



Research Article

Antidiabetic activities of 96% ethanol extract of *Abelmoschus esculentus* on Balb-c mice strain

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ABSTRACT

Diabetes mellitus (DM) is a progressive metabolic disease that continues to increase every year. Treatment therapy using oral hypoglycemic drugs to treat DM has side effects, and alternative approaches must be taken. *Abelmoschus esculentus* has been known to be used as an alternative to DM because it has antioxidant activity due to its high flavonoid content. The purpose of this study was to determine the antidiabetic activity of 96% ethanol extract of Okra leaves against male mice of balb-c strain. Male mice were acclimatized for seven days and divided into five test groups and then fasted for 18 hours. Then the mice were induced with 150 mg / 20 g BW alloxan and measured on the 3rd day. Ethanol extract 96% okra leaves were then given in a dose of 5.6 mg / 20g BW; 11.2 mg / 20g BW; 22.4 mg / 20g BW, CMC Na 0.5% as negative control and glibenclamide 0.026 mg / 20g BBs as positive control for 14 days. The results of therapy with 96% ethanol extract of okra leaves can reduce blood glucose levels in mice so that the okra leaves contain flavonoid compounds that have antioxidant activity and can reduce blood glucose levels.

INTRODUCTION

Diabetes mellitus (DM) is a progressive metabolic disease characterized by an increase in blood sugar levels (hyperglycemia) and is caused by abnormalities in the insulin hormone (Sukandar et al., 2008). Factors for the occurrence of these hormone abnormalities can occur due to various effects, both internal and external factors of the patient. However, these conditions can be categorized based on the causative factors, including DM type 1 (insulin-dependent DM) due to autoimmune conditions, type 2 DM due to resistance or decreased insulin secretion, and gestational DM due to glucose intolerance during pregnancy (Dipiro et al., 2016).

The incidence of DM globally is expected to continue to increase from year to year. The results showed the



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percentage of people with DM was at 4% in 1995 and estimated to be 5.4% in 2030 dominated by patients aged 45-64 years in developing countries (Kumar et al., 2008; Dubey & Mishra, 2017; Idris, Hasyim & Utama, 2017; Muhammad et al., 2017). DM incidence rate in Indonesia, according to WHO, ranks 4th in the world, and East Java Region ranks 10th largest (Lathifah, 2017). The incident is also expected to continue to increase from 5 million sufferers in 2010 and increase to 21.3 million in 2030 (Simanjuntak, 2018).

Clinical therapy that is currently used to reduce blood sugar levels is using Oral Hypoglycemia and insulin administration (Muhammad et al., 2017). However, both of these therapies cause side effects such as hypoglycemia, liver toxicity, and expensive costs, so we must start looking for alternative approaches to overcome DM conditions (Pasaribu, Sitorus & Bahri, 2012). One of the plants used to treat hereditary DM conditions is okra (*Abelmoschus esculentus* L.) (Dubey & Mishra, 2017).

Traditionally, okra is used as a source of food fiber in tropical and subtropical regions (Sindhu & Puri, 2016). Okra plants are often used because they have different contents such as calcium, carbohydrates, fiber, and unsaturated fats (Kumar et al., 2013; Durazzo et al., 2019; Peng, Lin, Lin, Wang & Huang, 2019). Okra is also used as an alternative therapy to reduce blood sugar levels. The activity is related to plant content such as phenolics and flavonoids found in leaves, seeds, and fruit (Torkpo, Danquah, Offei & Blay, 2006). These compounds can reduce glucose levels and increase insulin levels in mice (Sindhu & Puri, 2016). Other flavonoid compounds from okra can absorb glucose and maltose in the intestine (Dubey & Mishra, 2017).

This research was conducted to know the antidiabetic activity of 96% ethanol extract of okra leaves against male mice of balb-c strain. Mice diabetes condition will be done by the induction of alloxan to damage the pancreatic beta cells of mice (Yuriska, 2009). This research is essential to do to find alternative treatments and the development of antidiabetic in the future.

RESEARCH METHOD

Material

Plants and Animals

The okra leaves were obtained from farmers in the Ambulu sub-district, Jember, Indonesia, and then identified at LIPI Purwodadi, Pasuruan. The okra leaves are then dried and then pulverized.

Twenty-five balb-c strain male mice were collected from the Laboratory of Pharmacology, Akademi Farmasi Jember, and acclimatized in the laboratory for seven days using room temperature.

Chemical material

Ethanol 96% as a solvent, Glibenclamide as a positive control, Alloxan as a diabetes inductor, CMC Na and Aquadest.

Method

Okra leaf extraction

Okra leaf powder is extracted using the maceration method in a closed container and protected from sunlight for 24 hours. The filtrate was then filtered, and the yield was remaceration for 24 hours with five repetitions. The results of maceration are then collected and evaporated using a rotary evaporator at a temperature of 45 °C to get a 96% ethanol extract of 45.572 g.

Preparation of 96% ethanol extract okra leaf sample

230 mg samples were prepared for a dose of 5.6 mg, 11.2 mg, and 22.4 mg for 20 g mice BW. The extracts were weighed for each dose then mixed with CMC Na 0.5% by 10 mL.

Antidiabetic testing of 96% ethanol extract of okra leaves

Balb-c strain male mice fasted for 18 hours, and husks in each group were taken and divided into 5 test groups (Table 1). Mice were then induced by alloxan at a dose of 150 mg/kg BW intraperitoneally, and an initial glucose level was measured using glucotest on the 3rd day. Furthermore, preparations with a dose of 5.6 mg, 11.2 mg, and 22.4 mg were given for 14 consecutive days, and measurements of glucose levels were measured on day 1 and day 15.

Data analysis

Glucose level values will be analyzed using the IBM SPSS Version 24.

RESULTS AND DISCUSSION

Balb-c male mice have carried out induction of alloxan at a dose of 150 mg/Kg BW intraperitoneally to make diabetic test animals. Alloxan, which has been induced to increase reactive oxygen species (ROS) that can damage pancreatic beta cells (Skudelski, 2001; Ighodaro, Adeosun & Akinloye, 2017). As a result of the damage, beta cells are not able to produce the insulin needed so that the body

Table 1. Grouping of Balb-c mice strain

Test Group	Treatment
K (+)	Hyperglycemic mice were given 0.026 mg/20 g BW glibenclamide
K (-)	Hyperglycemic mice were given CMC Na 0.5%
D1	Hyperglycemic mice were given 5.6 mg /20 g BW extract
D2	Hyperglycemic mice were given 11.2 mg/20 g BW extract
D3	Hyperglycemic mice were given 22.4 mg/20 g BW extract

Table 2. Measurement of blood glucose levels before treatment

No	Blood Glucose Level (mg/dL)				
	K(+)	K(-)	D1	D2	D3
1	600	229	356	202	600
2	600	600	397	201	600
3	600	600	600	209	600
4	600	600	600	211	282
5	600	600	600	600	579
Average	600	525,8	510,6	284,6	532,2
SD	0,00	165,92	123,27	176,37	140,16

Table 3. Measurement of blood glucose levels after treatment

No	Blood Glucose Level (mg/dL)				
	K(-)	K(+)	D1	D2	D3
1	600	167	226	86	298
2	433	123	280	107	55
3	482	89	202	131	175
4	600	88	237	113	116
5	522	196	349	109	190
Average	527,4	132,6	258,8	109,2	166,8
SD	73,39	47,92	57,80	16,07	90,69

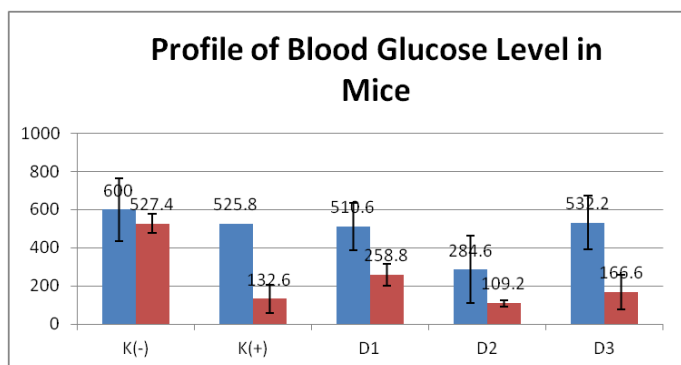


Figure 1. Profile of blood glucose level in mice .

experiences hyperglycemia (Yuriska, 2009; Lailiyah, 2016). Measurement of blood glucose levels was carried out after three days of alloxan induction using Glucotest (Table 2)

The average blood glucose level before treatment is more than 200mg/dL for each group that shows mice have experienced hyperglycemia. Mice in the five groups, then each received CMC Na 0.5% induction as the negative control, glibenclamide as the positive control, and also treated using okra leaf ethanol extract at various doses of 5.6 mg, 11.2 mg, and 22.4 mg. The result was a change in the blood glucose level of mice after treatment and was observed to decrease after measuring using Glucotest (Table 3). The results of table 3 illustrate

the percentage decrease in blood glucose levels 14 days of treatment in the positive control group as well as the D1, D2, and D3 groups.

The positive control group using glibenclamide therapy with a dose of 0.026mg/20g BW in mice had a percentage reduction in glucose levels with an average of 68.88 %. Groups D1, D2, and D3 used 96% ethanol extract therapy with okra leaves in mice. Group D1 used a dose of 5.6mg/20g BW had an average percentage reduction in blood glucose levels of 46.93 % and successively had an average percentage reduction of 53.96 % with a dose of 11.2 mg/20 g BW and had an average percentage reduction of 67,68 % at a dose of 22.4 mg/20 g BW. LSD analysis showed a significant difference (p > 0.05) between treatment groups using ethanol extract of okra leaves of all doses against negative controls. However, the treatment group using ethanol extract of okra leaves did not have a significant difference (p < 0.05) on positive control. The use of glibenclamide therapy or ethanol extract 96 % okra leaves can reduce blood glucose levels in mice after 14 days (Figure 1).

Decreased glucose levels by Glibenklamid is by stimulating the release of pancreatic β cells to release insulin (Mycek, Harvey & Champe, 2001). Receptors in pancreatic β cells bind to glibenclamide and then increase the cell's potential for insulin production (Lorenzati, Zucco, Miglietta, Lamberti, & Bruno, 2010). Insulin released from pancreatic β cells then regulates blood glucose levels close to normal (Dipiro et al., 2011).

Different mechanisms are found with 96% ethanol extract of okra leaves, which contain flavonoid compounds and act as antioxidant compounds (Torkpo et al., 2006). Flavonoid compounds are thought to reduce oxidative stress and inflammatory processes caused by alloxan induction. As a result, β cells of the pancreas can repair damaged cells and can produce the needed insulin (Testa, Bonfigli, Genovese, de Nigris & Ceriello, 2016). Flavonoid compounds are also thought to increase insulin sensitivity to metabolize mice glucose levels (Sangeetha, 2019).

CONCLUSION

Ethanol extract 96 % of okra leaves have the ability to reduce blood glucose levels. The flavonoid content of the okra leaf acts as an antioxidant compound, inhibiting oxidative stress that can damage pancreatic β cells caused by free radicals.

REFERENCES

- Dipiro, J. T., Talbert, R. L., Yee, G. C., Matzke, G. R., Wells, B. G., & Posey, L. M. (Eds.). (2016). *Pharmacotherapy: A Pathophysiologic Approach* (10th ed.). New York, NY: McGraw-Hill Medical.
- Dubey, P., & Mishra, S. (2017). A Review on: Diabetes and Okra (*Abelmoschus esculentus*). *Journal of Medicinal Plants Studies*, 5(3), 23-26.
- Durazzo, A., Lucarini, M., Novellino, E., Souto, E. B., Daliu, P., & Santini, A. (2019). *Abelmoschus esculentus* (L.): Bioactive Components' Beneficial Properties—Focused on Antidiabetic Role—For Sustainable Health Applications. *Molecules*, 24(1), 38.
- Idris, H., Hasyim, H., & Utama, F. (2017). Analysis of Diabetes Mellitus Determinants in Indonesia: A Study from the Indonesian Basic Health Resesarch 2013. *Acta Medica Indonesiana*, 49 (4), 291-298.
- Ighodaro, O. M., Adeosun, A. M., & Akinloye, O. A. (2017). Alloxan-induced Diabetes, A Common Model for Evaluating the Glycemic-control Potential of Therapeutic Compounds and Plants Extract in Experimental Studies. *Medicina*, 53(6), 365-374.
- Kumar, A., Ilavarasan, R., Jayachandran, T., Deecaraman, M., Aravindan, P., Padmanabhan, N., & Krishan, M. R. V. (2008). Anti-diabetic activity of *Syzygium cumini* and its isolated compound against streptozotocin-induced diabetic rats. *Journal of Medicinal Plants Research*, 2(9), 246-249.
- Kumar, D. S., Tony, D. E., Kumar, A. P., Kumar, K. A., Srinivasa, D. B., & Nadendla, R. (2013). A Review On: *Abelmoschus esculentus* (Okra). *International Research Journal of Pharmaceutical and Applied Sciences*, 3(4), 129-132.
- Lailiyah, I. Y. (2016). Uji Aktivitas Antidiabetes Ekstrak Etanol 96% Kulit Pisang Mas (*Musa acuminata* Colla) pada Mencit Jantan Galur Babi-C.(Undergraduate Thesis). Akademi Farmasi Jember, Jember, Indonesia.
- Lathifah, N. L. (2016). Hubungan Durasi Penyakit dan Kadar Gula Darah dengan Keluhan Subjektif Penderita Diabetes Mellitus. *Jurnal Berkala Epidemiologi*, 5(2), 231-239.
- Lorenzati, B., Zucco, C., Miglietta, S., Lamberti, F., & Bruno, G. (2010). Oral Hypoglycemic Drugs: Pathophysiological Basis of Their Mechanism of Action. *Pharmaceuticals*, 3(9), 3005-3020.
- Muhammad, I., Matazu, K. I., Yaradua, A. I., Nasir, A., Matazu, N. U., Zainab, A. S., ... & Abbas, A. Y. (2017). Antidiabetic Activity of *Abelmoschus esculentus* (Ex-Maradi Okra) Fruit in Alloxan-induced Diabetic Rats. *Nigerian Journal of Biochemistry and Molecular Biology*, 32(1), 44-52.
- Mycek, M. J., Harvey R.A., & Champe P.C. (2001). *Farmakologi Ulasan Bergambar*. Jakarta, Indonesia: Widya Medika.
- Pasaribu, F., Sitorus, P., & Bahri, S. (2012). Uji Ekstrak Etanol Kulit Buah Manggis (*Garcinia Mangostana* L.) Terhadap Penurunan Kadar Glukosa Darah. *Journal of Pharmaceutics and Pharmacology*, 1(1), 1-8.
- Peng, C. H., Lin, H. C., Lin, C. L., Wang, C. J., & Huang, C. N. (2019). *Abelmoschus esculentus* subfractions improved nephropathy with regulating dipeptidyl peptidase-4 and type 1 glucagon-like peptide receptor in type 2 diabetic rats. *Journal of Food and Drug Analysis*, 27(1), 135-144.
- Sangeetha, R. (2019). Luteolin in the Management of Type 2 Diabetes Mellitus. *Current Research in Nutrition and Food Science*, 7(2), 393-398.
- Simanjuntak, H. A. (2018). Pemanfaatan Tumbuhan Obat Diabetes Mellitus di Masyarakat Etnis Simalungun Kabupaten Simalungun Provinsi Sumatera Utara. *BIOLINK (Jurnal Biologi Lingkungan Industri Kesehatan)*, 5(1), 59-70.
- Sindhu, R. K., & Puri, V. (2016). Phytochemical, Nutritional and Pharmacological Evidences for *Abelmoschus esculentus* (L.). *The Journal of Phytopharmacology*, 5(6), 238-241.
- Sukandar, E. Y., Andrajati R., Sigit J. I., Adnyana I, K., Setiadi, A. A. P., Kusnandar. (2008). *ISO Farmakoterapi*. Jakarta, Indonesia: ISFI.

- Szkudelski, T. (2001). The Mechanism of Alloxan and Streptozotocin Action in B Cells of the Rat Pancreas. *Physiological Research*, 50(6), 537-546.
- Testa, R., Bonfigli, A. R., Genovese, S., de Nigris, V., & Ceriello, A. (2016). The Possible Role of Flavonoid in Prevention of Diabetic Complication. *Nutrients*, 8(5), 310.
- Torkpo, S. K., Danquah, E. Y., Offei, S. K., & Blay, E. T. (2006). Esterase, total protein and seed storage protein diversity in Okra (*Abelmoschus esculentus* L. Moench). *West African Journal of Applied Ecology*, 9(1), 1-7.
- Yuriska, F. (2009). Efek Aloksan Terhadap Kadar Glukosa Darah Tikus Wistar (Undergraduate Thesis). Universitas Diponegoro, Semarang, Indonesia.