ANTIDIABETIC AND HYPOLIPIDEMIC EFFECT OF CENTELLA ASIATICA EXTRACT IN STREPTOZOTOCIN-INDUCED DIABETIC RATS

Akbar Satria Fitriawan[1*], Ririn Wahyu Widayati[1], Wiwit Ananda Wahyu Setyaningsih[2], Nur Arfian[2], Dwi Cahyani Ratna Sari[2]

[1]School of Nursing, Faculty of Health Science, University of Respati Yogyakarta, Jl. Raya tajem, Yogyakarta, Indonesia.

[2]Department of Anatomy, Faculty of Medicine, Public Health, and Nursing, Universitas Gadjah Mada, Jl. Farmako, Sekip Utara, Yogyakarta, Indonesia

*corresponding author: akbarsatriafitriawan12831@gmail.com

Abstract

Diabetes mellitus is one of the major health problems and tends to increase throughout the years. Uncontrolled diabetes mellitus causes both microvascular and macrovascular complication. One of the active compounds of *Centella asiatica* (CeA) extract is madecassic acid which acts as an agonist of PPAR-γ. Through PPAR-γ activation, CeA enhances the expression of the lipolysis regulator proteins such as perilipin and Angptl-4, reduces NEFA production, prevents meta-inflammation, and increases insulin sensitivity. But no study has been conducted to evaluate the effect of long-term administration of CeA extract on chronic diabetes mellitus. We aim to elucidate the effect of long-term of CeA extract administration on blood glucose levels and lipid profiles in diabetic rats. Diabetes mellitus induced through single dose injection of Streptozotocin 60 mg/kgBW intraperitoneally for 1 month (DM1M) and two months (DM2M). *Centella asiatica (CeA)*-treated groups (400 mg/KgBW/day) were administered per-orally for 1 month (DM1C) and 2 months (DM2C) to diabetes mellitus rats. After the due date, the rats were sacrificed and the blood was taken from retro-orbital vein to assess blood glucose, cholesterol, triglyceride, and LDL levels. CeA-treated groups significantly diminished blood glucose and cholesterol levels compared to diabetes mellitus groups (p<0.05). Two months but not a month CeA-treated groups showed significantly decreased of LDL level (p<0.05) compared to diabetes mellitus groups. Moreover, the triglyceride level significantly increased (p<0.05) in CeA-treated groups compared to diabetes mellitus groups. *Centella asiatica* extract exerts antidiabetic and hypolipidemic activity on chronic streptozotocin-induced diabetic rats.

Keywords: antidiabetic, hypolipidemic, Centella asiatica, diabetes mellitus

1. INTRODUCTION

Diabetes mellitus and the complications are one of the major health problems and contribute to an increase of morbidity and mortality rates [1]. In 2015, *the International Diabetes Federation* (IDF) predicts 1 of 11 adults aged 20-79 years (415 million people) in the worldwide suffered from diabetes mellitus [2]. In 2010, diabetes mellitus and the complications caused 3.9 milliion death (6.8% global mortality), and in 2015 increased by 5 million mortality. Globally predicted in 2040, the number of people who suffered from diabetes mellitus were increased by 642 million. Research showed more than 90% diabetes mellitus problems are diabetes mellitus type II [1]. The expanded incidence of diabetes mellitus occurs in the countries experiencing the economic transition from low-income countries to middle-income countries [2].

Indonesia is one of the 10 countries with the highest number of diabetes melitus [3]. The prevalence of diabetes mellitus in urban is 5.7% and impaired glucose tolerant is 10.2% [3].

According to the Riset Kesehatan Dasar, Idris et.al suggested in 13% from 38.052 respondents suffered from diabetes mellitus [4].

Chronic hyperglycemia caused by diabetes mellitus contributes to the development diabetic complication which lead to various life-threatening complications. Diabetes mellitus complications divided into 2 groups, microvascular complications (nephropathy, neuropathy, and retinopathy) and macrovascular complication (stroke and cardiovascular disease) [2].

Diabetes mellitus characterized by type 1 diabetes mellitus and type 2 diabetes mellitus. Type 1 diabetes mellitus caused by absolute deficiency of insulin. In type 1 diabetes mellitus, an autoimmune pathological process found in islet de Langerhans pancreas [5]. Type 2 diabetes mellitus is a condition marked by chronic hyperglycemia combine with low insulin expression and peripheral insulin resistance in insulin-targeted cells (adipocyte, hepatocyte, skeletal muscle cells) [6]. In type 2 diabetes mellitus, the failure of the β pancreas cells to compensate for an increase of blood glucose level caused by insulin resistance in target cells fail (adipocyte, hepatocyte, skeletal muscle cells). The key of the molecular pathogenesis of type 2 diabetes mellitus is insulin resistance, in which the normal levels and functions of blood insulin cannot cope and affects the phenotype of the target cells (adipocyte, hepatocyte, skeletal muscle cells) then caused hyperglycemia [7]. Insulin resistance is characterized by a decrease or inactivation of Insulin-*Insulin Receptor signaling pathway* in insulin-targetted cells, especially on IR-IRS- PI3K-Akt-GLUT4 *axis*. It causes a decrease on translocation of GLUT 4 (Glucose Transporter 4) from endosomal vesicle in the cytoplasm to membrane cells and decreases passive facilitated glucose diffusion from extra cells into the cytoplasm of the target cell [8]. Insulin resistance is the main causes of hyperglycemia due to a decrease in glucose uptake to hepatocyte, adipocyte, and skeletal muscle cells. In addition, β cell dysfunction results in a decreased in insulin production which causes failure to maintain normal blood glucose levels. Insulin resistance and β cell dysfunction occur in the early stages of the pathogenesis of T2DM [8].

One of the main risk factors for T2DM is obesity [9]. One of the molecular mechanisms that connect obesity with insulin resistance and T2DM is nutrient-induced metabolic inflammation in insulin-targeted tissues [10]. Metabolic inflammation due to obesity induced by high levels of NEFA (Non-esterified Fatty Acid). NEFA produced by adipocyte and skeletal muscle cells through the process of lipolysis or the breakdown of Triacylglycerol on Lipid droplet. In obese individuals, there is an increase in the basal lipolysis process so that this causes an increase in NEFA production [11].

NEFA-produced obesity activates TLR-4, PRR lipopolysaccharide, which expressed by plasma membrane innate immune cells such as macrophage [12]. TLR-4 *signaling pathway* playing an important role in obesity-induced inflammation. The TLR signaling pathway in downstream inflammatory cells causes activation of IKK-α and IKK-ß, inactivation of the IƙB, and activation of NFƙB. The active form of NFƙB binds to the DNA *ƙB site* sequence which is a promoter sequence of the TNF-α gene and several proinflammatory cytokine genes and will activate the transcription of these genes [13]. In type 2 diabetes mellitus shows upregulation of the TNF-α in skeletal muscle cells [14]. TNF-α stimulates skeletal muscle cells and contributes to the insulin resistance phenotype. TNF-α enhances activation of JNK and IKKß [15]. JNK and IKK-ß phophorilate IRS-1 in Serine amino acids and caused the deactivation of IRS-1 during stimulation of insulin in skeletal muscle cells and deactivation of the PI3K-AKT-GLUT4 *axis* [16].

Gotu Kola *(Centella asiatica/*CeA) is a herbaceous plants composed with numerous bioactive compunds mostly *pentacyclic triterpenes* which consists of *madecassic acid* and *asiatic acid* [17]. *Centella asiatica* extract has exerted a hypoglycemic effect in numerous research. Kabir *et al.* (2014) found that *Centella asiatica* extract exert anti-hyperglycemic effect when administered

perorally in type 2 diabetes mellitus rat. Partially, anti-hyperglycemic effect of CeA mediated by inhibition of α-Amylase and disaccharidase. Therefore, it decreases glucose absorption in the intestine. This research elucidated that ethanolic extract of CeA 1000mg/kgBW is the most effective in lowering blood glucose levels, inhibition of disaccharidase, and repairing serum lipid profile [18]. Research conducted by Sasikala et al. showed an ethanolic extract of CeA with administered per-orally daily single doses 300 mg/KgBW and 400 mg/KgBW enhanced blood glucose lowering in STZ induced-diabetes mellitus rat [19].

Besides that, madecassic acid from CeA has agonist PPARγ (Peroxisome Proliferator-Activated Receptor- γ) effect [20]. PPARγ is a nuclear receptor protein which is expressed by majority cells in the body, including adipocyte cells and skeletal muscle cells. The specific ligand from PPARγ is eicosanoid. This research showed madecassic acid acts as a ligand which activates PPARγ. Molecular docking study showed madecassic acid dock into Ligand Binding Domain of PPARγ protein [20] which stimulates dimerization of PPARγ with *Retinoic X Receptor* (RXR) protein and works as a heterodimer transcription factor. This transcription factor adjacent in PPRE (*PPAR Responsive Regulatory Element*) sequence in DNA genome before regulates the expression of the target genes.

The target genes activated by heterodimer transcription factor PPARγ-RXR are Perilipin and Angptl-4 [21,22]. Promoter regions sequences of Perilipin contain PPRE sequence. The PPARγ found binds to the PPRE sequence and activates the transcription of Perilipin [21]. Angptl-4 is one of the target genes regulated by Peroxisome Proliferator-Activated Receptor family and widely use as targeted-drug of anti-diabetes and lowering lipid. Liu et al elucidated that PPARγ agonist enhances upregulation of the Angptl-4 dose-dependent manner [22]. This research explained that heterodimerization of the PPARγ-RXR binds specifically in Angptl4 genes promoter and enhanced activation of the Angptl-4 [22].

Perilipin is one of the proteins which compiled by Lipid Droplet Coating Protein [23]. Perilipin 2 protein, a family of Perilipin protein, is expressed by the skeletal muscle cells. Meanwhile, perilipin 1 is expressed by adipocyte cells. Either in the adipocyte cells or in the skeletal muscle cells, the lipolysis process is regulated by *Lipid Droplet-coating protein* such as Perilipin. Perilipin has an important role in the prevention of basal lipolysis in consequences prevent the production of NEFA in adipocyte cells. Overexpression of the perilipin 2 in skeletal muscle cells enhances IMCL (*Intramyocite Lipid*) Storage, Lipid Droplet formation, a decrease of production and oxidation of the free fatty acid, and insulin resistance [24].

Angptl-4 (Angiopoietin-like protein 4) is one of the target genes activated by PPARγ-RXR [22]. Angiopoietin-like protein 4 (Angptl-4) known as Peroxisome Proliferator Activated-Receptor γ angiopoietin-related (PGAR), fasting-induced adipose factor, or hepatic fibrinogen/angiopoietin-related protein. Angptl4 is adipokine which is expressed in adipose tissue, liver, and skeletal muscle cells [25,26]. The protein of Angptl-4 localized in the liver and secreted into the blood circulation (circulating protein) [27]. It regulates triglycerides level through inhibition of the biological function of LPL (Lipoprotein Lipase) [28]. The LPL is an endothelium-related enzyme which acts in catalysis hydrolysis reaction of the triacylglycerol compounds in chylomicron and very low-density lipoproteins (VLDL) in the circulation into non-esterified fatty acid (NEFA) and 2-monoacylglycerol which use in the tissue [27, 29]. Through inhibition of LPL, Angptl-4 reduce the level of NEFA and intake NEFA in the tissues. Research conducted by Wang *et al.* illustrated the overexpression of Angptl-4 enhance glucose tolerance and reduce insulin resistance if high fat diet-induced obesity in the mouse [30]. The overexpression of LPL stimulates an increase of NEFA through enhancement of triglycerides hydrolysis in circulation into NEFA, induces vascular

inflammation [30] and insulin resistance [31,32]. In the skeletal muscle cells, the overexpression of LPL provokes insulin resistance through cellular inhibition of glucose intake which is mediated by IRS-1-PI3K signaling pathway. Moreover, the overexpression of LPL in the liver caused insulin resistance due to the failure of insulin to inhibit endogenous glucose and mediated by inhibition of the IRS-2-PI3K activity [31].

Centella asiatica has an important role in inhibiting blood glucose. Unless there are a few numbers of research which contribute to elucidate the prolong effect of *Centella asiatica* in chronic diabetes mellitus. Therefore, this research aims to elucidate the long-term effect of Centella asiatica reduce blood glucose level and lipid profile in streptozotocin-induced diabetic rats.

2. METHODS
2.1 Animal experiment and diabetes mellitus model

Male Wistar rats (3 months-old) weighing 160-270 grams were obtained from Experimental Animal Care Unit of Universitas Gadjah Mada, Yogyakarta, Indonesia. Rats were acclimatized for 7 days and maintained in the standardize cage with temperature 18-22˚C, humidity 50-70%, and dark: light cycle 12 hours. Rats were fed with AIN-93A and water ad libitum. The animal studies were approved by the Ethical Committee of Faculty of Medicine, Public Health, and Nursing, Universitas Gadjah Mada.

Twenty-five rats were divided into 5 groups consisted of the control group, diabetes mellitus for a month (DM1M), diabetes mellitus for two months (DM2M), *Centella asiatica*-treated diabetes mellitus group for a month (DM1C), *Centella asiatica*-treated diabetes mellitus group for two months (DM2C). Diabetes mellitus was performed with single intraperitoneally (i.p) injection of streptozotocin (Nacalai, Cat. No. 32238-91) 60 mg/kgBW dissolved in 0.1M citrate buffer pH 4.5. Rats were allowed to drink 10% of glucose solution to counteract hypoglycemia. At the following day, blood glucose was measured to assure the elevation (>300 mg/dL). Ethanol extract of *Centella asiatica* (400 mg/kg BW) was administered by using oral gavage daily for a month (DM2C group) and two months (DM1C group) consecutively.

2.2 Sample collection

Blood samples were collected and centrifuged at 10.000 rpm for 10 minutes to obtain the serum. Blood serum was analyzed in Clinical Pathology Laboratory of Faculty of Medicine, Public Health, and Nursing, Universitas Gadjah Mada to assess blood glucose level, low-density lipoprotein (LDL), cholesterol, triglyceride, high-density lipoprotein (HDL), and creatinine.

2.3 Statistical analysis

The comparison test was analyzed using OneWay ANOVA test and Kruskall Wallis test.

3. RESULTS AND DISCUSSION
3.1 Effect of Centella asiatica Extract on Body Weight

The weight of rats in the CDM group increased significantly during the study. At the first month, the body weight increased significantly by $207,4 \pm 27,3$ grams (p<0.05). At the end of study there was a significantly increase of body weight into $239,8 \pm 41,5$ grams (p<0.05). While the the body weight from diabetic mellitus rats decreased throughout the study. After suffered from diabetic mellitus for a month (DM1M group), the body weight was fall significantly into $188 \pm 20,03$ grams (p<0.05). While, the body weight of DM2M group was fall significantly into $163 \pm 19,81$ grams (p<0.05).

The clinical manifestation of insulin deficient and hyperglycemia are polyphagia and loss of body weight [33]. Some research proved that diabetes mellitus enhanced loss of body weight [18, 19, 34, 35, 36]. Abnormal loss of body weight caused by diabetes mellitus stimulated by an increase of lipid metabolic conversion into energy [37].

At the first month of the treatment, the DM2C group showed significantly decrease of body weight by $145,6 \pm 31,3$ grams (p<0.05), then it increased significantly to $206,6 \pm 29,3$ gram (p<0.05). *Centella asiatica* treatment for 2 months prevent body weight loss in group DM1C (figure 1). The DM1C group showed a significant increase in body weight from 204.2 ± 21.6 grams to 212.2 16.14 grams in the 1st month, and increased again to $227 \pm 14,5$ grams in the second month (p<0.05). The previous study found that CeA extract was able to maintain normal body weight in diabetic rats [18,19,36].
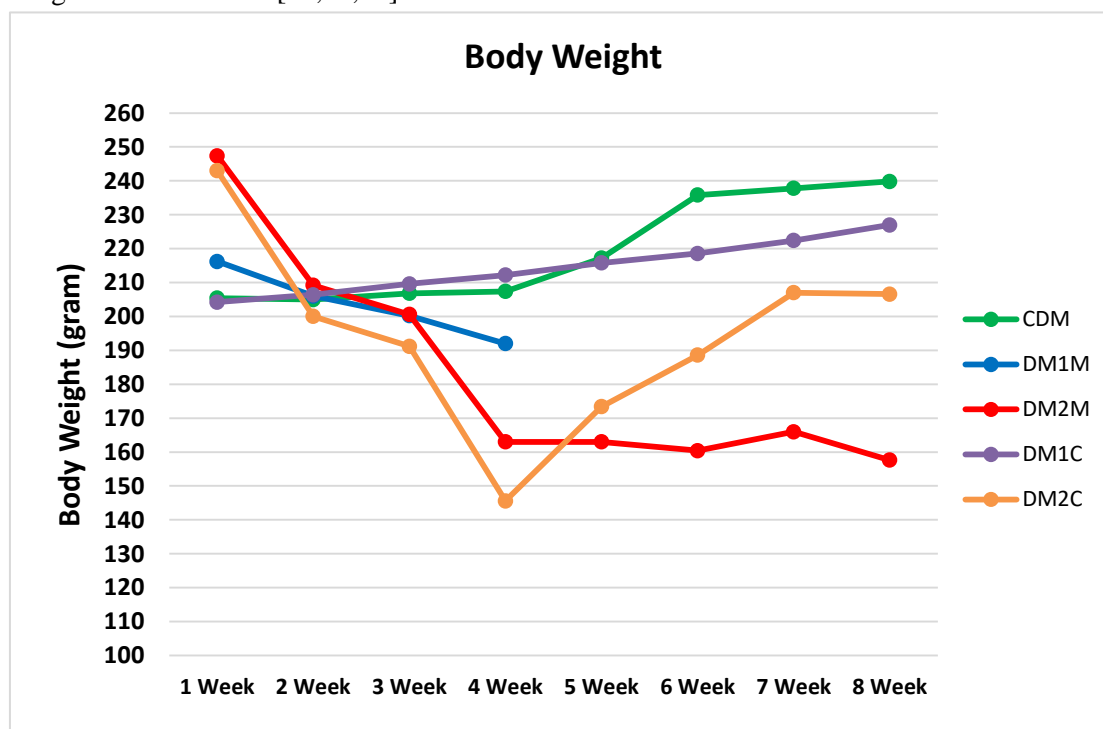

Figure 1. The alteration of body weight during treatment

Administration of CeA extract enhances body weight into the normal state. Even though it preceded with body weight loss. On the other hand, CeA treatment since the first-time diabetes mellitus diagnosed prevents body weight loss and stimulates an increase in body weight. Our results reinforced the previous study that CeA extract has the effect of preventing weight loss in diabetes mellitus [18,19,34,35,36]. Chronic diabetes mellitus-treated CeA extract showed an ability to restore body weight and BMI into normal levels, and reduce the condition of polyphagia and polydipsia [18]. The CeA contains many bioactive compounds consisting mainly of pentacyclic triterpenes such as madecassic acid [17]. Previously research suggested that madecassic acid increase body weight in diabetes mellitus [38]. This happened because blood glucose levels were better in the CeA group than in the diabetic group.

3.2 Effect of Centella asiatica Extract on Blood Glucose Level

The blood glucose levels of the CDM group increased to 88.4±11.8 mg/dL in the 2 months. The CDM group had normal blood glucose levels and did not undergo significant changes in blood

glucose levels either 24 hours after induction of DM (p>0.05). The DM1M and DM2M groups showed hyperglycemia conditions 24 hours after STZ injection as indicated by a blood glucose level of ≥ 300 mg/dL. The blood glucose levels of the DM1M group increased significantly to 492.6±43 mg/dL after a month STZ-injection (p<0.05). The DM2M group also experienced a significant increase in blood glucose levels from 530.4 ± 65.7 mg/dL 24 hours after STZ injection to 615.2 ± 14.17 mg/dL after 1 month (p<0.05), but interestingly experienced a significant decrease from 615.2 ± 14.17 mg/dL in the 1st month to 552.2 ± 22.6 mg/dL in the second month (p<0.05).

The group that received CeA extract 2 months (DM2C) and 1 month (DM1C) also developed diabetes mellitus. In the DM2C group, blood glucose levels 24 hours after STZ injection were $532,2 \pm 38,69$ mg/dL indicating the condition of hyperglycemia. This blood glucose level increased significantly to $609 \pm 6,04$ mg/dL in the 1st month (p<0.05), then decreased significantly in the second month to $473,2 \pm 31,86$ mg/dL (p<0.05). DM1C group experienced diabetes mellitus 24 hours after STZ injection with blood glucose level $482 \pm 26,62$ mg/dL. The blood glucose level of the DM1C group decreased in the 1st month to $474 \pm 34,37$ mg / dL, and became $397,4 \pm 51,66$ mg/dL (p<0.05).
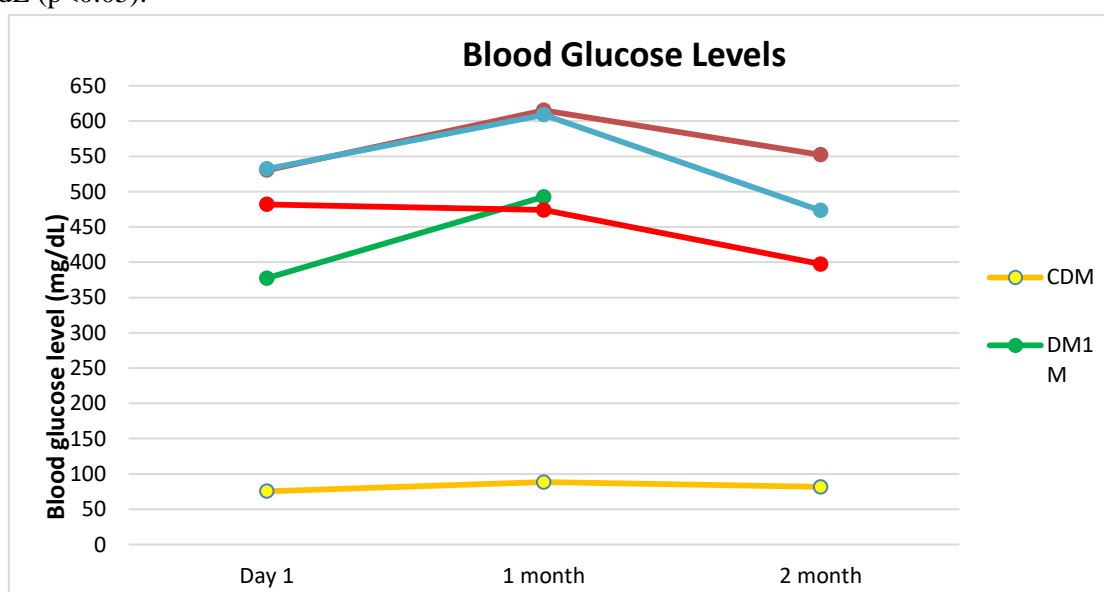

Figure 2. The alteration of blood glucose level during the treatment

Previous research found that CeA extract had an antihyperglycemic effect [18,19,34,35,36]. Kabir et al found that the anti-hyperglycemic effect of CeA extract was mediated by the inhibitory activity of α-Amylase and intestinal disaccharidase [18]. Hsu et al found that madecassic acid, which is one of the active compounds in CeA, reduces blood glucose levels through upregulation of insulin expression [35].

Study phytochemical screening indicates the presence of tannins, saponins, flavonoids, alkaloids and terpenoids in CeA [19]. Most plants with antidiabetic properties have been found to contain secondary metabolites such as tannins, saponins, alkaloids, and flavonoids [39]. The available literature reveals that flavonoids, terpenoids, alkaloids, saponins and tannins have been shown to possess hypoglycemic activity [40]. Flavonoid and tannins isolated from other antidiabetic medicinal plants has been found to stimulate insulin secretion from pancreatic β-cells or possess insulin like effect [41]. It has been reported that some alkaloids possess

antihyperglycemic activity which is mediated by the inhibition of α-glucosidase or stimulation of insulin secretion [40]. Tannins are also known to stimulate insulin secretion from β-cells [42].

3.3 Effect of Centella asiatica Extract on Cholesterol Level

The DM1M group and DM2M caused a significant increased in cholesterol levels compared to the CDM group ($p<0.05$). In the CDM group, cholesterol levels at month 1 were 72.2 ± 7.9 mg/dL and increased to $136.4\pm7,6$ mg.dL in the second month ($p<0.05$). The DM1M group had a significant increase of cholesterol level than the CDM ($177,6$ 21 mg/dL vs. 72.2 ± 7.9 mg/dL, $p<0.05$). While the DM2M group showed an increase in cholesterol levels compared to the CDM group ($p<0.05$). The DM2M group has a cholesterol level of 1 month 195.2 ± 39.7 mg/dL and 2 months cholesterol level 184.2 ± 25.9 mg/dL.

The DM2C group experienced a significant increase in the first month cholesterol level (203.4 ± 23.8 mg/dL) compared to CDM ($p<0.05$). Giving CeA extract for 1 month succeeded in causing a significant reduction in the cholesterol level of the DM2C group in the second month to 140.2 ± 27.8 mg/dL ($p<0.05$). The second-month cholesterol level of the DM2C group was also significantly lower than the DM2M group ($p<0.05$), and not significantly different from the CDM group ($p> 0.05$). Administration of CeA extract since the first day of DM in the DM1C group successfully prevented an increase in cholesterol levels in the 1st and 2nd month. The 1st-month cholesterol level in the DM1C group is 139.8 ± 0.8 mg/dL, and this is lower than the 1st-month cholesterol level in the DM1M, DM2M and DM2C groups ($p <0.05$). The cholesterol level of the DM1C group in the second month decreased slightly compared to the first month to 133 ± 9.7 mg/dL ($p>0.05$). This month's DM1C cholesterol level was significantly lower than the DM2M group ($p<0.05$).
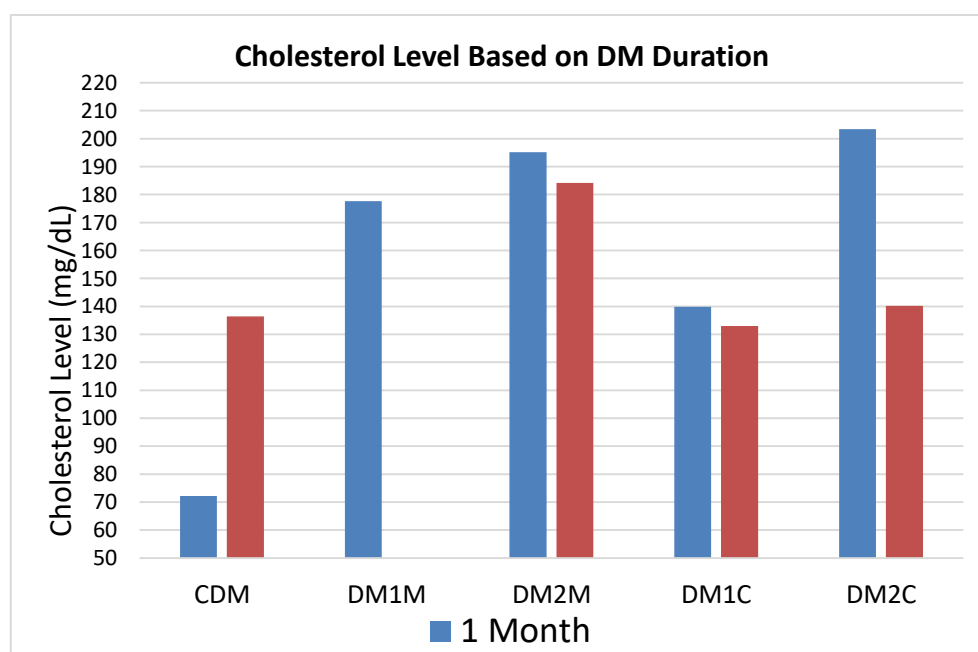

Figure 3. Cholesterol level between group based on DM duration

Previous study found that in untreated diabetic rats, serum cholesterol level increased significantly [36]. Altered lipid metabolism is a hallmark of type 2 diabetes induced dyslipidemia, which is characterized by reduced HDL and increased LDL, triglycerides, and total cholesterol [43]. Hyperglycemia and altered lipid status, in tandem, poses a significant threat of cardiovascular

complications in diabetic patients. Uncontrolled hyperlipidemia might give rise to both micro and macro vascular complications in type 2 diabetic patients [18]. Therefore, alleviated lipid profile in patients might improve diabetes induced secondary complications.

In this study, treatment with 400 mg/kg.b.w CeA extract for 1 month and 2 month duration decreased serum cholesterol level when compared to positive control. Previous study also found that CeA extract 200 mg/kgbw administration significantly decreased the serum cholesterol level, sugessting that CeA extract elicit anti-cholesterol effect in diabetes mellitus [36]. Kabir et.al suggested that 1000 mg/KgBW which consumed twice a day for 28 days decreased significantly of cholesterol levels [18]. Kumari et al., showed that CeA extract treatment reduced cholesterol level in the high cholesterol-fed (HCF) rats [40].

3.4 Effect of Centella asiatica Extract on Triglyceride Level

Serum triglyceride levels were measured in the second month of treatment. Serum triglyceride levels in the DM1M group were significantly higher in the CDM group (140.33 gr/dL vs. 61.79 gr/dL, $p < 0.05$). The DM2M group demonstrated an increase in triglyceride levels compared to CDM (100.97 gr/dL vs. 61.79 gr/dL, $p > 0.05$). The triglyceride level of the DM2C group was 237.77 gr/dL, whereas the triglyceride level of the DM1C group was 271.8 gr/dL. Triglyceride levels of the DM2C and DM1C groups were significantly higher than the CDM, DM1M, and DM2M groups ($p < 0.05$). This result suggested that CeA extract causes a significant increase in serum triglyceride levels.

This increase in serum triglyceride levels in CeA extract is probably caused by madecassic acid, one of the pentacyclic triterpenes compounds contained in CeA [17]. The study found that madecassic acid is a PPAR-on agonist [20]. PPAR-ɣ is a family of nuclear receptor proteins that are expressed in many body cells including adipocyte cells and skeletal muscle cells. The docking study showed that madecassic acid attaches to the Ligand Binding Domain section of the PPAR-protein-protein [20]. When the ligand binds to the Ligand Binding Domain of PPARɣ, PPARɣ will undergo dimerization with the Retinoic X Receptor (RXR) protein to form a transcription factor of heterodimer and will attach to the PPRE (PPAR Responsive Regulatory Element) sequence in the Genome DNA and regulate the expression of the target gene [21].
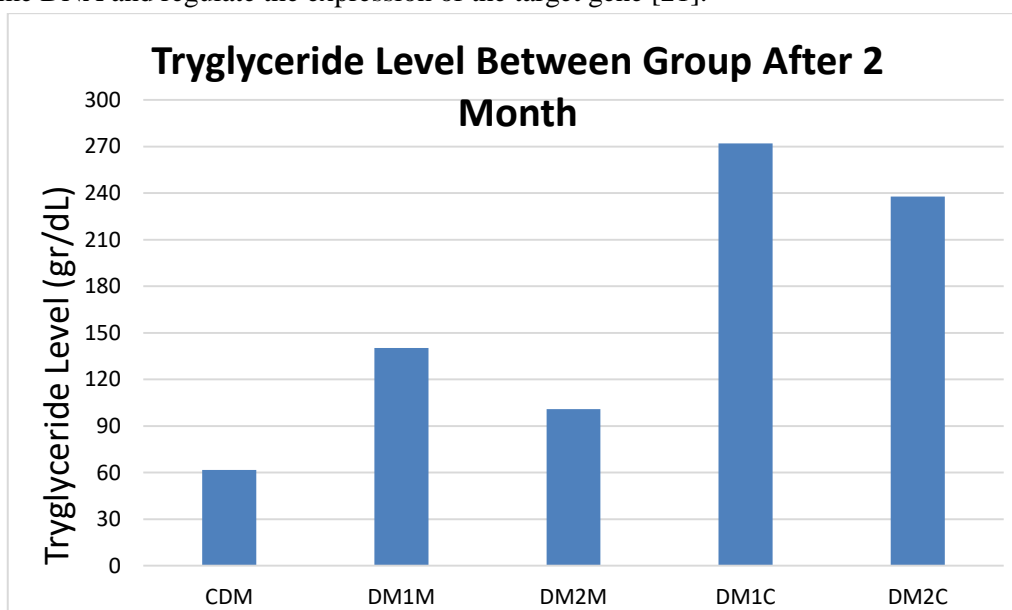

Figure 4. Serum triglycride level after 2 month

Angptl-4 (Angiopoietin-like protein 4) is one of the targets of the transcription-activated gene by the family of the transcription factor Peroxisome Proliferator-Activated Receptor, which is widely used as a target of anti-diabetes drugs and lipid-lowering drugs [25]. Angiopoietin-like protein 4 (Angptl-4) is adipokine which is mainly expressed in adipose, liver, and skeletal tissues [25,26,27]. Angptl-4 is a protein whose localization is secreted to the blood circulation (circulating protein) in the form of glycosylated, oligomerized, both in native and truncated isoforms [27]. Liu et al found that PPAR-ɣ agonists cause upregulation Angptl-4 expression, and this depends on the dose-dependent manner [22]. The study also found that PPARɣ-Retinoic X Receptor heterodimer specifically attaches to the promoter of the Angptl4 gene and increases transcription activation of the Angptl-4 gene [22] .

Angptl-4 regulates triglyceride levels by inhibiting the biological function of LPL (Lipoprotein Lipase) [28]. LPL is an enzyme associated with the endothelium which which act as a catalyst in the hydrolysis reaction of the triacylglycerol component in chylomicron and very low-density lipoproteins (VLDL) in the circulation into non-esterified fatty acids (NEFA) and 2-monoacylglycerol for use in tissues [27,29].

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Mice adenovirus-mediated Angptl-4 overexpression caused a marked elevation of serum triglycerides [45]. An earlier study from Yoshida *et al* also demonstrated that injection of recombinant ANGPTL4 acutely induces hypertriglyceridemia in mice possibly by inhibition of lipoprotein lipase activity [46]. More recently, Ge *et al* reported that adenovirus-mediated expression of ANGPTL4 raises plasma triglyceride levels by suppressing the clearance of very-low-density lipoprotein from the circulation [47].

3.5 Effect of Centella asiatica Extract on Low Density Lipoprotein (LDL)

The serum LDL level of the 1-month diabetes mellitus group (DM1M) was 8.24 2.2 gr/dL while in the 2-month diabetes mellitus group (DM2M) it was 11.82±3.2 gr/dL. DM1M and DM2M groups had lower LDL levels than CDM but this was not significant ($p > 0.05$). Administration of CeA extract for 1 month in the DM2C group caused a decrease in LDL levels compared to DM2M although this was not significant ($p > 0.05$). Whereas CeA extract for 2 months in the DM1C group caused the lowest LDL level compared to the other group, namely 5.76±2.7 gr/dL. LDL levels in the DM1C group were significantly lower than DM2M ($p < 0.05$), but not significantly different from DM1M and DM2C ($p > 0.05$).

Kabir et al in his study found that the CeA extract dose of 1000 mg / KgBB daily 2 times for 28 days significantly reduced serum LDL levels [18]. Another study by Kumari et.al found that CeA extract was able to significantly reduce LDL levels in high cholesterol-fed (HCF) rats [44].
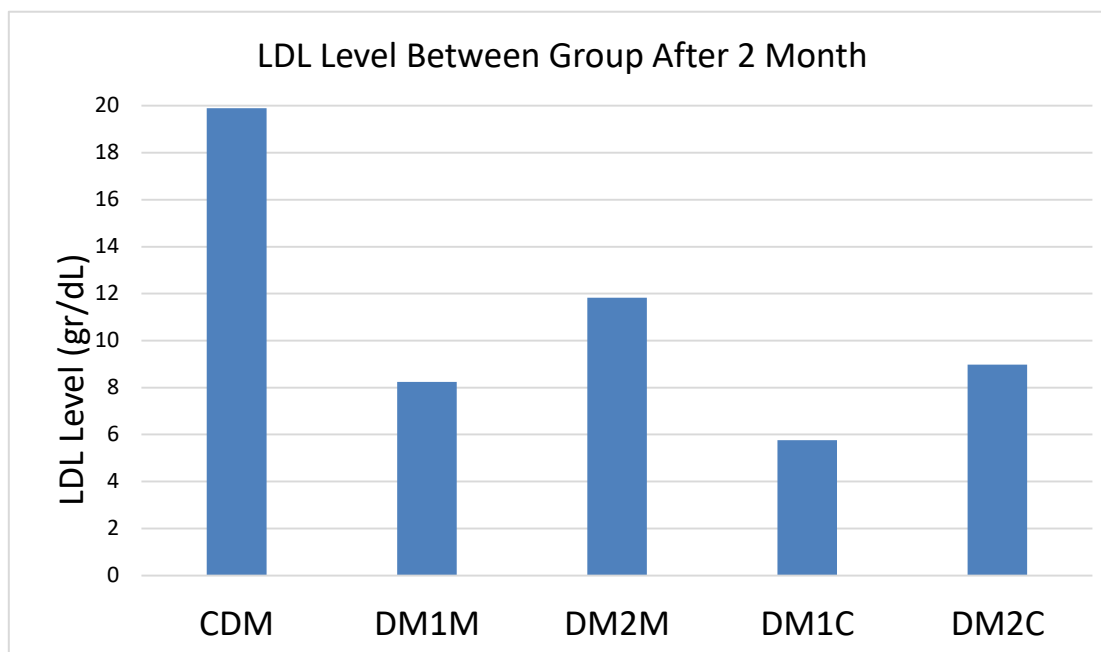
Figure 5. Serum LDL level after 2 month

3.6 Effect of Centella asiatica Extract on High Density Lipoprotein (HDL)

Serum HDL levels 2 months after intervention were shown in figure 6. HDL levels in the negative control group (CDM) were 76.8±10.1 gr/dL. The DM1M and DM2M had significantly lower HDL levels than CDM (p <0.05). This shows that the condition of diabetes mellitus, both 1 month and 2 months duration, causes a significant decrease in serum HDL levels. HDL levels in the DM1C group (65.7±8.9 gr/dL) were higher than the DM1M group (58.48±9.1 gr/dL) and DM2M (61.8±3.8 gr/dL), but this difference was not significant (p>0.05). This shows that the CeA extract dose of 400 mg/kgBW/day from the beginning of the process of diabetes mellitus is not effective in increasing serum HDL levels after 2 months of intervention. Research conducted by Kabir et al found that CeA extract dose of 500 mg/kgBW and 1000 mg/kgBW twice a day for 28 days significantly increased serum HDL levels [18].
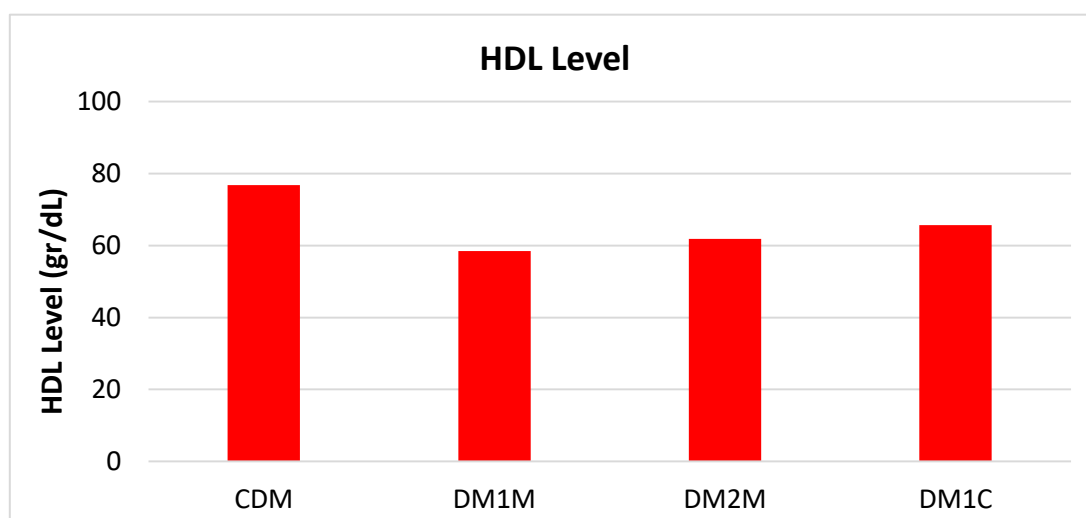

Figure 6. Serum HDL level between group after 2 month

3.7 Effect of Centella asiatica Extract on Serum Creatinine Level

Serum creatinine levels are one of the biomarkers for assessing kidney function in clinical practice. In this study, we measured serum creatinine levels at the end of the intervention, to assess whether CeA extract in diabetes mellitus can protect against kidney damage. In this study it was found that serum creatinine levels in the CDM group were normal values of 0.55±0.13 mg/dL. The 1-month diabetes mellitus group (DM1M) also had normal serum creatinine levels of 0.88±0.57 mg/dL, and this value was not significantly different from the CDM group (p>0.05). This shows that the duration of diabetes mellitus for 1 month has not caused kidney injury. The 2-month diabetes mellitus group (DM2C) experienced a significant increase in serum creatinine levels compared to CDM and DM1M with a value of 4.33±1.5 mg/dL (p<0.05). Serum creatinine levels of 4.33±1.5 mg/dL indicate kidney damage. This shows that the duration of diabetes mellitus for 2 months has been able to cause kidney damage.

Diabetic nephropathy (DN) is one of the most serious complications in diabetic patients and the primary cause of end-stage renal failure. It is characterized by the accumulation of extracellular matrix (ECM) proteins, including predominantly various collagens, laminin and fibronectin in the mesangial and renal tubulointerstitial as well as thickening of basement membranes [48,49]. This increased deposition of ECM subsequently leads to renal fibrosis, which can culminate in tubulointerstitial fibrosis, glomerulosclerosis, infiltration of inflammatory mediators, and the de novo activation of alpha-smooth muscle actin (α-SMA)-positive myofibroblasts. It has been shown that the number of myofibroblasts is inversely correlated with renal function in human DN [50,51]. It is widely accepted that these activated myofibroblasts are the principal effector cells that are responsible for the excess deposition of interstitial ECM [52].

The condition of hyperglycemia in diabetes mellitus can induce inflammation [53,54]. Chronic hyperglycemia also triggers an increase in the production of advanced glycation end products (AGE) which can bind and activate AGE Receptor (RAGE) [55,56]. Activation of RAGE will cause a signaling downstream cascade that causes activation of NFƙB transcription factors and increased expression of proinflammatory cytokine, chemokine, and adhesion molecule proteins, as well as reactive oxygen species [55,56,57]. Hyperglycemia causes upregulation of expression of proinflammatory cytokine proteins such as IL-1β, IL-6, TLR-4 and MCP-1 where this depends on the NFƙB pathway and Inflammasome [53,54]. Previous research found that inflammation was able to induce Epithelial to Mesenchymal Transition (EMT) in the kidneys, where tubular epithelial cells change to transition to myofibroblast cells.

Upregulation of pro-inflammatory cytokines in diabetes can induce epithelial tubular cells to experience Epithelial to Mesenchymal Transition (EMT) and endothelial cells to undergo Endothelial to Mesenchymal Transition (EndMT) to myofibroblast cells (58). This formation of myofibroblast causes accumulation of ECM and the occurrence of tubular interstitial fibrosis (TIF) which ultimately causes chronic kidney disease [59].

It was obviously found that DN associated with functional and morphological alterations of podocyte, mainly including podocyte hypertrophy, podocyte detachment, and podocyte apoptosis. In the progression of disease, we found that in the same stage of DN, some podocytes became hypertrophy and detached from the basement membrane. Decreased nephrin expression is observed during the early stages of DN, playing an important role in accelerating the development of DN. Previous research has found that inflammation and AGE can induce hypertrophy of podocyte with cell cycle arrest and podocyte apoptosis in diabetic conditions and this is one of them through activation of CXCL-9 mediated JAK2-STAT3 pathway [60]. This leads to

progressive alteration in the common architecture and loss of renal function in patients, resulting in a thick membrane and thick expansion, hallmarks of DN [61].

Increased levels of non-esterified fatty acids (NEFA) in the blood circulation have long been proven in diabetes mellitus [62]. Enlarged fat cells in obese adipose tissue diminish capacity to store fat and are resistant to the anti-lipolytic effect of insulin. Failure of packaging of excess lipid into lipid droplets causes chronic elevation of circulating fatty acids. In nonadipose tissues, excess cytosolic FFAs lead to cell dysfunction and death by promoting endoplasmic reticulum stress and excess production of reactive oxygen species, processes collectively designated as lipotoxicity [63]. In diabetes condition, filtered albumin has attached to FFAs; evidence suggests that it is the FA component in albuminuric states that exerts proinflammatory and profibrotic effects. D36, proposed to be a PTC receptor for FFA bound to albumin, was found to be overexpressed in human DN; *in vitro*, this receptor mediated PTC apoptosis and proinflammatory and profibrotic responses. This suggesting that elevated NEFA can cause apoptosis in PTC mediated by albumin-FFA-D36 interaction. Another study found that lipotoxicity can cause altered lipid composition of the lipid rafts. Podocin and nephrin must be spatially inserted within the cholesterol-rich segments (lipid rafts) of the podocyte slit diaphragm to maintain the proper podocin-nephrin and podocin-transient receptor potential cation channel 6 [TRPC-6] interactions and podocyte function. FA overload can cause altered lipid composition of the lipid rafts and therefore interfere with network signaling of the podocin-nephrin-TRPC-6 actin cytoskeleton, triggering podocyte detachment [64].

We then try to evaluate whether CeA extract in diabetes mellitus can protect against kidney damage. The DM2C group had serum creatinine levels of 1.75 0.13 mg / dL which showed abnormal values. It can be concluded that the administration of CeA extract for 1 month after having had diabetes mellitus for 1 month was not effective in protecting against kidney damage. Nonetheless, the serum creatinine level of the DM2C group was significantly lower than the DM2M group (p<0.05), which indicates that the administration of Cea Extract after previously experiencing diabetes mellitus was able to slow down kidney damage that occurred. Unlike the DM2C group, the DM1C group had normal serum creatinine levels of 0.46±0.53 mg/dL. This shows that the administration of CeA extract from the onset of the onset of diabetes mellitus is renoprotective and can protect against kidney damage. Serum creatinine levels in the DM1C group were significantly lower than the DM2M and DM2C groups (p<0.05).
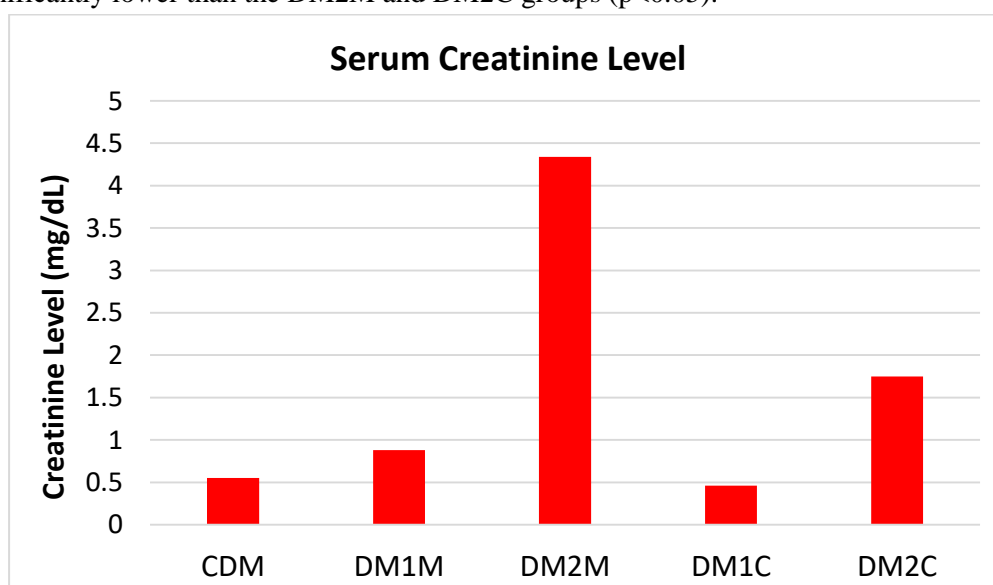

Figure 7. Serum creatinine level between group after 2 month

Research conducted by Hawaz et al found that CeA extract has an anti-inflammatory effect [65]. Masola et al found that CeA extract caused significant downregulation of expression of proinflammatory cytokine proteins such as TNF-α and IFN-ɣ and reduced MDA levels in renal diabetic rats [66]. One of the active compounds that has an anti-inflammatory effect on CeA is madecassic acid. Previous research has found that madecassic acid causes downregulation of expression of IL-1β, IL-6, TNF-α and MCP-1 in kidney diabetic mice [38]. This indicates that CeA has anti-inflammatory and antioxidant effects, and this might mediate the effect of renoprotective CeA on the condition of diabetes mellitus. Through inhibition of this inflammation, CeA extract can prevent various mechanisms involved in DN such as EMT, EndMT, TIF, and podocyte apoptosis and podocyte detachment. CeA extract can also reduce blood glucose levels [18,19,34,35,36]. Better control of blood glucose levels contributes to inflammation and oxidative stress reduction in the kidneys [38]. Previous study by Kumari et.al found that CeA extracts remarkably increases the activity of superoxide dismutase (SOD), enzyme antioxidant that catalyzes superoxide metabolism into hydrogen peroxide, suggesting that CEA may have the antioxidant activity [44]. Decreasing blood glucose levels and antioxidant activity by giving CeA extract is possible to reduce AGE production, prevent EMT, EndMT, TIF, podocyte hypertrophy and apoptosis.

4. CONCLUSION

The administration of CeA extract (400 mg/kgBW/day) from the onset of diabetes mellitus over a period of 2 months lowers blood glucose levels and improves serum lipid profiles such as lowering cholesterol, decreasing LDL, increasing HDL and increasing triglyceride in streptozotocin-induced diabetic rats. This suggest that CeA extracts elicit anti-hyperglycemic and hypolipidemic activity in diabetes mellitus. Furthermore, CeA extract (400 mg/kgBW/day) from the onset of diabetes mellitus over a period of 2 months reduced serum creatinine levels to normal values in streptozotocin-induced diabetic rats, suggesting that CeA extract may have renoprotective activity. Further research needs to be done to determine the cellular and molecular mechanisms of the antihyperglycemic, hypolipidemic, and renoprotective activities of CeA extract in diabetes mellitus.

5. ACKNOWLEDGMENT

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