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Original Article

NANOEMULSION OF OKRA FRUIT EXTRACT AS ANTIDIABETIC TREATMENT

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ABSTRACT

Objective: Okra (*Abelmoschus esculentus* (L.) Moench) has potential antidiabetic activity. This study created a nanoemulsion of okra extract (NOE) and examined its activity on alloxan-induced diabetes mellitus in mice.

Methods: Okra was macerated with 70% ethanol and dried in a rotary evaporator into the crude extract. The extract was encapsulated in a solution of glyceryl caprylate, propylene glycol, and glycerine to form a nanoemulsion. To determine the antihyperglycaemic effect of okra extract, 35 male mice (*Mus musculus* L.) were divided into seven groups: a non-diabetic normal control group and six diabetic mice groups (untreated negative control, glibenclamide-treated positive control, and four treatments with okra ethanol extract (OEE) at 200 and 400 mg/kg BW and NOE at 200 and 400 mg/kg BW).

Results: The group treated with NOE at 400 mg/kg BW (NOE400) had the lowest average blood glucose level of 93.4 mg/dL among hyperglycaemic mice. The decrease in blood glucose levels in NOE400 (52.05%) was significantly different from those in the positive control (42.63%) and OEE treatments (39.32%). The nanoemulsion used in this study fulfills quality requirements, with a mean particle size of 134.7 nm, a polydispersity index of 0.512, and a zeta value of −26.72 mV.

Conclusion: NOE reduced blood glucose levels in alloxan-induced hyperglycaemic mice better than OEE. Nanoemulsion can improve the antidiabetic activity of okra extract by increasing penetration of active compounds into interstitial space so that their delivery and bioavailability are higher.

Keywords: *Abelmoschus esculentus* (L.) Moench, Alloxan-induced hyperglycaemic mice, Diabetes mellitus, Nanoparticles, Okra fruit

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INTRODUCTION

Diabetes mellitus (DM) is a public health problem characterized by chronic hyperglycemia and impaired carbohydrate, fat, and protein metabolism caused by the dysfunction in insulin secretion, insulin action, or both [1]. In long-term damage, DM causes the failure and deterioration in the functioning of various organs of the human body [2]. In 2010, an estimated 285 million people suffered from DM, and this number is expected to increase to 439 million by 2030, with the majority of cases emerging in developing countries [3]. DM remains a leading cause of mortality and morbidity in the world, although various antidiabetic drugs are currently available [2, 4].

Okra (*Abelmoschus esculentus* (L.) Moench) is a functional food. It is used in traditional medicine for its phytochemical compounds that have many pharmacological activities [5]. Previous studies have found that some compounds in this plant have antidiabetic and antihyperglycaemic activity [6]. Okra is rich in dietary fibers with natural hypoglycaemic action that can lower blood sugar levels by limiting the rate of sugar absorption in the intestinal vili [7]. According to Anjani *et al.*, diabetic rats given okra extract showed a significant reduction in blood glucose levels [8].

Nanoparticle technology has grown rapidly in the last few years and has been widely applied for medicine due to the role of nanomaterial in different biomedical applications [9]. Nanoparticles are microscopic and excellent for increasing the bioavailability of biomolecules because of their diffusion ability and better penetration into the mucus layer [10]. The use of okra fruit as an antidiabetic agent can be improved by forming nanoemulsions with fruit extracts.

In a previous study, we showed that a nanoemulsion of okra fruit at pharmaceutical dosage had higher therapeutic effectiveness as an anticholesterol agent than a crude extract [11]. This study is aimed at determining the effects of an ethanol extract of okra fruit (OEE) and nanoemulsion of okra fruit extract (NOE) on alloxan-induced DM in mice.

MATERIALS AND METHODS

Samples

Okra Fruit was obtained from the Indonesian Spice and Medicinal Crops Research Institute (BALITRO) at Bogor, West Java, Indonesia. Okra has the taxonomic plant identification number 591/IPH.1.01/lf.07/III/2018 at the Herbarium Bogoriense, Botanical Division, Research Center for Biology, Indonesian Institute of Sciences (LIPI) Bogor.

Chemicals and reagents

Sodium hydroxide (≥99.0% purity), ammonium hydroxide, sodium acetate, chloroform (purity>99%), hydrogen chloride (37%, analytical grade), amyl alcohol (99.8%, analytical grade), ferric chloride (97%, reagent grade), ether, acid acetate anhydride (pharmaceutical secondary standard), hydrogen sulphate (99.99%, analytical grade), magnesium powder (turnings powder, 98%, reagent grade), ethanol (pure, d=0.789 g/ml), aquadest, propylene glycol, capmul and glycerine (99.5% purity) were purchased from Merck Indonesia Company. Mayer's reagents, dragendorff's reagent, and Stiasny reagent (formalin 30%, concentrated HCl 1/0.5) were obtained from Q-Lab Faculty of Pharmacy, Pancasila University.

Extraction of okra fruit

The okra fruit was extracted with 70% ethanol with kinetic maceration and dried in a rotary vacuum evaporator into a crude extract according to the method described by Ratna Djamil *et al.* (2020) [11].

Phytochemical screening

Phytochemical screening was performed according to Materia Medika Indonesia (1995) and Farnsworth's methods [12, 13]. Qualitative phytochemistry tests were conducted to analyze secondary metabolites, such as alkaloids, flavonoids, saponins,

tannins, quinones, steroids/terpenoids, coumarins, and volatile compounds.

Preparation of the nanoemulsion of okra fruit extract (NOE)

The NOE was prepared by the cosolvent method according to the method described by Ratna Djamil *et al.* (2020) [11]. A 100-mg crude extract of okra fruit was dissolved in a cosolvent consisting of 1:2.5:2:10 (v/v) capmul (glyceryl caprylate), propylene glycol, glycerine, and aqua dest. The mixture was homogenized by shaking gently to obtain an emulsion of nanoparticles. The stability of the nanoparticle emulsion was then observed for five days; this included observation of color, turbidity, and sediment.

Evaluation of the nanoemulsion of okra fruit extract

The size, distribution, and zeta potential of NOE particles were measured using a particle size analyzer (DelsaNano™, Beckman Coulter, Brea, CA, USA). The morphology of nanoparticles was evaluated with transmission electron microscopy (TEM; JEOL 1010, JEOL Ltd., Tokyo, Japan).

Ethical approval

Ethical approval to conduct the study was granted by the Health Research Ethics Committee, Faculty of Medicine, Universitas Indonesia-Cipto Mangunkusumo Hospital Jakarta Indonesia (No. KET-772/UN.2. FI/ETIK/PPM.00.02/2019), with protocol number 19-07-0825.

Experimental design of antidiabetic activity *in vivo*

The mice were acclimated to laboratory conditions for a week before behavioral monitoring, under conditions of 12 h light-dark cycle, 70%±2 relative humidity, and a temperature of 22±2 °C. Thirty-five male mice were divided into seven groups of five mice each. The normal control group (Group 1) consisted of non-diabetic mice. For the induction of hyperglycemia, mice in the diabetic groups were intraperitoneally injected (i. p.) with alloxan at a concentration of 200 mg/kg body weight (BW). The hyperglycaemic mice were divided into six equal groups of five animals each: an untreated group as a negative control (Group 2); a group treated with 0.62 mg/kg BW of glibenclamide as a positive control (Group 3); two groups that received OEE, one at a dose of 200 mg/kg BW (Group 4) and the other at 400 mg/kg BW (Group 5); and two groups that received NOE, one at a dose of 200 mg/kg BW (Group 6) and the other at 400 mg/kg BW (Group 7). Glibenclamide, OEE, and NOE were administered orally for 14 d.

Measurement of fasting blood glucose (FBG) levels and body weight (BW)

There were two stages to this experiment; in the first induction phase, mice were induced with alloxan at 200 mg/kg BW i. p., while in the second treatment phase, mice with hyperglycemia were administered different treatments as described above. BW and fasting blood glucose (FBG) levels were measured to assess the overall condition of the mice. The mice fasted for approximately 16 h each time before measuring blood glucose levels. Mouse blood was taken from the tail and measured using a glucometer. The data were then statistically analyzed using ANOVA.

RESULTS AND DISCUSSION

Phytochemical screening

The results of the qualitative phytochemistry tests for flavonoids, steroids, triterpenoids, coumarins, and saponins of the dried okra fruit and OEE are shown in table 1. The phytochemical content of okra fruit that we obtained was similar to that reported by Islam (2019). Fresh okra fruit is rich in pectin and mucilage, protein, fat, minerals, carbohydrate, flavonoids, and some important vitamins. Ethanolic extracts of okra fruit contain carbohydrates, gums and mucilage, proteins, phytosterols, flavonoids, tannins, phenolic compounds, volatile oils, and triterpenoids [14].

Table 1: Phytochemical screening of dried okra fruit and OEE

Phytochemical compound	Dried okra fruit	OEE
Alkaloid	+	+
Flavonoid	+	+
Saponin	+	+
Quinone	-	-
Tannins	-	-
Steroids	+	+
Triterpenoids	+	+
Volatile oil	-	-
Coumarine	+	+

Note.+: detected,-: No detected

Evaluation of the nanoemulsion of okra extract

The droplets of NOE appeared dark and spherical and were more or less 100 nm in size. TEM confirmed that the nanoparticles were homogenously spherical with an average size of about 134.7+2 nm (fig. 1). The results of our morphological analysis are similar to those for a cationic nanoemulsion of indomethacin for ophthalmic delivery studied by Ajmeera *et al.* [15]. The polydispersity index of the particle size distribution, which reflects the polydispersity of the emulsion, ranged from 0 to 1. Lower polydispersity index values indicate a more monodispersed suspension. The NOE has a polydispersity index of 0.512; an index value<0.5 indicates a relatively homogeneous dispersion. The zeta potential of NOE was −26.72 mV. The okra fruit nanoemulsion formulation has a negative zeta potential due to the anionic fractions of the ingredients. Nanoparticles with a zeta potential greater than+30 mV or less than −30 mV are considered strongly cationic and strongly anionic, respectively [16]. The zeta potential value shows that the particles in the NOE are stable because the electric charge of the droplets is strong enough to repel the droplets dominant in the nanoemulsion suspension system. High zeta potential is considered the cause of lower aggregation of particles. The charge on the nanoparticle surface will affect its distribution in the body and increase the uptake of nanoparticles into cells [17].

The phytochemicals in OEE contain beneficial antihyperglycaemic compounds that may be developed into commercially rewarding modern formulations of traditional medicine. However, in their natural form, they possess certain undesirable characteristics that can be remedied by encapsulation into nanoemulsions. The characterization of NOE in our study may be of use in the further development of useful tools and/or products with okra fruit extract.

Bodyweight analysis

The changes in BW from day 0 to 14 are shown for the different treatment groups in table 1. The data are presented as the mean±standard deviation for each group of five mice. The BW of the diabetic mice began decreasing three days after hyperglycemia was induced with alloxan. This loss in BW was the result of a reduction in the formation of fats and proteins, which can be broken down into energy [18]. BW then increased after 14 d of treatment. Overall, the treatments reduced BW loss in diabetic mice. Diabetic mice treated

with NOE experienced lower BW reductions compared to the other treatments. The reduction in BW of diabetic mice treated with OEE was close to that of the glibenclamide-treated group (positive control). In our study, the administration of treatments to diabetic mice affected BW, demonstrating that it triggered the formation of fat, which is generally the main cause of BW gain [19]. Meanwhile, treatment with OEE, NOE, or glibenclamide was shown to increase BW in diabetic mice compared to untreated diabetic mice.

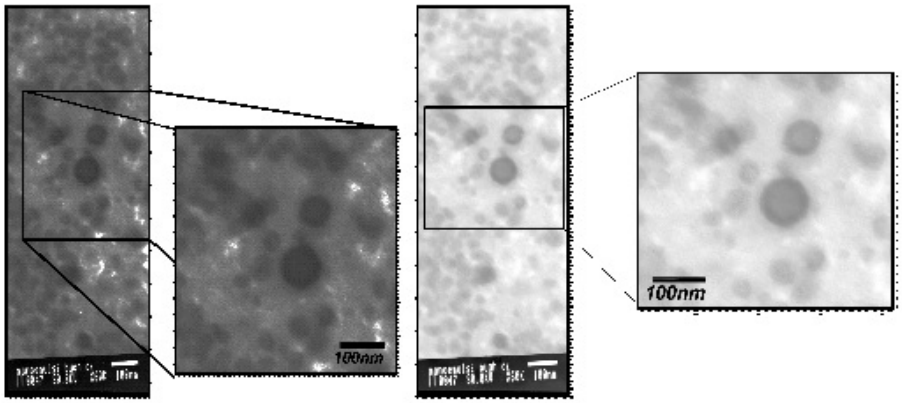

Fig. 1: The morphology of NOE

Table 2: Mice body weight average

	Mice body weight average (gram+SD)			
Animal group	**Day 0**	**Day 3**	**Day 7**	**Day 14**
Normal control	22.28+0.33	21.94+0.57	22.22+0.44	22.24+0.32
Negative control	22.08+0.50	21.54+0.73	21.92+0.87	22.44+0.82
Positive control	22.30+0.32	22.04+0.72	22.28+0.62	22.86+0.65
OEE 200	21.88+0.79	21.50+0.65	21.94+0.52	22.26+0.34
OEE 400	22.38+0.79	21.54+0.65	21.74+0.67	22.24+0.93
NOE 200	22.70+0.51	22.16+0.83	22.78+0.94	23.28+0.66
NOE 400	22.52+0.59	21.96+0.25	22.44+0.36	23.30+0.51

Note: The data was written in mean±SD from 5 mice

Changes in fasting blood glucose levels

The changes in FBG levels in the animals are shown in fig. 2. The development of diabetic conditions was evaluated on the third day after alloxan injection. All diabetic mouse groups developed DM, as is shown by their higher FBG levels compared to the non-diabetic mice (normal control). The mice in the normal control had an average normal blood glucose level of 96 mg/dl. In our study, insulin deficiency in the animal model was induced by injection of alloxan, which is diabetogenic due to selective damage to pancreatic β cells that cause a decrease in insulin secretion, increasing the blood glucose level. The mechanism of alloxan-induced DM involves oxidation of sulphhydryl groups, inhibition of glucokinase enzyme, production of free radicals, and disruption of intracellular calcium homeostasis [20].

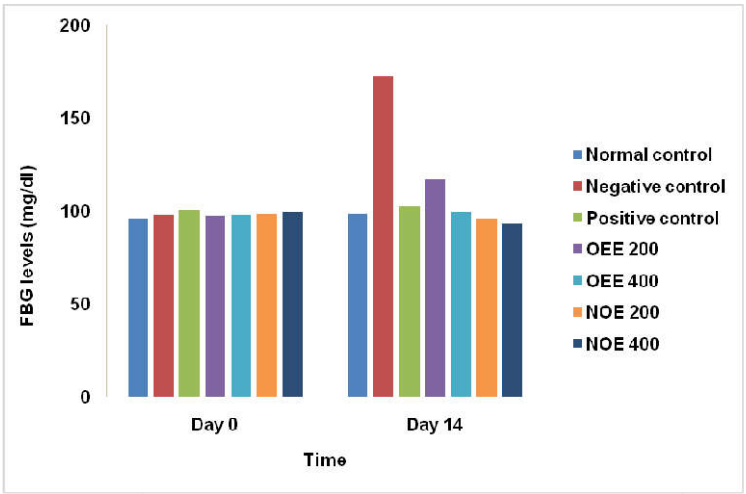

Fig. 2: The changes in FBG levels in normal conditions and after treatment, note: the data was given in mean±SD from 5 experiment

The FBG level of the negative control (untreated diabetic mice) increased to 189+3.08 mg/dl on day 3, with only a slight decrease to 180.0+5.10 mg/dl on day 7 and 172.6+4.98 mg/dl on day 14. The FBG levels decreased from day 3 to day 14 in all diabetic mice in the positive control and all OEE and NOE treatment groups. The FBG level of the positive control was 179.2+8.87 mg/dl on day 3; then, after treatment with glibenclamide, the FBG levels decreased to 146.0+8.51 and 102.8+5.07 mg/dl on days 7 and 14, respectively. Glibenclamide is an antihyperglycaemic agent effective in lowering blood glucose levels. The FBG levels of hyperglycaemic diabetic mice decreased after treatment with OEE and NOE. On day 14, the FBG levels decreased significantly to 93.4+1.14 until 117+5.79 mg/dl. NOE at 400 mg/kg BW showed the highest reduction in FBG levels among all treatments. Diabetic mice treated with OEE at 200 and 400 mg/kg BW had FBG levels almost the same as the glibenclamide-treated positive-control diabetic mice. This suggests that OEE and NOE can increase insulin secretion by pancreatic β cells to a similar extent to glibenclamide.

Table 3: Mice FBG measurement

Group	Mice FBG levels (mg/dl)			Percentage of decrease FBG levels (%)
	Day 3	Day 7	Day 14	
Normal control	97.0+4.00*#	97.8+2.90*#	98.6+2.51*#	-1.65
Negative control	189.0+3.08	180.0+5.10#	172.6+4.98#	8.68
Positive control	179.2+8.87	146.0+8.51*	102.8+5.07*	42.63
OEE200	192.8+3.70	165.8+2.59*#	117.0+5.79*#	39.32
OEE400	192.0+5.10	159.6+5.13*#	99.4+8.02*	48.23
NOE200	193.8+6.87	164.6+10.5*#	96.2+1.30*#	50.36
NOE400	194.8+5.45	154.4+8.02*	93.4+1.14*#	52.05

Note: The data were presented as mean±SD from 5 experiments, * significantly different compared to the negative control, #significantly different compared to the positive control. OEE200: Okra extract ethanol at dose 200 mg/kg BW, OEE400: Okra extract ethanol at dose 400 mg/kg BW, NOE200: Nanoemulsion okra extract ethanol at dose 200 mg/kg BW, NOE400: Nanoemulsion okra extract ethanol at dose 400 mg/kg BW. $p<0.05$, n = 5 mice/group.

Table 3 shows that all diabetic mouse groups were given glibenclamide or OEE and NOE at doses of 200 and 400 mg/kg BW had decreased FBG levels from day 7. This was a statistically significant result compared to the negative control group. Until day 14 of treatment, all groups of diabetic mice experienced a significant decrease in FBG levels compared to the negative control group. This means that OEE and NOE at all doses have the potential to reduce FBG levels. The FBG levels at day 7 in the group receiving NOE at 400 mg/kg BW were not statistically significant from that of the glibenclamide group. This means that NOE at this dose has the potential to reduce blood glucose levels as well as glibenclamide does. On day 14, the FBG levels in groups receiving NOE at doses 200 and 400 mg/kg BW were significantly different compared to that of the glibenclamide group. NOE appears to have better activity than glibenclamide due to the higher percentage decrease in FBG levels in the groups receiving it. The percentage decrease in FBG levels from the highest to the lowest order was NOE 400>NOE 200>OEE 400>positive control>OEE 200. Based on the percentage decrease in FBG levels, NOE at 400 mg/kg BW has the highest blood-glucose-reducing activity.

OEE at a dose of 400 mg/kg BW was the most similar in activity to glibenclamide. In addition, NOE was more effective at decreasing FBG levels than OEE. This demonstrates that the nano emulsification of OEE could improve its activity. This study confirms that okra fruit has hypoglycaemic activity. It may thus be a useful alternative medicine to treat different kinds of DM [21]. Nanoparticle encapsulation has been reported to increase the bioavailability of active compounds in plant extracts [22, 23]. Therefore, nanoencapsulation can improve the pharmacological activity of active compounds in okra fruits.

CONCLUSION

Oral administration of okra fruit could reduce blood glucose levels of mice with alloxan-induced hyperglycemia. A nanoemulsion of okra extract reduced the FBG levels of diabetic mice better than the standard extract. The nanoemulsion of okra, formed by mixing propylene glycol, capmul, and glycerine, could enhance the antidiabetic activity of okra fruit. The nanoemulsion contained spherical particles 134.7 nm in size with a zeta potential of −26.72 mV. The nanoemulsion at a dose of 400 mg/kg BW was the most potent antidiabetic agent among all the types examined in this study. Nanoemulsion of okra fruit may be suggested as an effective herbal therapy for diabetes.

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AUTHORS CONTRIBUTIONS

All the author have contributed equally.

CONFLICTS OF INTERESTS

The author has no conflicts of interest to declare.

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