2** Means (±SE) of Uric Acid Level, MDA dan SOD Rats' Blood

Treatment Groups	Means of Uric Acid levels (mg/dL)	Means of MDA levels (pg/mL)	Means of SOD levels (%)
N (untreated)	$1.983 \pm 0.1682^a$	$22.82016 \pm 4.605019^a$	$47.05883 \pm 7.565573^a$
C- (OP)	$3.467 \pm 0.3018^b$	$46.91350 \pm 9.546585^b$	$48.23520 \pm 10.979368^a$
C+ (OP + All)	$1.833 \pm 0.1542^a$	$34.16115 \pm 6.404795^{a,b}$	$64.46083 \pm 3.529713^{a,b}$
TE (OP + BLE)	$1.950 \pm 0.1384^a$	$23.43454 \pm 3.199519^a$	$77.45100 \pm 1.296985^b$

Notes:

1. The means with the same letters at the same column shows insignificant differences ( $p > 0.05$ ).
2. The means with different letters at the same column indicates significant differences ( $p < 0.05$ )
3. N = Normal, K- = negative control, K+ = positive control, TE = Treatment with betel leaf extract
4. OP = Oxonic Potassium, All = Allopurinol, BLE = Betel leaf extracts



Notes : N = Normal, C- = Control Negative, C+ = Control Positive, TE = Treatment with Extract, OP = Oxonic Potassium, All = Allopurinol, BLE = Betel leaf extract

**Figure2.**

Uric Acid Levels (A), MDA Levels (B) and SOD Levels (C) in various treatment group.

There is a positive correlation between of rats' blood uric acid levels with rats' blood MDA levels ( $r_{\text{count}} = 0.498 (*) > r_{\text{table}} = 0.404$  at  $\alpha = 0.05$  and  $n = 24$  or  $p < 0.05$ ) and a negative correlation, although not significantly with the rats' SOD blood levels ( $r_{\text{count}} = -0.273 < r_{\text{table}} = -0.404$  at  $\alpha = 0.05$  and  $n = 24$  or  $p > 0.05$ ).

## DISCUSSION

From the chromatogram analysis results of GCMS, betel leaf extract (Figure 1 and Table 1) shows the presence of phenol compounds detected i.e. of the 2<sup>nd</sup> until the 6<sup>th</sup> respectably are compounds of isochavibetol, chavibetol, eugenol, isoeugenol, cis-isoeugenol. A previous study reported that compounds contained in this phenol of betel leaf extract causes betel leaf extract to have anti-inflammatory activity, antioxidant and as an inhibitor of the enzyme of XO.<sup>5,12,13</sup>

The administration of oxonic potassium can increase the uric acid levels, increasing the levels of MDA and not lowering the rats' SOD blood levels compared to the normal rats. Increasing the levels of uric acid because oxonic potassium is an enzyme inhibitor of uricase / urate oxidase in degrading uric acid into allantoin, leading to the accumulation of uric acid (hyperuricemia) as a result of the metabolism of purines compounds into uric acid by enzymes of XO.<sup>7,14</sup> Oxonic potassium has been widely used to increase blood uric acid levels in the experimental rats.<sup>6,15</sup> Increasing the levels of MDA due to increased uric acid (hyperuricemia) increase the formation of ROS<sup>16</sup> through enzymatic oxidation reactions with enzyme NADH / NADPH oxidase,<sup>17,18</sup> xanthine oksidase (XO)<sup>19,20</sup> and endothelial NO synthase (eNOS).<sup>6</sup> Oxidative stress (ROS) causes oxidation of plasma LDL and release MDA,<sup>19</sup> by lipid peroxides, as a result of hydrolysis of acid lipid peroxides which is formed by free radical attack on plasma lipoproteins. Therefore, MDA is used as indirect measures of LDL oxidation<sup>7</sup> or oxidative stress

marker.<sup>21</sup> Increased oxidative stress (free radical) also decreases the activity of SOD.<sup>22</sup>

The administration of oxonic potassium together with the administration of allopurinol or betel leaf extract can prevent an increase in uric acid levels or lowering the uric acid levels because allopurinol is an enzyme inhibitor of XO, likewise betel leaf extract that contains phenol compound that functions as an enzyme inhibitor of XO i.e. inhibiting the enzyme activity of XO<sup>5</sup> in the oxidation reaction of hypoxanthine into xanthine and oxidation of xanthine into uric acid in the purine degradation pathway into uric acid,<sup>7</sup> so that there is no accumulation of uric acid. The administration of oxonic potassium together with a betel leaf extract also prevents an increase (decrease) of the levels of MDA and prevents a decrease (increase) of SOD because betel leaf extract contains phenol compounds that can act as inhibitors of XO enzymes<sup>5</sup> and it serves as an antioxidant<sup>23</sup> that can lower the uric acid levels and also ROS. Betel leaf extract can also increase the levels of SOD with activation of Nrf2-ARE<sup>24</sup> signaling pathway which further increases the expression of antioxidant enzymes including SOD and CAT.<sup>25,26</sup>

An interesting result that the administration of oxonic potassium together with allopurinol did not significantly reduce the levels of MDA and increase the SOD, this is likely due to allopurinol, although it can lower the uric acid levels but it cannot decrease ROS, since allopurinol is not only as an enzyme inhibitor of XO only, but when inhibiting XO, allopurinol produces free radicals (superoxide) during the conversion it becomes oxipurinol. These results are supported by the results of previous.<sup>6</sup>

## CONCLUSION

Based on the results of the study, it can be concluded that the betel leaf extract as anti-hyperuricemia lowers the uric acid levels and

oxidative stress by lowering the levels of MDA and increase the levels of rats' SOD blood hyperuricemia.

## REFERENCES

1. Nadkarni, A.K., and Nadkarni, K.M. 2007. *Indian Materia Medica*. Eastern Book Corporation. Mumbai. Two Volumes.
2. Kumar, N., Misra, P., Dube, A., Bhattacharya, S., Dikshit, M., and Ranade, S. 2010. Piper Betel Linn. a Malignant Pan-Asiatic Plant with an Array of Pharmacological Activities and Prospects for Drug Discovery. *Current Science* 99(7):922-932.
3. Arambewela, L.S.R., Arawwawala, L.D.A.M., Ratnasooriya, W.D. 2005. Antidiabetic Activities of Aqueous and Ethanolic Extracts of *Piper betle* Leaves in Rats. *Journal of Ethnopharmacology* 102:239-245.
4. Nalina, T. and Rahim, Z.H.A. 2007. The Crude Aqueous Extract of *Piper betle* L. And its Antibacterial Effect Towards *Streptococcus mutans*. *American Journal of Biotechnology and Biochemistry* 3(1): 10-15.
5. Pin, K.Y., Lukman, C.A., Abdul, R.A., Mazura, M.P., Fadzureena, J., Vimala, S. & Rasadah, M.A. 2010. Antioxidant and Anti-inflammatory Activities of Extracts of Betle Leaf (*Piper betle*) from Solvents with Different Polarities. *Journal of Tropical Forest Science* 22.4: 448-455.
6. Schwartz, I.F., Grupper, A., Chernichovski, T., Grupper, A., Hillel, O., Engel, A., Schwartz, D. 2011. Hyperuricemia Attenuates Aortic Nitric Oxide Generation, through Inhibition of Arginine Transport, in Rats. *J Vasc Res* 48:252-260.
7. George, J. and Struthers, A.D. 2009. Role of Urate, Xanthine Oxidase and the Effects of Allopurinol in Vascular Oxidative Stress. *Vascular Health and Risk Management* 5:265-272.
8. So, A. and Thorens, B. 2010. Uric Acid Transport and Disease. *Journal of Clinical Investigation* 120(6):1791-1799.
9. Kutzing, K. and Firestein, B.L. 2008. Altered Uric Acid level and Disease States. *The journal of Pharmacology and Experimental Therapeutics* 324(1):1-7.
10. Risdian, C., Widowati, W., Mosef, Tj., Wargasetia, T.L., Khiong, K. 2011. Free Radical Scavenging Activity of Ethanol Leaves Extract and Its Different Solvent fractions of *Piper betle* L. In Vitro. *Indonesian Journal of Cancer Chemoprevention* 2(1):141-145.
11. Uzel, A., Sorkun, K., Öncü, Ö., Gogulu, D., Gencay, Ö., Salih, B. 2005. Chemical Compositions and Antimicrobial Activities of Four Different Anatolian Propolis Samples. *Microbiological Research* 16:189-195.
12. Ganguly, S., Mula, S., Chattopadhyay, S., and Chatterjee, M. 2007. An Ethanol Extract of *Piper betle* Linn. Mediates its Anti-Inflammatory Activity Via Down-regulation of Nitric Oxide. *JPP* 59:1-8.
13. Deshpande, S.N., Kadam, D.G. 2013. GCMS Analysis and Antibacterial Activity of *Piper betle* (Linn) Leaves Against *Streptococcus Mutans*. *Asian J Pharm Clin Res* 6(5):99-101.
14. Haidari, F., Rashidi, M.R., Keshavarz, S.A., Mahboob, S.A., Eshraghian, M.R., and Shahi, M.M. 2008. Effects of Onion on Serum Uric Acid Level and Hepatic Xanthine Dehydrogenase/Xanthine Oxidase Activities in Hyperuricemic Rat. *Pakistan Journal of Biological Sciences* 11(14):1779-1784.
15. Mazzali, M., Hughes, J., Kim, Y.G., Jefferson, J.A., Kang, D.H., Gordon, K.L., Lan, H.Y., Kivlighn, S., Johnson, R.J. 2001. Elevated Uric Acid Increases Blood Pressure in the Rat by a Novel Crystal-Independent Mechanism. *Hypertension* 38:1101-1106.
16. Chao, H.H., Liu, J.C., Lin, J.W., Chen, C.H., Wu, C.H., Cheng, T.H. 2008. Uric Acid Stimulated Endotelin-1 Gene Expression Associated with NADPH Oxidase in Human Aortic Smooth Muscle Cells. *Acta Pharmacol Sin* 29(11):1301-1312.
17. Griendling, K.K., Minieri, C.A., Ollerenshaw, J.D., Alexander, R.W. 1994. Angiotensin II Stimulates NADH and NADPH Oxidase Activity in Cultured Vascular Smooth Muscle Cells. *Circ Res* 74(6):1141-1148.
18. Sautin, Y.Y., Nakagawa, T., Zharikov, S. and Johnson, R.J. 2007. Adverse Effects of the Classic Antioxidant Uric Acid in Adipocytes: NADPH Oxidase-mediated Oxidative/Nitrosative Stress. *Am J Physiol Cell Physiol* 293:C584-C596.
19. Farquharson, C.A., Butler, R., Hill, A., Belch, J.J., Struthers, A.D. 2002. Allopurinol Improves Endothelial Dysfunction in Chronic Heart Failure. *Circulation* 106:221-226.
20. Glantzounis, G.K., Tsimoyiannis, E.C., Kappas, A.M. and Galaris, D.A. 2005. Uric Acid and Oxidative Stress. *Current Pharmaceutical Design* 11:4145-4151.
21. Abo-youssef, A.M. 2015. Protective effects of Rosiglitazone, Quercetin, and Their Combination on Fructose-Induced Metabolic Syndrome in Rats. *Indian Journal of Pharmacology* 47(6):620-626.
22. Damasceno, D.C., Volvato, G.T., de Mattos Paranhos Calderon, I., Aguilar, R. and Cunha Rudge, M.V. 2004. Effect of *Bauhinia forficata* Extract in Diabetic Pregnant Rat:

- Maternal Repercussions. *Phytomedicine* 11:196-201.
23. Abraham, N.N., Kanthimathi, M.S., Abdul-Aziz, A. 2012. Piper betle Shows Antioxidant Activities, Inhibits MCF-7 Cell Proliferation and Increases Activities of Catalase and Superoxide Dismutase. [cited 2016 Maret 4] Available from:URL: <http://creativecommons.org/licenses/by/2.0>.
24. Hasan, W.N.W., Kwak, M-K., Makpol, S., Ngah, W.Z.W. and Yusof, Y.A.M. 2014. Piper betle Induces Phase I & II Genes Through Nrf2/ARE Signaling Pathway in Mouse Embryonic Fibroblasts Derived from Wild Type and Nrf2 Knockout Cells. *BMC Complementary and AlternanativeMedicene* 14:72.
25. Xing, H-Y., Cai, Y-Q., Wang, X-F., Wang, L-L., Li, P., Wang, G-Y., Chen, J-H. 2015. The Cytoprotective Effect of Hyperoside Against Oxidative Stress is Mediated by the Nrf2-ARE Signaling Pathway through GSK-3 $\beta$  Inactivation. *PLoS One* 10(12):e0145183.
26. Liao, J.C., Lee, K.T., You, B.J., Lee, C.L., Chang, W.T., Wu, C.Y., and Lee, H-Z. 2015. Raf/ERK/Nrf2 Signaling Pathway and MMP-7 Expression Involvement in the Trigonellin-mediated Inhibition of Hepatocarcinoma Cell Migration. *Food & Nutrition Research* 59:29884.

