



## Antihyperglycemic effects of carrageenan from *eucheuma spinosum* in alloxan-induced diabetic rats

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### Abstract

Carrageenan is widely used in the pharmaceutical, textile and cosmetic industries as a filler, emulsifier, thickener, and gelling agent. Carrageenan fiber is well recognized for slowing down the absorption of fat and glucose from the solution of the gastrointestinal tract into the bloodstream, which is good for decreasing blood glucose levels. In this study, male white Wistar breed rats with pancreatic injury from alloxan induction were used as test subjects to assess the *in vivo* hypoglycemic effect of iota carrageenan from *Eucheuma spinosum*. Five test groups were divided for this treatment. Group I (negative control) was given alloxan induction without therapy, group II (positive control) was treated with metformin; group III was given carrageenan suspension at a dose of 25 mg/KgBW, group IV was given carrageenan suspension at a dose of 50 mg/KgBW, group V Carrageenan suspension was given at a dose of 75 mg/KgBW. The Normality, Homogeneity, and Kruskal-Wallis tests were used in statistical analysis, followed by the Mann-Whitney test. According to the finding, iota carrageenan had an effect on reducing blood sugar levels in alloxan-induced rats. The most influential group showing anti-diabetic effect was treatment group III (75 mg/kgBW) of 35.38% when compared to treatment group II (50 mg/kgBW) of 32.80% and treatment group I (25 mg/kgBW) of 21.31%.

**Keywords:** iota carrageenan, blood glucose level, rat, alloxan

### Introduction

Red algae (Rhodophyceae), one of the marine organisms, can offer a plentiful source of natural resources and is easy to cultivate. According to Haniffa, some active compounds from algae have been discovered to have antibacterial and other properties such as antiviral, antifungal, and cytotoxic (Safitri *et al.*, 2018; Hanapi *et al.* 2013) [2, 3]. *Eucheuma spinosum* is red algae utilized as the primary ingredient in flour, gell, carrageenan, and alginate production. Especially carrageenan, it is widely used in textile, cosmetic, and other industries. Carrageenan functions as a stabilizer, emulsifier, thickener, filler, and gelling agent (Nurhuda *et al.*, 2017) [4].

Carrageenan is also a hydrocolloid compound consisting of potassium, sodium, magnesium and potassium sulfate esters with galactose 3,6 anhydrogalactose copolymers (Fatmawati *et al.*, 2013) [5]. Carrageenan is made from a red seaweed extract, a complex polysaccharide molecule with a linear chain and sulfate galactan. It is made up of galactose and 3.6-anhydrogalactose units that are linked together by sulfite groups. Carrageenan may be categorized into three different varieties based on the sulfate substituent in each monomer: Kappa, Iota, and Lambda carrageenan. Carrageenan is frequently used as a gelling agent, thickener, and stabilizer in various industries, including food, pharmaceuticals, cosmetics, printing, and textiles, because it can form a thermoreversible gel or a thick solution when added to a salt solution (Diharmi *et al.*, 2011) [6].

Dietary fiber, such as carrageenan, can slow glucose absorption from the solution in the gastrointestinal tract into the bloodstream and affect fat absorption. Red algae fiber products, such as carrageenan extracted from seaweed *Eucheuma spinosum* are helpful for people with diabetes mellitus (Wikanta *et al.*, 2002) [7]. In hyperglycemic rats, the action of carrageenan has been demonstrated to be advantageous for lowering blood sugar levels (Wikanta, 2005) [8]. Given that lignin and plant cell wall polysaccharides cannot be digested by digestive enzymes of mammals, including those found in humans, the benefits of consuming fibrous foods, especially those that are water-soluble, include slowing or preventing the rate at which blood glucose levels rise suddenly (Wikanta *et al.*, 2008) [9].

This study examined the *in vivo* hypoglycemic effects of carrageenan dietary fiber from *Eucheuma spinosum* in male Wistar rats and contrasted them with the effects of the commercially available oral diabetes medication metformin.

### Tools, Materials and Methods

#### Materials

The seaweed used as a carrageenan extraction material was harvested from the southern sea of Madura Island in 2020. The test preparation studied was the type of iota carrageenan extracted from the red seaweed *Eucheuma spinosum*.

### Sample Preparation

The cleaned dried *Eucheuma spinosum* seaweed was weighed as much as 100g and chopped and soaked in distilled water for 24 hours at a ratio of 1:50. Following the crushing of the *Eucheuma spinosum* immersion results using a blender, the extraction process was carried out in a microwave using a 0.5% NaOH base solution and seaweed at a ratio of 1:10 to the solvent. The extraction results were then filtered under hot conditions to prevent gel formation (Desiana *et al.*, 2015) <sup>[10]</sup>. The obtained filtrate was then subjected to a deposition process. To get carrageenan fiber precipitate, ethanol is added in a ratio of filtrate: ethanol (1:2) while being stirred for around 15 minutes. The precipitate was then filtered and dried in an oven set at 60°C until it reached a consistent weight. When completely dry, it was milled into carrageenan powder and sieved using a 60 mesh sieve.

### Experimental Animal

Creating the necessary circumstances for hyperglycemic rats by intraperitoneally administering alloxan at a dosage of 150 mg/kg BW/day for seven days. The experimental animals used were 30 *Rattus norvegicus* Wistar Strain rats, male sex, aged 3-5 months, with body weight between 250-300 grams (Covington *et al.*, 1993) <sup>[18]</sup>. A simple touch instrument was used to measure blood sugar levels.

### Metformin Dosage Calculation

For an adult human weighing, the 50 kg dose was 100 mg/day. The dose for rats, multiplied by the conversion factor, which was 0.018, became:  $140 \text{ mg} \times 0.018 = 2.52 \text{ mg/day/200 g BW rats}$ . Dosage calculation of dosage orientation for carrageenan preparations was obtained based on the calculation conversion from the usual dose for the treatment of Ulcerative colitis in humans with a body weight of 70 kg (Reynolds, 1982) <sup>[11]</sup>.

### Carrageenan Dosage Calculation

The typical dose deemed safe for ongoing therapy was 10 g/day (Reynolds, 1982) <sup>[11]</sup>. The dose conversion for humans to mice was 0.018. The dose of carrageenan used was 25 mg/KgBW, 50 mg/KgBW, 75 mg/KgBW.

### Test Method

First, the hyperglycemic rats were prepared by induced alloxan for 7 days. On the seventh day following alloxan induction, blood sugar levels in rats were once more measured to make sure the rodents had developed diabetes permanently. If a rat's blood sugar level was greater than 200 mg/dl, diabetes mellitus was diagnosed (Pagliassoti *et al.*, 2000) <sup>[12]</sup>. The dose of carrageenan used was 25 mg/KgBW, 50 mg/KgBW, and 75 mg/KgBW given orally, as shown in Table 1. Observations were conducted during the seven days of treatment. Blood samples were taken before and after treatment.

**Table 1:** Test treatment of each group

No	Group	Treatment	Number of Rat (s)
1	Control-Negative	Alloxan induced	5
2	Control-Positive	Alloxan + Metformin induced	5
3	Dosage 1	Alloxan induced at a dose of 32 mg/200g BW and carrageenan 25 mg/KgBW	5
4	Dosage 2	Alloxan induced at a dose of 32 mg/200g BW and carrageenan 50 mg/KgBW	5
5	Dosage 3	Alloxan induced at a dose of 32 mg/200g BW and carrageenan 75 mg/KgBW	5

Each rat was given one type of oral test preparation at the dose described above. All preparations were administered once daily for 1 week (7 days). Before and after 2.5 hours of administration of the test preparation, rat blood was sampled on days 3, 5, and 7 from each group. All of the rats were put to death when the experiment was over. SPSS 22.00 for Windows was used to analyze the data. Hypothesis testing used the One Way ANOVA parametric test. The true confidence of this test was set at 95%, and with  $p < 0.05$ , a significant difference was obtained.

### Result and Discussion

#### Yield

Yield is the weight (weight) of carrageenan from seaweed. The percentage value of the extracted carrageenan *Eucheuma spinosum* is obtained by calculating the ratio between the carrageenan product obtained and the dried *Eucheuma spinosum* seaweed sample.

**Table 2:** Percentage of Extracted Carrageenan Yield

Weight of <i>Eucheuma spinosum</i> powder (g)	Weight of Carrageenan (g)	Yield (%)	Colour
100,08	21,83	21,81%	Light Brown

The yield obtained in this study was 21.81%. The age of the seaweed and the extraction method used to produce carrageenan are two of several factors that determine the quantity of yield obtained (Gerung *et al.*, 2019) [13]. Systems for heating, drying, and storage also impact how much the yield is produced (Dirhami *et al.*, 2011) [6].

### Phytochemical Screening

**Table 3:** Results of Carrageenan Phytochemical Screening

Group/Compound	Reactor	Results of Phytochemical Screening
Alkaloids	Mayer	-
Flavonoids	Concentrated Mg + HCl powder	+
Saponins	Concentrated water/HCl	-
Steroids and terpenoids	Lieberman Burchard	-

According to the phytochemical screening results of carrageenan shown in table 3, Carrageenan from *Eucheuma spinosum* is positive for flavonoids, as shown by the presence of red-orange colour indicating the presence of flavones, bright red-red indicating flavanols, and bright red-purple red indicating flavanones. The red colour in the flavonoid test is due to the formation of flavilium salts (Achmad, 1986) [1].

### Anti-diabetic Activity Test

Anti-diabetic activity test of carrageenan from *Eucheuma spinosum* was carried out on Wistar strain rats in hyperglycemic condition after alloxan was induced at a dose of 150 mg/kg BW for 7 days of treatment. Alloxan was chosen as the diabetes inducer in this investigation because it undergoes oxidation-reduction metabolism in the body, generating free radicals and alloxan radicals, which cause harm to pancreatic beta cells (Santika, 2017) [14]. Wistar strain, as the test animal, was said to be hyperglycemic if the blood sugar level was 200 mg/dL after alloxan induction. Carrageenan from *Eucheuma spinosum* was tested for 7 days. During this period, all rats received treatment according to their respective test groups. The following is a table and graph of the effectiveness of *Eucheuma spinosum* carrageenan on rat blood sugar levels for 7 days.

**Table 4:** The average results of measuring blood sugar levels in rats

Group	Blood sugar level (mg/dL) $\pm$ standard deviation				
	Before Induction	After Induction	Day -3	Day -5	Day -7
Negative	88.05 $\pm$ 9.76	288.08 $\pm$ 65.36	277.67 $\pm$ 93.64	269.07 $\pm$ 36.37	240.33 $\pm$ 97.33
Positive	97.78 $\pm$ 8.56	279.67 $\pm$ 72.74	212.67 $\pm$ 97.38	177.67 $\pm$ 64.67	120.33 $\pm$ 15.82
25 mg/kgBW	107.34 $\pm$ 9.23	264.33 $\pm$ 133.47	257.00 $\pm$ 130.15	232.33 $\pm$ 114.60	208.00 $\pm$ 67.91
50 mg/kgBW	90.84 $\pm$ 7.42	271.33 $\pm$ 169.18	245.67 $\pm$ 88.28	217.00 $\pm$ 108.88	182.33 $\pm$ 18.77
75 mg/kgBW	89.11 $\pm$ 7.91	260.00 $\pm$ 75.28	236.33 $\pm$ 77.52	194.67 $\pm$ 23.00	168.00 $\pm$ 25.15

\*) Data is displayed in the form of mean  $\pm$  SD with total data (n) = 5

According to Table 4 above, the first blood sugar level before induction is typically between 88.05 and 107.34 mg/dL on average. Because no therapy has been administered, this glucose level covers normal blood sugar levels. After alloxan was induced, all treatment groups experienced an increase in blood sugar of around 260.00-288.08 mg/dL. It is due to the particular damage that alloxan causes to the insulin-secreting pancreatic cells, which suggests that alloxan induction attempts to train animals in an experimental state of diabetes by damaging the pancreatic structure. The experimental conditions of diabetes will cause normal rats to become rats with DM, marked by one of the characteristics of the clinical diagnosis of weight loss (Santika, 2017) [14].

The data on rat blood sugar levels in this study were statistically analyzed using One Way Anova which was then continued with the Bonferroni Post Hoc Test method ( $p < 0.05$ ). According to the results of the One Way Anova test, there was at least one pair of treatment groups with the treatment group at the dose I, the treatment group at dose II, and the treatment group at dose III that were significantly different from one another ( $p < 0.05$ ) in the test data on blood sugar levels in rats. According to the analysis, carrageenan from *Eucheuma spinosum* has an effect on lowering blood sugar levels in a manner equivalent to the positive control (metformin). According to data table 4, the negative control group's blood sugar levels did not significantly reduce from the first day of therapy until the seventh day. The positive group in this study was Metformin. The dose of Metformin used was 2.52 mg/day/200 g BW rats. The dosage was determined using the 5 mg/day effective oral human dose, which was then translated to a dose for rats. In the positive group, there was a decrease in blood sugar levels of 159.34 mg/dL for 7 days. Metformin therapy was chosen as a carrageenan comparison treatment since it can increase insulin secretion from the pancreatic gland (Chicco *et al.*, 2003) [15].

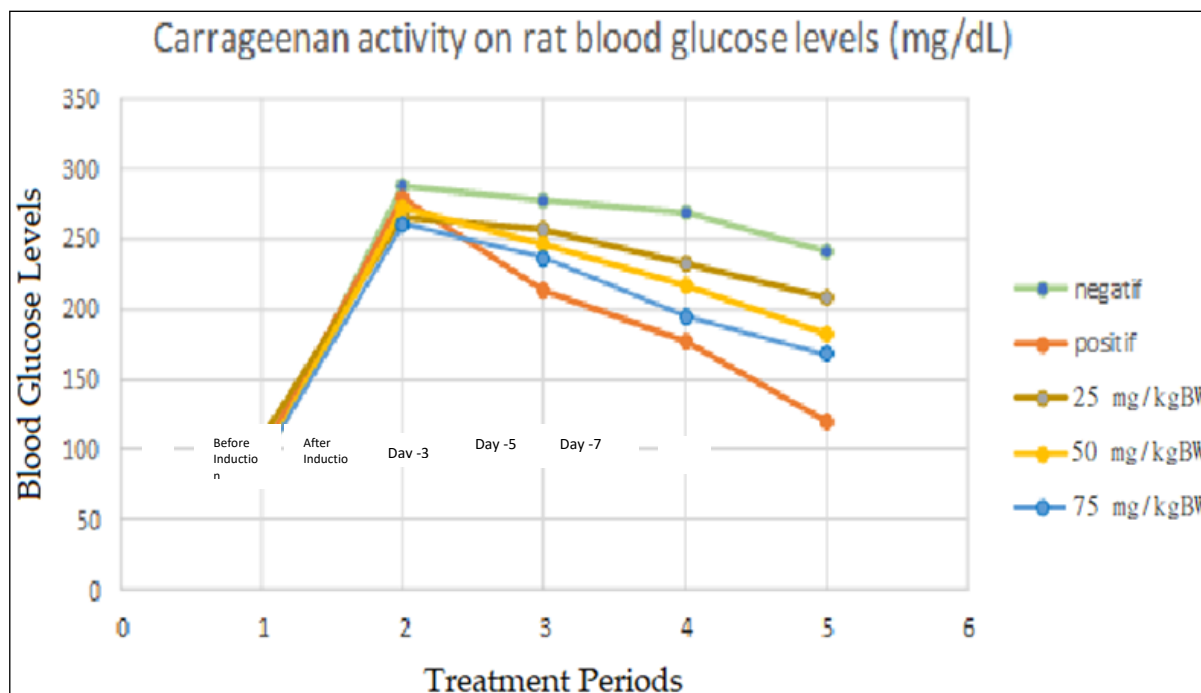


Fig 1

The treatment of carrageenan lowered blood sugar levels by 56.33 mg/dL within 7 days in the dosage group I hyperglycemic rats, which received carrageenan therapy from *Eucheuma spinosum* at a dose of 25 mg/kgBW, as well as other groups of hyperglycemic rats. Within 7 days, the diabetic rats in the second dosage group—the group that received carrageenan at a dose of 50 mg/kgBW—had their blood sugar levels drop by 89.00 mg/dL. Carrageenan was administered to diabetic rats in the third dose group at a dose of 75 mg/kgBW, and this group also experienced a decrease in blood sugar levels that was comparable to that seen in the second dose group, where carrageenan administration reduced blood sugar levels by 92.00 mg/dL within 7 days. Analysis of the data on the anti-diabetic activity of carrageenan as a whole was indicated by the percent decrease in sugar content (%GL). Table 4 displays the results of the %GL computation.

Table 5: Data analysis % Glucose Lowering (%GL)

No	Treatment Group	Average Blood Sugar			
		After Induction (X)	Day -7 (Y)	(X-Y)	% GL
1	Control (-)	288.08	240.33	47.75	16.57
2	Control (+)	279.67	120.33	159.34	56.97
3	25 mg/kgBW	264.33	208.00	56.33	21.31
4	50 mg/kgBW	271.33	182.33	89.00	32.80
5	75 mg/kgBW	260.00	168.00	92.00	35.38

This description leads to the conclusion that carrageenan can lower blood sugar levels in hyperglycemic rats. However, if it turns out that metformin therapy results in a greater decrease in blood sugar levels, this suggests that carrageenan's ability to treat diabetes is only moderately effective. However, if the dosage is considered, the higher the dose given, the greater the decrease in blood sugar levels obtained. Consequently, the carrageenan dosage must be increased to achieve a higher fall in blood sugar levels.

The presence of bioactive chemicals in carrageenan, which can prevent oxidation in pancreatic cells so that damage can be reduced, maybe the reason for the reduction in blood glucose levels brought on by its administration. According to the findings of phytochemical screening studies, some of the molecules found in carrageenan, especially flavonoid compounds, have the potential to be used as medications to treat diabetes mellitus. According to research conducted by Rochman *et al.* (2016) [16], flavonoid activity as a natural anti-diabetic can occur due to the activity of the  $\alpha$ -glucosidase inhibitor enzyme. The  $\alpha$ -glucosidase enzyme has a mechanism to break down carbohydrates into smaller sugar particles called glucose, which will then be absorbed systemically. Alpha-glucosidase is an intestinal enzyme inhibited by compounds from the  $\alpha$ -glucosidase Inhibitor (AGI) group competitively and reversibly. This results in a delay in the absorption of glucose and a reduction in the rate at which blood glucose levels rise after meals throughout the day. Besides carrageenan fiber can slow glucose absorption from the solution in the gastrointestinal tract into the bloodstream, it also affects fat absorption (Wikanta *et al.*, 2003). The digestive enzymes of animals, including humans, are known to be unable to digest the polysaccharides and lignin found in plant cell walls; thus, the advantages of consuming fibrous

foods, especially those that are water-soluble, include reducing or inhibiting the rate of a sudden increase in blood glucose levels (Dalimartha, 2002) <sup>[17]</sup>.

### Conclusion

According to the phytochemical screening test and anti-diabetic activity of carrageenan, the active ingredient component found is a flavonoid. Based on testing, the anti-diabetic activity of carrageenan has the effect of reducing blood sugar levels. The most effective group giving anti-diabetic effect was treatment group III (75 mg/kgBW) when compared to treatment group I (25 mg/kgBW) and treatment group II (50 mg/kgBW), with a decrease in blood sugar levels of 21.31%, 32.80%, and 35.38%.

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