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Original Article

The Effect of Robusta Coffee (Coffea canephora) on the Expression of SOD and Nrf-2 in Diabetes Mellitus condition (Animal Model)

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ABSTRACT

Hyperglycemia is a condition that occurs when Diabetes Mellitus (DM) is uncontrolled, which causes an increase in free radicals and disruption of cell function and endogenous antioxidants. Coffee, especially robusta coffee, is known to have benefits as an exogenous antioxidant that can increase the activity of endogenous. This study aims to examine the effect of giving robusta coffee on the expression of Nuclear Factor-erythroid-2 Related Factor 2 (Nrf-2) and Superoxide Dismutase (SOD) in DM model mice. The design of this study was experimental with the Post Test Control Group Design method. A total of 24 male Rattus norvegicus mice aged 3 months with a body weight of 230-250 grams were randomly divided into four groups. After undergoing acclimatization for one week, the mice were given robusta coffee once a day for 14 days. On the 15th day, Nrf-2 and SOD expression were examined. The results showed that the average expression of Nrf-2 in each group was: 23.15 ± 1.96 (normal group/KN), 62.14 ± 1.30 (positive DM group/KP), 50.66 ± 2.18 (DM group with low dose coffee/KK1), and 71.13 ± 1.51 (DM group with high dose coffee/KK2). Meanwhile, SOD expression was: 4.95 ± 0.62 (KN), 8.14 ± 0.54 (KP), 6.10 ± 0.79 (KK1), and 9.26 ± 0.31 (KK2). The results of the ANOVA and Post Hoc LSD tests showed a p value <0.05, which indicated a significant difference between groups. In conclusion, administration of robusta coffee (Coffea canephora) was able to increase the activity of the Nrf-2 and SOD enzymes in mice suffering from diabetes mellitus.

INTRODUCTION

Hyperglycemia is a condition in which Diabetes Mellitus (DM) in a patient's body is not controlled. Diabetes Mellitus (DM) is a chronic metabolic disease in which the body cannot produce sufficient insulin or utilize insulin properly to increase blood sugar levels.[1,2] The 2018 Riskesdas report also showed an increase of 1.5% from the 2013 Riskesdas, which was diagnosed by a doctor in the population aged ≥ 15 years.[3] Research (HE et al., 2021) has shown that complications of DM often occur in the male reproductive system, described by decreased sperm quality in the form of decreased motility, sperm concentration, normal sperm morphology, sperm volume, production of seminal plasma material, and sperm DNA damage.[4]

Infertility is a disease that affects the reproductive system, resulting in failure to achieve pregnancy after sexual intercourse without contraception.[5] Data shows that 10-12% of married couples experience infertility, which is classified based on gender factors: women (41%), men

(24%), both parties (24%), and the remaining 11% have unknown causes.[6]

The relationship between hyperglycemia and infertility based on research (HE et al., 2021) shows that male reproductive dysfunction caused by DM is based on hyperglycemia, which affects diabetic vascular neuropathy, oxidative stress injury, abnormal zinc metabolism, and insulin resistance syndrome.[4] Insulin deficiency and resistance in diabetes can damage the hypothalamus, pituitary gland, gonads, and perigonads. Therefore, it can reduce the secretion of sex hormones including *gonadotropin-releasing hormone, Follicle Stimulating Hormone* (FSH), *Luteinizing Hormone (LH),* and testosterone. LH plays a role in regulating the number of Leydig cells, so if LH secretion decreases, the number of Leydig cells will also decrease.[7]

Treatment of DM, especially complications, is still expensive, and an alternative rich in antioxidants is needed, one of which is coffee. Various chemical components in coffee include caffeine, flavonoids, tannins, saponins, chlorogenic acid, trigonellin, carbohydrates, fats, amino acids, organic acids, volatile aromas, and minerals.[6] Coffee is one of the most widely consumed drinks worldwide, with consumption reaching more than 400 billion cups per year. Apart from the stimulant effect of caffeine, coffee contains various bioactive compounds, especially polyphenolic compounds, which act as antioxidants. The antioxidant activity of coffee has been widely studied and reported to have various health benefits, such as reducing the risk of cardiovascular disease, type 2 diabetes, and several types of cancer.[8]

Robusta coffee (*Coffea canephora*) is known to contain higher amounts of the antioxidant compound chlorogenic acid than other coffees, especially Arabica coffee (Coffea arabica). This study tested the antioxidant activity of water macerate from Robusta green coffee bean. The results showed that chlorogenic acid, which is soluble in air, functioned as an antioxidant. Robusta green coffee beans contain more chlorogenic acid than other coffee beans do. Chlorogenic acid has moderate antioxidant activity, which was measured using the DPPH (2,2-diphenyl-1-picrylhydrazyl) method.[9] The mechanism of chlorogenic acid in reducing blood glucose levels is inhibiting fatty acid synthesis either in vitro or in vivo, similar to the mechanism of action of metformin.[10] Both compounds have antimutagenic, anticancer, and antioxidant activities that affect *Reactive Oxygen Species* (ROS).[11] ROS are formed enzymatically via the xanthine oxidase pathway, causing oxidative stress to increase, resulting in a decrease in the activity of the enzyme *Superoxide dismutase* (SOD).[12] *Nuclear Factor-erythroid-2 Related Factor 2* (Nrf-2) is a transcription factor that plays a key role in regulating cellular responses to oxidative stress. When cells experience stress due to increased free radicals, Nrf-2 is activated and moves from the cytoplasm to the nucleus. In the nucleus, Nrf-2 binds to antioxidant response elements (AREs) in DNA, which induces the expression of various antioxidant genes, including genes encoding SOD enzymes. Therefore, researchers want to know the effect of robusta coffee (*Coffea canephora)* on the expression of Nrf-2 and SOD in rats with DM.

METHODS

The type of research carried out was a laboratory experiment on experimental animals with a *Randomized Control Pretest Posttest Group Design Research Design*. This research was carried out in April 2024 at the IBL Laboratory of Sultan Agung Islamic University and received ethical approval number 0546/EA/KEPK/2024 from the Health Polytechnic of the Ministry of Health, Semarang. The research sample used 24 male Wistar rats *(Rattus norvegicus* which were randomly divided into four groups: the negative group, DM condition rats (KN), positive group, DM condition rats and administered metformin (KP), treatment group 1, DM condition rats given 1 ml/grBB coffee (KK1), treatment group 1, DM condition rats given 2 ml/grBB coffee (KK2). In this study, we used a single bean robusta coffee solution or *peaberry coffee.*

The research phase began with the preparation of experimental animals aged 8-12 weeks with a body weight of 230-250 grams and acclimated for 1 week. Subsequently, the experimental animals were grouped and *streptozotocin* (STZ) was induced at a dose of 40 mg/KgBB. After Stz induction, blood tests were carried out before administering robusta coffee with a glucometer to all rats (*Rattus norvegicus*) to monitor the effect of streptozotocin administration. Rats were declared to have hyperglycemia if their blood glucose levels were > 127 mg/dL. Controlled Trials

are used to ensure that each experimental animal has the same conditions before treatment. This includes comparisons of the average body weight, age, sex, and health conditions. Controlling for confounding factors helps reduce variability in research and ensures that each experimental animal has an equal chance of obtaining a good outcome.

A pure Robusta coffee (*Coffea canephora*) solution was prepared in a 1:1 ratio. Robusta coffee powder was weighed using an analytical balance (100 g), placed in a measuring cup, and dissolved in a 1:1 ratio. Robusta coffee powder was weighed using an analytical balance of 100 g, placed in a measuring cup, and dissolved in 100 mL of distilled water. Administration of robusta coffee solution was carried out in the negative control group at 1 mL/250 gramsBB (low dose of treatment), group P1 at 1 mL/250 gramsBB, and group P2 at 2 mL/250 gramsBB of coffee solution for 14 days using an oral needle or sonde. On the 15th day after coffee administration, blood glucose was examined and ELISA examined SOD and Nrf-2 expression. The data obtained were processed using one-way ANOVA and post-hoc LSD tests.

RESULTS

This study aimed to determine the effect of robusta coffee content on the expression of Nrf-2 and SOD. The research was conducted on 24 rats divided into four groups: negative group, DM condition rats (KN); positive group, DM condition rats and given metformin (KP); treatment group 1, DM condition rats given 1 ml/grBB coffee (KK1); treatment group 1, DM condition rats given 2 ml/grBB coffee (KK2). The average results for the expression of NRF-2 and SOD are shown in Table 1.

Table 1. The average results of Nrf-2 and SOD expression in DM condition rats

Variables	Group				*p Anova*
	KN	KP	KK1	KK2	
SOD (%)	23.15± 1.96	62.14± 1.30	50.66± 2.188	71.13± 1.51	0.000*
NRF-2 (pg/ml)	4.95± 0.62	8.14± 0.54	6.10± 0.79	9.26± 0.31	0.000*

***=Significant (*p*<0.05)**

Table 1. shows that the average expression of Nrf-2 and SOD in the control group (KN) was 23.15 and 4.95%, respectively. The results of NRF-2 and SOD expression showed an increase in each dose of treatment, with the highest results in the KK2 group 71.13 and 9.26%, respectively). The ANOVA test results showed p=0.000 (p<0.05), which means that there was a significant difference in each group.

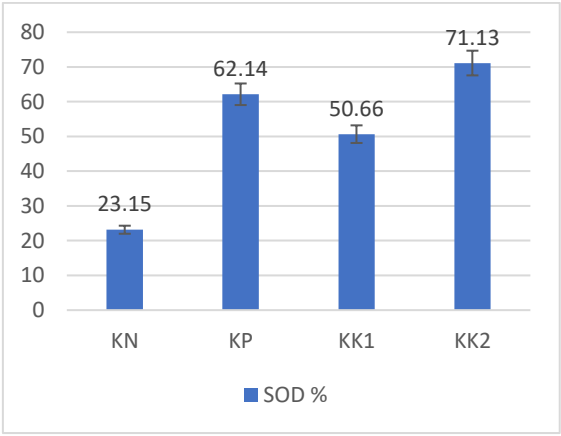

Figure 1. Graph of SOD expression in each group.

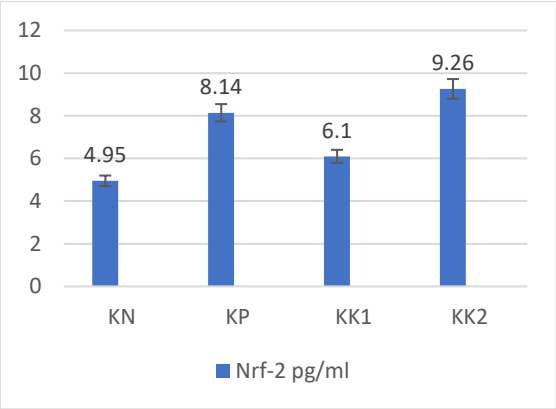

Figure 2. Graph of Nrf-2 expression in each group.

Nrf-2 (4.95pg/ml) and SOD (23.15%) expression were lowest in the KN group and highest in the KK2 group (Nrf-2 9.26pg/ml, SOD 71.13%) with the treatment of DM rats administered robusta coffee 2 ml/grBB. Furthermore, in KP, DM rats were administered metformin, and KK1 in DM rats with 2 ml/grBB coffee administration.

Table 2. Post Hoc LSD of Nrf-2 and SOD expresssion

Group	*Post Hoc p(sig)*		Result
	SOD	Nrf-2	
KN-KP	0.000*	0.000*	Significantly different
KN-KK1	0.000*	0.003*	Significantly different
KN-KK2	0.000*	0.000*	Significantly different
KP-KK1	0.000*	0.000*	Significantly different
KP-KK2	0.000*	0.004*	Significantly different
KK1-KK2	0.000*	0.000*	Significantly different

***=Significant (p<0.05)**

In the Post Hoc LSD test, differences between groups were observed. The results of Nrf-2 and SOD expression showed significant differences between the groups (p <0.05).

DISCUSSION

In examining the expression of SOD and Nrf-2 in DM rats treated with robusta coffee for 14 days, it was proven that robusta coffee could increase the expression of SOD and Nrf-2. This is in line with Al Megrin's 2022 study, which states that green coffee can be used as an exogenous antioxidant.[13] The increase in Nrf2 expression in each treatment was due to the administration of coffee at different doses.

Coffee contains flavonoids that can bind to esterogen receptors on the cell surface. This is followed by a cascade reaction that activates protein kinases, which then carry out oxidative phosphorylation reactions on Nrf2, which binds to the Keap1 protein.[14] ROS also modify the cysteine residue of Keap1. This causes the release of the bond between Nrf2 and Keap1. Nrf2 then enters the cell nucleus and binds to the *Antioxydant Response Element*) receptor in the gene with the MAF protein. This binding leads to the production of antioxidant enzymes such as SOD.[15]

Nuclear factor-erythroid-2 related factor 2 (Nrf-2) is a transcription factor that regulates the activation of endogenous antioxidant enzymes produced in the cytoplasm. According to Layal (2016), under physiological conditions, oxidative stress triggers upregulation of endogenous antioxidants and cytoprotective proteins to prevent or limit tissue damage.[16] The mechanism of action of Nrf2 involves the formation of various antioxidant enzymes such as *Glutathione peroxidase*, heme oxygenase, and SOD.[17] In normal conditions, Nrf2 binds to the Keap1 protein for degradation. Diabetes mellitus, which disrupts metabolism in the mitochondria, causes an increase in the amount of ROS in the body and can cause cell damage. Giving a 2 ml/grBB dose of

robusta coffee can increase the expression of the highest Nrf2 antioxidant enzyme.

In treatment group 1, at a dose of 1 ml/grBB, there was an increase in Nrf2 expression when compared to the negative control group. However, at this dose, there was a decrease in the average value of expression compared to the positive control group. This is because Nrf2 bound to the gene promoter not only produces antioxidant enzymes, but also produces Nrf2 and Keap1, so that the amount of Nrf2 is sufficient to produce antioxidant enzymes, and the bond between Keap1 and Nrf2 is maintained and there is no release of Nrf2 to the cell nucleus.[18]

An antioxidant can be defined as any substance that, when present at low concentrations compared to an oxidizable substrate, significantly delays or inhibits the oxidation of that substance. Radicals can react indiscriminately, causing damage to almost all cellular components. A wide variety of antioxidant defenses, both endogenous and exogenous, are present to protect cellular components from damage caused by free radicals (Kolb et al., 2021) *Superoxide dismutase* (SOD) is widely distributed in oxygen-metabolizing cells and has been thought to protect those cells from the damaging actions of superoxide radicals.[19]

Coffee, an exogenous antioxidant ingredient, can increase endogenous antioxidant enzymes such as SOD, catalase (CAT), and glutathione peroxidase (GPx). The flavonoid content in coffee works as an antioxidant by increasing SOD levels by donating hydrogen ions and electrons to superoxide anions, so that they become more stable to protect lipoproteins and DNA from oxidation.[20] The CGA content in coffee works by scavenging free radicals, and the metal mineral content inactivates reactive components.[21]

Various chemical components in coffee include caffeine, flavonoids, tannins, saponins, chlorogenic acid, trigonellin, carbohydrates, fats, amino acids, organic acids, volatile aromas, and minerals. Chlorogenic acid is the most dominant acid, with a content of 8% in coffee beans or 4.5% in coffee through the roasting process.[11] The mechanism of chemical compounds in the form of steroids, alkaloids, and flavonoids as aphrodisiac ingredients occurs through vasodilation, nitric oxide formation, increased levels of testosterone, and gonadotropins to increase sexual activity in men.[22] The mechanism of chlorogenic acid in reducing blood glucose levels is inhibiting fatty acid synthesis either in vitro or in vivo, such as the mechanism of action of metformin. Both compounds have antimutagenic, anticancer, and antioxidant activities that affect *Reactive Oxygen Species* (ROS).[11]

ROS are enzymatically formed by the xanthine oxidase pathway, causing oxidative stress to increase, which reduces the activity of superoxide dismutase (SOD). Research conducted by Daniela et al. (2017) stated that administering caffeine at a dose of 5 mg/kg/day in two daily doses can reduce the level of oxidative stress (D. Metro et al., 2017). Research by Fitriana & Jatmiko (2019) reported that administering coffee at a dose of 0.108 grams/200 gramsBB and 0.162 grams/200 gramsBB can increase the number of normal spermatozoa morphology.[23] The antioxidant content of coffee can reduce free radical levels in the body and prevent oxidative stress, and coffee can also simultaneously reduce blood glucose levels.

In recent research on robusta coffee in rats with DM, robusta coffee was shown to reduce blood glucose in DM rats and increase the number of pancreatic beta cells.[8,24] Another benefit of administering chlorogenic acid in coffee is that it improves streptozotocin-induced rat sperm morphology.[25]

In this study, it was necessary to add a group of healthy mice to determine whether there was a decrease or increase that could be compared with other groups. The use of a fat diet is also recommended to stabilize the condition of type 2 DM.

CONCLUSION

Giving coffee at a dose of 1 ml/gr BB, 2 ml/grBB given for 14 days can increase the activity of Nrf-2 and SOD enzymes in rats with diabetes mellitus.

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