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# Effect of *Clitoria ternatea L*. Extract on Melatonin, NRF2, and MDA Levels in Diabetic RAT

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# ABSTRACT

Oxidative stress is one of the mechanisms that causes pancreatic damage and the complications of diabetes mellitus (DM). Activating Nuclear Factor Erythroid 2-Related Factor 2 (NRF2) is important in protecting pancreatic cells from oxidative stress and inflammation. *Clitoria ternatea L*. (CT) flower was studied for its antioxidative effects in diabetic rats. Indeed, there has not been a lot of study on how CT impacts melatonin levels and NRF2 activity. CT flower was extracted using maceration procedures with 96% ethanol. The male Wistar rats (n=25) were randomly divided into five groups, including the control group, which received normal saline orally for 14 days. Diabetes groups received intraperitoneally (i.p.) streptozotocin (STZ) as a single dose (45 mg/kg, i.p.). Normal saline, CT (100 and 200 mg/kg), and metformin (150 mg/kg) were orally administered to diabetic rats for 14 days. The melatonin, NRF2, and malondialdehyde (MDA) levels were measured in serum. CT flower extract had no effect on melatonin levels in diabetic rats (p>0.05), but significantly increased NRF2 levels and decreased MDA levels (p<0.05). CT can activate NRF2 and reduce oxidative stress in DM, and its administration may prevent diabetes complications.

Keywords: Clitoria ternatea L; Diabetes Mellitus; Melatonin; MDA; NRF2.

# **INTRODUCTION**

Reactive oxygen species (ROS) are one of the main causes of insulin resistance, cell dysfunction, and diabetes complications (Bhatti et al., 2022). Overproduction of ROS makes the liver, muscles, and fat resistant to insulin. This leads to hyperglycemia, which promotes markers of chronic inflammation and stimulates the production of additional ROS (Luc et al., 2019; X. Wang & X. Hai, 2011).

Reactive oxygen species are produced in the pancreas as a byproduct of oxidative phosphorylation in mitochondria. Protecting the pancreas from ROS requires endogenous antioxidant defenses. Pancreatic beta cells have a lesser antioxidant defense than the liver to counter the continuously produced superoxide anion (J. Wang & Wang, 2017). This makes beta cells very sensitive to ROS signaling and very vulnerable to oxidative stress. Persistent oxidative stress that is not mitigated by antioxidants leads to the destruction and death of pancreatic beta cells, which in turn causes GSIS diseases. (Gerber & Rutter, 2017; X. Wang & X. Hai, 2011).

The neurohormone melatonin has antioxidant action. It prevents oxidative stress from

occurring and maintains constant levels of glutathione and glutathione peroxidase activity in diabetic animals' pancreases (Abdulwahab et al., 2021). Based on research with diabetic rat models. melatonin and insulin have been found to have an antagonistic relationship. Type 2 diabetic rats have low melatonin levels and rising insulin levels, whereas type 1 diabetic rats have high melatonin levels and low insulin levels (Peschke et al., 2015). Research shows that melatonin plays a part in reducing problems by activating the Sirtuin 1 (SIRT1)/NRF2/Heme oxygenase-1 (HO-1) pathway, specifically acute kidney ischemia (Shi et al., 2019).

Nuclear Factor Erythroid 2-Related Factor 2 is a transcription factor that controls the production of several cytoprotective genes in response to oxidative stress or inflammation (Ahmadi & Ashrafizadeh, 2020; Francisqueti-Ferron et al., 2019). Activation of the NRF2 pathway protects and preserves pancreatic cell mass under hyperglycemia-induced conditions of increased ROS (Baumel-Alterzon et al., 2021). It has been well demonstrated that reducing oxidative stress can be achieved by increasing NRF2 activity when there are elevated ROS (Hendrawati, 2017).

The antioxidant properties of natural products and their bioactive constituents have been demonstrated in several in vivo and in vitro investigations (Bhatti et al., 2022). Clitoria ternatea L.(CT), also known as 'Kembang telang' and 'Flower pea,' is an antioxidant plant whose various parts have been traditionally utilized in Ayurvedic (SK et al., 2018). CT flower medicine. phytochemicals consist of kaempferol, quercetin, myricetin, glycosides, and anthocyanin. (Jeyaraj et al., 2021). Studies on CT extract in rats induced by STZ showed that pancreatic regeneration and decreased oxidative stress contributed to the restoration of cell function (Verma et al., 2013). Healthy volunteers who were administered sucrose and CT flower extract demonstrated that CT could increase plasma antioxidant capacity and lower plasma MDA concentrations (Chusak et al., 2018).

# RESEARCH METHOD

**Extraction procedures** 

Dried flowers of CT were collected from the garden in Cipicung, Kuningan, as much as 600 grams in Mei 2022. CT flowers have been identified by BRIN (B-1912/II.6.2/DI.05.07/6/2022). Extraction was carried out at the Laboratory Herbal Yarsi using the maceration method. The dried flower of CT (approximately 600 g) was added to Ethanol 96 percent (6000 mL) and vortexed until homogeneous. The filtrate was evaporated using a 40°C rotary evaporator so that an extract of 212.4 grams was obtained.

# Animal studies

Twenty-five male adult Wistar rats (175-200 gr) were used to study the antioxidant activity of the CT flower extract. The Institutional Research Ethics Committee of Universitas Yarsi has approved the animal study for this study (155/KEP-UY/BIA/V/2022). The animals were kept at 35-37°c, given standard feed, and drinking ad libitum. Experimental animals were placed individually with sufficient light conditions and good air circulation. Constant 12-hour light/dark timing. This adaptation is intended so that all experimental animals are not under stressful conditions and are in the same conditions as when the study started.

Rats were randomly divided into five groups of five rats each. The first group received a single daily dose of aquadest orally, group II was given STZ and a single dose of aquadest orally, group III was given STZ and single dose CT 200 mg/ kg weight orally (Verma et al., 2013), group IV was given STZ and single dose CT 100mg/ kg weight orally, and group V was given STZ and single dose metformin generic Hexapharm Jaya Kalbe 150 mg/kg weight orally.

# Melatonin test

The Elabscience Melatonin KIT was used to measure melatonin levels. A five-time dilution of the serum was used. The sample and standard were placed in the well, followed by the addition of the available biotinylated Ab working solution. Incubate at 37 degrees Celsius for 45 minutes, then aspirate and wash the plate. Add the HRP conjugate working solution and incubate at 37 °C for 30 minutes. Before introducing the substrate reagent, aspirate and clean the well. Before the optical density (OD value) is measured with the Tecan Elisa reader at 450 nm, the reaction will be stopped with a stop solution.

# NRF2 test

The Elabscience brand NRF2 KIT was used to measure NRF2 levels. A five-time dilution of the serum was used. The wells were filled with samples and standards, which were then incubated before the working solution of biotinylated AB was added. Incubate at 37c for 45 minutes, then aspirate and wash dishes. Add the HRP conjugate working solution and incubate at 37 °C for 30 minutes. Just before adding the substrate reagent, aspirate and thoroughly wash. Before the optical density (OD) is measured using the Tecan Elisa reader at 450 nm, the reaction will be stopped with a stop solution.

# MDA test

The lipid peroxidization study was carried out using the Thiobarbituric Acid Reactive Substances (TBARS) method developed by Ganta et al., 2021. The reagent material consists of 0.5 cc of serum that has been diluted 20 times, 20% TCA, 0.5% TBA, and 2.5N HCL placed in a test tube. The solution is heated in a water bath for 20 minutes and then centrifuged at 2,000 rpm for 10 minutes. Take supernatant to be evaluated the bv spectrophotometry at 532 nm and compare it to a blank containing all reagents but no biological sample. The MDA of the sample was computed utilizing an extinction coefficient of 1.56 105 M 1 cm 1. (Ganta et al., 2021)

# RESULTS

Table 1 shows that the mean melatonin levels were higher in the diabetic group than in the normal group. The mean difference between the diabetic and diabetic receiving CT groups was not statistically significant. This shows that there is no effect of CT in diabetic rats.

Parameter	Normal rats	Diabetic rats	Diabetic rats treated with CT 200	Diabetic rats treated with CT 100	Diabetic rats treated with metformin 150
Melatonin	1401.96±65.47	1955.12±343.70	1787.95±157.61	1851.53±55.59	1856.34±224.49
NRF2	274.18±42.12	223.67±34.95	$299.60{\pm}40.40^{*}$	$285.97 \pm 55.59^*$	$287.38{\pm}48.02^*$
MDA	0.22±0.15	1.24±0.56	$0.55{\pm}0.23^{*}$	$0.42{\pm}0.26^{*}$	$1.08{\pm}0.40$

Table 1. Effect of C. ternatea L. extract on Melatonin, NRF2, and MDA

Results are expressed as mean±Stdev; \*Statistically significant compared to STZ-induced rats' group (p<0.05)

The NRF2 levels in the five groups of rats had significant average differences, as shown in Table 1. The highest average NRF2 levels were in the CT 200 group and the lowest in the diabetic group. Based on table 1, it was concluded that the CT 100 and CT 200 groups had significant value in increasing NRF2 levels.

The MDA levels of the five groups of rats had a significant average difference, as shown in Table 1. The diabetic rats had the highest MDA levels, while CT supplementation significantly decreased MDA levels in the diabetic rats. There was no significant difference in the mean between the metformin and diabetes groups.

# DISCUSSION

Streptozotocin promotes hyperglycemia by decreasing the release of insulin and damaging pancreatic cells (Graham et al., 2011; Koksal, 2015). Several studies demonstrate that the use of STZ is an effective method for significantly reducing plasma insulin levels in rats and mice (Koksal, 2015).

The stimulation of insulin secretion through modulation of intracellular cAMP has been confirmed to have an inhibitory effect on melatonin (Peschke et al., 2015). Research has established that insulin and melatonin work in antagonism each other. (Banihani et al., 2020; Espino, 2011; Peschke et al., 2015; She et al., 2014). Rats with type 1 diabetes have extremely low insulin levels and increased plasma melatonin levels, according to a Wistar rat study (Peschke et al., 2015). The increase in melatonin in this study may have occurred because there is no negative feedback mechanism from insulin to the pineal gland, which secretes melatonin.

Computerized Tomography administration did not affect melatonin levels in the STZ-induced group. Even though the administration of CT flower extract decreased oxidative stress, it was insufficient to restore melatonin levels. In contrast to a previous study, providing fresh pomegranate juice to patients with impaired glucose tolerance resulted in a decrease in melatonin levels and an increase in insulin. (Banihani et al., 2020). When analyzed further with the existing literature, it can be concluded that giving CT flower extract is not enough to restore melatonin levels.

This study shows that CT can activate NRF2 in STZ-induced rats. Antioxidant-rich components of CT may be the reason for this. Mukherjee et al. and Kazuma et al. reported the phytochemical content of CT flowers as ternatin anthocyanins and various flavonol glycosides of kaempferol, quercetin, and myricetin (Jeyaraj et al., 2021). Giving quercetin to STZ-induced rats showed a low Nrf2 fluorescence density in the DM group, which increased with increasing quercetin doses (Dong et al., 2022). The administration of anthocyanins to rats induced by STZ shows that anthocyanins can activate NRF2 signaling in the retinal tissue of mice compared to the diabetes group (Song et al., 2016). Several studies have shown that anthocyanins activate NRF2 factors and antioxidant enzymes, such as superoxide dismutase, catalase, and glutathione peroxidase (Salehi et al., 2020).

The results of this study provide a basis for further investigation into the role of antioxidants and their potential application in the prevention of and the management of diabetic disease complications using CT. A study by Yagishita employing four genetically altered mice models showed that reactive species do not harm pancreatic cells when the NRF2 pathway is activated (Yagishita et al., 2014). Pterostilbene's activation of NRF2 has been shown in studies on mice caused by STZ to protect pancreatic cells from oxidative stress (Sireesh et al., 2017). Through Nrf2/HO-1 signaling, the usage of blueberry anthocyanins in STZ-induced rats demonstrated a protective effect against oxidative stress and inflammation on retinal cells. (Song et al., 2016). NRF2 plays a role in regulating cellular responses to combat harmful levels of oxidative stress (Baumel-Alterzon et al., 2021). The activation of the NRF2 pathway plays a part in defending and maintaining pancreatic cell mass when there is an increase in ROS generation due to hyperglycemia. (Baumel-Alterzon et al., Increased insulin sensitivity and cell 2021). protection provided by NRF2 activation can improve glucose regulation and delay the onset of micro- and macrovascular problems associated with diabetes. (Jiménez-Osorifddeho et al., 2014)

Diabetes development can be predicted using the biomarker MDA's measurement of the generation of lipid peroxidation. The results showed that the formation of the diabetes group was higher than the normal group; this is in accordance with several studies in experimental animals and diabetic patients, which showed higher MDA levels in the diabetes group compared to the normal group (Jiménez-Osorio et al., 2014; Sunita et al., 2020; Tsounapi et al., 2019). High MDA levels in diabetics are an indication of their poor glycemic control (Fatani et al., 2016). Intake of CT flowers during the postprandial phase can decrease MDA levels and increase plasma antioxidant levels. (Chusak et al., 2018; Vidana Gamage et al., 2021). This study also showed that giving CT flowers could decrease the levels of MDA. Giving had lower levels of MDA than metformin, demonstrating that using CT is more efficient at lowering MDA levels.

The antioxidant activities of CT were evaluated using free radical scavengers (Zhang et

al., 2021). Free radicals can be scavenged by rutin content, one of the constituents in CT that contains several OH substitutions, by being given electrons (Ghorbani, 2017). It is also suspected that CT activation of NRF2 contributed to the reduction in MDA levels seen in this study. NRF2 regulates the antioxidant activity of superoxide dismutase (SOD), catalase (CAT), heme-oxygenase 1 (HO-1), glutathione peroxidase 1 (GPx-1), and NAD(P)H: quinone oxidoreductase 1. (Da Costa et al., 2019; Francisqueti-Ferron et al., 2019)

#### CONCLUSION

The results show that CT as a potential antioxidant can reduce free radicals in diabetic rats. CT reduces free radicals by increasing the activity of NRF2. Even though oxidative stress has decreased, melatonin levels have not returned to their normal levels.

# ACKNOWLEDGMENTS

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# **CONFLICT OF INTEREST STATEMENT**

The authors declare no conflict of interest.

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