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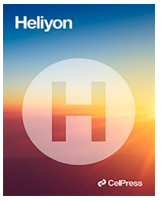

Research article

Antidiabetic and hepatoprotection effect of butterfly pea flower (*Clitoria ternatea* L.) through antioxidant, anti-inflammatory, lower LDH, ACP, AST, and ALT on diabetes mellitus and dyslipidemia rat

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ABSTRACT

This study explores the antidiabetic and hepatoprotective potential of Butterfly pea flower extract (*Clitoria ternatea* L.) (CTE) in diabetic and dyslipidemia rat models. Diabetes Mellitus (DM) is a chronic metabolic disorder marked by high levels of blood glucose, which can cause dyslipidemia and liver damage as a result of oxidative stress. CTE, a natural substance, is recognized for its positive attributes, such as anti-inflammatory, antioxidant, anti-diabetic, anti-dyslipidemia, antibiotic, and liver tissue protection capabilities. Dyslipidemia was induced in rats using a high-fat diet (HFD) and propylthiouracil (PTU) for 28 days. DM was induced using streptozotocin (STZ) and nicotinamide (NA). Rats were treated with varying doses of CTE for 28 days, along with glibenclamide and simvastatin. The research showed that CTE raised the levels of SOD, CAT, and liver proteins while lowering the levels of MDA, LDH, ACP, AST, ALT, IL-1β, and CRP in rats with DM and dyslipidemia. This suggests that CTE might be useful for treating DM.

1. Introduction

Diabetes mellitus (DM) is a persistent metabolic condition marked by elevated blood sugar levels (hyperglycemia) resulting from deficiencies in insulin production or insufficient insulin effectiveness. This condition leads to disruptions in carbohydrate, lipid, and protein metabolism [1]. Insulin resistance causes the liver to produce excess glucose through glycogenesis, contributing to hyperglycemia [2]. Consequently, the body experiences an energy deficiency, leading to the breakdown of fats through lipolysis, which can develop in the release of fatty acids into the bloodstream, the accumulation of triglycerides, and increased cholesterol levels in the liver, ultimately causing dyslipidemia [3]. The International Diabetes Federation (IDF) reports that currently, approximately 463 million individuals aged 20–79 are afflicted with diabetes. The projections indicate that there will be a rise to 578 million by the year

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2030 and a further escalation to approximately 700 million by 2045.

In individuals with DM and dyslipidemia, hyperglycemia can trigger oxidative stress, leading to inflammation and tissue damage [4,5]. The liver, being susceptible to hyperglycemia-induced oxidative stress, is particularly prone to damage [6]. Oxidative stress further stimulates lipid peroxidation, resulting in the production of Malondialdehyde (MDA) [7]. Elevated levels of free radicals cause cellular damage and also impact tissues, leading to increased levels of Lactate Dehydrogenase (LDH) and Acid Phosphatase (ACP) [8]. Additionally, heightened levels of Alanine Aminotransferase (ALT) and Aspartate Aminotransferase (AST) occur as a result of oxidative stress [9].

Interleukin-1β (IL-1β) has been shown in numerous studies to be vital to the development of inflammation and the elevated cytokine production linked to oxidative stress-induced liver damage [10]. Additionally, C-reactive protein (CRP), a protein produced by the liver during acute-phase response, shows increased levels in reaction to inflammation [11].

Three primary enzymes are part of the antioxidant defense system: catalase (CAT), glutathione peroxidase (GPx), and superoxide dismutase (SOD) [12]. Since the human body lacks excessive antioxidant reserves to counteract free radical activity, antioxidant therapy can be considered for patients with DM and dyslipidemia [13]. Natural products, compared to chemical drugs, offer the advantage of minimal side effects and high safety. One such product is the extract derived from *Clitoria ternatea* L. or the butterfly pea flower (CTE), which has been recognized for its antioxidant, anti-inflammatory, anti-diabetic, anti-dyslipidemia, antibiotic, and liver-protective properties [14]. This claim is further supported by Talpate et al., who demonstrated significant antihyperglycemic effects of CTE at 200 and 400 mg/kg BW [15]. These doses resulted in reduced Fasting Serum Glucose (FSG) levels, decreased Nitric Oxide (NO) levels, and increased SOD and CAT activities.

The purpose of this study is to assess the hepatoprotective and antidiabetic benefits of CTE through *in vivo* in rats with dyslipidemia and DM. The study will investigate the effect of CTE on various parameters, including MDA, SOD, CAT, LDH, ACP, AST, ALT, IL-1β, CRP, and rat liver protein levels.

2. Materials and methods

2.1. Plant extraction

The butterfly pea flower (*C. ternatea* L.) is sourced from the Herbal Village of Sukolilo Village, Prigen District, Pasuruan, East Java. The identification of the butterfly pea flower was conducted at The Indonesian Institute of Sciences in Bogor. In accordance with Good Manufacturing Practices (GMP), PT FAST processed the butterfly pea flower extract (CTE) in Depok, Indonesia (CoA Batch 00103211072). The resulting CTE extract adheres to the standardized Cara Pembuatan Obat Tradisional yang Baik (CPOTB). The extraction process involved using 70 % ethanol through the maceration method. The resulting extract was transformed into a paste and combined with lactose to create CTE powder, which was stored at room temperature [14]. CTE powder is known to contain inositol, delphinidin-3-*O*-(6-*O*-*p*-coumaroyl) glucoside-pyruvic acid, 2-hydroxycinnamic acid, and (+) catechin 7-*O*-β-glucoside based on our previous research [14].

2.2. Animal experimental

The research protocol received approval from the Ethical Committee of Maranatha Christian University, Bandung, Indonesia (147/KEP/VI/2021). Male Sprague Dawley rats, with an average weight ranging from 120 to 140 g and approximately 6 weeks old, were attained from IratCo Laboratory in Bogor, Indonesia. They were acclimatized for 7 days in individual cages under controlled conditions of 23 ± 2 °C and a light-dark cycle. The rats were administered regular food and had unlimited access to water during the acclimation phase [16]. After the acclimatization period, dyslipidemia was induced in the rats by feeding them 50 g of High Fat Diet (HFD) from PT Indofeed and providing drinking water containing 0.01 % Propylthiouracil (PTU) from Dexa Medica for 28 days [17]. Dyslipidemia was verified by assessing serum cholesterol levels with the use of a cholesterol kit (Elabscsi, E-BC-K109-M), with a threshold of >200 mg/dL. The rats were given 120 mg/kg body weight (BW) of intraperitoneally (IP) nicotinamide (NA) from Sigma Aldrich (N3376) to develop diabetes. After 60 min, streptozotocin (STZ) from Sigma Aldrich (SO130) at a dosage of 60 mg/kg BW was injected NA. After 5 days, the rats had a 6-h fast. Autocheck was used to determine their blood glucose levels. Rats exhibiting blood sugar levels exceeding 250 mg/dL and serum cholesterol levels≥ 200 mg/dL were identified as having both DM and dyslipidemia. After the confirmation of both DM and dyslipidemia in the rats, they were given oral doses of CTE, glibenclamide (Generic, GKL9520905004A2), and simvastatin (Generic, GKL131670271A). The rats were separated into eight groups, each group consisted of four rats, as follows: Group I: Negative control (normal rats) (NC). Group II: Positive control (rats with DM and dyslipidemia) (PC). Group III: Positive control + CTE 200 mg/kg BW (CTE200). Group IV: PC + CTE 400 mg/kg BW (CTE400). Group V: PC + CTE 800 mg/kg BW (CTE800). Group VI: PC + Simvastatin 0.9 mg/kg BW (SV). Group VII: PC + Glibenclamide 0.45 mg/kg BW (GC). Group VIII: PC + Glibenclamide 0.45 mg/kg BW and Simvastatin 0.9 g/kg BW (GS) [14].

2.3. Termination and liver Collection

The rats were put to sleep by intracardial injection of 10 mg/kg BW xylazine (Interchemie, 361,453) and 100 mg/kg BW ketamine HCl (Ikapharmindo Putramas) at the end of their 28-day treatment period. The rat liver was carefully excised, rinsed with chilled saline solution, and preserved in 10 % formalin for subsequent investigation. Liver samples were stored at −80 °C for further analysis [14].

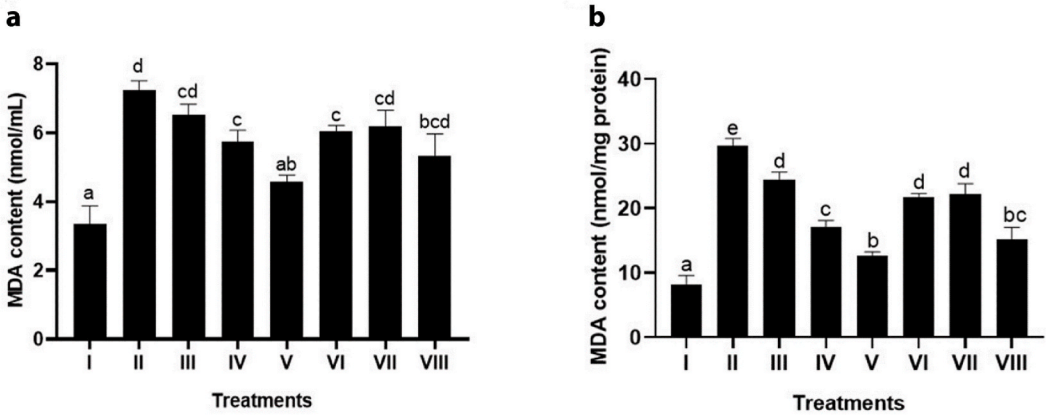

Fig. 1. Effect of CTE on liver MDA levels in DM and Dyslipidemia Rat Model. (a) MDA level (nmol/mL); (b) MDA level (nmol/mg protein)
⁎The data are reported as means±SD and were obtained from four repetitions. The experimental groups: Group I: Negative control (NC) consisting of normal rats, Group II: Positive control (PC) comprising rats with DM and dyslipidemia, Group III: PC+CTE 200 mg/kg BW (CTE200), Group IV: PC+CTE 400 mg/kg BW (CTE400), Group V: PC+CTE 800 mg/kg BW (CTE800), Group VI: PC+Simvastatin 0.9 mg/kg BW (SV), Group VII: PC+Glibenclamide 0.45 mg/kg BW (GC), and Group VIII: PC+Glibenclamide 0.45 mg/kg BW+Simvastatin 0.9 mg/kg BW (GS). Superscript signs on Figure 1a (a, ab, bcd, c, cd, d) and Figure 1b (a, b, bc, c, d, e) indicate significant differences ($p<0.05$) among samples obtained from Tukey's HSD.

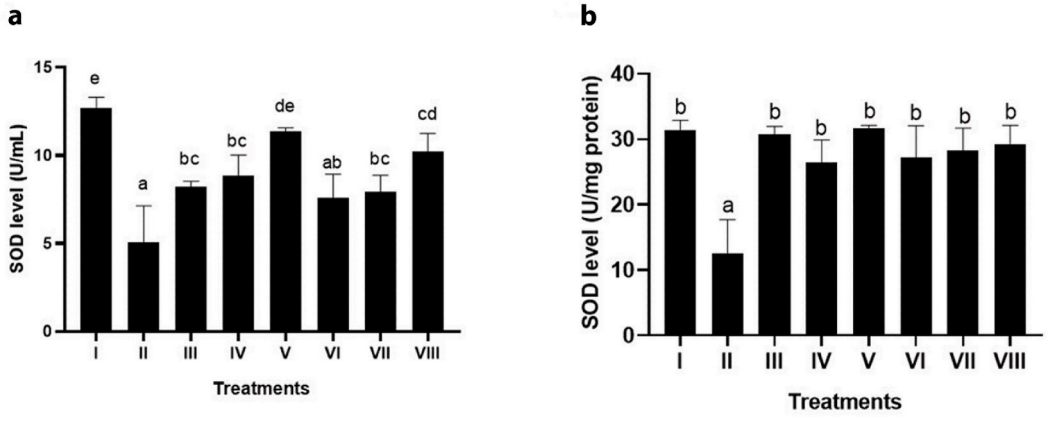

Fig. 2. Effect of CTE on liver SOD levels in DM and Dyslipidemia Rat Model. (a) SOD level (U/mL); (b) SOD level (U/mg protein)
⁎The data are presented as means±SD and were obtained from four repetitions. The experimental groups: Group I: Negative control (NC) comprising normal rats, Group II: Positive control (PC) consisting of rats with DM and dyslipidemia, Group III: PC+CTE 200 mg/kg BW (CTE200), Group IV: PC+CTE 400 mg/kg BW (CTE400), Group V: PC+CTE 800 mg/kg BW (CTE800), Group VI: PC+Simvastatin 0.9 mg/kg BW (SV), Group VII: PC+Glibenclamide 0.45 mg/kg BW (GC), and Group VIII: PC+Glibenclamide 0.45 mg/kg BW+Simvastatin 0.9 mg/kg BW (GS). Superscript signs on Figure 2a (a, ab, bc, cd, de, e) and Figure 2b (a, b) indicate significant differences ($p<0.05$) among samples obtained from Tukey's HSD.

2.4. Measurement of MDA, SOD, CAT, LDH, ACP, AST, ALT, IL-1β, and CRP liver levels

Utilizing the guidelines provided by the manufacturer, the Elisa Kit was employed to quantify many parameters. The measurements included MDA levels (Elabscience, E-BC-K025-S), hepatic SOD levels (Elabscience, E-BC-K020), CAT levels (Elabscience, E-BC-K031), hepatic LDH levels (E-BC-K046-M), hepatic ACP levels (E-BC-K010-M), hepatic AST levels (Elabscience, E-BC-K236), hepatic ALT levels (Elabscience, E-BC-K235), hepatic IL-1β levels (E-EL-R0012), and hepatic CRP levels (Elabscience, E-EL-R0506) [14,18].

2.5. Protein assay

For plate preparation, Quick Start Dye Reagent 1X (200 μL) (Biorad, 5,000,205) was applied to each well at room temperature. Sample (liver homogenate) and Standard solutions of Bovine Serum Albumin (BSA) (Sigma Aldrich, A9576) (1000 μL) were added and put into incubation for 5 min. A microplate reader was used to detect the absorbance of the samples (595 nm) [14].

2.6. Data analysis

The statistical analysis was carried out using the SPSS 20.0 software. One-way ANOVA was used for data that showed homogeneity

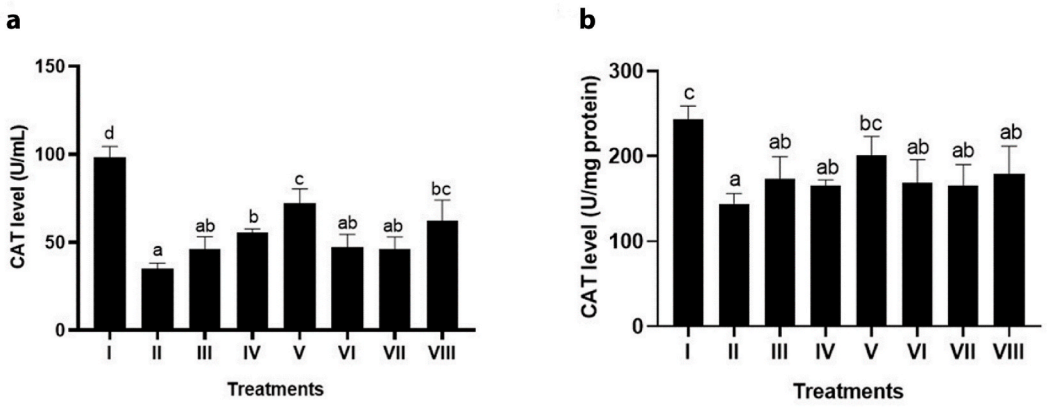

Fig. 3. Effect of CTE on liver CAT levels in DM and Dyslipidemia Rat Model. (a) CAT level (U/mL); (b) CAT level (U/mg protein)
⁎The data are presented as means±SD and were obtained from four repetitions. The experimental groups: Group I: Negative control (NC) comprising normal rats, Group II: Positive control (PC) consisting of rats with DM and dyslipidemia, Group III: PC+CTE 200 mg/kg BW (CTE200), Group IV: PC+CTE 400 mg/kg BW (CTE400), Group V: PC+CTE 800 mg/kg BW (CTE800), Group VI: PC+Simvastatin 0.9 mg/kg BW (SV), Group VII: PC+Glibenclamide 0.45 mg/kg BW (GC), and Group VIII: PC+Glibenclamide 0.45 mg/kg BW+Simvastatin 0.9 mg/kg BW (GS). Superscript signs on Figure 3a (a, ab, b, bc, c, d) and Figure 3b (a, ab, bc, c) indicate significant differences ($p<0.05$) between samples obtained from Tukey's HSD.

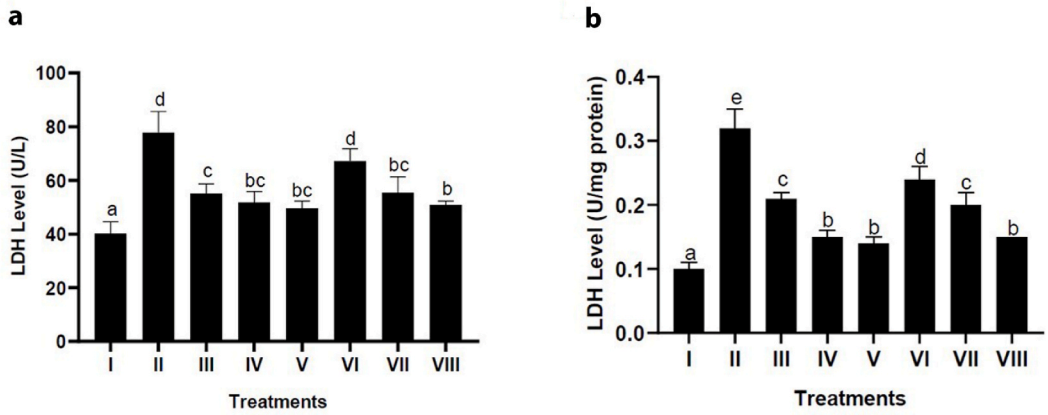

Fig. 4. Effect of CTE on liver LDH levels in DM and Dyslipidemia Rat Model. (a) LDH level (U/L); (b) LDH level (U/mg protein)
⁎The data are presented as means±SD and were obtained from four repetitions. The experimental groups: Group I: Negative control (NC) comprising normal rats, Group II: Positive control (PC) consisting of rats with DM and dyslipidemia, Group III: PC+CTE 200 mg/kg BW (CTE200), Group IV: PC+CTE 400 mg/kg BW (CTE400), Group V: PC+CTE 800 mg/kg BW (CTE800), Group VI: PC+Simvastatin 0.9 mg/kg BW (SV), Group VII: PC+Glibenclamide 0.45 mg/kg BW (GC), and Group VIII: PC+Glibenclamide 0.45 mg/kg BW+Simvastatin 0.9 mg/kg BW (GS). Superscript signs on Figure 4a (a, b, bc, c, d) and Figure 4b (a, b, c, d, e) indicate significant differences ($p<0.05$) among samples obtained from Tukey's HSD.

and a normal distribution, and the Tukey Post Hoc test was then run. The Independent Sample T-Test was used when the data were normally distributed but not homogeneous. All data are provided as mean ± standard deviation from three replications and statistical significance was set at $p < 0.05$ [14].

3. Results and discussion

The butterfly pea (*C. ternatea* L.) has been used traditionally to treat a variety of health problems due to its rich composition of phytochemicals. Based on research done by Widowati et al. using LC-MS/MS analysis, it was found that CTE (butterfly pea tea extract) contains compounds such as (+) catechin 7-*O*-β-glucoside, inositol, 2-hydroxycinnamic acid, and delphinidin-3-*O*-(6-*O*-*p*-coumaroyl) glucoside-pyruvic acid [14]. According to this research, it is established that CTE includes flavonoids and phenols that exhibit antioxidant action. Studies in the past have demonstrated the efficacy of inositol and catechin in the treatment of DM [19]. Another study by Al-Snafi et al. reported that CTE is also rich in flavonoids, phenols, tannins, and glycosides [20]. Additionally, CTE is known to contain anthocyanins, triterpenoids, alkaloids, and saponins, which contribute to its antioxidant properties. Widowati et al. also suggest that CTE exhibits both antioxidant and anti-inflammatory effects [14].

Diabetes and dyslipidemia are identified by high levels of glucose and cholesterol in the serum. In this study, induction of STZ and NA, as well as PTU and HFD, was used to create rat models of DM and dyslipidemia. The results of the induction showed that the blood glucose levels of the rats reached >250 mg/dL and cholesterol levels reached >200 mg/dL. Treatment with CTE can reduce the glucose

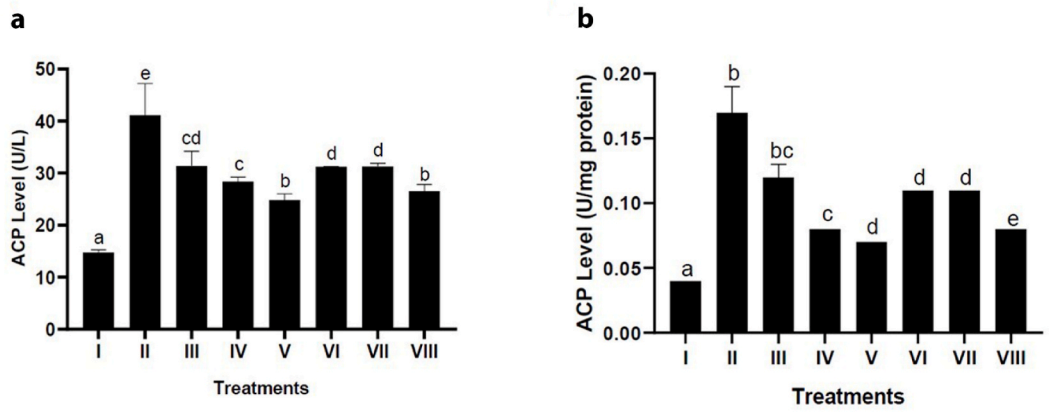

Fig. 5. Effect of CTE on liver ACP levels in DM and Dyslipidemia Rat Model. (a) ACP level (U/L); (b) ACP level (U/mg protein)
⁎The data are presented as means±SD and were obtained from four repetitions. The experimental groups: Group I: Negative control (NC) comprising normal rats, Group II: Positive control (PC) consisting of rats with DM and dyslipidemia, Group III: PC+CTE 200 mg/kg BW (CTE200), Group IV: PC+CTE 400 mg/kg BW (CTE400), Group V: PC+CTE 800 mg/kg BW (CTE800), Group VI: PC+Simvastatin 0.9 mg/kg BW (SV), Group VII: PC+Glibenclamide 0.45 mg/kg BW (GC), and Group VIII: PC+Glibenclamide 0.45 mg/kg BW+Simvastatin 0.9 mg/kg BW (GS). Superscript signs on Figure 5a (a, b, c, cd, d, e) and Figure 5b (a, b, bc, c, d, e) indicate significant differences ($p<0.05$) among samples obtained from Tukey's HSD.

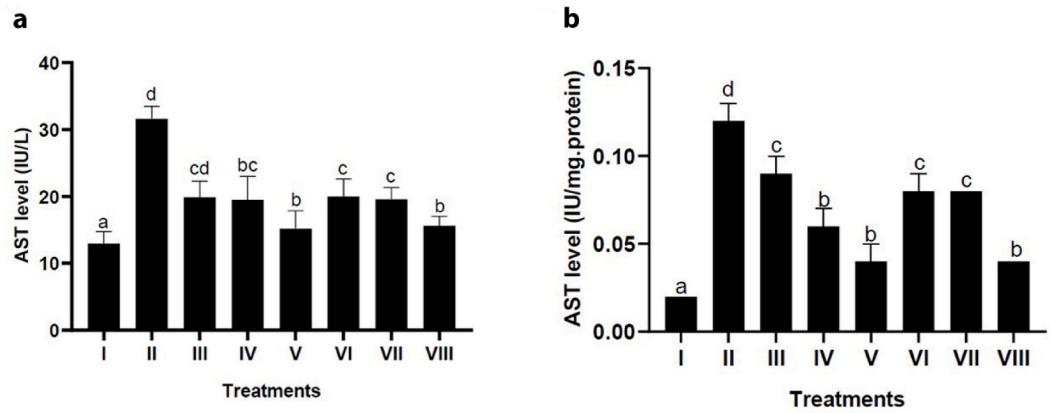

Fig. 6. Effect of CTE on liver AST levels in DM and Dyslipidemia Rat Model. (a) AST level (IU/L); (b) AST level (IU/mg protein)
⁎The data are presented as means±SD and were obtained from four repetitions. The experimental groups: Group I: Negative control (NC) comprising normal rats, Group II: Positive control (PC) consisting of rats with DM and dyslipidemia, Group III: PC+CTE 200 mg/kg BW (CTE200), Group IV: PC+CTE 400 mg/kg BW (CTE400), Group V: PC+CTE 800 mg/kg BW (CTE800), Group VI: PC+Simvastatin 0.9 mg/kg BW (SV), Group VII: PC+Glibenclamide 0.45 mg/kg BW (GC), and Group VIII: PC+Glibenclamide 0.45 mg/kg BW+Simvastatin 0.9 mg/kg BW (GS). Superscript signs on Figure 6a (a, b, bc, c, cd, d) and Figure 6b (a, b, c, d) indicate significant differences ($p<0.05$) among samples obtained from Tukey's HSD.

levels of rats. These results have been published in our previous study [14].

Studies have demonstrated that hyperglycemia conditions in DM and dyslipidemia lead to oxidative stress, which in turn causes damage to pancreatic β cells. This process involves the activation of Protein Kinase C (PKC) and Nuclear Factor kappa B (NF-kB) within the cells, resulting in the overexpression of Nicotinamide Adenine Dinucleotide Phosphate (NADPH) and the subsequent production of excessive amounts of superoxide. Ultimately, Kumawat et al. state that oxidative stress induces lipid peroxidation, leading to the formation of Malondialdehyde (MDA), a marker of tissue damage [7]. Fig. 1a–b illustrates the effect of CTE on liver MDA levels in rats with DM and dyslipidemia. Results showed that MDA levels in the PC group (7.45 nmol/mL) (29.69 nmol/mg protein) ($p < 0.05$) had significantly increased. Moreover, treatment with CTE at 800 mg/kg BW (4.46 nmol/mL) (12.71 nmol/mg protein) effectively reduced MDA levels compared to other CTE treatments. CTE at 800 mg/kg BW exhibited a stronger effect on reducing liver MDA levels compared to SV, GC, and GS. Thus, CTE was deemed effective in decreasing liver MDA levels. The presence of anthocyanins in CTE contributes to its antioxidant activity, and it can decrease MDA levels [21]. Furthermore, the saponin content in CTE acts as an antioxidant and exerts a hepatoprotective effect. Its mechanism involves inhibiting lipid peroxidation and preserving antioxidant capacity, thereby preventing liver necrosis [22].

Critical antioxidant defense systems like superoxide dismutase (SOD) and catalase (CAT) become less active when people with diabetes and dyslipidemia have elevated levels of free radicals in their bodies. These enzymes play a vital role in preventing cellular damage because they produce molecular oxygen and hydrogen peroxide from superoxide radicals produced during oxygen metabolism [23,24]. Fig. 2a–b demonstrates the impact of CTE on liver SOD levels in rat models of DM and dyslipidemia. The PC group (5.08

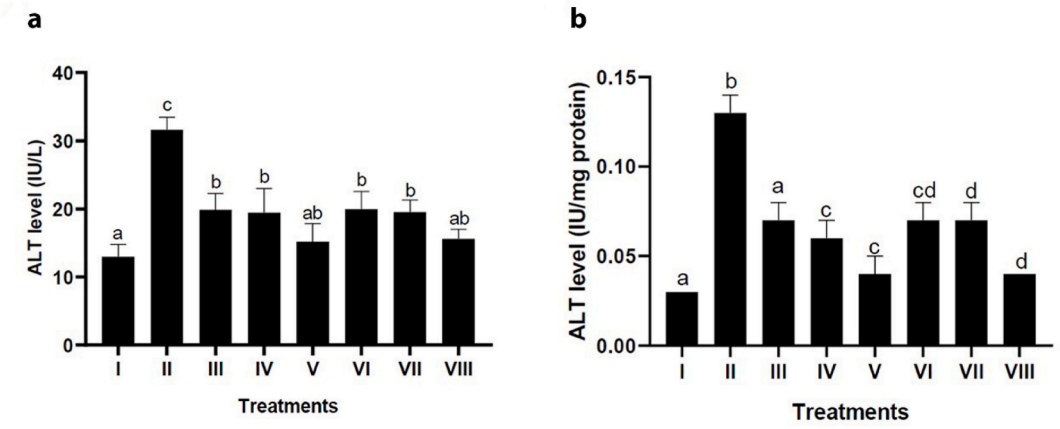

Fig. 7. Effect of CTE on liver ALT levels in DM and Dyslipidemia Rat Model. (a) ALT levels (IU/L); (b) ALT level (IU/mg protein)
⁎The data are presented as means±SD and were obtained from four repetitions. The experimental groups: Group I: Negative control (NC) comprising normal rats, Group II: Positive control (PC) consisting of rats with DM and dyslipidemia, Group III: PC+CTE 200 mg/kg BW (CTE200), Group IV: PC+CTE 400 mg/kg BW (CTE400), Group V: PC+CTE 800 mg/kg BW (CTE800), Group VI: PC+Simvastatin 0.9 mg/kg BW (SV), Group VII: PC+Glibenclamide 0.45 mg/kg BW (GC), and Group VIII: PC+Glibenclamide 0.45 mg/kg BW+Simvastatin 0.9 mg/kg BW (GS). Superscript signs on Figure 7a (a, ab, b, c) and Figure 7b (a, b, c, cd, d) indicate significant differences ($p<0.05$) among samples obtained from T−Test.

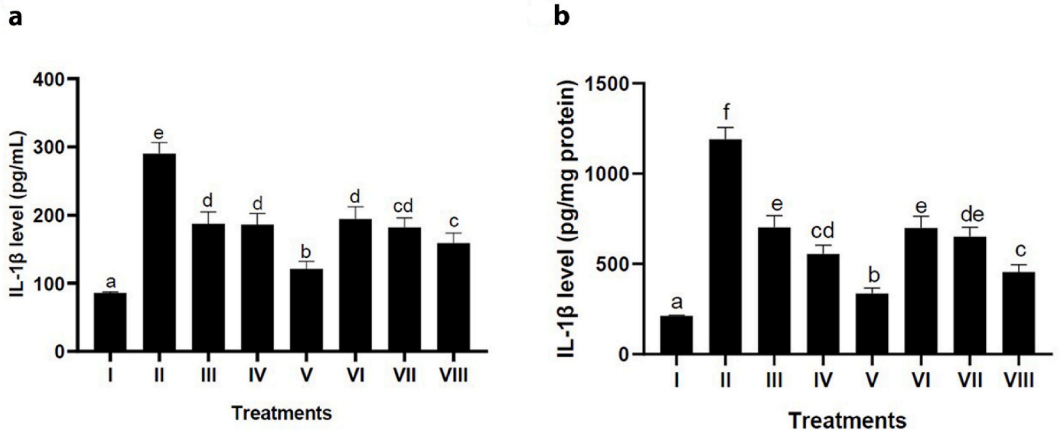

Fig. 8. Effect of CTE on liver IL-1β levels in DM and Dyslipidemia Rat Model. (a) IL-1β levels (pg/mg); (b) IL-1β level (pg/mg protein) ⁎The data are presented as means±SD and were obtained from four repetitions. The experimental groups: Group I: Negative control (NC) comprising normal rats, Group II: Positive control (PC) consisting of rats with DM and dyslipidemia, Group III: PC+CTE 200 mg/kg BW (CTE200), Group IV: PC+CTE 400 mg/kg BW (CTE400), Group V: PC+CTE 800 mg/kg BW (CTE800), Group VI: PC+Simvastatin 0.9 mg/kg BW (SV), Group VII: PC+Glibenclamide 0.45 mg/kg BW (GC), and Group VIII: PC+Glibenclamide 0.45 mg/kg BW+Simvastatin 0.9 mg/kg BW (GS). Superscript signs on Figure 8a (a, b, c, cd, d, e) and Figure 8b (a, b, c, cd, de, e, f) indicate significant differences ($p<0.05$) among samples obtained from Tukey's HSD.

U/mL) (12.59 U/mg protein) exhibited significantly reduced SOD levels ($p < 0.05$). However, treatment with CTE increased SOD levels, with the most effective treatment observed with CTE at 800 mg/kg BW (11.39 U/mL) (31.64 U/mg protein). Notably, CTE at 800 mg/kg BW did not significantly differ from the negative control group, suggesting that it was effective in raising SOD levels. Fig. 3a–b illustrates the effect of CTE on liver CAT levels in rat models of DM and dyslipidemia. The findings indicated a significant reduction in CAT levels within the PC group (35.19 U/mL) (144.23 U/mg protein) ($p < 0.05$). However, treatment with CTE at 800 mg/kg BW (72.16 U/mL) (200.45 U/mg protein) ($p < 0.05$) demonstrated having the capacity to elevate CAT levels. Additionally, CTE at a dosage of 800 mg/kg BW exhibited a stronger effect on elevating liver CAT levels compared to SV, GC, and GS. Thus, CTE was considered effective in increasing liver CAT levels. These results are in line with the study carried out by Jayaraman et al., which demonstrated that the presence of flavonoids in CTE promotes the elevation of SOD and CAT levels in STZ-induced DM rats [25]. The increased levels of these antioxidant enzymes can help reduce damage to pancreatic β cells, normalize insulin levels, and subsequently enhance SOD and CAT activities (see Fig. 4).

Oxidative stress-induced cell damage is often characterized by elevated levels of intracellular enzymes such as LDH and ACP [8,26]. In the current research, treatment with CTE increased LDH levels in the PC group (77.78 U/L) (0.32 U/mg protein), whereas CTE treatment resulted in a decrease in LDH levels ($p < 0.05$). The optimal doses of CTE were 400 mg/kg BW (51.74 U/L) (0.15 U/mg protein) and 800 mg/kg BW (49.62 U/L) (0.14 U/mg protein), which showed no significant difference compared to GC and GS. Therefore, CTE is effective in reducing liver LDH levels in rats with DM and dyslipidemia. Fig. 5a–b displays the effect of CTE on liver

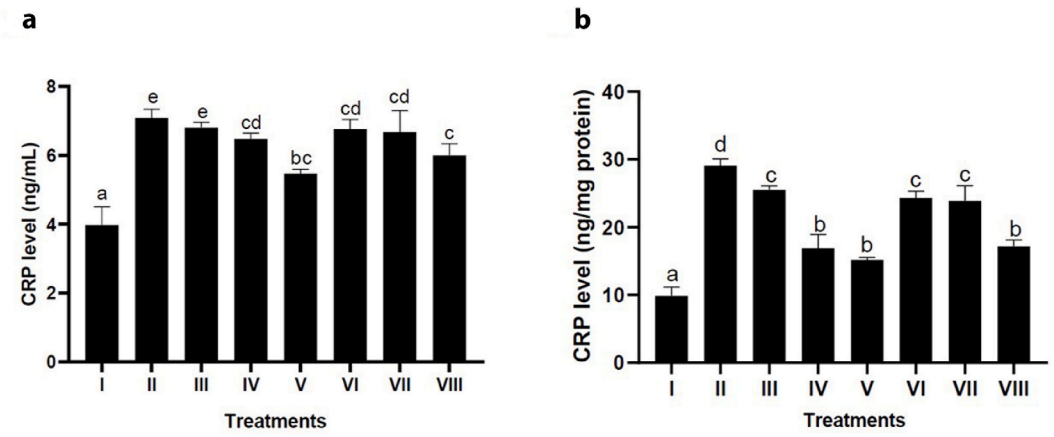

Fig. 9. Effect of CTE on liver CRP levels in DM and Dyslipidemia Rat Model. (a) CRP levels (ng/mL); (b) CRP level (ng/mg protein)
*The data are presented as means±SD and were obtained from four repetitions. The experimental groups: Group I: Negative control (NC) comprising normal rats, Group II: Positive control (PC) consisting of rats with DM and dyslipidemia, Group III: PC+CTE 200 mg/kg BW (CTE200), Group IV: PC+CTE 400 mg/kg BW (CTE400), Group V: PC+CTE 800 mg/kg BW (CTE800), Group VI: PC+Simvastatin 0.9 mg/kg BW (SV), Group VII: PC+Glibenclamide 0.45 mg/kg BW (GC), and Group VIII: PC+Glibenclamide 0.45 mg/kg BW+Simvastatin 0.9 mg/kg BW (GS). Superscript signs on Figure 9a (a, bc, c, cd, e) and Figure 9b (a, b, c, d) indicate significant differences ($p<0.05$) among samples obtained from Tukey's HSD.

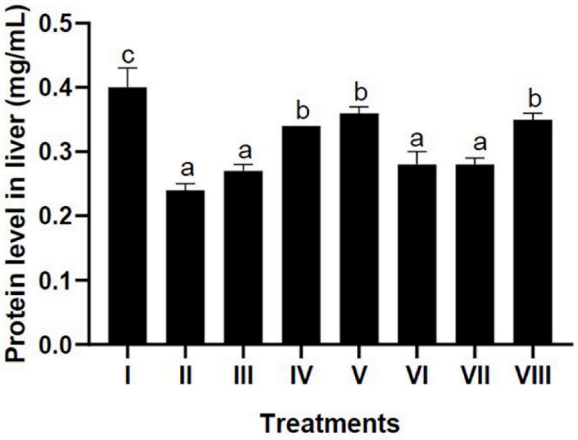

Fig. 10. Effect of CTE on Protein Level in DM and Dyslipidemia Rat Model
*The data are presented as means±SD and were obtained from four repetitions. The experimental groups: Group I: Negative control (NC) comprising normal rats, Group II: Positive control (PC) consisting of rats with DM and dyslipidemia, Group III: PC+CTE 200 mg/kg BW (CTE200), Group IV: PC+CTE 400 mg/kg BW (CTE400), Group V: PC+CTE 800 mg/kg BW (CTE800), Group VI: PC+Simvastatin 0.9 mg/kg BW (SV), Group VII: PC+Glibenclamide 0.45 mg/kg BW (GC), and Group VIII: PC+Glibenclamide 0.45 mg/kg BW+Simvastatin 0.9 mg/kg BW (GS). Superscript signs (a, b, c) indicate significant differences ($p<0.05$) among samples obtained from Tukey's HSD.

ACP levels in rats with DM and dyslipidemia. According to the findings, the PC group's ACP levels significantly increased (41.10 U/L) (0.17 U/mg protein). However, treatment with CTE at 800 mg/kg BW (24.83 U/L) (0.07 U/mg protein) significantly declined ACP levels ($p < 0.05$). Furthermore, the findings demonstrated that CTE at a dosage of 800 mg/kg BW did not exhibit a statistically significant difference compared to the SV, GC, and GS groups. Hence, CTE proved to be potent in reducing liver ACP levels in rats with DM and dyslipidemia. These effects can be attributed to the antioxidant activity of CTE, which is due to the inclusion of flavonoids and anthocyanins. Flavonoids and anthocyanins act as potent antioxidants, capable of counteracting free radicals and protecting cells and tissues from damage [27].

In addition to the increase in LDH and ACP enzymes, elevated levels of ALT and AST in the liver are indicative of cellular apoptosis and damage [15]. Based on the research findings, the PC group exhibited increased AST and ALT levels ($p < 0.05$). Treatment with CTE at 800 mg/kg BW demonstrated effectiveness in reducing liver AST levels (Fig. 6a–b) and ALT levels (Fig. 7a–b). The optimal level of CTE that yielded minimal change in results compared to GS was found to be 800 mg/kg BW, resulting in 15.46 IU/L (0.04 IU/mg protein) (Fig. 6a–b). In liver ALT levels results, the PC group (31.61 IU/L) (0.13 IU/mg protein) also exhibited increased ALT levels ($p < 0.05$). The most active dose of CTE observed was CTE 800 mg/kg BW (15.16 IU/L) (0.04 IU/mg protein) (Fig. 7a–b). These findings are consistent with the study conducted by Nithianantham et al., which highlighted the hepatoprotective effects of CTE. The study showed that CTE significantly decreased the levels of serum bilirubin, ALT, and AST [28]. This hepatoprotective effect can be

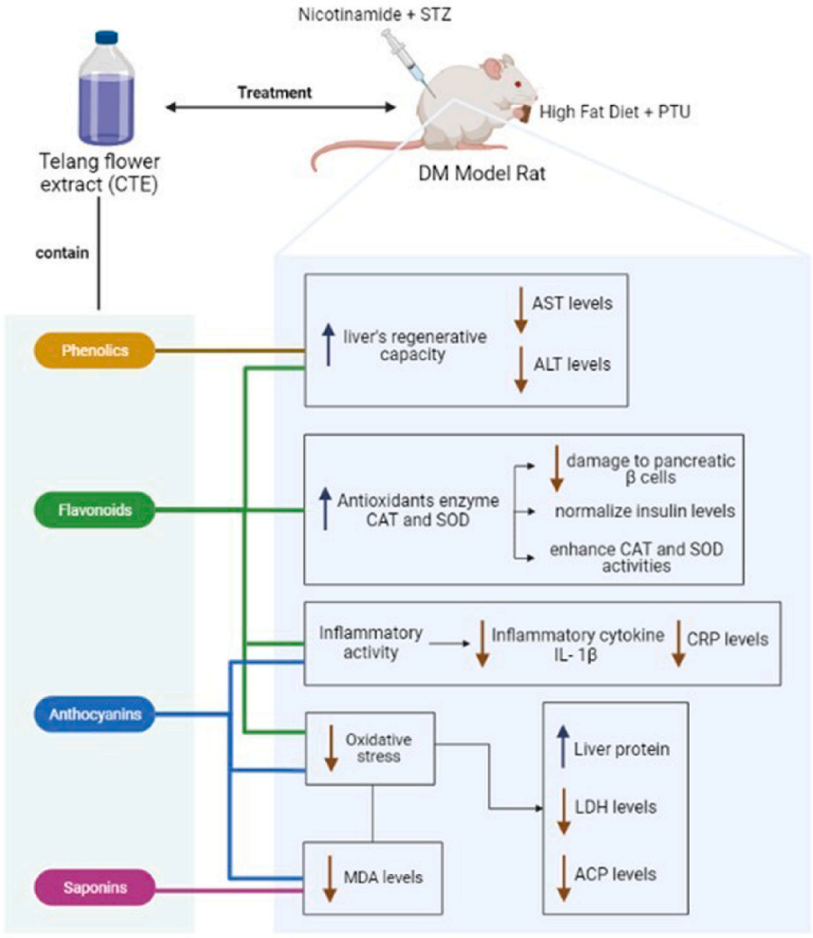

Fig. 11. Proposed mechanism on how CTE influence metabolic syndrome rats' model
⁎CTE is believed to have a hepatoprotective effect with phenolics and flavonoids content playing a crucial part in scavenging elevated free radicals. Also, flavonoids in CTE have shown the ability to increase the activity of antioxidant enzymes such as CAT and SOD. By elevating the activity of these enzymes, damage pancreatic β cells can be prevented, helping to maintain normal insulin levels in the body. CTE combats oxidative stress by countering free radicals, thereby protecting cells and tissues from damage. Consequently, levels of ACP and LDH, which indicate cell death and cellular injury, are reduced, suggesting decreased injury to the liver and an elevation in the level of liver protein. Additionally, the antioxidant anthocyanins and saponins present in CTE can further contribute to reducing the level of MDA, another indicator of oxidative stress. CTE also can suppress the pro-inflammatory cytokine IL−1β and reduce the levels of CRP, a protein that increases during inflammation, by inhibiting secondary enzymes.

explained by the presence of different flavonoid and phenolic components in CTE, which enhance the liver's regenerative capacity.

Free radicals are produced when oxidative stress, hyperinsulinemia, and hyperglycemia are present. This causes inflammation and cellular necrosis. Previous studies have demonstrated a direct correlation between markers of lipid peroxidation, diabetes, and inflammation cytokine expression, especially for TNF-α and IL-1β [29]. In DM and dyslipidemia, inflammation results in the bloodstream release of IL-6, which in turn triggers the liver's production of CRP and other acute-phase proteins [30]. CTE treatment at 800 mg/kg BW (Fig. 8a–b) lowers IL-1β levels, demonstrating its anti-inflammatory properties due to the presence of flavonoids like quercetin and anthocyanins. Furthermore, CTE at 400 and 800 mg/kg BW (Fig. 9a–b) reduces CRP levels through the contribution of flavonoids and anthocyanins [31,32]. Additionally, the results demonstrate that CTE 800 mg/kg BW reduces IL-1β levels to a greater extent compared to SV, GC, and GS. These results can be explained by the fact that CTE has anti-inflammatory properties. Research carried out through *in vivo* by Jeyaraj et al. has demonstrated the anti-inflammatory activity of CTE [33]. CTE exerts its anti-inflammatory effects by inhibiting secondary enzymes like nitric oxide synthase (NOS), lipooxygenase (LOX), cyclooxygenase (COX), and phospholipase A2 (PLA2) [34] (see Fig. 10).

The liver is essential in preserving the body's homeostasis and engages in several metabolic activities, including the metabolism of proteins, fats, and cholesterol, among others [35]. However, oxidative stress, in addition to causing damage to cells and tissues, can also lead to protein damage [36]. In DM and dyslipidemia, oxidative stress causes a decrease in liver protein levels. However, the study's findings reveal that CTE at 400 and 800 mg/kg BW dosages increased liver protein levels in rats with DM and dyslipidemia. These results indicate the hepatoprotective effects of CTE. Previous research has shown that flavonoids possess antioxidant properties, which can reduce lipid peroxidation and oxidative stress, thereby preventing liver damage [37,38]. As a result, liver protein levels are

increased. The overall mechanism for CTE treatment in DM model rats is shown in Fig. 11.

4. Conclusions

CTE exhibits the ability to increase levels of liver protein, CAT, and SOD. Additionally, CTE has the potential to decrease levels of IL-1β, CRP, AST, ALT, LDH, and MDA in the liver of rats with DM and dyslipidemia. Overall, these discoveries indicate that CTE shows potential as a viable treatment for DM.

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Ethics statement

The research protocol received approval from the Ethical Committee of Maranatha Christian University, Bandung, Indonesia, and Immanuel Hospital, Bandung, Indonesia (147/KEP/VI/2021).

CRediT authorship contribution statement

Wahyu Widowati: Writing – review & editing, Writing – original draft, Supervision, Funding acquisition, Conceptualization. **Lusiana Darsono:** Writing – review & editing, Writing – original draft, Conceptualization. **Herry S. Utomo:** Writing – review & editing, Writing – original draft, Resources, Conceptualization. **Adilah Hafizha Nur Sabrina:** Writing – review & editing, Writing – original draft, Methodology, Data curation. **Maria Rizka Natariza:** Writing – review & editing, Writing – original draft, Methodology, Data curation. **Albert Christoper Valentinus Tarigan:** Writing – review & editing, Writing – original draft, Methodology, Data curation. **Novaldo Wahid Waluyo:** Writing – review & editing, Writing – original draft, Methodology, Data curation. **Abigail Maydaline Gleyriena:** Writing – review & editing, Writing – original draft, Visualization, Methodology. **Berlian Haifa Siahaan:** Writing – review & editing, Writing – original draft, Visualization, Methodology, Conceptualization. **Reza Oktaviani:** Writing – review & editing, Writing – original draft, Methodology, Data curation.

Declaration of competing interest

The authors declared the following financial interests/personal relationships which may be considered as potential competing interests: Wahyu Widowati reported financial support was provided by Maranatha Christian University. Wahyu Widowati reported a relationship with Maranatha Christian University that includes: employment and funding grants. The other authors declared that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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