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EFFECT OF *ARTOCARPUS ALTILIS* DECOCTION UNRIPE ON ADVANCED GLYCATION END PRODUCTS IN HYPERGLICEMIA INDUCED RATS *(RATTUS NORVEGIUS)*

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Abstract: Hyperglycemia is a condition in which an excess of sugar (glucose) circulates in the blood. Hyperglycemia promotes oxidative stress. Oxidative stress to the continuance to end the formation Advanced Glycation End Products (AGEs) that may accelerate the formation of advanced glycation end products (AGEs). The increase in AGEs formation may damage organs and tissues such as kidney, liver, brain, lung and nerve. It is also known to be an antioxidant capable of protecting cell membrane and may help prevent AGEs accumulation. This research is aimed to study the effect of decoction of unripe *A. altilis* on AGEs inhibitor liver of hyperglycemic male rats (*Rattus norvegicus*). It was a experimental study with posttest-only with control design, consisted of five groups of treatment. Group 1: control rats given only buffer; group 2: diabetic control (STZ 55mg/kg body weight of rats);group 3,4,5: diabetic rats treated with decoction of unripe *A.altilis* to dose of 0.266; 0.533; 1.066 mg/g body weight of rats/day/100ml) in aqueous solution orally for 30 days. The average of AGEs level groups 1, 2, 3, 4, 5 were 0.724 (mol l^{-1}), 1.128 (mol l^{-1}), 0.937 (mol l^{-1}), 0.805 (mol l^{-1}), 1.262 (mol l^{-1}), respectively. In conclusion, *A. altilis* decoction of unripe was capable of inhibiting AGEs formation in hyperglycemia induced rats in dose of 0.266 mg/g, and 0.533 mg/g body weight of rats in 100 ml.

***Keywords** : AGEs, Artocarpus altilis, streptozotocin*

INTRODUCTION

Hyperglycemia is a condition in which an excess of sugar (glucose) circulates in the blood, therefore it plays a role in promoting complication of diabetes mellitus. Chronic hyperglycemia in diabetes contributes to long-term disorders, disfunction or failure of some body organs especially eyes, kidney, neurons, heart and blood vessels (Peppa, 2003). Diabetes can stimulates the increase of Oxygen Free Radicals (OFRs) (Lawrence, 2004) such as superoxide anion (O_2), hydroxyl radical (OH), and Hydrogen peroxide (H_2O_2) (Halliwell, 1999; Ceriello and Motz, 2004). Formation of OFRs in diabetes are caused by the involvement auto-oxidation of glucose, non-enzymatic protein glycosylation (AGEs), and activation of poliol line (Setiawan and Suhartono, 2005). OFRs cause the increase of oxidative stress of various tissues which indicated by the damage of protein, lipid and DNA (Evans *et al.*, 2002). Some researches have revealed the occurrence of oxidative stress caused by diabetes. According to Lonn, diabetes is a condition which correlates with the increase of oxidative stress as a consequence of hyperglycemia (Atamer *et al.*, 1996). Singh et al reported that there is a strong positive correlation among the metabolism control, the length of diabetes mellitus and the seriousness of oxidative stress induction (Singh *et al.*, 1997). Various studies have shown deficiency of total antioxidant defense status of diabetes patients caused by hyperglycemia, one of which is the glutathione compound which plays a role in the activity of glutathione peroxidase (GPx) (Setiawan and Suhartono, 2005; Lightfoot, 2006). GPx is in peroxide group which can be found in erythrocyte, plasma, liver and other tissues in human body (Winarsi, 2007). GPx acts as an endogenous antioxidant

compound which plays a role of protecting cells, not only from H_2O_2 but also from other organic peroxides (Chen and Schopfer, 1999). Besides GPx, other antioxidant enzyme which takes part in catalyzing H_2O_2 is catalase. To reduce the negative impact of oxidant, as well as to inhibit and terminate the oxidative damage, antioxidants intake is required. Antioxidants can be found in various nature plants, one of which is *A. altilis*. There is an empirical proof related with the use of unripe *A. altilis* in reducing blood-sugar levels of diabetes mellitus patients.

In Indonesia, *A. altilis* has been used as a traditional medicine for diabetes mellitus, high blood pressure, and chronic kidney failure, as well as a cure for swollen or itchy skin. Many beneficial substances such as vitamins and minerals are found in *A. altilis* plant. The leaves and fruits contain flavonoid and vitamin C (Hakim, 2007; Ersam *et al.*, 2003). Flavonoid and vitamin C are known as exogenous antioxidants which act to reduce free radicals (Kirsh *et al.*, 2006). The flavonoid group consists of flavon, isoflavon, flavonol, flavonon, and antocyanin. Two isoprenilflavon compounds from santon, i.e. artonol B and from furanohidrobenzosanton i.e. cycloartobilosanton are new compounds discovered in *A. altilis* with the highest oxidation level (Hakim, 2007; Ersam *et al.*, 2003). Antioxidants in *A. altilis* fruit are suspected to be beneficial in reducing oxidative stress in diabetic condition. However there is no scientific research that has proven this expectation hence this research is conducted. This research aimed to prove the influence of decoction of unripe *A. artilis* to the levels of AGEs of male rats (*R. norvegicus*) with diabetes. It is expected that this research can provide some information on the effects of decoction of unripe *A. altilis* to oxidative stress in hyperglycemic condition and as an antioxidant supplement to prevent further complication of diabetic sufferer.

METHODS

Streptozosin was purchased from CV. Kristalindo Biolab Surabaya. All the other chemicals used were of analytical grade and purchased from commercial sources.

Fruit of *A. altilis* were collected from Banjarbaru,South Kalimantan. Identification of samples was done by using standard botanical monographs. They were further confirmed with the Department of Biology, MIPA Faculty of Lambung Mangkurat University. *A. artilis* started with a certain weight of unripe material which is boiled with aquades until the volume of the water becomes 1/3 of its initial volume. The concentration of decoction was set to be 0.266 , 0.533 and 1.066 mg/g body weight/day/100 ml. Male *Rattus novergicus* rats, weighing 300-330 g obtained from Research Unit of animal Yogyakarta. The animal were maintained on standard rat feed and ad libitum water. The animals fasted overnight and diabetes was induced by single intraperitoneal injection of freshly-prepared STZ (55 mg/Kg body weight of rats) in 0.1 M citrate buffer (pH 4.5).The animals were allowed to drink 5% glucose solution overnight to overcome the drug-induced hypoglycaemia. Control rats were injected with citrate buffer alone. The animals were considered as diabetic, if their blood glucose values were above 200 mg/dL on the third day after the STZ injection. The treatment was started on the fourth day after the STZ injection and this was considered the first day of treatment. The Treatment was continued for 30 days (Kaleem *et al.*, 2006). The rats were divided into five groups comprising six animals in each group as follows: Group I: control rats given only buffer; group II: diabetic control (STZ 55mg/kg body weight of rats);group III: diabetic rats treated with *A.altilis* (0.266 mg/g body weight of rats/day/100ml) in aqueous solution orally for 30 days; group IV: diabetic rats treated with *A.altilis* (0.533mg/g body weight of rats/day/100ml) in aqueous solution orally for 30 days; group V: diabetic rats treated with *A.altilis* (1.066 mg/g body weight of rats/day/100ml) in aqueous solution orally for 30 days. After completion of treatment, the animals were sacrificed. Blood was collected in tubes containing potassium oxalate and sodium fluoride. Plasma was used for estimation of glucose using the easy touch.

The liver tissue were excited and rinsed in ice-cold saline. Tissue were cut into small pieces and homogenized in Tris-HCL buffer (pH 7.4). The homogenate was centrifuged and supernatant was used for AGEs measurement. AGEs was estimated using the method of Voziyan and coworkers (2003). AGEs spectrophotometric were carried out in UV-visible at λ=340 nm (genesys 20) spectrophotometer. All data were statistically evaluated using computer. Hypothesis testing methods included one way analysis of variance (ANOVA) followed by least significant differences test Tukey HSD. P-values of less than 0.05 were considered to indicate statistical significances. All the result were expressed as mean±standard deviation (SD) for six animals in each group.

RESULTS

Based on the research, data of AGEs level of liver rats with diabetic after given decoction of unripe *A. altilis* are shown in table 1 and figure 1 below:

Table 1. Effect of treatment Breadfruit A.altilis for 30 days on AGEs of control and experimental groups of rats

Group	AGEs
1	0.724 ± 0.593
2	1.128 ± 0.211[a]
3	0.937 ± 0.230[b]
4	0.805 ± 0.193[b]
5	1.346 ± 0.253[b]

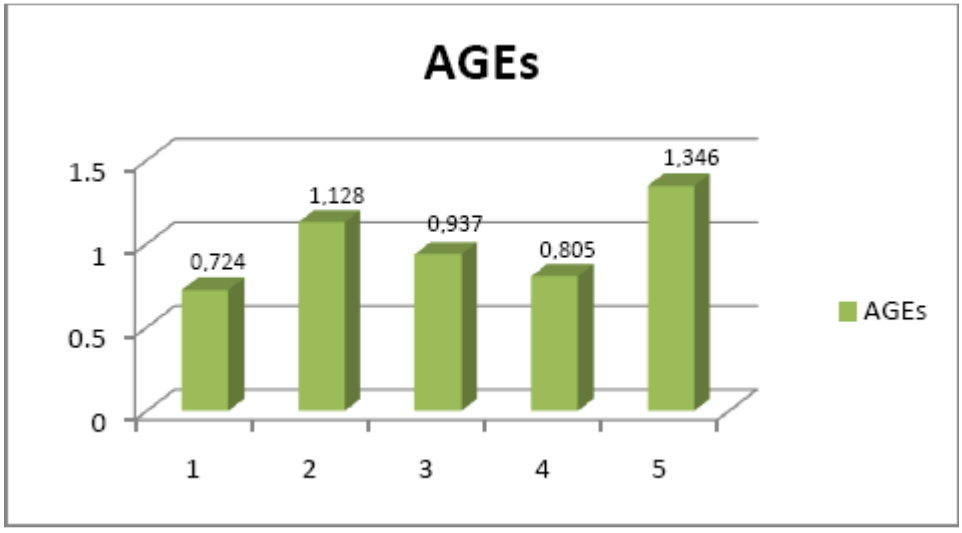

Figure 1. Diagram effect of treatment Breadfruit *A.altilis* for 30 days on AGEs of control and experimental groups of rats

1: control
2: diabetic
3: diabetic+*A.altilis* 0.266 mg/g body weight/day/100ml
4: diabetic+*A.altilis* 0.533 mg/g body weight/day/100ml
5:diabetic +A.altilis 1.066 mg/g body weight/day/100ml

Table 1 and figure 1 show that there is a significant increase of AGEs ($p<0,05$) in the diabetic group compared with the control (a). The given of decoction of unripe *A.altilis* with the concentration of 0.266 and 0.533 mg/g body weight of rats/day/100ml (P3, P4) to the diabetic rats have reduced AGEs significantly compared with the diabetic group (b).

DISCUSSION

Oxidative stress has an important role in diabetic, that is in diabetes condition, OFRs are produced from stimulation of H_2O_2 in cell-β of pancreas. Many researches have shown that concentration of lipid peroxides and hydroperoxide increase in diabetic rats. This indicates the increase of free radicals formation. The increase of lipid peroxides in diabetic may cause oxidative stress as a consequence of the decrease of endogenous antioxidant enzyme system (Kaleem *et al.*, 2005). Results of pre-clinic research have given many convincing evidence on the role of medicinal plant on the decrease of oxidative stress for diabetics.

The decrease of endogenous antioxidants and the increase of peroxidase in mellitus diabetics have emphasized the urgency of keeping the potential of antioxidants. The increasing production in mellitus diabetics are caused by glucose autooxidase, protein glicase, and activation of poliol line (Evans *et al.,* 2002). OFRs are the cause of the increase of oxidative stress of various tissues which indicated by the damage of proteins, lipids and DNA (Atamer *et al.*, 1996).

The high level of glucose in STZ induced rats can cause oxidative stress. STZ works selectively in rats (*R.norvegicus*) by damaging cell- β of pancreas, hence inhibiting the synthesis of insulin hormone creating the hyperglycemia. This is in accordance with the research result of Matsuoka *et al.* (1997) which stated that the increase of reactive oxygen compound in pancreas' cell-β can inhibit the transcription of insulin gene. The given of decoction of unripe *A. altilis* facilitates the decrease of free radicals produced during diabetes. This is supported by the level of AGEs in P3 (dose of 0.266 mg/g body weight of rats/day/100ml) and P4 (dose of 0.533 mg/g body weight of rats/day /100ml) which are significantly lower than P2 group (without the given of *A. altilis*). With the dose, the exogenous antioxidant contained in unripe *A. altilis* is able to show a significant effect in inhibiting the formation of AGEs. Therefore the decoction of *A. altilis* with doses of 0.266 mg/g body weight of rats/day/100ml and 0.533 mg/g body weight of rats/day/100ml can partially reduce imbalance between OFRs and scavenging enzyme activity. Hence the excess formation of AGEs caused by the influence of various glycation reactions can be reduced. It is assumed that this phenomenon is caused by the influence of various antioxidant contained in unripe *A. artilis*, namely some micronutrients such as vitamin B1, vitamin C, and minerals, flavonoid and steroid (Hakim, 2007; Ersam *et al.*, 2003). Micronutrients and flavonoid act as inhibitors for AGEs. Micronutrients as vitamins and minerals are needed by the body to run specific functions i.e. the continuation of metabolism reactions and cellular reactions such as glycolysis, lipid cycle, and acid metabolism to retain the production of body energy. Micronutrients are famous for their role in preventing and dealing with complication that often found in type 1 and 2 diabetes patients (O'Connel, 2001). Derivate of vitamin B1, vitamin B6, pyridoxamin and pyrophosphate thiamin are known to be the inhibition of protein modification. According to Voziyan, there are 2 inhibition mechanisms, that is by electrofilic reaction of –NH2 cluster with dicarbonyl cluster and metal chelating agent (Culbertson *et al.*, 2003).

Flavonoid in A.altilis fruit is a compound discent of prenilased flavonoid as artoindonesianin-F (stilben type), artoindonesianin- AN (arilbenzofuran discent). Flavonoid is one of the contents of medicinal plants which can be function as antioxidants (Hakim, 2007). Flavonoid can reduce free radicals (…), alkoksil, (ROO), and (OH) (Kirsh *et al.*, 2006; Pourmorad *et al.*, 2006). The work mechanism of anti-free radicals consists of (3): (1) reducing of formation of free radicals or reactive oxygen species, either by enzyme inhibition or metal ion chelating which involved in the production of free radicals. (2) scavenging of free-radicals. Based on the research by Culbertson and coworkers (Tuminah, 2003), continuous formation of Amadori product which end with the formation of AGEs in glycocilase can be reduced or limited by giving chelating agent and radical trap by antioxidants.

Vitamin C as a micronutrient contained in unripe fruit of *A. altilis* can inhibit the formation of AGEs through its role as a reduction-equivalent donor (Beckman *et al.,* 2001). The role is done by ascorbate acid which is a form of active vitamin C, as an equivalent donor it is capable of playing role as free-radicals reducer and reacting directly with superperoxide anion (O_2), hydroxyl radical (OH) and lipid peroxide. Vitamin C can inhibit formation of O_2, OH, peroxil radical (ROO), singlet oxygen (O_2) and (H_2O_2). This is in line with the research of Beckman and coworkers (2001). Intake of vitamin C can repair the function of decreasing endothel by hyperglycemia. In vitro, vitamin C also plays a role as a co-antioxidant in the regeneration form of α-tocoferol, gluthation, and β -caroten. The role of the vitamin C is beneficial for maintaining cells membrane integrity. Intakes of exogenous antioxidant of vitamin C and flavonoid which contained in unripe *A. altilis* show the influence of AGEs levels of rats hepar toward group P4 ($p<0,05$) indicated by the decrease of AGEs levels compared with the positive control which STZ induced without the intake of decoction of unripe *A. altilis*.

Diabetes rats with the decoction of unripe fruit of *A. altilis* for group 5 showed an interesting result. Compared with group 4, there is an increase of AGEs levels in group 5. therefore it is suspected that the active compounds in the decoction of unripe fruit of *A. altilis* have potentials as antioxidants in certain range of dosage. However, in higher dosage there is a possibility that the active compounds may change into pro-oxidants which need endogenous antioxidants to neutralize them. In line with this finding, active compounds such as vitamin C will be oxidized fast with the presence of metal catalyst, especially Cu. Vitamin C oxidation inducted by Cu can produce peroxide hydrogen (H_2O_2) and hydroxyl radical (OH). The hydroxyl radical can draw hydrogen atom from the membrane lipid causing lipid peroxidation (Halliwell, 1999). The iron-ascorbate binding even can produce much more hydroxyl radical and activate some enzyme including catalase (Ceriello and Motz, 2004; Ivanova and Ivanov, 2000). From the statement it can be assumed that high ascorbate acid consumption may trigger the formation of high hydroxyl radical (OH).

CONCLUSION

From this research it can be concluded that decoction of unripe A.altilis can reduce free radicals caused by diabetes which is indicated by the decrease of liver AGEs especially at the concentration of 0.266 and 0.533 mg/gbody weight of rats/day/100ml, however, higher concentration showed a potential as pro-oxidant. Therefore the use of decoction of unripe *A.altilis* needs further chemical and pharmacology investigations to be used as an antioxidant supplement to prevent further complication of diabetes.

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