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Effect of cardamom leaves extract as antidiabetic, weight lost and hypocholesterolemic to alloxan-induced *Sprague Dawley* diabetic rats

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Abstract

Current study was proposed to know the potency of Cardamom leaves as antidiabetic, weight lost, and hypocholesterolemic on alloxan-induced Sprague-Dawley rats. The Cardamom leaves were prepared as extract using ethanol and its termed as ethanolic cardamom leaves extract (ECLE). Thirty of 45 rats were induced with alloxan at the dose of 120 mg/kg body weight, intraperitoneal. Blood glucose level of the rats were measured after 7 days of alloxan induction and forced to fast overnight. For this purpose, we split the rats into three different groups consisted of 15 rats each. Group I; the diabetic rats were given fed added by the ECLE, Group II; the diabetic rats were only given fed, and Group III; were non diabetic rats and given only fed. Intervention was observed for 14 days, and blood sample were taken 3 times i.e.: 0, 7 and 14 days after being intervented. One mL blood was taken from eyes sub-orbital using hematocrit capillary tube then transferred to eppendorf tube containing 10% EDTA. Samples were then centrifuged at 3,000 rpm, for 10 minutes. Blood glucose and cholesterol level were quantified after blood plasma was obtained. The rats body weight were measured simultaneously with that of blood sampling. Data were then analyzed by analysis of variance, and followed by Duncan test whenever there were a 5% significant different. Data showed that blood glucose level of rats group I decreased from 201.7 to 102. 8 mg/dl (P = 0.017), cholesterol level from 77.6 to 56 mg/dl (P = 0.025), but not to the rats body weight which increased as also noted from the non-diabetic rat (P = 0.92). ECLE is potential to be used as functional food component for therapeutic of diabetic patient.

Introduction

Diabetes mellitus is caused by metabolic disorders. This diseases is characterized by hyperglycemia due to insulin secretion disorders, production or both (ADA, 2006). Diabetic is a progressive disease and strongly related to more complicated diseases. Moreover, diabetic has been known as the main cause of death in either human or animal (Avizeh *et al*., 2010). There was no modern medicinal is safe to be used by patients, but, there are more than 800 plants/herbs are containing antidiabetic components (Alarcon-Aguilera *et al.*, 1998). The plant could be grouped as traditional antidiabetic when it has potential to cause hypoglycemia. Several medicines are chategorized capable to reduce hyperglycemic toward the diabetic patients, however, this group will cause side effects like diarrhoea lactic acidosis, and body weight. This situation is therefore, forcing us to search newer antidiabetic medicine or component with minimum risks to diabetic patients.

Diabetic patients in most of the cases will also facing problems of hyperlipidemic and hypercholesterolemic. Alloxan-induced diabetic rats showed high level of lipid leads to becomes phospholipid and cholesterol. The high cholesterol level, the high risk for atherosclerosis, therefore, solving the problem of hypercholesterol of diabetic rats becomes a prerequisite.

Induction of alloxan to the guinea pigs causes also negatively to their weight due to their hyperglycemic (Szkudelski, 2001) and defects the protein and lipid tissues. The situation where body has a limit amount of energy, these two important components are then used by the body as energy sources, meanwhile the body has lots of glucose in its blood tissues (Shirwaikar *et al*., 2004). This condition will then cause reduction of weight while it needs to be controlled.

The cardamom leaves are never being used by the growers or farmers, however, Winarsi *et al.* (2012) reported that the cardamom leaves contain 129.6±6.9 mg/g flavonoid and vitamin C 19.22±1.1 mg/g. It has been reported that flavonoid has antioxidant (Coskun *et al*., 2005), antidiabetic (Dewanjee *et al.*, 2008),

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hypocholesterolemic characteristic (Fuhrman *et al.*, 2000), and capable to control body weight (Yuji *et al.*, 2006). Winarsi *et al.* (2012) were also noted that the IC_{50} of cardamom leaves extract was smaller than that of ascorbic acids. In the current times, however, there is no available data showing the potential of ECLE as antidiabetic, hypocholesterolemic and controlling body weight *in vivo*. Currently, this study was done to overcome the potential characteristic of ECLE on reducing blood glucose and total cholesterol level, and therefore, controlling body weight of alloxan induced-diabetic rats.

Methods

Preparation of ECLE

Ethanol Cardamom Leaves Extract (ECLE) was prepared as in Winarsi *et al.* (2013). Cardamom leaves were cut into small pieces and grind to powder (cardamom leaves powder, CLP). The CLP was then immersed in ethanol 96% for several times until a clear filtrate was obtained, then evaporated to let get the extract.

Induction of alloxan to the rats

Fourty five males *Rattus norvegicus* L. strain Sprague Dawley rats, age of 2-3 months old, 220-250 gram weight, collection of the LPPT UGM Yogyakarta. During the acclimatisation, these rats were pellet fed of 10% from their body weight, the amount of water is ad libitum. After being forced to fast overnight, 30 of those 45 rats were induced with monohydrate alloxan in physiological NaCl sterile at the dose of 120 mg/kg body weight, intraperitoneal (Kim *et al*., 2006; Winarsi *et al*., 2013). Blood glucose level of the rats were measured with Glucotest meter (Nesco) after 7 days of alloxan induction and forced to fast overnight.

Grouping of treated rat and intervention

The diabetic rats were then grouped into 2 different groups consisting of 15 rats each. The rest 15 rats were grouped as control group. Total was three groups. Group I was diabetic rat given for feed plus ECLE, Group II was diabetic rat given for feed only, and Group III was non-diabetic rat given for feed only.

Intervention of ECLE to the diabetic rat

Intervention was done for a period of 14 days. The ECLE was given to the treated rat at the dose of 100 mg/kg body weight, pellet feed, however, given for only 10% of their body weight, and water at ad libitum (Winarsi *et al*., 2013).

Preparation of blood samples

One ml of blood was taken from each rat for three times, i.e.: at the baseline, 7, and 14 days after having intervention. Blood was taken from eye sub orbital using the hematocrite tube then transferred into eppendorf tube containing of 10% EDTA. Part of the blood was separated for measuring the glucose level, while the rest was centrifuged to obtain the plasma for cholesterol level determination.

Determination of blood glucose level

Blood glucose level of the treated rat was determined using a Glucotest meter (Nesco). Before being used, the Glucotest meter was calibrated as written on the test-stripes code number. Every measurement need a single strip-test. Standard liquid for calibration is availbale in the kit. Strip-test was placed on the Glucotest meter and a sign of blood will appear on the screen to be ready for a further use. Blood was placed on to the strip-test and left for about 5 second until the screen shows the number of glucose level which was noted in mg/dl.

Determination of total cholesterol level

Blood plasma was determined for its cholesterol level using an end point method (Stanbio Cholesterol Liquicolor procedure no 1010). For this purpose, one ml reagent was added by one ml standard solution and one ml blood plasma are placed into cuvette-I and used as Blanko Reagent (BR). One ml of this reagent was then added by 0.01 ml standard solution and placed into the cuvette-II, this is called as standard solution (S). Another one ml of reagent was added by 0.01 ml blood plasma and placed in the cuvette-III and used as sample solution (U). All cuvettes containing different material were then incubated for 5 minutes at 37^{o}C or alternativelly incubation time of 10 minutes at the room temperature. Each sample was then read for the absorbance using spectrophotometer at 500 nm. The total cholesterol level can be calculated with the following equation:

$$\text{Total cholesterol (mg/dl)} = \frac{Au}{As} \times 200$$

Notes:
Au = the absorbance value of the plasma
As = the absorbance value of the standarad solution
200 = standard for total cholesterol (mg/dl)

Determination of the treated rat body weight

The treated rat body weight was determined in the different times namely: baseline, 7, and 14 days after intervention using a weigh scale of Cook Master Kitchen Scale Model CMK6602, ISO 9001: 2008

Certified by SGS.

Statistical analysis

Data obtained were then analyzed by Analysis of Variance (Anova), and followed by Duncan test when there is significant different at the level of 5%.

Results

Rats which were induced by alloxan showed hyperglycemic, characterized by high level of glucose level in their blood 351.5 mg/dl and so called as diabetic rat, this number was higher than that of non induced rat which was only 110.3 mg/dl (P = 6.18E-08). With total cholesterol of 75.5 mg/dl, which was higher than the non diabetic ones 45.5 mg/dl. On the contrary, body weight of the diabetic rat were only 220.8±23 g, a slightly lower than that of non-diabetic rat 247.6±28 g (P < 0.0005) (Winarsi *et al.*, 2012).

Discussion

Blood glucose level of the diabetic rats

High blood glucose level in the treated rats were related with the induction of alloxan which is known as diabetogenic compound and toxic, selective to beta-pancreatic cells, leads to insulin defficiency. Pari *et al.* (2002) stated alloxan could decrease insulin secretion by beta-pancreatic cells and therefore, causing hyperglycemic. This situation will cause glucose can not be inserted in the metabolisms process but stucking in the blood.

Through a redox reaction, the high level of blood glucose will increase the formation of reactive oxygen species (ROS), leads to more NADH and FADH2 enter to electron transport chain (Suarsana *et al.*, 2011). Increase of electron transport rate leads to the contribution of free radicals formation and cause a more severe diabetic.

Alloxan has an analog glucose character and therefore, this compound is known by the GLUT2, then entered to bilayer membrane of lipid plasma where beta-pancreatic cells are accumulated, and cause damage in granules those produce insulin (Lenzen, 2007). The cytotoxic activity of alloxan is mediated by free radicals formed during the redox reaction. Alloxan and its reduction product (dialuric acid) through redox cycles will form superoxide radicals and cause dismutation to become hydrogen peroxide. The more free radicals in the blood, the higher concentration of cytosol calcium leads to the faster damage of beta-pancreatic cells.

The beta-pancreatic cells are the glucose censorship, work to reduce blood glucose level by

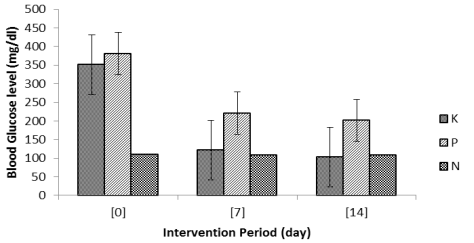

Figure 1. Blood glucose level of diabetic rats during intervention. K, diabetic rats were given pellet + ECLE; P, diabetic rats were given pellet; N, non diabetic rats were given pellet.

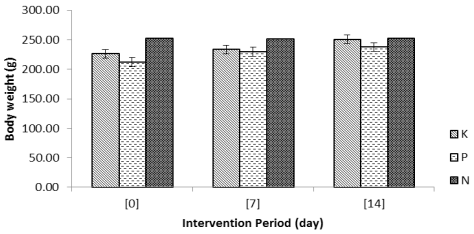

Figure 2. Body weight of diabetic rats during intervention. K, diabetic rats were given pellet + ECLE; P, diabetic rats were given pellet; N, non diabetic rats were given pellet.

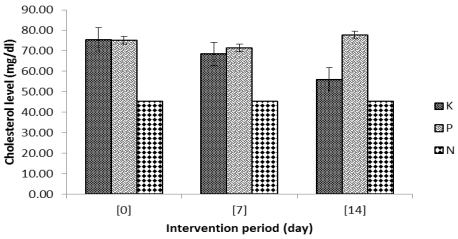

Figure 3. Cholesterol level of diabetic rats during intervention. K, diabetic rats were given pellet + ECLE; P, diabetic rats were given pellet; N, non diabetic rats were given pellet.

releasing insulin. When glucose passed the membrane plasma, it will be fosforilated by glucosidase and start to glycolysis activity. The ATP which was formed during the metabolism in the mitochondrion will force to close channel of KATP, leads to depolarization of plasma membrane. This situation will cause Ca^{2+} enter the blood through gradient concentration, and increase the Ca^{2+} concentration in the cytosol, leads to the exocytosis of insulin. The high concentration of Ca^{2+} in the cytosol, the high level of insulin secreted, and will damage beta-pancreatic cells (Maechler *et al.*, 2006). The chronic condition of hyperglycemic due to oxidative stress may cause damage, disfunction, and failure of several organs (Likidlilid *et al*., 2007), therefore, decreasing or minimalizing blood glucose level of diabetic patients becomes a prerequisite.

Current study showed that the diabetic rats given the ECLE for 7 consecutive days, decreased in their blood glucose level from 221.6 to 122.2 mg/dl (P = 0,019). The blood glucose level of this treated rats were continuously decrease when the ECLE was given to a longer time (14 days) of 201.7 to 102. 8

mg/dl (P = 0,017) in compare to those of diabetic group rat given feed only (Figure 1).

The ECLE contains flavonoid of 129.6±6.9 mg/g (Winarsi *et al*., 2012). Flavonoids are group of plant phenolic secondary metabolite which are abundantly present in nature. Eventhough, in their research did not define the type of flavonoids in the ECLE, but it is clear that this phenolic compound has similar structure to flavone, flavanone, flavonol, flavanonol, cetechin, anthocyanidin, and isoflavone. Several researchers stated that function of the flavonoid as antidiabetic (Brahmachari, 2008; Brahmachari, 2009). This compound reduced blood glucose level of diabetic rats. Using several kind of guinea pigs, they showed the positive hypoglycemic affect of flavonoid, and therefore, it can be used to reduce absorption of glucose or to increase glucose tolerance. A similar results have been reported by Bhathena and Velásquez (2002) who explore the use of isoflavone extract in vitro, they found this compound could stop glucose absorption on brush border colon of rabbit vesicula membrane. Naringenin was also reported to reduce uptake the rate of glucose within the vesicle membrane brush border of diabetic rat, to the same amount of non-diabetic rat (Li *et al*., 2006). Several types of flavonoids like epicatechin gallate, myricetin, quercetin, apigenin, epigallocatechin gallate, and epigallocatechin were also can be used as competitive inhibitor on the glucose absorption against the sodium dependent glucose transporter-1. Further study by Johnston *et al*. (2005) and Zhao *et al.* (2004) noted that non-glycosylase of flavonoid clarify reduction of absorption activity of sodium-dependent glucose both *in vitro* or *in vivo*.

Besides reducing absorption of glucose, in order to control blood glucose level, flavonoid might also take another route by slowering down glucosidase-alpha activity in the colons. Effect on slowering down activity of the enzyme could be seen when luteolin, kaempferol, chrysin or galangin is taking place in the absorption and metabolism of carbohydrate (Matsui *et al*., 2002). Kim *et al.* (2000) also proofed if activity of glucosidase-alpha was inhibited by flavonoid luteolin, amentoflavone, luteolin 7-o-glucoside and daidzein which runs as glycation inhibitors. Another possibility is by stimulating glucose absorption in the peripheral tissues and regulating enzymes of carbohydrate metabolism.

Hypoglycemic effects of flavonoid was also showed by pruning (naringenin 7-O-β-D-glucoside) which deliberately given intraperitoneally to diabetic rat. Moreover, it was also stated that flavonoid could not only to cause hypoglycemic but also potential to be used as anti-hyperglycemic especially of those chrysin and its derivates, like silymarin, isoquercetin, and rutin (Hnatyszyn *et al.*, 2002). In a long period of intervention of this ECLE containing flavonoid, given to the diabetic rats per oral, showed a decrease of glucose in the blood plasma up to 70.7%, but in the diabetic rats were not given the compound reduced to 47%. Similar with this research that other types of flavonoids, hesperidin and naringin were also can reduce blood glucose level of the diabetic rats (Jung *et al*., 2004). Those of flavonoid might take either cellular or molecular mechanism.

Flavonoids could also take a role in the secretion of insulin due to their insulin mimetic characteristic which might be related with pleitropic mechanism (Winarsi and Purwanto, 2010). This action could help to suppress complication due to diabetic. This phenomenon showed that flavonoid is potential to be used as drugs to stimulate glucose absorption on peripheral tissues, and control enzymes activities within the carbohydrate metabolism pathway. In their study found that genistein was activated beta-pancreatic cells directly (Liu *et al.*, 2006), and cause the activation of cAMP/PKA signaling route leads to the insulinotropic effects. It might be concluded that flavonoid is a natural compound fit to be used as an alternative medicines in diabetic therapy.

Another study noted that flavonoid given routinely per oral to the diabetic rat caused reduction of glucose level in the blood plasma, increasing insulin, glycogen content, and activity of hexokinase enzyme. Prince and Kamalakkannan (2006) supported the previous study by saying that flavonoid which was given routinely reduced glucose 6-phosphatase and fructose 1,6-bisphosphatase significantly in the liver and tissues of diabetic rat.

The mechanism of antidiabetic effect of apigenin-6-C-β-fucopyranoside and L-apigenin-6-C-(2-O''-α-L-rhamnopyranosyl)-β-l-fucopyranoside by stimulating or inducing secretion of insulin in the hyperglycemic rat (Cazarolli *et al*., 2009a; 2009b). Flavonoid was also reported that capable to stimulate synthesis of glycogen in the rat tissues through mechanism of transduction of insulin signals, then showed a double effect as anti-hyperglycemic or insulin secretion, and insulin mimetics or glycogen synthesis. Flavonoid is an antioxidant compound which can reduce cell damage due to diabetic. Quercetin, a strong type of flavonoid, was able to reduce blood glucose level normalize antioxidant level, superoxide dismutase, vitamin C, and vitamin E. The quercetin was reported to be more effective to control diabetic which induced by oxidative stress in a low dose (Mahesh and Menon, 2004).

The potential of alloxan, a diabetogenic

compound, will reduce in the present of glutathione, especially when alloxan radicals are changed into dialuric acid. The oxidative radical compound formed can destroy beta-pancreatic cells in the langerhans. Quercetin is potential to minimize the damage of beta-pancreatic cells either as cells scavengers or taking role as chelating agent (Mira *et al.*, 2002; Anjaneyulu and Chopra 2004).

Intervention of ECLE orally showed a reduction in blood glucose level of the diabetic rat. Clinical study by Knekt *et al.* (2002) showed that improving condition on the diabetic rat might help to reduce risks of diabetic complications as retinopathy, nephropathy, neuropathy and foot diabetic, and also inhibit the possibility of atherosclerosis especially that of related with myocard infark and stroke. Several research reports also noted the significant role of flavonoid in reducing hyperglycemic (Anjaneyulu and Chopra, 2004; Dene *et al*., 2005) and glycation of non-enzymatic protein on the guinea pigs.

Flavonoid is proven to be able to stimulate immune system. This statement was due to antioxidant character of flavonoid which was able to recover the beta-pancreatic cells damage caused by alloxan. The flavonoid glycosidic component takes place as suppressor of the hydroxyl radicals, and therefore, capable to block development of diabetic. It was also reported that flavonoid could interact with several protein transporter then it will inactivate some enzymes and causing concentration of hormones in the tissues. Quercetin capable to block the formation of free radicals and therefore, can be used as antidiabetic. Using ethanolic extract of ginger (*Zingiber officinale*) Elshater *et al.* (2009) reported this extract was able to reduce blood glucose level of diabetic rat. This extract was explored to contain phenolic compounds like gingerol, shogaol, zingerone, and paradol, which are capable to repair insulin secretion by beta-pancreatic cells. Raj *et al.* (2011) supported that ethanolic extraction of other rhizome (*Alpinia calcarata*) which contains flavonoid, alcaloid, and coumarin was capable to reduce blood glucose level through increasing secretion of insulin after repairing the beta-pancreatic cells, glycogenolysis and gluconeogenesis.

Hyperglycemic of the diabetic patient will reduce the system of antioxidant defence. Previous reports noted that this condition will be followed by low level of enzymatic antioxidants like glutathione peroxidase, superoxide dismutase, glutathione reductase, and vitamin C in both blood plasma and tissues (Kalaivani *et al.*, 2008; Middha *et al.*, 2011). Defficiency of vitamin C in blood plasma of diabetic patients might cause severe damage of the tissues due to the presence of ROS. As a potential antioxidant, vitamin C is known capable to suppress the ROS. Dakhale *et al.* (2011) showed the combination of vitamin C and metformin could control blood glucose level of the diabetic patients. In this case, metformin repairs transport of glucose into the muscle cells, while vitamin C reduce the ROS.

Vitamin C (ascorbic acids) helps body to produce collagen and basic component for connective tissues. Collagen is an important element of blood vessels membrane, gum, and bone; and this material is strongly important to those who are in a recovery phase after having surgery. Though it mechanisms is not clear, but vitamin C is functioned as antioxidant by removing free radicals, improving immune system, protection of cancer, cataract, and slowering down degenerative diseases due to aging, macula retina, and other chronic diseases. Vitamin C is an important antioxidant in human and suppress the formation of oxygen radicals (McRae, 2007). Due to its similar structure to glucose, vitamin C could also take over the position of glucose in some reactions. Knekt *et al.* (2004) stated vitamin C was effective in blocking non enzymatic proteins. Besides that, in the guinea pigs, vitamin C is capable to regulate catabolism of cholesterol becomes acids, and even proofed to play significant role in lipid regulation. Several studies done previously, showed reduction of vitamin C level in the diabetic patients will increase oxidative stress (Després *et al*., 2000; Franceschini, 2001).

Body weight of diabetic rats

The present of alloxan in the blood, was still debatable to be the main cause of losing weight of the treated rat. Though Diniz *et al.* (2008) reported the present of alloxan was not affecting the rat weight, but several reports stated differently. Iranloye *et al.* (2011) and Raj *et al*. (2011) noted alloxan which is potential diabetogenic compound could change the non-diabetic rat to become diabetic ones as showed by their blood glucose level of 351.5 mg/dl, and therefore, called hyperglycemic.

The diabetic rat will show a symptom of consume more feed than non-diabetic rat, but they showed a reduction in body weight. This might be because of insulin deficiency and glucose is not able to enter the cells, leads to the use of lipid and protein as energy sources instead of glucose itself. Losing of body weight, is due to severe damages in protein of the tissues. Kamalakkannan and Prince (2006) supported in previous reports by saying dehydration process is related with situations of polydipsia and polyuria, which promoted by high level of lipid and protein catabolism. Weight loss in the diabetic is similar to

gluconeogenesis, where in this pathway, the muscle size will be smaller than normal and therefore, lose of protein in the tissues. Gradual losing of body weight to the diabetic patients which related with chronic hyperglycemia is also severe to infection, maintaining body weight is therefore, very important to the diabetic.

Current study was capable to control weight loss of the treated rat, where 7 days after intervention of ECLE, their body weight was increase as in the non-diabetic rat (P = 0.22). Figure 2 showed the body weight of treated rat of the diabetic and non-diabetic ones was similar after 14 days of intervention with ECLE (P = 0.92). The increase of body weight did not automatically means as obesity or overweight, and therefore, it might be conclude that the ECLE might be used to control body weight of the diabetic rat due to alloxan induction.

Winarsi *et al*. (2012) noted ECLE contains 129 mg/g flavonoid, capable to control body weight. Flavonoid which is also contained in the green tea was reported to have biological function; consuming this compound in high dose will therefore control body weight, adipose tissues, and regulate of lipid metabolism (Ashida *et al.*, 2004). Lee *et al.* (2011) supported this statement by saying several natural compounds like catechins, capsaicin, conjugated linoleic acids, fucoxanthin, isoflavone of soy bean, glabridin, astaxanthin, and cyanidin-3-glucoside was effective to maintain body weight through varies mechanism.

Glabridin, the main flavonoid content of licorice (*Glycyrrhiza glabra*), was known capable to maintain body weight of both treated animals and human by spinning up differentiation of adipose tissues. This type of flavonoid was effective in reducing intra-abdominal fat through activation of peroxisome proliferator-activated receptor-γ (PPAR-γ) (Nakagawa *et al*., 2004). Astaxanthin, a natural carotenoid showed pharmacological effects like: anti-tumor, anti-cancer, anti-diabetes, and anti-inflammation. Astaxanthin was also noted to reduce the weight of liver, triacylglycerol levels in liver and plasma, and total cholesterol (Ikeuchi *et al*., 2007). Astaxanthin could stimulate the use of fatty acid of the treated rat, though its function in reducing body weight is still not clear yet.

The ECLE was not containing only flavonoid but also vitamin C (Winarsi *et al*., 2012), which was reported to affect lipid oxidation. Blaak and Saris (2002) reported oxidized lipids takes a role in obesity and failure in weight loss. Defficiency of vitamin C, therefore, could cause metabolic disorders and potential to affect body weight. There was a significant paradox effects between the presence of vitamin C and gaining body weight. Means the sufficient amount of vitamin C in the blood plasma will reduce the obesity level. Vitamin C is a cofactor in carnitine biosynthesis, a molecule to cause oxidation of fatty acid. The higher consumption of vitamin C the more fatty acids will be oxidized, leads the contribution of reduction of adipose cells and therefore, losing body weight. The current study, might then conclude that the ECLE could repair body weight of the treated rat through its contents which were flavonoids and vitamin C.

Cholesterol level of diabetic rats

The high level of lipid in the serum of diabetic patient is mainly due to blockade in sensitivity of lipase by insulin and leads to mobilization of fatty acid from lipid stock in the peripheral. Hyperlipidemic, which is also known to be one among the characteristics of diabetic, as a consequence of non blocking activity of lipolytic enzymes in the peripheral lipid. Over content of fatty acids in the alloxan-induced treated rats, promoted the conversion of lipid compound in the liver to become phospholipid and cholesterol. However, these two compounds, together with high level of triglycerides could be thrown as lipoprotein in the blood, to cause readable total cholesterol is low.

After 7 days of observation, total cholesterol levels of diabetic rats treated ECLE were not significantly different from diabetic rat group which was given feed only (P = 0.74), but higher than that of non-diabetic rat group (P = 1.61E-05). Observation done in 7 days following to the first obervation, noted a reduce in total cholesterol from 77.6 to 56 mg/dl (P = 0.025) of the diabetic rat group with ECLE treatment, this level was still higher than that of non-diabetic rat group (P = 0.009) (Figure 3).

Decreasing of total cholesterol level in the alloxan-induced treated rat was due to the high level of flavonoid contained in the ECLE. Several studies showed that the use of flavonoids reduce risk of cardiovascular disease (Graf *et al*., 2005; Scalbert *et al*., 2005) and atherosclerosis (Aviram and Fuhrman, 1998). Mechanism flavonoids inhibit lipid lesion development by reducing the formation of oxidized LDL. Fuhrman *et al*. (2000) also reported that polifenol of flavonoid, both *in vitro* and *in vivo*, can reduce the number of macrophages and oxidized LDL. The role of flavonoids *in vitro* in oxidized LDL modification proves that high levels of known compounds in the ECLE can prolong the lag time of LDL oxidation.

Apart from, blocking the production of oxidized

LDL, flavonoid is capable suppress proliferation of plain muscle cells which normally take part in atherosclerosis, and reduce total cholesterol. Flavonoid that comes from a natural compounds, has also antioxidant characteristic and it function as scavenger of free radicals, along the redox reaction it is also donating hydrogen atom to the free radicals. It might be concluded that flavonoid has more potential than tocopherol.

The current study, however, has not identified the particular flavonoid or types of it that effective in reducing blood glucose level, reducing total cholesterol, and repairing body weight. This might be because of all components which include in the ECLE which were not soludified in water. Nakagawa *et al.* (2004) reported that hydrophobic flavonoids could reduce total amount of abdominal fat and causing hypoglycemic through activation of PPAR-γ. And therefore, that flavonoid is not only potential to be used as antioxidant but also to activate PPAR-γ, leads to anti-atherosclerotic, because the PPAR-γ also taking place in reducing cholesterol level of the macrophage of spone cells.

In a general situation, the diabetic has a disorders in lipid metabolism charaterized with a low level of HDL-cholesterol but high level of triglycerides. It has already reported that vitamin C can protect the HDL-cholesterol from oxidation process and therefore, might include in the process of reverse transport (Hillstrom *et al.*, 2003). A reverse transport process of cholesterol includes in excluding cholesterol before esterification in the membrane of extrahepatic cells, and takes the cholesterol to the esterification of lecithin to become acyltransferase. Ester cholesterol in lipoprotein would then retransferred into liver to be processed further and excreted through bile. The oxidized HDL might also modify structure of apolipoprotein AI. The potential of HDL lipoprotein in activating lecithin as acyltransferase cholesterol will cause blocking in esterification and excluding the extrahepatic cholesterol.

Conclusion, ethanolic cardamom leaves extract was capable to reduce blood glucose level as well as total cholesterol, and also repair body weight of alloxan-induced diabetic rats. In the future, the cardamom leaves could be used as functional foods/ drinks compound for diabetic patients.

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