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RESEARCH ARTICLE

The Potency of Beligo Seeds (*Benincasa hispida* (Thunb.) Cogn.) as Antihyperlipidemic in L-NAME-induced Hyperlipidemic Rats

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The Potency of Beligo Seeds (Benincasa hispida (Thunb.) Cogn.) as Antihyperlipidemic in L-NAME-induced Hyperlipidemic Rats

SUMMARY

Beligo or Bligo is the name of Benincasa hispida (Thunb.) Cogn. in Indonesian, empirically used in the treatment of cholesterol and hypertension. The part of the plant used is the seeds. This study aimed to determine the antihyperlipidemic activity of beligo seeds in hyperlipidemic rats induced by L-NAME. The method of this study, male Wistar albino rats (n = 25) were measured for their initial levels of total cholesterol (TC), high density lipoprotein (HDL), triglyceride (TG), very low density lipoprotein (VLDL) and low density lipoprotein (LDL) using a human analyzer (Thermo Scientific Indico®). All rats were induced by L-NAME 40 mg/kg body weight (BW) for four weeks and then the TC, HDL, TG, VLDL, and LDL levels were measured again. After the discontinuation of L-NAME administration, the treatment was carried out and all rats were divided into five groups consisting of group I as negative control which was given sodium carboxy methyl celulose (CMC) 1%; groups II, III, and IV were given beligo seeds ethanol extract (BSEE) each dose of 100 mg/kg BW, 200 mg/kg BW, 300 mg/kg BW; and group V as the positive control group which was given Simvastatin 10 mg/kg BW. The results showed that the beligo seeds ethanol extract (BSEE) had an antihyperlipidemic activity where doses of 100 mg/kg BW, 200 mg/kg BW, and 300 mg/kg BW could significantly reduce levels of TC, TG, LDL, and VLDL (p<0 .05) and significantly increased HDL levels (p<0.05).

Key Words: *Benincasa hispida (Thunb.) Cogn., Hyperlipidemic, L-NAME, Lipid profile*

Beligo Tohumlarının (Benincasa hispida (Thunb.) Cogn.) L-NAME ile İndüklenen Hiperlipidemik Sıçanlarda Antihiperlipidemik Olarak Potansiyeli

ÖZ

Beligo veya Bligo, Endonezce'de kolesterol ve hipertansiyon tedavisinde ampirik olarak kullanılan Benincasa hispida (Thunb.) Cogn.'un adıdır. Bitkinin kullanılan kısmı tohumlarıdır. Bu çalışma, L-NAME ile indüklenen hiperlipidemik sıçanlarda beligo tohumlarının antihiperlipidemik aktivitesini belirlemeyi amaçlamaktadır. Bu çalışmanın yönteminde, erkek Wistar albino sıçanlarının (n = 25) başlangıç total kolesterol (TK), yüksek dansiteli lipoprotein (YDL), trigliserit (TG), çok düşük dansiteli lipoprotein (ÇDDL) ve düşük dansiteli lipoprotein (DDL) seviyeleri bir insan analizörü (Thermo Scientific Indico®) kullanılarak ölçülmüştür. Tüm sıçanlar, dört hafta boyunca L-NAME 40 mg/kg BW ile uyarılmış ve ardından TK, YDL, TG, ÇDDL ve DDL seviyeleri tekrar ölçülmüştür. L-NAME uygulaması kesildikten sonra tedaviye devam edilmiş ve negatif kontrol olarak sodyum karboksimetilselüloz CMC %1 verilen I. grup; her bir dozu 100 mg/kg vücut ağırlığı (VA), 200 mg/kg VA, 300 mg/kg VA olan beligo tohumları etanol ekstresi (BSEE) verilen gruplar II, III ve IV. grup; Simvastatin 10 mg/kg VA verilen pozitif kontrol grubu V. grup olmak üzere tüm sıçanlar beş gruba ayrılmıştır. Sonuçlar, beligo tohumları etanol ekstraktının (BSEE), 100 mg/kg VA, 200 mg/kg VA ve 300 mg/kg VA dozlarının antihiperlipidemik aktiviteye sahip olduğunu, TK, YDL, TG, ÇDDL, DDL düzeylerini önemli ölçüde azaltabileceğini (p<0.05) ve YDL düzeylerini önemli ölçüde artırabileceğini (p<0.05) göstermiştir.

Anahtar Kelimeler: *Benincasa hispida (Thunb.) Cogn., Hiperlipidemik, L-NAME, Lipit profili*

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INTRODUCTION

Hyperlipidemia is considered to be a major risk factor for cardiovascular diseases. Cardiovascular disease is responsible for the highest disease burden in the world. It is the leading cause of death, morbidity, and health costs in both developed and developing countries, accounting for approximately 30% of annual global deaths and 10% of the world's health burden (Cosenza et al., 2019).

An increase in plasma lipids, including total cholesterol and triglycerides, is one of the main factors causing cardiovascular disease defined as hyperlipidemia. Hyperlipidemia has also been reported as the most widespread marker for susceptibility to atherosclerotic heart disease (Surya et al., 2017).

One of the plants that can be used as a treatment for hyperlipidemia is the seeds of beligo (*Benincasa hispida* (Thunb.) Cogn.). Beligo plant belongs to the Cucurbitaceae family, it is known to be widely available in Asia such as India, China, Malaysia, Japan, and Indonesia, and tropical countries. Beligo is a popular vegetable plant, especially among Asian people to fulfill nutrition and medicine (Al-snafi, 2013).

Beligo or Bligo is the name of *Benincasa hispida* (Thunb.) Cogn. in Indonesian, is empirically used in the treatment of cholesterol and hypertension. The part of the plant used is the seed. Previous research conducted showed that the secondary metabolites contained in beligo seeds are alkaloids, flavonoids, fatty acids, phenolics, and saponins (Aqilah et al., 2010). Meanwhile, according to Samad et al. (2013), beligo seed extract has a high total phenolic and flavonoid content, therefore beligo seed extract can be used as a natural antioxidant. Saponins have broad activities such as the ability to lower cholesterol in the blood. Meanwhile, according to Samad et al. (2013), beligo seed extract has a high total phenolic and flavonoid content, therefore beligo seed extract can be used as a natural antioxidant. Saponins have broad activities such as the ability to lower cholesterol in the blood. Phenolic compounds and flavonoids donate hydrogen to free radicals and thereby break the lipid oxidation chain reaction so that they can act as excellent radical scavengers (Alim et al., 2021, 2022), which is one of the most prominent and medically useful properties, especially in preventing or treating cardiovascular diseases. Fatty acids can lower blood lipid profiles (Burdge & Calder, 2015), namely lowering high levels of cholesterol, triglycerides (TAG), low-density lipoprotein (LDL) and increasing levels of low high-density lipoprotein (HDL) cholesterol (Harris et al., 2018; Kontostathi et al., 2021). Therefore, the content of secondary metabolites can be used in the treatment of hyperlipidemia. And this property has not been evaluated for *Benincasa hispida* (Thunb.) Cogn before.

Beligo seed ethanol extract has a hypoglycemic effect on male Wistar rats (Maryati et al., 2019). The stem alcoholic extract of *Benincasa hispida* has hypoglycemic and antihyperglycemic effects in normal and in alloxan-induced diabetic rats at doses 50,100, 200 mg/kg body weight (BW), per oral (p.o.). The maximum reduction in blood glucose levels with stem extract of *Benincasa hispida* was recorded at a dose of 200 mg/kg BW (Battu et al., 2007). The stem chloroform extract of *Benincasa hispida* has significantly decreased elevated levels of serum glucose, cholesterol, LDL, and triglyceride and increased serum levels of HDL in diabetic rats (Patil et al., 2011). *Benincasa hispida* in a dose of 250 and 500 mg/kg in mice induced a dose-dependent decrease in glucose, triglyceride, and insulin levels in plasma (Al-snafi, 2013). So, this experiment used doses of 100 mg/kg, 200 mg/kg, and 300 mg/kg body weight Wistar rats.

This experiment was conducted to determine the antihyperlipidemic activity of beligo seed ethanol ext-

ract (BSEE) to L-NAME induced hyperlipidemic rats by measurement of increase the levels of HDL and reduction of total cholesterol, triglycerides, very low-density lipoprotein (VLDL) and LDL to compare with positive control of simvastatin which is a synthetic drug.

Simvastatin, a cholesterol-lowering agent, has been widely used in the treatment of hyperlipidemia**.** As a comparison, simvastatin was used, which is included in the statin class and is the first-line drug to treat hypercholesterolemia. Comparison as a positive control was used to get a clearer picture of the effect of beligo seed ethanol extract on hyperlipidemic rats. Simvastatin works by inhibiting cholesterol synthesis in the liver, and by inhibiting 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase. As a result of this reduction in cholesterol synthesis, the sterol regulatory element binding proteins (SREBPs) contained in the membrane are then transported to the nucleus. Transcription factors will then bind to the LDL receptor gene, increasing LDL receptor synthesis. Increasing the number of LDL receptors on the hepatocyte cell membrane will reduce cholesterol levels even more. Apart from LDL, VLDL, and IDL also decreased, while HDL increased (Bhattarai et al., 2020; Welty et al., 2016) significantly at 10 mg/kg and 20 mg/kg orally (Verma et al., 2022).

MATERIAL AND METHODS

Chemical Material

N(G)-nitro-L-arginine methyl ester hydrate (L-NAME) (Sigma Aldrich), reagent HDL, triglycerides, total cholesterol for human analyzer (Thermo Scientific Indico®), simvastatin (Sigma Aldrich), 70% ethanol and other chemical material obtained by the official chemical store in Makassar, South Sulawesi, Indonesia.

Plant Collection

Beligo fruit is collected from farmers in South Sulawesi, Indonesia. Determined in the Biology laboratory of Makassar State University. The sample herbarium is stored in the Pharmacognosy-Phytochemical Laboratory of the Islamic University of Makassar.

Extraction

The sample of beligo seeds (as shown in Figure 1) was extracted by maceration method (Rusdi, M. et al., 2017) using 70% ethanol. Beligo seeds that have been separated from the rest of the fruit, are dried and powdered. Extracted using 70% ethanol, evaporated to produce a thick extract, and freeze-dried to obtain a dry extract (Samad et al., 2013). Stored in a refrigerator at 2-8° Celsius until used in the test (Tata et al., 2019).

Figure 1. Beligo fruit and seed

Animal Preparation

Male Wistar albino rats (n=25) aged 2-3 months, weighing 200-250 g were obtained from animal breeders in the City of Bandung, West Java Province, Indonesia, and were declared healthy and free from infectious animal diseases by the Department of Food Security and Agriculture of the City of Bandung, certified with Number: TN.01.01.11 /4543-DKPP/XI/2021. Male Wistar albino rats (n=25) aged 2-3 months, weighing 200-250 grams were obtained from animal breeders in the City of Bandung, West Java Province, Indonesia, and were declared healthy and free from infectious animal diseases by the Department of Food Security and Agriculture of the City of Bandung, certified with Number: TN.01.01.11 /4543-DKPP/XI/2021.

All rats were adapted for seven days before the study was conducted to get used to the experimental environment and placed in animal cages with 12 hours of day and night lighting each. The animals had free access to food and water. All animal protocols were performed by the Guide for the Care and Use of Laboratory Animals. This experiment was carried out after obtaining an ethical approval recommendation from the Health Research Ethics Commission, Faculty of Medicine, Muslim University of Indonesia, and IBNU SINA YW-UMI Hospital with Number: 073/A.1/KEPK-UMI/2021 on February 25, 2021.

Experimental Protocol

This protocol is based on the modification of Tata et al., (2019) and Salam, et al., (2016). After being adapted for seven days, the levels of TC, HDL, TG, VLDL, and LDL were measured. All rats were induced by L-NAME 40 mg/kg BW for four weeks and then the total cholesterol (TC), HDL, triglyceride (TG), VLDL, and LDL levels were measured again. After discontinuation of L-NAME administration, the treatment was carried out.

Animals were randomized and divided into five groups of five animals per group (n = 5) as follows:

Group I: L-NAME + Sodium CMC 1% (Sodium Carboxy Methyl Cellulose 1%)

Group II: L-NAME+BSEE 100 mg/kg BW

Group III: L-NAME+BSEE 200 mg/kg BW

Group IV: L-NAME +BSEE 300 mg/kg BW

Group V: L-NAME + SIMVASTATIN 10 mg/kg BW

All treatment groups were given via the oral route.

SOD. CMC 1% is used as a negative control because the ethanol extract BSEE is insoluble in water, so a suspension is needed so that BSEE is suspended homogeneously. BSEE is a plant extract as a test sample. Simvastatin is a synthetic drug as a positive control.

After four weeks of treatment, TC, HDL, TG, VLDL, and LDL levels were measured. Measurement of lipid profile levels using a human analyzer (Thermo Scientific Indico®).

Measurement of the lipid profile of rat blood

Animals are anesthetized first by inhalation using ether. Blood was drawn from the lateral vein in the rat's tail and through the orbital sinus in the eye with a microhematocrit pipette. Blood was collected in a microtube and allowed to stand for 5 minutes and centrifuged for 20 minutes at a speed of 3000 rpm until serum was obtained.

Measurement of total cholesterol (TC) levels

Pipette blood serum as much as 500 µL into the sample cup, and place the sample according to the position of the data inputted on the Thermo Scientific Indico® instrument. After pressing start, the sample reagent needle will take the total cholesterol reagent after it is incubated and then read at a wavelength of 550 nm.

Measurement of high-density lipoprotein (HDL) level

Pipette blood serum as much as 500 µL into the sample cup, and place the sample according to the position of the data inputted on the Thermo Scientific Indico device. After pressing start, the sample reagent needle will take the HDL reagent after it is incubated and then read at a wavelength of 600 nm.

Measurement of triglyceride (TG) and very low-density lipoprotein (VLDL) levels

Pipette blood serum as much as 500 µL into the sample cup, and place the sample according to the position of the data inputted on the Thermo Scientific Indico device. After pressing start, the sample reagent needle will take the triglyceride reagent after it is incubated and then read at a wavelength of 510 nm.

Measure VLDL levels, it is calculated by the formula of the Friedewald equation as described by Vuilleumier et al., (2010):

VLDL = TG/5.

Measurement of low-density lipoprotein (LDL) levels

Measure LDL levels, it is calculated by the formula of the Friedewald equation as described by Vuilleumier et al., (2010):

TC = HDL + LDL + VLDL

Which, VLDL = TG/5.

So , LDL = TC - HDL - VLDL

Data analysis

The results are presented in the form of mean ± standard error of the mean (SEM). Statistic analysis using the paired T-test (comparing before-induced, and induced-post treatment) to determine differences. Before the data analysis, the normality and homogeneity of the data were first tested. *One-way* analysis of variance (ANOVA) followed by Tukey's HSD *posthoc* test for multiple comparisons was performed to determine differences between treatment groups. Statistical tests were carried out at a 95% confidence level and this difference was significant if the *p*-value was less than 0.05.

RESULTS AND DISCUSSION

Table 1a. Lipid profile of TG and VLDL of rats, before and after L-NAME induced for four weeks, and post-treatment for four weeks

Group	Parameter (mg/dL)					
	TG			VLDL		
	Before induced	L-NAME induced	Post Treatment	Before induced	L-NAME induced	Post Treatment
LN + SOD. CMC 1%	38.40±1.14	86.60±3.78*	79.60±4.83##	7.68±0.23	17.32±0.75*	16.76±0.63##
LN+ BSEE 100 mg/kg BW	46.80±3.83	82.60±3.78*	49.40±1.14**#	9.36±0.08	16.52±0.76*	9.80±0.24**#
LN+ BSEE 200 mg/kg BW	47.80±0.84	89.00±0.00*	48.80±1.48**#	9.56±0.17	16.60±0.00*	9.76±0.29**#
LN+ BSEE 300 mg/kg BW	45.20±1.48	98.40±5.41*	45.20±1.09**#	9.16±0.33	19.68±1.08*	8.68±0.27**#
LN + Simvastatin 10 mg/kg BW	45.40±3.28	97.60±0.55*	46.80±1.48**#	9.12±0.63	19.52±1.11*	8.80±0.25**#

LN= L-NAME, BSEE= Beligo seed ethanol extract, BSEE = Beligo Seed Ethanol Extract; LN + SOD.CMC = L-NAME + Sodium Carboxy Methyl Cellulose control group; LN+Simvastatin = L-NAME + Simvastatin control group;

*p<0.05 compared to before induced and **p<0.05 compared to L-NAME induce by the paired T-Test;

#p< 0.05 compared to LN + SOD.CMC 1% control group and ##p < 0.05 compared to LN + Simvastatin by Tukey's HSD *posthoc*

Table 1b. Lipid profile of LDL and TC of rats, before and after L-NAME induced for four weeks, and post-treatment for four weeks

Group	Parameter (mg/dL)					
	LDL			TC		
	Before induced	L-NAME induced	Post Treatment	Before induced	L-NAME induced	Post Treatment
LN + SOD. CMC 1%	4.72±2.67	39.16±1.12*	37.32±1.78##	58.20±1.30	96.20±1.30*	94.60±1.14##
LN+ BSEE 100 mg/kg BW	5.24±2.29	40.48±4.31*	6.20±3.69**#	56.20±2.39	92.00±1.58*	74.80±3.96**#
LN+ BSEE 200 mg/kg BW	4.04±2.50	43.00±4.09*	5.24±3.69**#	56.40±3.05	98.40±2.97*	79.20±5.54**#
LN+ BSEE 300 mg/kg BW	5.44±1.56	38.92±2.41*	1.52±1.05**#	58.20±1.64	95.60±2.79*	73.00±2.44**#
LN + Simvastatin 10 mg/kg BW	7.48±2.30	37.48±1.49*	1.20±0.51**#	57.60±1.82	92.00±1.00*	74.40±1.51**#

LN= L-NAME, BSEE= Beligo seed ethanol extract, BSEE = Beligo Seed Ethanol Extract; LN + SOD.CMC = L-NAME + Sodium Carboxy Methyl Cellulose control group; LN+Simvastatin = L-NAME + Simvastatin control group;

*p<0.05 compared to before induced and **p<0.05 compared to L-NAME induce by the paired T-Test;

#p < 0.05 compared to LN + SOD.CMC 1% control group and ##p < 0.05 compared to LN + Simvastatin by Tukey's HSD *posthoc*

Table 1c. Lipid profile of HDL of rats, before and after L-NAME induced for four weeks, and post-treatment for four weeks

Group	Parameter (mg/dL)		
	HDL		
	Before induced	L-NAME induced	Post Treatment
LN + SOD. CMC 1%	45.80±1.30	39.60±0.55*	40.00±1.00##
LN+ BSEE 100 mg/kg BW	42.40±0.55	37.00±1.00*	58.60±0.55**#
LN BSEE 200 mg/kg BW	44.40±0.55	39.80±1.09*	65.00±1.00**#
LN+ BSEE 300 mg/kg BW	43.60±0.55	37.00±2.74*	65.40±0.55**#
LN + Simvastatin 10 mg/kg BW	41.00±1.00	35.00±1.08*	60.60±1.00**#

LN= L-NAME, BSEE= Beligo seed ethanol extract, BSEE = Beligo Seed Ethanol Extract; LN + SOD.CMC = L-NAME + Sodium Carboxy Methyl Cellulose control group; LN+Simvastatin = L-NAME + Simvastatin control group;

*p<0.05 compared to before induced and **p<0.05 compared to L-NAME induce by the paired T-Test;

#p < 0.05 compared to LN + SOD.CMC 1% control group and ##p < 0.05 compared to LN + Simvastatin by Tukey's HSD *posthoc*

Table 2. The percentage of decrease of TG, VLDL, LDL, TC, and increase of HDL post-treatment in L-NAME-induced hyperlipidemic rats

GROUP	Parameter (mg/dL)				
	TG	VLDL	HDL	LDL	TC
LN + SOD. CMC 1%	8.80%	5.42%	1.00%	4.69%	1.66%
LN+ BSEE 100 mg/kg BW	67.20%	40.68%	36.86%	84.68%	18.70%
LN+ BSEE 200 mg/kg BW	82.38%	41.20%	38.77%	87.81%	19.51%
LN+ BSEE 300 mg/kg BW	117.70%	55.89%	43.43%	96.09%	23.64%
LN + Simvastatin 10 mg/kg BW	100.00%	54.92%	45.48%	96.80%	19.13%

Plant Sample

This study used a sample of beligo seeds. The health benefits of the *Benincasa hispida* (Thunb.) Cogn. seeds may be related to their rich fatty acids, flavonoid, phenolic, and saponin contents. Indeed, studies have demonstrated the usefulness of plant fatty acids, flavonoids, and phenolic content in the prevention of hyperlipidemia (Ramchoun et al., 2020) and these properties have been evaluated in this experiment.

Lipid profile

Administration of L-NAME for four weeks in this experiment can improve lipid profile. L-NAME is an inhibitor of endothelial nitric oxide synthase (eNOS), which is widely used as an inducer of the hypertension model but also has an effect on increasing lipid profiles so that it can be used as an inducer of the hyperlipidemia model (Aluko et al., 2020; Tata et al., 2019). Treatment with L-NAME resulted in decreased HDL significantly ($p<0.05$) and increased TC, LDL, TG, and VLDL significantly ($p<0.05$) in all groups compared to baseline as shown in (Table 1a., Table 1b., and Table 1c.), thus corroborating the findings of (Salam, et al., 2016) who showed that L-NAME treatment harmed lipid profiles in treated rats. L-NAME treatment raised the concentration of TC, LDL, TG, and VLDL and reduced the concentration of HDL which in turn was capable of interfering with eNOS activity. Under normal conditions, eNOS is associated with cholesterol-enriched caveolae in endothelial cells, where its activity can be carefully regulated (Shu et al., 2015). However, in hyperlipidemia, LDL, especially oxidized LDL (ox-LDL) negatively affects the activity and subcellular distribution of eNOS hence leading to a decrease in NO bioavailability (Förstermann & Münzel, 2006; Shaul, 2003) which is generated by eNOS. On the other hand, HDL causes the activation of eNOS within the caveolae, with the resultant generation of NO (Talas et al., 2014). The result of our experiment showed that BSEE at the dose of 100 mg/kg BW, 200 mg/kg BW and 300 mg/kg BW reduced significantly ($p<0,05$) levels of TC, TG, LDL, and VLDL and increased significantly ($p<0,05$) HDL levels compared to simvastatin as a positive control and Sod. CMC 1% is used as a negative control (shown in Table 1a., Table 1b. and Table 1c.). The percentage reduction of all groups is shown in Table 2. These results indicate that BSEE can be reducing of TC, LDL, TG VLDL, and increasing of HDL by increasing eNOS activity compared to simvastatin (Tata et al., 2019).

CONCLUSION

The results showed that administration of BSEE has an antihyperlipidemic activity which at a dose of 100 mg/kg BW, 200 mg/kg BW, and 300 mg/kg BW can reduce significantly ($p<0,05$) levels of TC, TG, LDL, and VLDL and can increase significantly ($p<0,05$) HDL levels.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

AUTHOR CONTRIBUTION STATEMENT

NA as the research leader is responsible for compiling and conceptualizing the research flow, carrying out research, interpreting data, and compiling and revising the manuscript. RH is responsible for extracting, collecting research data, and conducting research. HR, AB, ND, and YYD are responsible as advisors and directors for conducting research, data interpretation, and manuscript revision.

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