The Effects of *Curcuma heyneana* Ethanolic Extract on the Superoxide Dismutase Activity and Histological Pancreas of Type 1 Diabetes Mellitus Rats

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Abstract

Diabetes Mellitus (DM) ordinarily cause anatomically and functionally.organ damage. Herbal therapy using *Curcuma heyneana* ethanolic extract is one of DM treatment. The object of the research in order to reveal effect of *C. heyneana* ethanolic extract on Superoxide Dismutase (SOD) activity and pancreatic beta cells repearing of DM rats. DM type 1 rats induced by 20 mg/kgBW streptozotocin (Multiple Low Dose) for 5 consecutive days use as animals model. *C. heyneana* ethanolic extract given administrated to DM rats in varying doses of 36, 72, and 108 mg/kgBW for 7 consecutive days. The result showed that *C. heyneana* ethanolic extract was able to increase SOD activity and repair the pancreatic beta cells damage on DM rats induced by MLD-STZ. 72 mg/kgBW ethanolic extract of *C. heyneana* is the optimal dose for therapy DM rats.

Keywords: *Curcuma heyneana*, MLD-STZ, SOD activity

Introduction

Diabetes Mellitus (DM) type 1 is an autoimmune caused by pancreatic beta cell damage. The damage of pancreatic beta cell is caused by mononuclear cells infiltration [10]. Destruction of pancreatic beta cells decreased insulin production so that blood glucose content increased called hyperglycemia [12].

Hyperglycemia triggers autooxidase on glucose so reactive oxygen species (ROS) generated. Accumulation of ROS especially in free radical form superoxide (O^{*}) characterized on *Superoxide Dismutase* (SOD) enzyme decreasing [8].

To make DM type 1 white rat (*Rattus norvegicus*) Wistar strain used as experimental animal by *Multiple Low Dose Streptozotocin* (MLD-STZ) induced [1]. STZ releases NO radical, superoxide radical and hydrogen peroksida radical in large amount, so it accelerate the destruction of pancreatic beta cells characterized by glucose blood level increasing [11]. Enzymatic antioxidant decrease if free radical increase [4].

C. heyneana is one of medicinal plant include in genus *Curcuma* having antioxidant activity. *C. heyneana* rhizome water extract in a previous study were reported lowered blood glucose content in rats exposed to MLD-STZ [6].

In previous study by Betty Lukiati (2010) unpublish known that *Curcuma heyneana* ethanolic extract contain antioxidant as curcumin and flavonoid. This research in order to prove the ability of antioxidant contained of that extract can catch free radical in pancreas of DM rats. So *C. heyneana* ethanolic extract can use as an alternative therapy on Diabetes Mellitus type 1.

Materials

- **Plants:** *C. heyneana* rhizome was obtained from BMM (Balai Materia Medica) Batu
- **Animals:** male Wistar Rats weighting 150 to 200 g weight, 60-70 days old, obtained Laboratory of Molecular Biology Brawijaya University.
- **Chemical compound:** Streptozotocin (MP Biomedicals, Inc Ohio), citrate buffer, pH 4,5, NaCl, Phosphat Buffer Saline (PBS), Paraformaldehide (PFA) 4%, xilol dilution, liquid paraffin, hematoxylin, eosin, ethanol, SOD assay kit Northwest Life Science USA (Cat. No. NWK-SODO2).

Methods

- **Extraction of *C. heyneana* Rhizome:** 2 kg of dried powder *C. heyneana* rhizome macerate with ethanol 95% in 24 hour. After filtered the residu were

macerated again and again until the clearing filtrate obtained. All of the filtrates were mixed and evaporate with rotary evaporator, so concentrate filtrates were obtained, according [7].

- **DM rat induction:** Rats with 150-200 g weight, 60-70 days old were injected with 20 mg/kgBW STZ for 5 consecitive days and incubated for 14 days. After incubation measured the blood glucose contain used glucometer(One Touch Ultra. Life Scan. Inc. USA Inverness Medical Ltd). The rats suffered DM if the blood glucose contain >300 mg/dl [5].
- **Experiment:** 30 rats devided into 5 group:
  (1) healthy group as a negative control
  (2) DM rats without *C. heyneana* ethanolic extract as a positive control
  (3) DM rats given 36 mg/kgBW *C. heyneana* ethanolic extract orally
  (4) DM rats given 72 mg/kgBW *C. heyneana* ethanolic extract orally
  (5) DM rats given 108 mg/kgBW *C. heyneana* ethanolic extract orally
  The administration *C. heyneana* ethanolic extract orally to group 3,4, and 5 on 7 concecitive days. 14 days after treatment all animals experiment were dissected. All of the pancreas were collected in each group, a part of the number of pancreas tested for SOD activity, the other part were made slide to search the histology of the tissue.
- **Histological Observation:** pancreatic tissue fixed in Bouin's solution, slice and stained with hematoxylen-eosin. The slide observed by light microscope and made it microphotographe.
- **SOD activity measured:** 15 µl of pancreatic homogenate dissolved in 30 µl dilution buffer. All ELISA well plate were filled 15 µl assay buffer then devided into 2 group. One group were added 5 µl assay buffer as a blank test, and the other group add 5 µl homogenate as sample test. Incubated for 2 minute, then add 5 µl reagent hematoxylen and absorbance measure at λ560 nm every 5 minute for 30 minute.

Result

C. heyneana ethanolic extract effect on SOD activity and beta cells repairing of DM rats.

- SOD activity in pancreas DM rats as effect of *C. heyneana* ethanolic extract showed at figure 1. SOD activity of rats induced by MLD-STZ decreased up to 55,60% compare of control group. SOD activity in DM rats pancreas increased after *C. heyneana* ethanolic extract dose 36 mg/kgBW, 72 mg/kgBW, and 108 mg/kgBW treatment. According to the One-way ANOVA, effect of *C. heyneana* ethanolic extract to the increasing of SOD activity in pancreas of DM rats is significant (p<0,01).

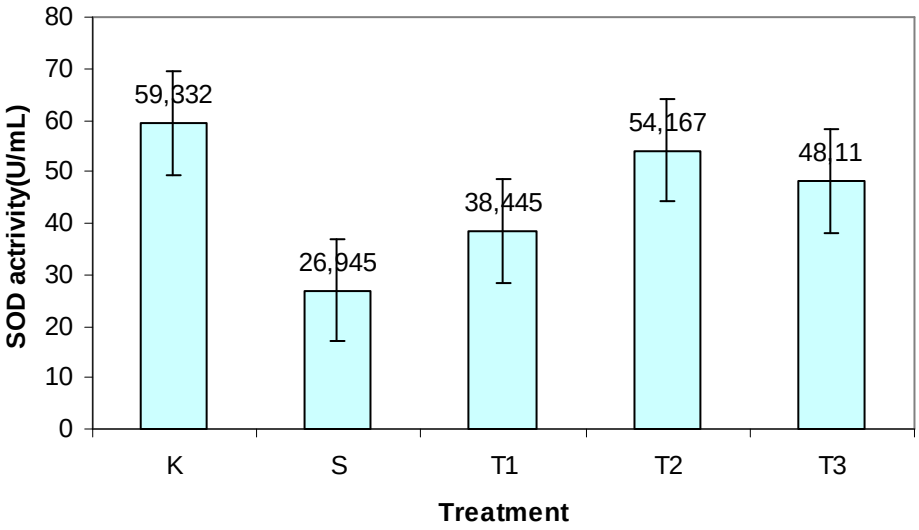

Figure 1: Effect of ethanolic extrac of *C.heyneana* to SOD activity in Pancreatic Rat (*Rattus norvegicus*). 5 wk after treatment.

K: control group, S: streptozotocin treated group, T1: streptozotocin + 36 mg/kgBW extract treated group, T2: streptozotocin + 72 mg/ kgBW extract treated group, T3: streptozotocin + 108 mg/kgBW extract treated group.

72 mg/kgBW *C. heyneana* ethanolic extract is an optimum dose for SOD activity increasing in DM rats.

- Oxidative stress in DM rats induced by MLD-STZ caused beta cells damage. The degrre of beta cells damage scores range 0-4 [2].
  Score 0 if not found beta cells damage,
  Score 1: if pancreatic beta cells damage <25%,
  Score 2: if pancreatic beta cells damage 25-50%,
  Score 3: if pancreatic beta cells damage 50-75%,
  Score 4: if pancreatic beta cells damage >75%.

Repairing the pancreatic beta cells damage as effect of *C. heyneana* showed at figure 2. The slide of pancreas tissue observed by light microscope appear the selectif destruction of pancreatic beta cells characterized by reducing a number of beta cells on Langerhans island central. The insulitis DM rats reach score 3 to score 4, it means 51% - >75% beta cells damage. *C. heyneana* ethanolic extract dose 36 mg/kgBW, 72 mg/kgBW, and 108 mg/kgBW inhibited pancreatic beta cells damage effectively, so beta cells repairing (Figure 3).

According to the One way ANOVA, effect of *C. heyneana* ethanolic extract to repairing beta cells of DM rats is significant ($p<0,01$). 72 mg/kgBW *C. heyneana* ethanolic extract is an optimum dose for pancreatic beta cells repairing in DM rats.

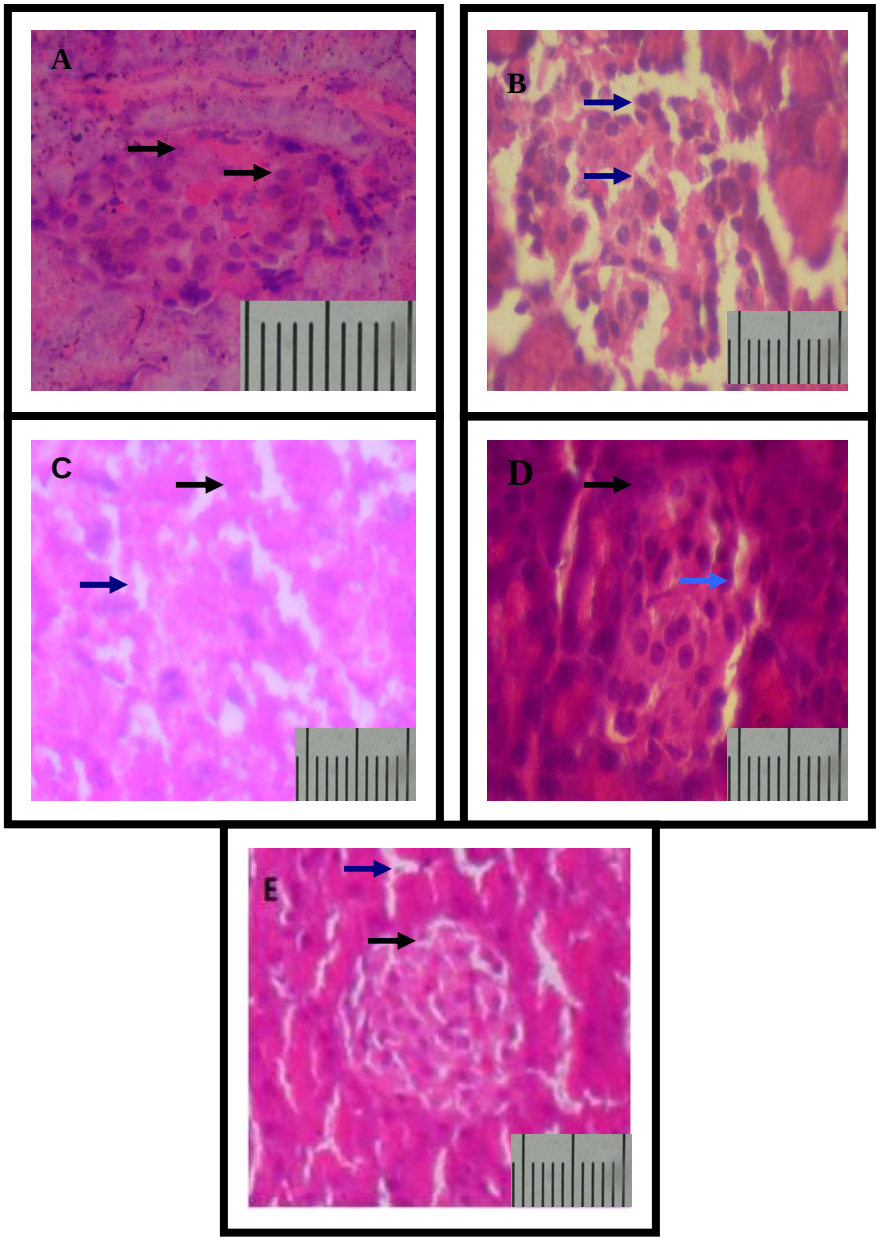

Figure 2: Histological of Langerhans island of rat (*Rattus norvegicus*).
K: control group, S: streptozotocin treated group, T1: streptozotocin + 36 mg/kgBW extrac treated group, T2: streptozotocin + 72 mg/kgBW treated group, T3: streptozotocin + 108 mg/kgBW extract treated group 5 wk after treatment. → : beta cell, → :inter cells space.

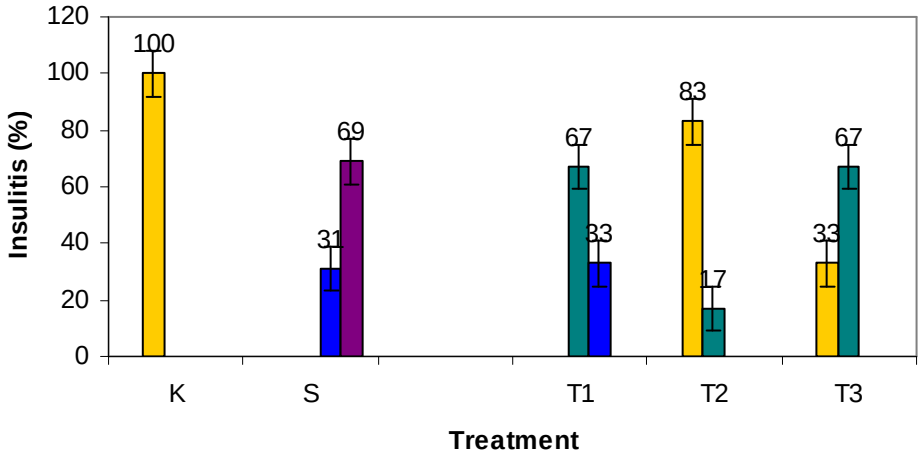

Figure 3: Score of insulitis of rat (*Rattus norvegicus*

K: control group, S: streptozotocin treated group, T1: streptozotocin + 36 mg/kgBW extrac treated group, T2: streptozotocin + 72 mg/kgBW treated group, T3: streptozotocin + 108 mg/kgBW extract treated group 5 wk after treatment.Score:1 < 25% damage, 2 25-50% damage, 3 51-75% damage, 4 >75%. Damage.

Discussion

Streptozotocin is a toxic compound that is specific to pancreatic beta cells. This compound is a source of NO^* radicals and OH^* radicals wich caused stress oxidative and pancreatic beta cells damage in DM rats. The destruction of beta cells are occur when macrophages as mononuclear migrate to the islet of Insula Langerhans, called insulitis. Insulitis is followed the pancreatic beta cells phagocytosis by macrophages, so pancreatic beta cells mass is reduced [11].

The increasing levels of NO^* destruct pancreatic beta cells too, because the peroxynitrie ($ONOO^-$) in cytoplasm increase. Peroxonytrite is highly reactive, destruct, and make pancreatic beta cells died [8].

The increasing of free radicals in the pancreas promote enzymatic antioxidant such as *Superoxide Dismutase* (SOD) activity decreased. According

[3], peroxynitrite radicals can make the amino acid tyrosin residues in SOD catch nitrit so it lead modification an inactivation of SOD.

C. heyneana ethanolic extract has an ability to catch free radical doe to the flavonoid and curcumin contain in ethanolic extract, so pancreatic beta cells damage can prevented by it action. Hydroxyl groups on the flavonoid and curcumin aromatic ring donate a hydrogen atom to free radicals. Resonance by the conjugated double bond caused the new radicals were less reactive. Free radicals decreased and reduced the macrophage activity trigger, so the risk of beta cells phagocytosis by macrophages prevented.

Reduction free radicals can prevent oxidative stress by *Reactive Oxygen Species* (ROS), so the process of inactivation SOD can be prevented. It make SOD activity increased. Flavonoid and curcumin also increase the enzymatic antioxidant activity *Glutation peroxydase* and *Superoxyde dismutase* .

Conclusion

C. heyneana ethanolic extract doses 36, 72, and 108 mg/kgBW were able to increase SOD activity and repaire the pancreatic beta cells damage in DM rats. 72 mg/kgBW *C. heyneana* ethanolic extract is an optimum dose for therapy DM rats induced by MLD-STZ. The research can be continued to reveal the effect *C. heyneana* ethanolic extract on insulin product of DM rats.

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