Published by DiscoverSys

1,4-bis-3,4,5-trimethoxy-phenyl-tetrahydro-furo(3,4-c) furan from mahogany (*swietenia macrophylla king*) seed significantly reduces glucose and malondialdehyde levels in diabetic wistar rats

CrossMark

Muthmainah[1*], Kristanto Yuli Yarso[2], Bambang Purwanto[3],
Ambar Mudigdo[4], Mustofa[5]

ABSTRACT

Background: Diabetes mellitus (DM) is an urgent global health issue with increased annual prevalence. The uncontrolled hyperglycemia may induce an oxidative stress state which could lead to the development of diabetic related complications. Several studies showed that mahogany seed contains 1,4-bis-3,4,5-trimethoxy-phenyl-tetrahydro-furo(3,4-c) furan which has glucose-lowering properties. However, its efficacy toward oxidative stress condition is yet to be investigated.

Objectives: This study aimed to examine the activity of 1,4-bis-3,4,5-trimethoxy-phenyl-tetrahydro-furo(3,4-c) furan in reducing the malondialdehyde (MDA) level as oxidative stress biomarker and blood glucose levels in diabetic rats.

Method: 36 male Wistar rats were used and divided randomly into six groups: Normal control, DM, DM+glibenclamide, DM+isolate10, DM+isolate20, and DM+Isolate40. The isolate and glibenclamide were given for 21 consecutive days. Streptozotocin

and nicotinamide were used to induce the diabetic model. Levels of glucose and MDA were measured in successive phases: Before induced by streptozotocin and nicotinamide; before treatment; and after 7, 14, and 21 days of treatment.

Results: Within 21 days of treatment, mean glucose and MDA levels in each therapy group: (DM+glibenclamide, DM+isolate10, DM+isolate20, and DM+Isolate40) showed significant decreases over time. Mean of glucose and MDA levels in the therapy groups were significantly lower than in DM (diabetic control) group. Mean of glucose level in DM+Isolate40 group was significantly different from the normal control group but the MDA level showed no significant difference. The results between the DM+Isolate40 group and DM+glibenclamide group showed no significant difference.

Conclusion: The 1,4-bis-(3,4,5-trimethoxy-phenyl)-tetrahydro-furo(3,4-c) furan had significant glucose lowering and anti-oxidant effect in diabetic rats.

[1]Doctoral Program in Medicine, Universitas Sebelas Maret, Surakarta, Indonesia

[2]Departement of Surgery Oncology Division Faculty of Medicine, Universitas Sebelas Maret, Surakarta, Indonesia Surakarta Indonesia

[3]Department of Internal Medicine, Dr. Moewardi Hospital, Surakarta, Indonesia

[4]Department of Anatomic Pathology, Faculty of Medicine, Universitas Sebelas Maret, Surakarta, Indonesia

[5]Department of Pharmacology, Faculty of Medicine, Universitas Gadjah Mada, Yogyakarta, Indonesia

*Corresponding Author:
Muthmainah; Doctoral Program in Medicine, Sebelas Maret University, Surakarta, Indonesia;
muthmain@gmail.com

INTRODUCTION

The prevalence of Diabetes Mellitus (DM) is continuously increased globally as well as in Indonesia. International Diabetes Federation (IDF) (2017)[1] reported that the prevalence of DM in adult (20-79 years old) in 2017 was 424.9 million people and predicted to increase to 628.6 million by 2045. In Indonesia, the prevalence of DM reach 10.3 million in 2017 and it was predicted to rise to 16.7 million in 2045.

Uncontrolled Diabetes Mellitus may result in many types of macrovascular and microvascular complication. The primary pathogenic process of these complications is the oxidative stress which induced by uncontrolled hyperglycemia that resulted from the excessive formation of Reactive Oxygen Species (ROS).[2] The oxidative stress level

can be assessed by measuring malondialdehyde (MDA), the final product of lipid peroxidation.[3,4] Multiple studies had reported increased MDA level in DM which are in accordance with the existing theory.[5,6] Therefore, controlling hyperglycemia and preventing oxidative stress are necessary to avert diabetic-related complication. The first line of control consists of education, diet, and physical training as well as medication therapy.[7,8]

Plant-based medication therapy is currently developed in many countries, including Indonesia. The development of medicines from plants is based on the fact that Indonesia is rich in biodiversity.[9] Also, people tend to use traditional remedies from plants to alleviate health problems partly due to the belief of the disadvantages of modern drugs, higher price, and many associated side effects.[10] One of the

cause of the expensive drugs in Indonesia is that most (approximately 90%) of the materials have to be imported. Therefore, the investigation of the local active agent to develop new domestic drugs is needed to overcome this problem.[11]

One of the potential medicinal plants in Indonesia is Mahogany (*Swietenia macrophylla* King) which is abundantly found in Indonesia and known for its rich therapeutic benefits.[12] Its seed is commonly used by the community from generation to generation to treat many diseases, including lowering blood sugar in DM patient.[13,14] Multiple studies showed that mahogany seed contains chemical compounds that could act as a glucose lowering agent. Mursiti (2009)[15] identified one saponin compound in the isolate, namely 1,4-bis-(3,4,5-trimethoxy-phenyl)-tetrahydro-furo(3,4-c) furan which proved to have glucose lowering capability in diabetic rats.[16] However, the study regarding the activity of this isolate in DM is still limited and no study investigated the activity of the isolate in reducing oxidative stress as well that could prevent the continued elevation of blood glucose levels. Therefore, we research to investigate the activity of mahogany seed isolate (1,4-bis-(3,4,5-trymethoxy-phenyl)-tetrahydro-furo(3,4-c) furan) in reducing MDA (as oxidative stress biomarker) and blood glucose levels in diabetic rats.

RESEARCH DESIGN AND METHODS

Animals

This research was conducted by using 36 8-10 weeks old male Wistar rats weighted between 200-250g. The rats were obtained from the Animal Center of Faculty of Pharmacy, Gadjah Mada University, Yogyakarta. The rats were housed in the Animal Laboratory of Food and Nutrition Study Center of Gadjah Mada University, Yogyakarta, Indonesia at room temperature (25°C) and 12/12 h light/dark cycle. They were fed a standard diet and water ad libitum. The experimental protocol was approved by Ethical Committee of Sebelas Maret University, Surakarta, Indonesia.

Chemicals and drug:

Streptozotocin (STZ) (Nacalai Tesque, Inc. Japan) was dissolved in freshly prepared sodium citrate buffer at pH 4.5. Nicotinamide (NA) (Sigma-Aldrich Chemical Co. USA) was dissolved in normal physiological saline while Glibenclamide (Kimia Farma Co. Indonesia) was dissolved in Na CMC 0.5%. The 1,4-bis-(3,4,5-trymethoxy-phenyl)-tetrahydro-furo(3,4-c) furan isolate of mahogany seed was dissolved in Na CMC 0.5%. The isolate was obtained from the Chemistry Laboratory of Mathematics and Natural Sciences Faculty,

Universitas Negeri Semarang, Indonesia. The kit for Glucose Oxidase-Phenol Aminoantypiryn (GOD-PAP) test was purchased from DiaSys and the kit for Thiobarbituric Acid Reacting Substances (TBARS) test was purchased from Sigma-Aldrich Chemical Co. USA.

Induction of type 2 diabetes in rats

Type 2 diabetes model in the rat was conducted in overnight-fasted experimental rats by administering NA 110 mg/kg b.w., intraperitoneal 15 minutes before a single dose of STZ 45 mg/kg b.w., intraperitoneal. Diabetes status was confirmed by measuring blood glucose levels 72 hours after streptozotocin injection. Rats were considered to be diabetic if they had fasting blood glucose levels >250 mg/dL.

Experimental design

After seven days of acclimation, the 36 rats were randomly divided into six groups. Each group consisted of six rats. The groups were:

N group	:	Normal control group, normal rats without treatment.
DM group	:	Diabetic control group, diabetic rats treated with vehicle (Na CMC 0,5%).
DM+Gliben group	:	Diabetic rats treated with glibenclamide 0.45 mg/kg b.w.
DM+Isolate-10 group	:	Diabetic rats treated with 10 mg/kg b.w of mahogany seed isolate.
DM+Isolate-20 group	:	Diabetic rats treated with 20 mg/kg b.w of mahogany seed isolate.
DM+Isolate-40 group	:	Diabetic rats treated with 40 mg/kg b.w of mahogany seed isolate.

Vehicle, glibenclamide, and mahogany seed isolate were given orally with gastric sonde once daily for 21 consecutive days.

The assessment of blood glucose levels and blood MDA levels were conducted in the successive period, namely:

Day(-)	:	Before rats induced with STZ and NA
Day(0)	:	After rats induced with STZ and NA but not yet received any intervention
Day(7)	:	After rats induced with STZ and NA and then received seven days of treatment
Day(14)	:	After rats induced with STZ and NA and then received 14 days of treatment
Day(21)	:	After rats induced with STZ and NA and later received 21 days of treatment

Assessment of blood glucose level and blood MDA levels

Blood was taken from a retroorbital vein of overnight-fasted experimental rats, with microcapillary pipe. Before taking the blood, rats were anesthetized using ketamine (45 mg/kg b.w, intramuscular). Blood samples were stored in a tube and subsequently centrifuged at 4000 rpm for 15 min in the room temperature. The serum that was in the upper part of the tube was taken for assessment of blood glucose level and blood MDA levels. Blood glucose levels were measured by Glucose Oxidase-Phenol Aminoantypiryn (GOD-PAP) test. Blood MDA levels in nmol/mL were measured by Thiobarbituric Acid Reacting Substances (TBARS) method.

Statistical analysis

The data on blood glucose levels and blood MDA levels were presented as the mean ± standard deviation (SD). One Way ANOVA test was used to analyze the difference in blood glucose levels and blood MDA levels between groups. If the result of the One Way ANOVA test was significant, then data were analyzed using the Tukey HSD test. Repeated ANOVA test was used to analyze the difference in blood glucose levels and blood MDA levels in each group over time from Day(-) to Day(21). If the result of the Repeated ANOVA test showed a significant difference, then data were analyzed using the LSD test. The value of $p < 0.05$ was considered significant. Statistical analysis was performed using the SPSS for Windows version 24.0 (IBM Corp.).

RESULTS

Blood glucose level

Blood glucose levels profile from Day (-) to Day (21) is presented in Table 1. The table showed that on Day(0) when rats had been induced with STZ and NA before obtaining therapy, the mean of blood glucose levels in all groups increased (>250 mg/dL), except in N group (Normal Control). Meanwhile, the mean blood glucose levels in DM groups treated with mahogany seed isolate significantly decreased over time from Day(0) to Day(21). At the end of the study (Day(21)), significant differences were observed in the mean blood glucose levels among all groups, except between DM+Isolate-40 group and DM+Gliben group (p = 0.182).

Blood MDA level

Blood MDA levels in each group from Day (-) to Day(21) is presented in Table 2. It showed that on Day(0), the mean blood MDA levels in all groups increased (> 2 nmol/mL), except in the N group (Normal Control). The mean blood MDA levels in DM groups that were given mahogany seed isolate significantly decreased over time from Day(0) to Day(21). After 21 days of the treatment, the comparison of mean blood MDA levels among all groups showed significant differences, except between DM+Isolate-40 group and DM+Gliben group (p=1.000), as well as between the DM+Isolate-40 group and N (normal control) group (p=0.865).

DISCUSSION

The mechanism of action of the isolate in decreasing blood glucose level is still very limited. Nugraha (2012)[16] reported that the isolate could reduce blood glucose level in diabetic rats by increasing insulin secretion by islet of Langerhans in the pancreas. Thus, this study may provide additional information regarding the isolate's mechanism of action in decreasing blood glucose level that is by reducing oxidative stress (reducing MDA level). The decrease of oxidative stress will prevent the continuous increase of blood glucose level so that it can control chronic hyperglycemia. The isolate's capability in reducing blood glucose level was equivalent to glibenclamide. Glibenclamide is an insulin secretagogue type agent that commonly used in the diabetic treatment and this drug exerts its effect by the same mechanism.[8,23]

Initially, when rats had been induced with STZ and NA before obtaining therapy, MDA levels of DM model groups (DM, DM+Gliben, DM+Isolate-10, DM+Isolate-20, and DM+Isolate-40 groups) were significantly higher than N group (normal control). This result indicated that the STZ and NA induction in DM model groups could induce oxidative stress. Oxidative stress could be observed by measuring blood MDA level. Malondialdehyde is the final product of lipid peroxidation that is often used as a biomarker of oxidative stress.[3,4]

In this study, an increase of blood MDA level in rat groups that were induced by STZ and NA could be due to excessive ROS production. Streptozotocin is known to have the ability to induce a state of hyperglycemia. This state will lead mitochondria to produce an excessive amount of ROS that potentially induce DNA damage and subsequent PARP activation to repair it. The activated PARP break NAD+ into nicotinic acid and ADP-ribose. In a further situation, polymer ADP-ribose is formed which then accumulated in glyceraldehyde-3 phosphate dehydrogenase (GAPDH) enzyme, inhibiting its activity.[2] The decrease of GADPH activity activates many biochemical pathways such as polyol pathway, protein kinase-C (PKC) pathway, hexosamine pathway, Advanced Glycation End Products (AGEs) formation, and AGEs receptor

Table 1. The mean of blood glucose levels on each group from Day(-) to Day(21)

Group	Blood glucose level (mg/dL) Mean ± SD				
	Day(-)	Day(0)	Day(7)	Day(14)	Day(21)
N	62.05 ± 2.25	62.81 ± 2.09	63.46 ± 1.95	64.16 ± 2.14	64.75 ± 1.72■
DM	60.99 ± 1.17	255.70 ± 1.37#	255.91 ± 1.36	256.49 ± 1.72	256.88 ± 1.63
DM+Gliben	63.87 ± 3.22	256.80 ± 2.74•,#	240.81 ± 2.54*	141.98 ± 2.48£	98.39 ± 2.42■,Ψ
DM+Isolate-10	63.31 ± 1.21	258.09 ± 2.19•,#	248.56 ± 2.70*	182.99 ± 2.83£	151.98 ± 1.75■,Ψ,‖
DM+Isolate-20	65.13 ± 0.96	257.72 ± 3.47•,#	240.22 ± 1.73*	157.25 ± 2.37£	130.11 ± 2.04■,Ψ,‖
DM+Isolate-40	65.55 ± 2.96	258.83 ± 4.63•,#	235.50 ± 3.80*	143.03 ± 2.00£	101.55 ± 3.47■,Ψ,=

N: Normal control group. DM: Diabetic control group. DM+Gliben: Diabetic rats treated with glibenclamide 0.45 mg/kg b.w. DM+Isolate-10, DM+Isolate-20, and DM+Isolate-40: Diabetic rats treated with 10 mg/kg b.w., 20 mg/kg b.w., and 40 mg/kg b.w. of mahogany seed isolate, respectively. Day(-): Before rats were induced by STZ and NA. Day(0): After rats were induced by STZ and NA but not yet treated. Day(7), Day(14), and Day(21): After rats were induced by STZ and NA then received 7 days, 14 days, and 21 days of treatment, respectively.

• $p < 0.01$ compared to the values of Day(7) in the same group, * $p < 0.01$ compared to the values of Day(14) in the same group, £ $p < 0.01$ compared to the values of Day(21) in the same group.

# $p < 0.01$ compared to the values of N in Day(0), ■ $p < 0.01$ compared to the values of DM in Day(21), Ψ $p < 0.01$ compared to the values of N in Day(21), ‖ $p < 0.01$ compared to the values of DM+Gliben in Day(21), = $p > 0.05$ compared to the value of DM+Gliben in Day(21).

Tabel 2. The mean of blood MDA levels of white rats in each groups from Day(-) to Day(21)

Group	Blood MDA level (nmol/mL) Mean ± SD				
	Day(-)	Day(0)	Day(7)	Day(14)	Day(21)
N	1.25 ± 0.16	1.42 ± 0.21	1.44 ± 0.18	1.56 ± 0.17	1.61 ± 0.16■
DM	1.22 ± 0.14	8.83 ± 0.12#	8.98 ± 0.18	9.06 ± 0.17	9.22 ± 0.22
DM+Gliben	1.44 ± 0.23	8.94 ± 0.28•,#	7.34 ± 0.14*	4.66 ± 0.17£	1.75 ± 0.17■,▲
DM+Isolate-10	1.40 ± 0.14	9.01 ± 0.22•,#	7.96 ± 0.18*	8.28 ± 0.16£	5.88 ± 0.20■,Ψ,‖
DM+Isolate-20	1.54 ± 0.07	9.00 ± 0.32•,#	7.22 ± 0.12*	5.83 ± 0.11£	3.11 ± 0.18■,Ψ,‖
DM+Isolate-40	1.47 ± 0.17	9.23 ± 0.42•,#	6.81 ± 0.22*	4.64 ± 0.12£	1.73 ± 0.13■,=,▲

N: Normal control group. DM: Diabetic control group. DM+Gliben: Diabetic rats treated with glibenclamide 0.45 mg/kg b.w. DM+Isolate-10, DM+Isolate-20, and DM+Isolate-40: Diabetic rats treated with 10 mg/kg b.w., 20 mg/kg b.w., and 40 mg/kg b.w. of mahogany seed isolate, respectively. Day(-): Before rats were induced by STZ and NA. Day(0): After rats were induced by STZ and NA but not yet treated. Day(7), Day(14), and Day(21): After rats were induced by STZ and NA then received 7 days, 14 days, and 21 days of treatment, respectively.

• $p < 0.01$ compared to the values of Day(7) in the same group, * $p < 0.01$ compared to the values of Day(14) in the same group, £ $p < 0.01$ compared to the values of Day(21) in the same group.

# $p < 0.01$ compared to the values of N in Day(0), ■ $p < 0.01$ compared to the values of DM in Day(21), Ψ $p < 0.01$ compared to the values of N in Day(21), ‖ $p < 0.01$ compared to the values of DM+Gliben in Day(21), = $p > 0.05$ compared to the values of DM+Gliben in Day(21), ▲ $p > 0.05$ compared to the values of N in Day(21).

(RAGEs) and its ligands expression. Activation of these biochemical pathways leads to further increase in ROS production, and thus, lipid peroxidation.[2,24] The rate of lipid peroxidation can be monitored by assessing MDA level in which increased level of MDA indicate an increased rate of lipid peroxidation.[19]

In this study, it was showed that the treatment with 40 mg/kg b.w. of mahogany seed isolate for 21 consecutive days significantly reduce blood MDA level (oxidative stress), although it couldn't lower the blood glucose level to normal. This result indicate that there may be other pathways that play a role to lower blood glucose levels, other than oxidative stress reduction. The most possible pathway is anti-inflammation which should be evaluated in the mahogany seed isolate. Blood glucose level is not only influenced by oxidative stress, but it could also

affected by inflammation. In DM, high blood glucose level induces oxidative stress which subsequently result in inflammatory status. The inflammation itself could further enhance the oxidative stress and result in vicious cycle of inflammation and oxidative stress.[25] The prolong oxidative stress and inflammation will augment pancreatic b cell death and result in further increase of blood glucose from insulin depletion.[19,20]

CONCLUSION

It can be concluded that the mahogany seed isolate (1,4-bis-3,4,5-trimethoxy-phenyl-tetrahydro-furo(3,4-c) furan) could reduce blood glucose and blood MDA levels in diabetic rats. The effective dose was found at 40 mg/kg b.w. which could reduce blood MDA level to normal although it had no significant effect on blood glucose level. The effect of mahogany seed isolate was also thought to be equivalent to glibenclamide. However, further studies with higher dose isolates are need to be performed and the investigation on the anti-inflammatory activity of this isolate are necessary with more representative parameters such as HbA1c and antioxidant enzymes (Superoxide Dismutase, Catalase, Glutathione Peroxidase) are needed.

CONFLICT OF INTEREST

There is no conflict of interest regarding this article

AUTHORS CONTRIBUTIONS

All authors contribute equally in writing and reviewing this article

REFERENCES

1. International Diabetes Federation (2017). IDF Diabetes Atlas. Eighth edition. pp: 40-41, 46.
2. Giacco F and Brownlee M (2010). Oxidative stress and diabetic complications. Circ Res, Vol. 107, pp: 1058-1070.
3. Ayala A, Munoz MF, Arguelles S (2014). Lipid peroxidation: Production, metabolism, and signaling mechanisms of malondialdehyde and 4-hydroxy-2-nonenal. Oxidative Medicine and Cellular Longevity, Vol. 2014, pp: 1-31
4. Ho E, Galougahi KK, Liu CC, Bhindi R, Figtree GA (2013). Biological markers of oxidative stress: Aplications to cardiovascular research and practice. Redox Biology, Vol. 1, pp: 483–491.
5. Judiono W, Djokomoeljanto, Hadisaputro S (2012). Biomolecular aspects of plain kefir antidiabetic potentials. International Journal of Food, Nutrition & Public Health, Vol. 5 No. 1/2/3.
6. Moussa SA (2008). Oxidative stress in diabetes mellitus. Romanian J. Biophys, Vol. 18, No. 3, pp: 225–236.
7. Perkeni (2015). Konsensus Pengelolaan dan Pencegahan Diabetes Melitus Tipe2 di Indonesia 2015. Pengurus Besar Perkumpulan Endokrinologi Indonesia, pp: 16-17.
8. Soegondo S (2014). Farmakoterapi pada pengendalian glikemia diabetes melitus tipe 2. In: Setiati S, Alwi I, Sudoyo AW, Simadibrata M, Setiyohadi B, Syam AF (eds). Buku Ajar Ilmu Penyakit Dalam. The second volume, the sixth edition. Publishing Center of Internal Medicine Jakarta, pp: 2328-2333.
9. Febrianty PP (2015). Ketergantungan Bahan Baku Obat Impor. Pharmacy Unhas. http://www.kompasiana.com (Accessed on: 4-4-2016).
10. Dewoto (2007). Pengembangan obat tradisional Indonesia menjadi fitofarmaka. Maj Kedokt Indon, Vol. 57, No. 7, pp: 205-211.
11. Hukormas Setditjen Farmalkes (2016). Menuju Indonesia Mandiri dalam Produksi Bahan Baku Obat. Kementerian Kesehatan RI. www.binfar.kemkes.go.id (Accessed on: 4-4-2016).
12. Krisnawati H, Kallio M, Kanninen M (2011). Swietenia macrophylla King: Ecology, Silviculture and Productivity. Bogor: CIFOR, pp: 1-5.
13. Eid AMM, Elmarzugi NA, El-Enshasy HA (2013). A review on the phytopharmacological effect of Swietenia macrophylla. Int J Pharm Pharm Sci, Vol 5, Suppl 3, pp: 47-53.
14. Moghadamtousi SZ, Goh BH, Chan CK, Shabab T, and Kadir HA (2013). Review: Biological activities and phytochemicals of Swietenia macrophylla King. Molecules,Vol.18, pp: 10465-10483.
15. Mursiti S (2009). Isolasi, karakterisasi, dan uji aktivitas hipoglikemik senyawa dalam biji mahoni bebas minyak dan minyak biji mahoni (Swietenia macrophylla King). Research Report. Semarang: UNNES.
16. Nugraha A (2012). Docking molekuler dan aktivitas antihiperglikemik senyawa aktif hasil isolasi dari ekstrak metanol biji mahoni (Swietenia macrophylla King) pada tikus diabetes setelah induksi streptozotocin. Study Program of Basic Medical Sciences and Biomedical Sciences, Faculty of Medicine, Gadjah Mada University, Yogyakarta. Thesis.
17. Eleazu CO, Elazu KC, Chukwuma S, Essien UN (2013). Review of the mechanism of cell death resulting from streptozotocin challenge in experimental animals, its practical use and potential risk to humans. Journal of Diabetes and Metabolic Disorders, Vol. 12, No. 60, pp: 1-7.
18. Lenzen S (2008). The mechanisms of alloxan and streptozotocin-induced diabetes. Diabetologia, Vol. 51, pp: 216–226.
19. Kumar V, Abbas AK, Aster JC (2013). Robbins Basic Pathology. Ninth edition. Canada: Elsevier Saunders, pp: 13-16, 19, 29-49, 58, 68-69.
20. Manaf A (2008). Genetical abnormality and glucotoxicity in diabetes mellitus: The background of tissue damage and infection. PDPI, Pekanbaru, Maret 2008.

21. Ghasemi A, Khalifi S, Jedi S (2014). Review: Streptozotocin-nicotinamide-induced rat model of type 2 diabetes. Acta Physiologica Hungarica, Vol. 101(4), pp: 408-420.
22. Szkudelski T (2012). Minireview: Streptozotocin-nicotinamide-induced diabetes in the rat. Caracteristics of the experimental model. *Experimental Biology and Medicine*, Vol. 237, pp: 481-490.
23. Suherman SK and Nafrialdi (2012). Insulin dan antidiabetik oral. In: Gunawan SG, Setiabudy R, Nafrialdi, Elysabeth (eds). *Farmakologi dan Terapi*. The fifth edition. Department of Pharmacology and Therapeutic, Faculty of Medicine, University of Indonesia, Jakarta, pp: 490-491.
24. Yao D and Brownlee M (2010). Hyperglycemia-induced Reactive Oxygen Species increase expression of the Receptor for Advanced Glycation End Products (RAGE) and RAGE ligands. Diabetes, Vol. 59, pp: 249-255.
25. Elmarakby AA and Sullivan JC (2012). Relationship between oxidative stress and inflammatory cytokines in diabetic nephropathy. *Cardiovascular Therapeutics*, Vol. 30, pp: 49–59.