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The effect of phlorotannin Sargassum sp. extract on colon profile of diabetic rats

F Faricha\textsuperscript{1,}, M Firdaus\textsuperscript{1,2}

\textsuperscript{1} Department of Fishery Product Technology, Faculty of Fisheries and Marine Science, Universitas Brawijaya, Jalan Veteran, Malang 65145, East Java, Indonesia
\textsuperscript{2} Bioseafood Research Unit, Faculty of Fisheries and Marine Science, Universitas Brawijaya, Jalan Veteran, Malang 65145, East Java, Indonesia

*Corresponding author: fafa.faricha@gmail.com

Abstract. Diabetes mellitus is a metabolic disorder marked by hyperglycemia. Polyphenol is capable of being scavenger of free radicals by transferring its hydroxyl atom. Polyphenol, which contained in brown algae, is phlorotannin. This study aimed to investigate the effects of phlorotannin from Sargassum sp. on blood glucose and histopathology of the colon of diabetic rats. Research steps were extraction of a phlorotannin, the establishment of the diabetic model, general histological staining, histopathology analysis, and determination of phlorotannin contain. This research was comprised of seven groups and five replications of treatment. Diabetic rats were prepared by injection of 40 mg/kg BW streptozotocin in intraperitoneal. The results showed that Sargassum sp. extracts were capable of diminishing blood glucose level, likewise repairing the histopathology of the colon. A dose of 600 mg/kg BW is the best dose of extract Sargassum sp. on improving the colon profile of diabetic rats.

1. Introduction

Diabetes Mellitus (DM) is a health disturbance which is marked by blood glucose levels increase due to lack of insulin [1]. This circumstance may produce an abnormally high level of free radicals that emerge oxidative degeneration into body cells [2]. Hence, clinical complications due to oxidative detriment might occur on the digestive system; one of them is colon [3].

Polyphenol is a phenol substance that has more than one hydroxyl cluster (OH) [4]. It has been widely known that polyphenol is capable of being scavenger of free radical by transfer its hydroxyl atom [5], eliminates HeLa cells [6], likewise protects biological tissue from free radical damage on diabetic [7]. Moreover, [8, 9, 10] stated that polyphenol of brown seaweed alleviates blood glucose level as antihyperglycemic.

Sargassum is one of the brown algae which comprises much nutritious substance and bioactive, namely polyphenol [11]. Brown algae, such as Sargassum sp. has a phlorotannin [12]. Phlorotannin is one of the tannin derivates that exists in nature, besides hydrolyzed tannin and condensed tannin. While both hydrolyzed and condensed tannin are numerous in water or terrestrial plants, phlorotannin is founded specifically in brown seaweed [13]. This compound could be gained by extraction using methanol. Methanol is a solvent in which highly used in extraction due to its capability in holding polyphenol substance [14].
The investigation of phlorotannin from *Sargassum* sp. on their capability to repair histopathology of the digestive system, particularly colon, which has not yet been done. Therefore, this research aimed to inspect the effect of phlorotannin *Sargassum* sp. on blood glucose levels and histopathology colon of diabetic rats

2. Methodology

2.1. Materials

Brown seaweed (*Sargassum* sp.) were collected from Madura Island, East Java, Indonesia.

2.2. Composing of extract

Seaweed (*Sargassum* sp.) washed thoroughly with fresh water to remove either sand or epiphytes, then dried indirectly under the sun. Afterward, the dried *Sargassum* sp. were ground to a fine powder and were macerated three times with methanol. Phlorotannin of *Sargassum* sp. was extracted according to the method of Firdaus [15] with modification. The extract was further concentrated to a semisolid form using a rotary vacuum evaporator. Then, it was flashed by nitrogen gas and freeze-dried by lyophilization to obtain the *Sargassum* methanol extract [16].

2.3. Establishment of the diabetic model

This research was conducted on male Sprague-Dawley rats weighing 150-200 g. All procedures were in accordance with the institutional guidelines for animal testing. They fed in normal diet and water *ad libitum* and also kept in dry and clean cages at Biosains Laboratory of Universitas Brawijaya. Diabetes was induced by a single injection of 40 mg/kg streptozotocin intraperitoneally, which dissolved in freshly prepared 0.1 M citrate buffer pH 4.5 which based on Erwin et al., [17] made from the mixture of 26.75 mL citric acid solvent and 23.25 mL sodium citric dissolved in 50 mL distilled water. Diabetes was confirmed seven days by monitoring blood glucose levels ≥200 mg/dL using a glucometer. The rats divided into seven groups, each group comprising of 5 rats:

- A: normal
- B: normal + oral hypoglycaemic agent (Gliclazide)
- C: diabetic rats
- D: diabetic rats + Gliclazide
- E: diabetic rats + *Sargassum* sp. extract 200 mg/kg BW
- F: diabetic rats + *Sargassum* sp. extract 400 mg/kg BW
- G: diabetic rats + *Sargassum* sp. extract 600 mg/kg BW

These treatments had been done for 45 days. The day after, all of the rats were sectioned in order to gain a colon organ. Colon washed in NaFis 0,9% and stored in formalin 10% before observed.

2.4. General histological staining

The colon was fixed in 10% phosphate-buffered formalin over 24 h. The specimens were dehydrated in a series of graded ethanol 70%, 80%, 90% respectively, and embedded in paraffin. Four-micron sections were cut, and the paraffin was cleared from the slides. The sections were rehydrated and stained with hematoxylin and eosin (H&E). Hematoxylin color nuclei while eosin colors cytoplasm. General histological staining had done based on modification method of Chen et al. [18]

2.5. Histopathology analysis

The observation of cell detriment comprise of necrosis, pyknosis, and karyolysis had been done in 5 fields of view. Necrosis is the death of the cell, which is marked by the damage of the whole structure; likewise, its function [19], Pyknosis is indicated by the irreversible shrinking of nuclei [20] karyolysis represented by the loss of nuclei’s affinity for basic stain [21]. Afterwards, scores had given based on Bayrak et al., [22] for histopathology understanding, 0 = normal, 1 = light, 2 = moderate, 3 = heavy.
This distortion will be marked as light if <1/3, moderate if 1/3 – 2/3, heavy if >2/3 in each field of view.

2.6. Determination of phlorotannin contain on Sargassum sp. extract and feces
2 g of extract and feces were dissolved in ethanol 85% (1:2). Both samples were incubated in dark room for 8 hours. 0.05 mL of this solution diluted in 4.95 mL H2O. Afterward, 1 mL of this mixture added 1 mL Folin-Ciocalteu 50% and 2 mL solvent of Na2CO3 20%. Both solutions were then incubated at room temperature for 45 minutes. The following was a separation of the solid and liquid substance by centrifugal action on 4000 rpm for 5 minutes. Phlorotannin concentration could be measured by sample absorbency on a standard curve

3. Result

3.1. Phlorotannin absorption
Phlorotannin is a polyphenol in Sargassum sp. In this research, phlorotannin was measured quantitatively. Analyzing data depicted that phlorotannin absorption was distinct in each treatment at the end of the observation period (P<0.05). Results revealed the absorption percentage of phlorotannin on Sargassum sp. extract for 0.67496 µg phloroglucinol/mg in each dose Figure 1.

![Figure 1. Percentage of phlorotannin absorption.](image)

Figure 1 relates that the proportion of phlorotannin raised as the dose of Sargassum sp. extract increased. It means that given doses 400 mg/kg BW and 600 mg/kg BW were more effective than 200 mg/kg BW. The amount of phlorotannin absorption on treatment G and F showed incompletely different. Huang et al., [23] stated that the absorption of polyphenol would be inclined along with the new number of given doses. Liu et al., [24] affirmed in which given concentration between 350-700 mg/kg could improve its capability to be absorbed.

3.2. Blood glucose level
Blood glucose level means the amount of glucose which is contained in blood circulation. Analyzing data related that the blood glucose level of each treatment (Figure 2) was considerably different (P < 0.05).
Figure 2. The blood glucose level in each treatment.

Blood glucose level on normal treatment (A) was higher than treatment B; likewise, the comparison between C and D due to the addition of gliclazide. The addition of gliclazide in the long term may bring some side effects of hypoglycemia [25]. Furthermore, Sarkar et al., [26] stated that gliclazide stimulates cell β pancreas to release insulin and decrease blood glucose levels.

Treatment of Sargassum sp extract 200 mg/kg BW and 400 mg/kg BW had similar effectiveness as an antihyperglycemic agent. This circumstance was shown by the number of blood glucose level on D, E, F in which were slightly different. The amount of blood glucose level on G was lower than that of D treatment. It depicted that the extract of Sargassum sp. 600 mg/kg BW was more effective as anti hyperglycemia and antioxidant, compared to gliclazide.

3.3. Histopathology of colon

Histopathology observation had done in terms of distinguishing the damage of colon tissue due to free radical, which is caused by diabetes. Figure 3 denoted histopathology of colon tissue in each treatment.
Based on figure 3 and figure 4, it can firmly be seen that cell detriment in normal condition (A) was lighter than a normal condition with gliclazide addition (B). According to Pratama et al., [27], the consumption of drugs in the long term affects inflammation, which causes cell death (necrosis). The

Data analysis of the colon histopathology score in each treatment was depicted in Figure 4.

Figure 3. Photomicrograph of Colon tissue in each treatment with a magnification of \( \times 30 \mu \)

- : Normal
- : Necrosis
- : Pyknosis
- : Karyolysis

Figure 4. The score of histopathology colon in each treatment.
given of gliclazide in normal conditions brings detrimental effects. On the other hand, gliclazide consumption on diabetic condition (D) diminished cell wreckage even though its effectiveness was slightly lower than Sargassum sp. extract.

Consumption of Sargassum sp. extract on diabetic condition (E, F, G) brought remarkable reparation of a degenerative condition in a cell. Figures 3 and 4 depicted that a given dose of 600 mg/kg BW holds its capability to alleviate cell damage, nearly normal condition. It meant that phlorotannin extract Sargassum sp. was more effective than gliclazide in terms of cell repairing. Heo et al., [28] stated that as the given dose of phlorotannin had increased, its reparation effect on cells grew better effectiveness.

4. Conclusion
Phlorotannin Sargassum sp. can reduce blood glucose levels and repair histopathology colon of diabetic rats. In the future, this finding will be utilized as repairing agents of deleterious complications on the digestive system, which is caused by diabetes. The best dose in declining blood glucose level and repairing histopathology colon was 600 mg/kg BW.

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