Test of Hypoglikemic Effect

by International Journal

Submission date: 25-Apr-2020 07:55AM (UTC-0700)
Submission ID: 1307492789
File name: 3._Test_of_Hypoglikemic_Effect.docx (32.99K)
Word count: 2570
Character count: 13368

Test of Hypoglikemic Effect of Brown Seaweeds Extract (*Padina* and *Sargassum binderi*) on Mice Induced by Alloxan

ABSTRACT

The aim of this study was to know the effectiveness of alginates were extracted from brown seaweed *Padina* and *Sargassum binderi* in decreasing blood glucose levels. This study was designed to obtain the optimum concentration of dietary fiber effects of alginate type *Padina* and *Sargassum binderi* in decreasing blood glucose levels of alloxan-induced mice test. This research method was experimental research approach with post-test only control group design. Alloxan concentration was used 125 mg / kg body weight of mice that given intraperitoneally. Mice were divided into 4 groups, i.e the negative control group, treated alginate 0.5%, 0.75% and 1%. Each group consisted of 5 mice. Whistar mice were used at 3 months old which weighed between 146-154 grams. The results showed that in all treatments except the negative control, decreased glucose level of alloxan mice. The highest decrease was 30 mg/dl and showed by the addition of alginate from *Padina* in all concentrations.

Keywords : *Padina*, *Sargassum binderi*, alginate, blood glucose

INTRODUCTION

The use of alginate as a food additive in food industries was related to its bio-physical properties such as a thickener in food [1][2][3], so that the product was more stable [4][5][6], as a stabilizer for mixtures, dispersions and emulsions related to its characteristic as a gelling agents and to increase the viscosity [7]. Alginate was the important constituent of brown seaweeds. Besides the alginate, the main constituent of seaweeds were dietary fiber [8]. A high diet of dietary fiber, could decreased the cholesterol concentration of hiperkolesterolemik patients, decreased the requirement of insulin in diabetics, decreased triglyceride serum concentration for patients with hypertriglyceridemia, good for obesity people, decreased the risk of atherosclerosis and also reduce the risk of certain cancers [9][10][11]. Have been developed a technique to extract brown seaweed from the rocky coast areas of Gunung Kidul Yogyakarta [12][13]. Although alginate has been successfully extracted from that areas, but it has never been used expecially for food additive. Moreover, its utilization in food that could completed the specifications of the food industry and their biophysical properties of chemical testing of alginates in the food, hasn't been known. There's still no research on the health testing effects and biophysical properties of alginate that extracted from brown seaweed in the rocky coast areas of Gunung Kidul Yogyakarta. Though the potention for *Sargassum* and *Padina* were quite alot and if explored further, it would have an impact for developing in the food industry. Brown seaweed potentially as a provider of natural dietary fiber. Given the importance of the role of dietary fiber in the prevention of degenerative diseases due to low consumption of dietary fiber, its necessary to optimize the utilization of seaweed. Analysis the effects of dietary fiber performed on mice [14] to determine the effectiveness of the alginate on decreasing blood glucose level. Therefore, its important to do this research.

MATERIALS AND METHODS

Materials

This research was conducted at the Laboratory of Process Engineering and Production in Tribhuwana Tunggadewi University of Malang. This research was an experimental research based on post-test only control group design. This research used whistar mice aged 3 months and has a weight of 146-154 grams. This experiment used 35 rats and used alginate that extracted from brown seaweed in the rocky coast areas of Gunung Kidul Yogyakarta. The drinking water for the mice, given ad libitum. Equipment used includes equipment for the extraction of alginate, whistar mice cage, termination tools for mice, and blood glucose analyzer equipment.

Methods

This research was devided into 3 stages: alginate extraction, the treatments for 37 days (Fig.1), termination and blood glucose analysis.

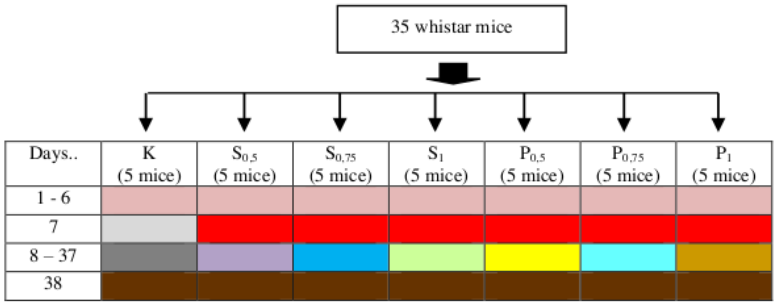

Fig. 1. Implementation of research

: Adaptation period (standard diet, *ad libitum*)
: Normal controls (without alloxan injection)
: Normal controls (standard diet)
: Mice induced by alloxan (125 mg/kg BW)
: Alginate diet (*Sargassum* 0,5%)
: Alginate diet (*Sargassum* 0,75%)
: Alginate diet (*Sargassum* 1%)
: Alginate diet (*Padina* 0,5%)
: Alginate diet (*Padina* 0,75%)
: Alginate diet (*Padina* 1%)

The methods used in this research was post test only control group design. Analysis of blood glucose level in mice conducted at the beginning and end of the study. On day 38, all mice had been terminated and blood taken for analysis of blood glucose levels. Centrifuge was used to separate blood serum. Glucose level was the amount of glucose that contained in 1 L or 1 dL blood of Wistar mice and examined quantitatively. Data analyzes were performed using SPSS for windows 16.00. Hypothesis testing using parametric One Way Anova test. Confidences true test set was 95%, and p <0.05 were obtained significant differences.

RESULTS AND DISCUSSION

Alloxan injection effect on Blood Glucose Levels

Based on the results of the study with 35 test mice, showed that a decrease in blood glucose levels of alloxan-induced mice by treatment with the addition of alginate to the diet, both types of alginate from Sargassum and Padina.

Table 1. Results of blood glucose levels in each group (mg / dL)

Groups	Average of Blood Glucose Levels (mg/dL)			Decrease in blood glucose levels (mg/dL)
	I	II	III	
$P_{0,5}$	80.8	115.6	85.6	-30
$P_{0,75}$	80.6	114.8	84.8	-30
P_1	86.6	149.0	119.0	-30
$S_{0,5}$	92.8	144.2	114.2	-30
$S_{0,75}$	89.0	131.8	103.8	-28
S_1	82.0	119.8	90.8	-29
K	86.2	150.0	91.0	-6

I and III, respectively are the results of blood glucose levels at the beginning of treatment, 2 weeks after alloxan injection and at the end of treatment (termination)

Blood glucose levels before the mice induced by alloxan in the range of 85.4 mg / dL and increased after induced with alloxan, which is in the range of 129 mg / dL. This has demonstrated the use of alloxan 125 mg / kg BW, has been able to increase blood glucose levels. The research that has been conducted [15], alloxan injection at a dose of 125 mg / kg BW intraperitoneally, has been able to raise mice blood glucose levels. Intraperitoneal injection has been effective as the entry of some chemicals and drugs into the body [16]. Alloxan was a diabetogenic chemicals, hydrophilic and unstable [17][18][19][20][21][22]. Alloxan monohydrate were administered intraperitoneally, resulted in experimental diabetic rats undergo trials and resulted in a state of hyperglycemic mice. Alloxan was a pyrimidine derivative simple essential substance that could causes damage and reduced number of insulin granules carrier in β-cell pancreatic. This damage resulted in no production of insulin that works to convert blood glucose into glycogen and the type of damage was irreversible. mice blood glucose levels increased to hyperglycemic conditions. In Table 2, the condition of hyperglycemia was seen striking, seen in 3 groups with glucose levels from 131.8 to 149 mg / dL. Blood glucose levels increased to 52.2 mg / dL. Based on research, the group of P_1, $S_{0,5}$ and $S_{0,75}$ have shown an increase in blood glucose levels that were higher than other treatment groups after injection with alloxan. It has been shown that alloxan as a diabetogenic substance has been successful and work more effectively to influence the performance of the beta cells in the pancreatic islets of Langerhans to produce insulin. Insulin was a hormone that plays a role in glucose metabolism that serves as an intermediary for the entry of glucose in the blood to the cells of other tissues. Impaired insulin secretion due to alloxan, resulting in high concentrations of glucose in the blood [23][24][25][26]. In the process of metabolism, insulin plays a role in glucose enter the cells. The release of insulin depending on the levels of glucose in the blood In the process of metabolism, insulin plays a role in glucose enter the cells. The release of insulin depending on the levels of glucose in the blood. Blood glucose levels above 70 mg / dL, stimulating the release of insulin. This insulin activates the enzyme tyrosine kinase, resulting in activation of protein synthesis, glycogen, lipogenesis and increase glucose transport into muscle and adipose tissue with the help of glucose transporter (GLUT 4). So if there was a lack of the insulin hormone, it would interfere with the body's metabolic system [17][27][28]. In the β cells, alloxan would result in mitochondrial depolarization due to excessive Ca^{2+} influx followed by excessive energy use resulting in a lack of energy in cells. Alloxan in blood binds to GLUT 4. GLUT 4 as a glucose transporter serves as a facilitating entry of alloxan to the pancreatic beta cell cytoplasm. Both of these mechanisms result in damage to the number and mass of pancreatic cells, so that would lead to a decrease in the amount of insulin released and will happen hyperglycemia [26][29]. In the group $P_{0,5}$, $P_{0,75}$ and S_1, there have been increasing blood glucose levels, but still lower than group P_1, $S_{0,5}$ and $S_{0,75}$. This was made possible because of alloxan at a dose of 125 mg / kg had not been too strong to give effect to β-pancreatic cell damage, so the effect was not as strong as in the hyperglycemia group P_1, $S_{0,5}$ and $S_{0,75}$. The influence of immune mice unequal both among mice in one group and between groups, has resulted in a blood glucose level was not uniform [28][30].

Alginate effect on Blood Glucose Levels Decrease

Based on the results of the study showed that administration of dietary alginate in all treatment groups except the control group, can lower blood glucose levels of alloxan-induced mice with decreased range of 28-30 mg / dL. Highest decrease in glucose levels were 30 mg / dL, which was obtained by a diet of $S_{0,5}$, $P_{0,5}$, $P_{0,75}$ and P_1. After injection with alloxan, blood glucose group $S_{0,5}$, $P_{0,5}$, $P_{0,75}$ and P_1 has experienced a higher increase than the other treatment groups. This was caused by the initial glucose levels in this group of relatively higher than other groups. In general, an increase in blood glucose levels after induced by alloxan was above 100 mg / dL. Based on the research, the initial condition was alloxan diabetic mice with glucose levels of 100-200 mg / dL [30].

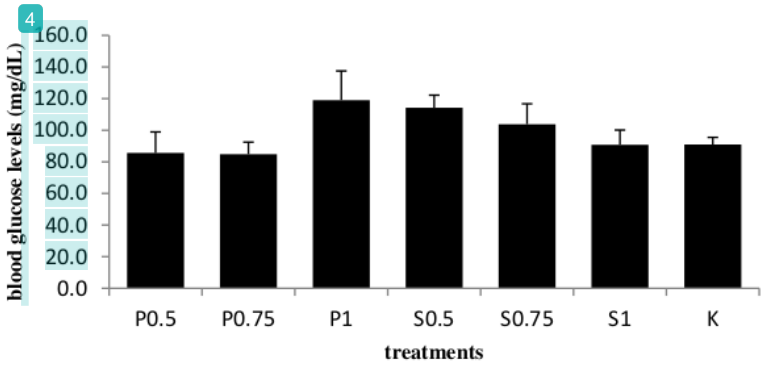

Fig 1. Blood glucose levels on post treatment

P_1 treated by *Padina* 1% (119 mg / dL), $S_{0,5}$ by *Sargassum* 0.5% (114.2 mg / dL) and $S_{0,75}$ treated by *Sargassum* 0.75% (103.8 mg / dL) were higher than the normal control group and other groups. This did not mean that the administration of alginate treatment in all three groups had no effect on lowering blood glucose levels. High levels of glucose at the end of this treatment was affected by high blood glucose levels after the injection of alloxan in the three groups. However, after the addition of alginate treatment on feed to these three groups, showed a decrease in blood glucose levels from the end of treatment on blood glucose levels after alloxan injection, with a range of 28-30 mg / dL. Group $P_1$and $S_{0,5}$ have shown decreased glucose levels are higher than other treatment groups, was 30 mg / dL. This has demonstrated the effectiveness of the use of dietary alginate types *Sargassum* 0.5% and *Padina* 1% of the standart feed weight 40 g, which has been able to lower glucose levels of alloxan diabetic mice. Have suggested that dietary doses of sodium alginate from *Sargassum ilicifolium* (Turner) C. Agardh amounted to 200 and 400 mg / kg BW [31]. This dose has given decrease in blood glucose levels were significantly different by administering metformin suspension 50 mg / kg. The main content of brown seaweed was alginate. Alginate was a polysaccharide that couldn't digested by the stomach and small intestine, and related biophysical and biochemical properties [1][32][33][334][35][36][37][38]. The existence of these fibers, resulting nutrients were released slowly into the small intestine or reduce the absorption rate of glucose, so the blood glucose levels would rise slowly. Moreover, alginate was able to assist in the improvement of pancreatic mice with alloxan-induced damage [39][40][41][42][43][44][45][46][47]. A decrease in blood glucose levels both *Padina* and *Sargassum* diet in this study, have shown that alginate has the ability to slow down the absorption of glucose into the blood. The increase in blood glucose after injection with alloxan with a mean 129.2 mg / dL, has shown that rats had hyperglycemia [48][49]. Blood glucose levels higher than 120 mg / dL was a condition of rats had hyperglycemia [50]. This condition was the result of alloxan administration that can damage β cells without damaging the α cells. Destruction of β cells affected the reduction of insulin secretion but glucagon secretion by α cells still persist and lead to disruption of the insulin-glucagon to increase blood glucose levels [51].

In the hyperglycemic condition, the amount of free radicals in the blood increased and the cause of the inflammation in the vessel wall. In this condition, there have been increasing levels of hydrogen peroxide and superoxide, as well as an increase in free radicals. Using seaweed diet that contain antioxidants, has been having an impact on reducing the formation of free radicals and has been helping to protect cells from damage caused by free radical exposure [25][52][53][54][55][56]. Therefore, the alginate was a constituent of glucose groups, namely guluronic acid and mannuronic acid, has been made possible with this glucose groups was able to capture hydroxyl radicals as well as the compound amygdalin and bay leaf extract [57][58], so as to prevent the diabetogenic action of alloxan. In addition, alginate also contain vitamins and minerals that were possible to act as an antioxidant to counteract the bad influence of hydroxyl free radicals. Therapy with alginate was thought to have hypoglycemic mechanism through inactivation of hydroxyl free radicals that attack pancreatic β cells, so that β pancreatic cells could work well for secreting insulin. Based on the research, in the form of mineral ash content of alginate *Sargassum* species ranged

from 18.20 to 21.78% [60]. *Sargassum crassifolium* J. Agardh containing Ca, Fe and P, respectively per 100g BK alginate were 1540.66 ± 6.99; 132.65 ± 3.47 and 474.0 ± 1.01 [61]. The content of vitamin A and C were 489.55 ± 8.4mg RE/100 g and 49.01 ± 0.75mg/100 g. Vitamin acts as an antioxidant compounds being inhibitors by inhibiting free radical oxidation by reacting with free radicals to form reactive and free radicals were not reactive and relatively more stable [62]. Vitamin C including that of the hydrophilic alginate was possible to vitamin antioxidants to stop the chain reaction of free radicals including hydroxyl free radicals generated from alloxan injection. Vitamin C could directly react with superoxide and hydroxyl anion [15][63]. This antioxidant compounds would react with reactive oxygen or decrease its concentration locally. Moreover, alginate was also a cleansing agent that functions as a free radical initiator such as hydroxyl. Therefore, dietary alginate from *Sargassum* and *Padina* species has been possible mechanism able to do this, with evidence of decreased blood glucose levels were quite noticeable up to 30 mg / dL. Effective concentration of the mechanism of reduction of blood glucose levels of alloxan diabetic mice were Sargassum 0,5% and Padina 1%.

CONCLUSION

Based on the research, concluded that alginate extracted from *Sargassum* and *Padina* species has the ability to decrease blood glucose levels of alloxan diabetic mice, with the optimal level of effectiveness, respectively 0.5% and 1%.

ACKNOWLEDGEMENT

This work was supported by Directorate of Research and Community Service, Kopertis 7, East Java Republic of Indonesia 2013.

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