ASIAN JOURNAL OF PHARMACEUTICAL AND CLINICAL RESEARCH

INNOVARE ACADEMIC SCIENCES
Knowledge to Innovation

Vol 11, Issue 11, 2018

Online - 2455-3891
Print - 0974-2441

Research Article

SARGASSUM POLYCYSTUM METHANOL EXTRACT AFFECTS THE NUCLEAR FACTOR-κ BETA AND INTERLEUKIN-6 EXPRESSION IN STREPTOZOTOCIN-INDUCED DIABETES RATS

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Received: 02 June 2018, Revised and Accepted: 18 July 2018

ABSTRACT

Objective: The objective of this study is to investigate the attenuation of the nuclear factor-κ beta (NF-κB) and interleukin (IL)-6 expression in streptozotocin-induced diabetic rats by *Sargassum polycystum* methanol extract.

Methods: Diabetic rats were intraperitoneally induced by streptozotocin (40 mg/kg b.w.). *S. polycystum* methanol extract was administered to diabetic rats for 45 days. The effect of *S. polycystum* methanol extract on blood glucose, hemoglobin A_{1c} (HbA_{1c}), NF-κB, and IL-6 expression was investigated on streptozotocin-induced diabetic rats. The gliclazide (30 mg/kg b.w., p.o.) was used as a reference antidiabetic drug.

Results: The administration of *S. polycystum* methanol extract on streptozotocin-induced diabetic rats decreased the blood glucose level, HbA_{1c}, NF-κB, and IL-6.

Conclusion: *S. polycystum* methanol extract indicates a promising anti-inflammation agent in diabetes.

Keywords: Diabetes mellitus, Free radical, Inflammation, *Sargassum polycystum*.

 DOI: http://dx.doi.org/10.22159/ajpcr.2018.v11i11.27658

INTRODUCTION

Hyperglycemia can provoke inflammation, where the complications in diabetes are always initiated by this occurrence [1,2]. The inflammation on diabetes can be induced by the formation of advanced glycation end products, such as hemoglobin A_{1c} (HbA_{1c}) [3], and then the formation of these radicals elicits the nuclear factor-κ beta (NF-κB) activation. This cytokine can activate pro-inflammatory cytokines such as interleukin (IL)-6 [4]. The administration of oral hypoglycemic agents or insulin has been known to be able to control blood glucose and effective to prevent inflammation in diabetics. However, its use chronically stimulates the side effects [5]. Efforts must be taken, therefore, to discover and develop new hypoglycemic agents that do not coincide with inflammation.

Sargassum polycystum is a brown seaweed that grows in lower intertidal zones of warm tropical waters. This seaweed grows to a height of around 1–2 m, having erect branches and stems with numerous spines [6,7]. The methanol extract of this seaweed contains numerous phytochemical compounds such as steroids, alkaloids, phenolics, flavonoids, saponins, and sterols [8]. Previous studies had shown that the methanol extract of this seaweed is considered non-toxic [9], having antioxidant activity [10], hypoglycemic action [11], and oxidative stress amelioration [12].

The evaluation of this brown seaweed methanol extract on the attenuation of inflammation in diabetes appears to lack information. Therefore, this study was undertaken to investigate the amelioration role of *S. polycystum* methanol extract on the inflammation in diabetic rats.

METHODS

Plant material

S. polycystum was collected from Talango coastline, Sumenep District, East Java, Indonesia, in March 2016. This seaweed was authenticated by AchmadKadi, the phycologist at the Research Center for Oceanography, Indonesian Institute of Sciences (Identification No. 498/IPK.2/LT.02.04/XII).

Preparation of extract

The collected sample was sun-dried, powdered by dish mill and passed through a sieve, and macerated by methanol (1:3; b/v) 3 times for 24 h at 4°C. The extract was further concentrated to a semisolid form using a rotary vacuum evaporator, and then, it was flashed by nitrogen gas and finally freeze-dried by lyophilization to obtain the SPME.

Standard drug

Gliclazide tablets (Diamicron 30 MR, Servier, Laboratories Ltd.) are one of the group of sulfonylurea compounds used as the standard drug. This was purchased from Kimia Farma Pharmacy, Malang. The tablets were suspended in sesame oil and used for the experiment.

Animals

Male species of *Rattus norvegicus* strain Wistar with body weights ranging from 180 to 200 g were used in this study. The animal experiments were conducted according to the Institutional Ethics Committee of Animal Care and Use, Brawijaya University, Malang, Indonesia (No: 510-KEP-UB). The rats were acclimatized for 7 days before the study and housed in polypropylene cages under an ambient temperature. The rats were kept on a standard pellet diet and water *ad libitum*.

Induction of diabetes

Rats were induced diabetes by single intraperitoneal injection of streptozotocin (bioWORLD) (40 mg/kg) in fresh citrate buffer (pH=4.5) after overnight fasting. After 10 days of injection, rats showing blood glucose level >200 mg/dl were selected in the study and considered as diabetic, while the rats with a blood glucose <200 mg/dl were excluded from this study.

Table 1: Effect of SPME on blood glucose in streptozotocin-induced diabetic rats

Groups	Mean blood glucose level in mg/dl at different time intervals						
	Treatment (mg/kg)	0 day	9th day	18th day	27th day	36th day	45th day
Normal control	Sesame oil, *p.o.*	119.8±8.2	118±8.1	118.4±8.4	121.2±8.8	112.48.0	114.0±7.9
Diabetic control	Sesame oil, *p.o.*	503.0±17.4*	506.0±16.9*	538.2±18.2*	545.4±17.8*	556.8±13.8*	566.8±13.9*
Gliclazide	30, *p.o.*	519.4±21.9*	512.4±19.6*	406.8±15.2*†	250.8±10.8*†	205.4±8.5*†	176.0±6.6*†
SPME	600, *p.o.*	507.4±19.2*	494.2±17.8*	454.6±16.5*†	363.4±14.4*†	280.8±10.7*†	206.4±7.5*†

Data were expressed as mean±SEM, (n=5). *p<0.05 versus NC, †p<0.05 versus DC. SEM: Standard error of the mean

Table 2: Effect of SPME on HbA_{1c}, NF-κB, and IL-6 in streptozotocin-induced diabetic rats

Groups	Treatment	HbA_{1c}	NF-κB	IL-6
	(mg/kg)	(%)	(ηg/ml)	(ρg/ml)
Normal control	Sesame oil, *p.o.*	5.37±0.10	1.5±0.03	13.6±0.51
Diabetic control	Sesame oil, *p.o.*	16.65±0.31*	4.8±0.08*	81.8±3.04*
Gliclazide	30, *p.o.*	7.11±0.13*†	2.3±0.04*†	24.6±0.93*†
SPME	600, *p.o.*	9.09±0.17*†	3.8±0.07*†	43.4±1.61*†

Data were expressed as mean±SEM, (n=5). *p<0.05 versus NC, †p<0.05 versus DC. HbA_{1c}: Hemoglobin A_{1c}, NF-κB: Nuclear factor-κ beta, IL: Interleukin, SEM: Standard error of the mean

Experimental design

Four groups of five rats each were distributed to the following treatments for 45 days - Group 1: Healthy normal rats received only sesame oil, Group 2: Diabetic control rats received only sesame oil, Group 3: Diabetic rats treated with gliclazide 30 mg/kg body weight/day orally, and Group 4: Diabetic rats treated with 600 mg/kg of SPME, respectively. The blood sample was obtained from the tails of rats. The blood glucose of rats was determined by glucometer (GlucoDr BioSensor AGM-2100) every 9 days during the experiment period. The blood of rats was acquired from the heart by puncture, and then, the serum was obtained by the centrifugation of the blood. The serum was stored at −80°C until it was used for cytokines analysis.

HbA_{1c}

HbA_{1c} of serum was determined using Cobas Integra 400 Plus, Roche Diagnostics Ltd., Switzerland.

NF-κB and IL-6

NF-κB and IL-6 levels of serum were determined by ELISA kits (Bioassay Technology Laboratory No E0287Ra for NF-κB analysis and Bioassay Technology Laboratory No E0135Ra for IL-6 determination [Shanghai, China]), and the protocol of assay followed the manual of kits. The absorbance of the cytokine solutions was read using Bio-Rad Model 680 microplate reader.

Statistical analysis

Data of this study were stated as mean±standard error mean. The difference among treatments was analyzed by the analysis of variance and followed by the least square difference test. The significance level was set at $p<0.05$.

RESULTS

Effect of SPME on blood glucose in streptozotocin-induced diabetic rats

The effect of SPME on blood glucose in streptozotocin-induced diabetic rats is shown in Table 1. This study showed the SPME treatments on diabetic rats significantly reduced the blood glucose level after the 18th day compared to the diabetic control. Diabetic control showed a significant increase in the blood glucose when compared to normal.

Effect of SPME on HbA_{1c} levels in streptozotocin-induced diabetic rats

Table 2 shows that SPME diminished significantly on the HbA_{1c} level of streptozotocin-induced diabetic rats when compared to diabetic control. The HbA_{1c} level on diabetic rats was diminished by the SPME treatments. Diabetic control exhibited the highest level of the HbA_{1c} among other treatments.

Effect of SPME on NF-κB and IL-6 levels in streptozotocin-induced diabetic rats

The decrease of NF-κB and IL-6 expression in streptozotocin-induced diabetic rats was affected by SPME (Table 2). The expression of NF-κB and IL-6 on diabetic rats was reduced by gliclazide as well. The inflammation level on diabetic control showed the strongest level compared to other treatments.

DISCUSSION

The blood glucose level of diabetic rats was higher than normal. The treatment of streptozotocin injection causes hyperglycemia on the normal rats [13]. Usage of gliclazide reduced the blood glucose level on the diabetic rats by enhancing insulin secretion. The blood glucose of diabetic rats which were treated with SPME showed a decrease. Phenolics in SPME reveal the insulin-mimicking effect [14], so the blood glucose level in diabetic rats decreased.

Hyperglycemia in diabetic rats caused the increase in advanced glycation end products formation, such as HbA_{1c} [15]. The hypoglycemic capability of gliclazide affected this Hb level in diabetic rats. This drug is also able to eliminate the free radical, so the production of this glycation product will decrease. SPME contains phenolics, where these compounds are capable as insulin-mimicking and antioxidant agents. The importance of these activities is the inhibition and reduction of glycation product in the Hb [16].

Inflammation can be induced by high blood glucose in diabetics. The NF-κB and IL-6 expression in the diabetic rats was the highest among the other treatments. Gliclazide belongs to insulin secretagogues, so the blood glucose level of diabetics decreased. This reduction will diminish the inflammation in diabetics. The diabetic rats treated with SPME showed the decrease of NF-κB and IL-6 expressions. These phenomena can be affected by the decrease of blood glucose by SPME in diabetic rats, where one of the inflammation causes in diabetes is hyperglycemia.

Thus, the present study proves that the *S. polycystum* methanol extract exhibits potency and demonstrates the prevention of inflammation in diabetes.

ACKNOWLEDGMENT

This study is financially supported by Penelitian Berbasis Kompetensi Grant No. 33/SP2H/LT/DRPM/II/2016 from the Ministry of Research, Technology and Higher Education, Republic of Indonesia.

AUTHORS' CONTRIBUTION

Dr. Muhamad Firdaus - He was the principal investigator of this present study. He prepared the manuscript drafting and treated the diabetic animal model. Dr. AniesChamidah - She contributed on the blood and cytokines analysis, data collection, analysis, and interpretation.

CONFLICTS OF INTERESTS

Authors declare no conflicts of interest.

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