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RESEARCH ARTICLE

Pancreatic histological studies in mice induced by alloxan and steeping okra coffee (*Abelmoschus esculentus [L.] Moench*)

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Abstract

Introduction: Those suffering from diabetes mellitus (DM) form one of the high-risk groups for developing severe illness from COVID-19. Steeping okra coffee (*Abelmoschus esculentus [L.] Moench*) is empirically used in DM treatment. **Objective**: To determine the pancreatic histology in mice induced by alloxan and steeping okra coffee (SOC). **Method**: This was an experimental research using 16 mice that were divided into four groups, 1) control group (alloxan and aquades) and SOC Group; 2-4), treated with steeping okra coffee at 1820, 3640, and 5460 mg/KgBW concentration for ten days. **Results**: The treatment groups 2 and 3 showed an improvement in the damage of acinar cells and islets of Langerhans by 100%. On the other hand, there was still 25% vacuolisation on the islets of Langerhans in the group 4 treatment group. **Conclusion:** The steeping okra coffee repaired the islets of Langerhans cells and acinar cells that were vacuolised.

Introduction

The COVID-19 pandemic puts persons with comorbidities at risk of infection. One of the comorbid groups is people with diabetes mellitus (DM). DM is a metabolic disease with hyperglycemia disorders that occurs when the body cannot produce any or enough insulin or cannot effectively use the insulin it produces (International Diabetes Federation [IDF], 2019). Changes in the histological structure of the pancreatic islets of Langerhans are one of the pathological features that are often found significantly in patients with diabetes mellitus (Andréen-Sandberg & Hardt, 2008). Therefore, research on the effect of hyperglycemia on the histological picture of the pancreas and its role in the pathogenesis of diabetes mellitus is fundamental. Hyperglycemia might worsen the prognosis and survival rate of COVID-19 (Wang *et al.*, 2020). Therefore, managing hyperglycemia would result in the reduction of the cytokines serum level and the improvement of the prognosis in COVID-19 patients (Tabatabaei-Malazy *et al.,* 2020).

Most of the natural resources have been used for various cures. In recent years, many herbal medicines such as black cumin (Wahid & Darmawan, 2020), pomegranate (Wahid, 2020a; Wahid, 2020b), and okra plants have been developed (Munawaroh *et al.*, 2019; Tandraini *et al.*, 2020). Okra (*Abelmoschus esculentus [L.] Moench*) has a chemical content of α-cellulose and hemicellulose, which can provide antidiabetic effects (Kumar *et al.*, 2013). Furthermore, research by Romdhane and colleagues (2020) found that okra contains several bioactive chemicals, the most abundant of which are oxygenated monoterpenes, sesquiterpene hydrocarbons, and phenylpropanoids. Eight flavonols were identified using High-Performance Liquid Chromatography coupled to a DAD detector and

electrospray ionisation mass spectrometry (HPLC-DAD-MS/ESI); quercetin-3-O-glucoside was the most abundant phenolic component, followed by quercetin-O-pentosyl-hexoside and quercetin-dihexoside (Romdhane *et al.*, 2020).

Coffee is drunk daily in the Indonesian community (29.3%) (Basic Health Research (Riskesdas), 2013), both in the lower and the upper-middle class, but the coffee that is commonly consumed contains caffeine. According to Bawazeer and colleagues (2013), 34.3% of energy drinks that contain caffeine have side effects (Juliano & Griffiths, 2004). Okra coffee is a type of coffee that does not contain caffeine, so it does not cause addiction. The making of okra coffee functions as a treatment for diabetes mellitus.

There is research on okra plants, but no research on using okra seeds to make coffee has been found. Therefore, the author wanted to conduct research entitled "Pancreatic Histological Studies in Mice Induced by Alloxan and Steeping Okra Coffee (*Abelmoschus esculentus [L.] Moench*)".

Method

Fresh okra plants that are ready for human consumption are obtained from Situbondo, East Java, Indonesia, and examined at the East Java Provincial Health Office, Indonesia. The okra seeds were separated from their fruit and dried in the sun. The dried materials were ground into a fine powder using a mixer grinder (Philips mixer, Model HR2223). Making the okra steeping coffee was done by boiling water into a water bath (Memmert WNB-14), then heated until the water temperature reached 70°C. As much as 0.5 mL of steeping water was poured into a measuring cup and mixed with okra coffee powder according to the concentration. They were stirred until homogeneous.

Sixteen male mice strain BALB/c (*Mus musculus*) with similar body weights and that had been induced with alloxan were used as DM models and randomly divided into one control and three steeping okra coffee (SOC) treatment groups (1820 mg/kg BW, 3640 mg/kg BW, and 5460 mg/kg BW). All mice were prepared from the Laboratory of Pharmacology and Pharmaceutical, Faculty of Health, University of Darussalam Gontor and histology tests were done in the Anatomical Pathology, Faculty of Veterinary Medicine, Universitas Gadjah Mada. The animals were acclimatised for seven days and fed with standard feed pellets (Feed Br-II), tap water ad libitum, at a temperature of 18 - 26°C, and the relative humidity was 40-70%. This procedure was examined and approved by the Health Research Ethics Committee of the Faculty of Medicine, Universitas Muhammadiyah Surakarta (No: 1785/A.1/KEPK-FKUMS/I/2019).

After ten days of the experimental period, the mice were starved for a day and then anaesthetised to remove their pancreatic organs. Histological examination was carried out through morphometric analysis by assessing the number of endocrine cells and acinar cells in the exocrine gland. Observations were made using a microscope with a magnification of 400 times to observe cell changes. Observations can be grouped by scoring. Scoring is carried out according to the criteria of normal cells and damaged cells. Score 0: if no cell damage is found; score 1: if there is damage to small cells with a degree of damage (1 - 25%); score 2: if there is moderate cell damage with degrees of damage (26 - 50%), and score 3: if large cell damage occurs with a degree of damage (> 50%). Data from the pancreatic histology observation were analysed using descriptive methods, while data from the weighing results were analysed using non-parametric Kruskal-Wallis with Mann-Whitney post hoc. The statistical programme used is the SPSS 16 programme.

Results

The histological features observed included the islets of Langerhans because it was the site of insulin production and acinar cells, which constituted 80% of the pancreatic components (Keighley & Williams, 2018). Data on the percentage of pancreatic damage can be seen in Table I. Table I shows the percentage value of acinar cells and islets of Langerhans which experienced vacuolisation and no change in the control group (CG), which was induced by alloxan and only given as much as 75% of aqua dest without pathological changes and as many as 25% experienced necrosis on the islets of Langerhans. In the histology description, the pancreatic organ of treatment by steeping coffee of okra had improved. In treatment Group 1, with a coffee concentration of 1820 mg/kg BW and Group 2, with a coffee concentration of 3640 mg/kg BW, the pancreatic organs as a whole were improved in the exocrine and endocrine glands with no vacuolisation found.

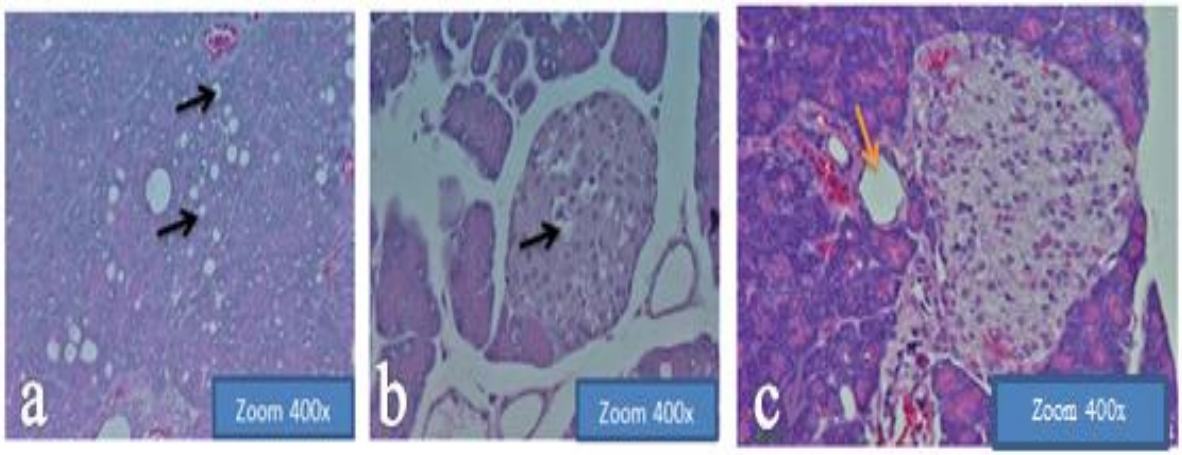
In the control group, there was more severe damage in acinar cells, namely as much as 87.5% vacuolisation. The results of this research indicate that the administration of alloxan does not damage the islets of Langerhans but damages the acinar cells of the pancreas. In the description of histology, the pancreatic organ had improved through treatment by okra steeping coffee. In treatment Group 1, with a coffee concentration of 1820 mg/kg BW and P2 with a coffee

concentration of 3640 mg/kg BW, the pancreatic organs as a whole were improved in the exocrine and endocrine glands, with no vacuolisation being found. Whereas in treatment Group 3 with a coffee concentration of 5460 mg/kg BW, the islets of Langerhans improved by 100%, and there was still 25% vacuolisation occurring in acinar cells. Significant pathological changes occurred in acinar cells of exocrine glands. This can be seen in Figure 1.

Table I: Average percentage of histological features

Treatment	Repeat	Acinar		Islets of Langerhans	
		Vacuolisation	No changes	Vacuolisation	No changes
CG	4	87.5%	12.5%	25%	75%
Group 1	4	-	100%	-	100%
Group 2	4	-	100%	-	100%
Group 3	4	25%	75%	-	100%

Note: CG (control group, induced by alloxan and given aquadest); Group 1 (SOC 1820 mg/KgBW); Group 2 (SOC 3640 mg/KgBW); Group 3 (SOC 5460 mg/KgBW).



Magnification x 400. (a) = control group, damage to acinar cells, (b) = control group, damage to the islet of Langerhans, (c) = the steeping okra coffee treatment group. Description: (→) = vacuolisation, (→) = capillary

Figure 1: Histopathological observations of liver tissues were performed with Hematoxylin and Eosin

Discussion

Based on the results of the above research, it can be seen that the administration of okra coffee can improve acinar cells and the pancreatic islets of Langerhans. Repair of the pancreatic islets of Langerhans will cause glucose levels in the blood of the mice to decrease. The research conducted by Munawaroh and colleagues (2019), using the same okra coffee concentration, showed that in group 1, with a concentration of 1820 mg/kg BW, group 2 with a concentration of 3640 mg/kg BW, and group 3 with a concentration of 5460 mg/kg BW blood glucose levels decreased by 61.3% ($p < 0.05$).

Based on this research, it is possible that the content of steeping okra coffee contains several important nutrients and other phytochemicals that can play a role in stimulating the regeneration of damaged pancreatic cells. The antioxidant property of phenolic compounds in okra might also be a part of the mechanisms for the repair and regeneration of the damaged pancreatic cells (Gemede *et al.*, 2015). So, okra coffee could be used as an alternative herbal therapy for people with diabetes mellitus. Additionally, this research demonstrated that therapy with steeping okra coffee improved the pancreas in general, particularly the acinar cells. According to the results of pancreatic histology in this research, the effective consumption of coffee brewing was 1820 mg/Kg BW and 3640 mg/Kg BW. Even at a concentration of 5460 mg/Kg BW, consumption leads to the repair of damaged acinar cells.

Research by Xiong and colleagues (2021) established that okra supplementation is effective at preserving intestinal barrier function and attenuating inflammatory responses induced by acute pancreatitis (Xiong *et al.,* 2021). In contrast, Tian and colleagues (2015) found that okra extract can suppress oxidative stress and insulin resistance as well as repair B cell damage in the pancreas through its antioxidant and anti-inflammatory effects (Tian *et al.,* 2015). Antioxidant enzymes in the body, such as nuclear factor erythroid 2-related factor 2 (Nrf2), superoxide dismutase (SOD), catalase, glutathione

peroxidase (GPx) and glutathione (GSH) vitamins A, C, and E are natural substances that are able to capture free radicals and prevent damage to cells (Sabitha *et al.,* 2012; Wahid & Darmawan, 2020).

Okra is a flavonoid and polysaccharide-rich plant that is also rich in vitamins and minerals. Okra plants have a flavonoid content that acts as an antioxidant that can prevent pancreatic β cell damage due to oxidative stress and can help increase insulin secretion (Abbas *et al.,* 2018). In normal rats, the polysaccharide content of okra has anticomplementary and hypoglycemic activity (Tomoda *et al.*, 1989). According to Liao and colleagues (2019), polysaccharides extracted from okra acted as an anti-T2DM agent by modulating oxidative stress via increased Nrf2 expression and promoting Nrf2-mediated heme oxygenase-1 (HO-1) and superoxide dismutase 2 (SOD2) expression (Liao *et al.,* 2019). Pancreatic islets are known to contain low levels of antioxidants which renders them vulnerable to oxidative stress. Nrf2 is a master antioxidant, cytoprotective regulator, and detoxifier. The treatment of human pancreatic islets with the potent synthetic Nrf2 activator dh404 significantly increased the expression of key antioxidant enzymes, decreased inflammatory mediators, and conferred protection against oxidative stress in beta cells (Masuda *et al.,* 2015).

Conclusion

The pancreatic histology in mice induced by alloxan and given okra (*Abelmoschus esculentus [L.] Moench*) steeping coffee showed improvement in islets of Langerhans (endocrine gland) and acinar (exocrine gland) cells which were vacuolated.

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