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RESEARCH ARTICLE

Effect of Garlic *(Allium sativum L.)* Bulb Skin Extract on Urea and Creatinine Levels in Alloxan-Induced Hyperglycemia Wistar Rat

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ABSTRAK
Latar Belakang: Ekstrak kulit umbi bawang putih memiliki kandungan flavonoid, alkaloid, saponin, kuinon dan polifenol. Kulit umbi bawang putih merupakan limbah dari umbi bawang putih yang masih sedikit dimanfaatkan dalam bidang kesehatan.
Tujuan: untuk mengetahui pengaruh ekstrak kulit umbi bawang putih *(Allium sativumL.)* terhadap kadar urea kreatinin pada tikus wistar hiperglikemia yang diinduksi aloksan.
Metode: Penelitian eksperimental menggunakan rancangan *post test control group design,* dengan analisis statistik non parametrik dengan *Kruskal Wallis* dan dilanjutkan dengan *uji Mann Withney.* Pembuatan tikus hiperglikemia dengan cara di induksi aloksan dengan dosis tunggal 160 mg/kgBB secara intraperitoneal. Dosis ekstrak kulit umbi bawang putih yang digunakan 200mg/200gBB, 400mg/200gBB, dan 800mg/200gBB selama 14 hari. Pengambilan sampel darah melalui vena orbitalis yang kemudian di uji kadar urea kreatinin.
Hasil : Uji urea pada tikus hiperglikemia dengan pemberian ekstrak kulit umbi bawang putih200mg/200gBB (rerata kadar urea 88,87 mg/dL) tidak memiliki perbedaan dengan kelompok negatif (p>0,05), dosis 400mg/200gBB dan 800mg/200gBB (rerata kadar urea 41,86 mg/dL dan 41,96 mg/dL) terdapat perbedaan yang signifikan (p<0,05). Uji kreatinin terdapat pengaruh signifikan (p<0,05) pada dosis ektrak 400mg/200gBB (rerata kadar kreatinin 1,16 mg/dL).
Kesimpulan: dapat ditarik kesimpulan ekstrak kulit umbi bawang putih dapat berpengaruh terhadap penurunan kadar urea pada dosis 400mg/200gBB dan 800mg/200gBB serta kreatinin pada dosis 400mg/200gBB.

Kata kunci : Kulit umbi bawang putih, hiperglikemia, kadar urea kreatinin.

ABSTRACT
Background: *Garlic bulb skin extract contains flavonoid, alkaloid, saponin, quinone and polyphenol. Garlic bulb skin is a waste of garlic bulb which has not been optimally used so far for medical purpose. Objective: to discover the influence of garlic (Allium sativum L.) bulb skin extract on urea and creatinine levels in alloxan-induced hyperglycemia wistar rat.*
Methods: *Experimental research using post-test control group design and its statistic analysis using Kruskal Wallis followed with Mann Witney test. The hyperglychemia rat is made by inducing alloxan at a single dose of 160 mg/kg bodyweight (BW) intraperitoneally. The garlic bulb skin extract doses used are 200mg/200gBW, 400mg/200gBW, and 800mg/200gBW for 14 days. The blood is sampled through vena orbitalis which is tested for its urea and creatinine levels.*
Results: *The urea test in hyperglychemia rat administered with 200mg/200gBB garlic bulb skin extract (average urea level 88.87 mg/dL) has no differencce from the negative group (p>0.05), while the 400mg/200gBB and 800mg/200gBB doses (average urea levels 41.86 mg/dL and 41.96 mg/dL) show significant difference (p<0.05). The creatinine test shows significant influence (p<0.05) in extract dose of 400mg/200gBB (average creatinine level 1.16 mg/dL).*
Conclusion: *it can be concluded that garlic bulb skin extract may have some influence in reducing urea level at 400mg/200gBB and 800mg/200gBB doses and creatinine level at 400mg/200gBB dose.*

Keywords: *Garlic bulb skin, hyperglycemia, urea and creatinine levels.*

INTRODUCTION

Hyperglycemia constitutes a symptom of the occurence of Diabetes Mellitus, a metabolic disease caused by insulin work disorder or by insulin secretion disorder or by both (American Diabetes Association, 2004). Diabetes Mellitus prevalence will increase up to 21.3 million lives, and this prevalence will keep on increasing around the world from 65 to 80% (Subroto, 2006).

Diabetic nephropathy (DN) is a microvascular complication of diabetes mellitus disease occuring in fine blood vessels. Diabetic nephropathy is one of main causes of kidney failure and leading cause of death among all complication of diabetes mellitus (Hendromartono, 2009). Approximately one third of type-1 Diabetes Mellitus patients and one sixth of type-2 Diabetes Mellitus patients will undergo diabetic nephropathy complication. According to

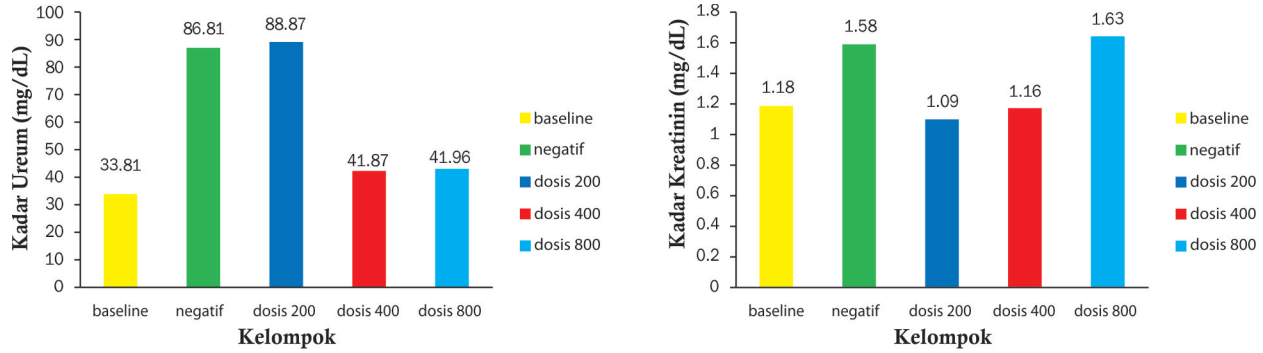

Figure 1. Urea & Creatinine level graphic

Basic Health Research (Riskesdas, 2007) the national Diabetes Mellitus prevalence is 1.1%, however, the diabetic nephropathy incidence in DM is not known with certainty just yet.

The parameter that the kidney function has been impaired in diabetic nephropathy includes increased urea and creatinine concentrations (Hendromartono, 2009), as well as increased Blood Urea Nitrogen (BUN) (Wulandari *et al.,* 2012). Impaired kidney in diabetic nephropathy needs to be controlled by decreasing its progressiveness through the proper diet management in order to maintain the optimal nutrition status, to control the blood lipid, blood glucose, blood pressure, antioxidant, and protein intake (Hakim and Ayustaningwarno, 2003). According to Nugroho (2006), alloxan is the chemical used to induce diabetes in experimental animals and constitutes a fast way to generate hyperglycemia condition in these experimental animals, and to generate a hyperglycemia rat a dose of alloxan of 160 mg/kgBW is used (Chougale *et al.* 2007).

Garlic bulb skin contains several active components capable of influencing physiologic functions. The active subtances contained in garlic bulb skin include alkaloid, quinone, flavonoid, saponin, and polyphenol (Wijayanti, and Rosyid, 2015)

RESEARCH METHOD

Research Design

This is an experimental research with post-test control group design. The urea and creatinine levels in hyperglycemia wistar strain rat are measured after garlic bulb skin extract at 200mg/200gBW, 400mg/200gBW, and 800mg/200gBW doses is administered.

Research Flow

This research is conducted in several stages, namely plant determination, the making of garlic bulb skin extract, phytochemical screening, test animal treatment for 14 days. The blood is sampled through sinus orbitalis which is first fasted for 12 hours and, then, an analysis of urea and creatinine levels is conducted using enzymatic colorimetric test.

Data analysis

To the data on urea creatinine level a statistic test is performed, namely normality test with Shapiro Wilk and homogeneity test with *Levene Test.* The data are distributed normally with value of $p>0.05$, yet, the data are not homogeneous since value of $p<0.05$ with confidence level 95%. Afterwards, a statistic non-parametic test with *Kruskal Wallis* is performed, and followed with *Mann Whitney test.*

RESULTS

The current study is conducted in December 2015-January 2016. From the plant determination, it is decided that the plant to be used is the skin of garlic bulb *(Allium sativum L.)*. The phytochemical screening results indicate that garlic bulb skin extract contains secondary metabolite compounds such as alkaloid, flavonoid, saponin, polyphenol, and tannin.

The urea and creatinin levels in rat after being administered with garlic bulb skin extract can be seen in Figure 1 below:

Figure 1 show that the administration of garlic bulb skin extract may influence urea and creatinine levels in hyperglycemia wistar rat.

To determine the significance of such influence of garlic bulb skin extract administration on the urea and creatinin levels in hyperglycemic wistar rat, a statistic analysis *Mann Whitney* is conducted resulting in as follows table 1.

DISCUSSION

The incidence of hyperglycemia will have some influence in impairing the kidney resulting from disorder in endothelium. Hyperglycemia caused accumulation of sorbitol and fructose, the endothelial gap gets wider and proteinuria occurs, then, the

Table 1. Statistic Analysis Mann-Whitney of Urea and Creatinine Levels

Groups	Urea Level	Creatinine Level
	P	*P*
1 and 2	0.009*	0.009*
1 and 3	0.028*	0.675
1 and 4	0.117	0.917
1 and 5	0.917	0.117
2 and 3	0.465	0.117
2 and 4	0.016*	0.009*
2 and 5	0.028*	0.465
3 and 4	0.465	0.754
3 and 5	0.175	0.175
4 and 5	0.602	0.117

***significantly different**

glomerular basement membrane is thickening due to the high blood glucose level as a result of type I, III, and IV collagen deposition, and glycoprotein, and the decreased glycoaminoglycans and cysteine levels. This causes the disappearance of anionic nature of glomerular basement membrane resulting from its increased permeability and the occuring albuminuria. The mesangium in hyperglycemia condition undergoes matrix production increase, thus, it is widening and the glomerular filtration surface shrinks (Waspadji, 2009). In addition, it causes increased glomerular filtration rate resulting from afferent arteriole dilatation affected by the amount of glucose in blood resulting in diabetic nephropathy as indicated by the increased urea and creatinine levels (Hendromartono, 2009).

From the statistic tests using non-parametric *test Kruskal Wallis,* followed with the use of *Mann Whitney test,* it is found that the comparison between group I (baseline) and group II (negative) for urea and creatinine levels yield significantly different result because the result obtained is 0.009 where the value of $p<0.05$, meaning that between group I and group II there is a significant difference in urea level and it indicates significant difference because the influence of alloxan induction on group II may cause high blood sugar level which will have some influence in impairing the kidney, resulting in an increased glomerular filtration rate triggerred by afferent arteriole dilatation affected by the amount of glucose in blood, thus, leading to diabetic nephropathy as indicated by the increased urea and creatinine levels (Hendromartono, 2009).

The comparison between group II (negative) and group III made by administering garlic bulb skin extract at 200mg/200gBW dose indicates that neither the obtained urea nor creatinine level is significantly different because the obtained urea level is 0.465 and the creatinine level is 0.117 where the value of $p>0.05$, meaning that the administration of garlic bulb skin extract at that dose has no influence on the urea and creatinine level decrease because the obtained levels are similar to that in group II (negative). The extract treatment at such dose cannot lower the urea level significantly because it is unable to hinder the progressiveness of diabetes complication due to the low administered dose, leading to the highly limited amount of compounds capable of stabilizing the reactive oxidant,and those compounds are flavonoid, polyphenol, and tannin (Shahidi, 1997).

The comparison between group II (negative) and group IV made by administering garlic bulb skin extract at 400mg/200gBW dose for urea and creatinine levels yield significantly different results because the obtained urea level is 0.016 and the creatinine level is 0.009 where the value of $p<0.05$, meaning that the administration of garlic bulb skin extract at that dose has some influence on the urea and creatinine level decreases because the obtained levels are different from group II (negative), which are capable of hindering the progressiveness of diabetes complication, and, thus, stabilizing the reactive oxidant, and those compounds are flavonoid, polyphenol, and tannin (Shahidi, 1997).

The comparison between group II (negative) and group V made by administering garlic bulb skin extract at 800mg/200gBW dose for urea levels yield significantly different results with the resulting level 0.028 where the value of $p<0.05$, meaning that the administration of garlic bulb skin extract at that dose has some influence on the urea level decrease because the obtained levelis different from group II (negative), which are capable of hindering the progressiveness of diabetes complication, thus, it may stabilize the reactive oxidant, and those compounds are flavonoid, polyphenol, and tannin (Shahidi, 1997). Meanwhile, for the creatinine level, the result obtained is not significantly different because

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the resulting level is 0.465 where p>0.05, meaning that at that dose it is unable to decrease the creatinine level and at 400mg/200gBW dose it has been able to decrease the creatinine level; this is because of several factors which cannot be controlled such as muscle mass change, excessive physical activities, increased tubular secretion and internal creatinine destruction (Aru et all., 2006). Creatinine is a muscle protein product constituting the end result of muscle metabolism excreted by the kidney through filtration and secretion processes (Corwin, 2001). Another factor which cannot be controlled is gastrointestinal inflammation related to protein intake and urinary tract infection (Noer, 2006).

The mechanism of flavonoid in stabilizing reactive oxidant resulting from alloxant induction can be seen in Figure 2.

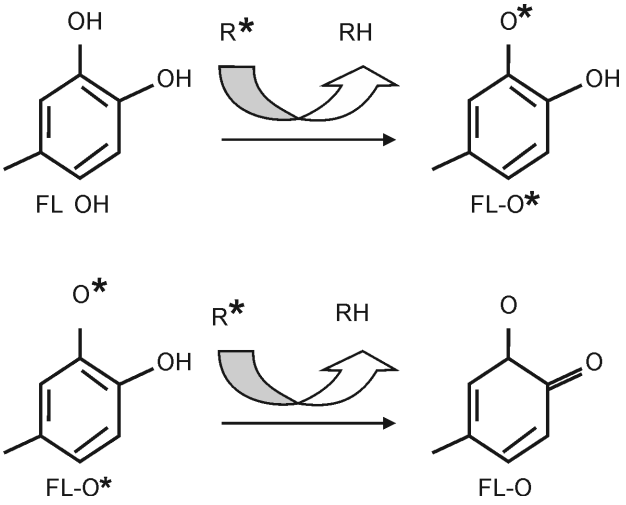

Figure 2. Flavonoid reaction

Note :
R : free radicals
FL-OH : flavonoid
FL-O* : flavonoid radicals
(Amic *et al,* 2003)

The flavonoid radicals can react with second free radical compound, forming a more stable quinone structure. Flavonoid radicals will undergo a termination reaction with free radicals, forming a stable and non-reactive radical flavonoid compound (Amic *et al,* 2003). The mechanism of polyphenol and tannin in stabilizing the reactive oxidant is by completing the free radical's electron defficiency, and preventing a chain reaction to form free radicals by donoring hydrogen atom from its hydroxyl group (Hattenschwiler and Vitausek, 2000).

The limitation of this research lies in those uncontrolable factors which may influence the research result, namely muscle mass change, excessive physical activities, increased tubular secretion and internal creatinine destruction, gastrointestinal inflammation related to protein intake and urinary tract infection. Additionally, there has been no positive control (patent medicine with synergic effect) as a comparison for garlic bulb skin extract.

CONCLUSION

Garlic bulb skin extract may have some influence in reducing urea level at 400mg/200gBW and 800mg/200gBW doses. At 800 mg/200gBW doses, it is more effective with average urea level of 41.962 mg/dL. The garlic bulb skin extract may also have some influence in the creatinine level decrease at 400mg/200gBW dose with average creatinine level of 41.962 mg/dL.

REFERENCES

American Diabetes Association, 2004. Diagnosis and classification of diabetes mellitus. *Diabetes care* 27(S1):5-10

Amic, D., Davidovic, A.D., Beslo, Trinajstc, 2003, Structure-Radical Scavenging Activity Relationship of Flavonoids, *Croatia Chemica Acta,* 76 (1), 55-61.

Aru, W.S., Setyohadi, B., Alwi, I., Simadibrata, M., Setiati, S., 2006. *Nefropati Diabetik.* Dalam :Buku Ajar Ilmu Penyakit Dalam Jilid III. Edisi IV. Jakarta: Pusat Penerbitan Departemen Ilmu Penyakit Dalam Fakultas Kedokteran Universitas Indonesia.

Chougale, A.D., Panaskar, S.N., Gurao, P.M. dan Arvindekar, A.U., 2007. Optimation Of Alloxan Dose is Essential To Induce Stable Diabetes For Prolonged Period. *Asian Journal of Biochemistry* 2 (6): 402-408.

Corwin, J.E. 2001. Buku Saku Patofisiologi. Penerbit Buku Kedokteran. Jakarta: EGC.

Hakim, V.P., dan Ayustaningwarno, F., 2003. Analisis Aktivitas Antioksidan, Kandungan Zat Gizi Makro Dan Mikro Snack Bar Beras Warna Sebagai Makanan Selingan Penderita Nefropati Diabetik, *Journal of Nutrition.* Vol.2, No.4.

Hattenschwiller, S., dan Vitousek, P. M. 2000.The Role of Polyphenols Interrestrial Ecosystem Nutrient Cycling. Review PII: S0169-5347(00)01861-9 *TREE* vol. 15. 15 Januari 2016.

Hendromartono, 2009. Nefropati Diabetik, dalam Buku Ajar Ilmu Penyakit Dalam jilid III edisi V, *Interna Publishing,* Jakarta.

Noer, MS. 2006.Evaluasi Fungsi Ginjal Secara Laboratorium. http:/www.pediatrik.co

Nugroho, A.E., 2006. Hewan Percobaan Diabetes Mellitus: Patologi dan Mekanisme Aksi Diabetogenik, *Biodiversitas*, 7, 4, 378-382.

Riset Kesehatan Dasar, 2007. Badan Penelitian dan Pengembangan Kesehatan Dasar Departemen Kesehatan Republik Indonesia.

Shahidi, F., 1997. *Natural Antioxidants Chemistry, Health Effects, and Applications.*AOCS Press. Champaign, Illinois.

Subroto, M.A., 2006. *Ramuan Herbal Untuk Diabetes Mellitus,* Jakarta Penebar Swadaya, pp: 3., 6-8.

Waspadji, S., 2009. Komplikasi Kronik Diabetes: Mekanisme Terjadinya, Diagnosis, dan Strategi Pengelolaan, dalam Buku Ajar Ilmu Penyakit Dalam jilid III edisi V, *Interna Publishing,* Jakarta.

Wijayanti, R., dan Rosyid, A., 2015, Efek Ekstrak Kulit Umbi Bawang Putih *(Allium sativum L.)* terhadap Penurunan Kadar Glukosa Darah pada Tikus Putih Jantan Galur Wistar yang Diinduksi Aloxan. *Journal of pharmaceutical science and clinical pharmacy,* Vol.12, No.1, 1-15.

Wulandari, A. D., Chasani, S., dan Ismail, A., 2012. Hubungan Dislipidemia Dengan Kadar Urea Dan Kreatinin Darah Pada Penderita Nefropati Diabetik, *Tesis.* Semarang: Universitas Diponegoro.