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Original Article

NANOPARTICLES OF BROWN SEAWEED (*SARGASSUM POLYCYSTUM*) EXTRACT AND ITS ANTIOXIDANT ACTIVITY IN RATS FED HIGH-FAT DIET

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ABSTRACT

Objective: This research aimed to formulate the nanoparticles of brown seaweed (*Sargassum polycystum*) and determine its antioxidant activity in rats on high-fat diets (HFD) with the parameters of superoxide dismutase (SOD), catalase, and malondialdehyde (MDA).

Methods: The nanoparticles of brown seaweed extract (NBSE) were formulated with chitosan and tripolyphosphate polymers using the ionic gelation method. The NBSE was evaluated for its particle size and potential zeta. Twenty-four male Wistar rats were divided into six groups of four rats each. One group of normal control groups was given a standard diet as a baseline. Rats were induced with a high-fat diet for 35 d. The HFD rats were divided into five equal subgroups of four animals each: an untreated group as a negative control; a group treated with vitamin E as a positive control, and three NBSE treatment groups that had oral administration of 50, 100, or 200 mg/kg BW, respectively, for 14 d. On days 0, 14, 35, and 49, blood samples were taken to determine antioxidant activity against antioxidant parameters such as MDA, SOD, and catalase.

Results: The NBSE has a particle size of 891.2±44.3 d. nm, a polydispersity index of 0.3820.03, and a zeta potential value of 11.7±0.2 mV with a positive charge. The oral administration of NBSE prevented oxidative stress on HFD rats by increasing SOD and catalase, with the highest inhibition percentage SOD parameters were 196.71% and catalase parameters were 218.73%. Formation of MDA was prevented by all doses with the highest inhibition percentage MDA parameters were 57.90%.

Conclusion: The NBSE has antioxidant activity and could prevent oxidative stress in HFD rats.

Keywords: Brown seaweed *(S. polycystum*), Antioxidants activity, Nanoparticles

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INTRODUCTION

Hepatic hypercholesterolemia is the most dangerous. Clamping is caused by a buildup of fat along blood vessels. Cardiovascular disease is primarily caused by atherosclerosis [1, 2]. Polyunsaturated fatty acids (PUFA) are unsaturated lipids (PUFAs) that are found in cell membranes. Uncontrolled lipid peroxidation causes cell membrane damage and causes diseases like coronary heart disease, stroke, diabetes, and aging [2]. Plasma malondialdehyde (MDA) levels are frequently used to detect lipid peroxidation. A low-cholesterol diet, weight loss, and antioxidants can help control lipid peroxidation [3-5].

They bind to free radicals and other reactive molecules to inhibit oxidation. Endogenous antioxidants, such as catalase and SOD, are produced by the body's immune system. A variety of natural and synthetic antioxidants are available (exogenous antioxidants). Brown seaweed, which contains phenolic compounds and flavonoids, is a rich source of natural antioxidants [6, 7].

In previous studies, BSE showed a high percentage antioxidant parameters of SOD, catalase, and MDA. Modifying BSE into nanoparticle preparation technology can increase its antioxidant activity. Physically, nanoparticles of BSE have been used to alter and improve drug pharmacokinetic and pharmacodynamic properties. *In vivo*, nanoparticles have been used to deliver BSE, increase bioavailability, and allow for continuous drug release [8, 9]. The objective of this research was to obtain brown seaweed nanoparticles (*Sargassum polycystum*) and to determine their antioxidant activity in rats fed a high-fat diet (HFD) using the superoxide dismutase (SOD), catalase, and malondialdehyde (MDA) parameters.

MATERIALS AND METHODS

Materials

The sample materials used in this study were brown seaweed (*Sargassum polycystum*) taken from Garut, West Java, Indonesia. The determination of brown seaweed was done at the Research Center for Oceanography, Indonesian Institute of Sciences, Ancol, North Jakarta, Indonesia (B-2716/I/IPK.2/IF/X/2016).

Chemical and reagent

Chitosan from shrimp shell with food and medical grade was purchased from CV. Bio Chitosan Indonesia (Jakarta Indonesia). Sodium Tripolyphosphate 0.1% was purchased from Hubei Xingfa Chemical Group (China). Water, Trichloroacetate (TCA) 20%, thiobarbiturate (TBA) 0.67%, phosphate buffer 0.05 M pH 7, carbonate buffer 0.518 M, epinephrine solution 0.5% M, ethanol solution 96%, chloroform, H_2O_2, and EDTA 10% were purchased from Merck Chemicals and Life Sciences Co (Jakarta, Indonesia).

Extraction process

The obtained brown seaweed was cleaned of impurities, washed, and dried in an oven at 40 °C for 3 d. It is extracted with ethanol 70 % using the maceration technique. The ethanolic extract filtrate was evaporated using a rotary vacuum evaporator (Rotavapor R-205, Büchi, Switzerland) to give a viscous residue and then dried using an oven vacuum.

Nanoparticles of brown seaweed extract

The nanoparticles of brown seaweed extract (NBSE) were made by ionic gelation according to Djamil *et al.* with slight modification [8, 9]. A 1% chitosan solution was prepared by dissolving 1 gram of chitosan in 100 ml of 1% glacial acetic acid with stirring using a magnetic stirrer. A total of 100 mg of thick brown seaweed extract was dissolved in 96% ethanol, 100 ml was taken, and then 70 ml was taken. Then 30 ml of chitosan solution was added so that the chitosan concentration was 0.3%. The mixture was homogenized gently to obtain a nanoemulsion. The mixture was stirred for 10 m using a magnetic stirrer. Then 16.5 ml of 0.1% sodium tripolyphosphate was added to the mixture at a speed of 1 drop every 3 seconds with stirring

using a magnetic stirrer at a stirring speed of 250 rpm until homogeneous turbidity was formed. Then 10 ml of tween 80 1% was added until it was homogeneous and stable. The nanoparticle suspension was stirred for 30 min on a magnetic stirrer.

Determination of particle size, polydispersity index, and zeta potential

The particle size diameter and polydispersity index (PDI) of NBSE were measured with the Delsa™ Nano Particle Size Analyzer (Beckman Coulter, Inc., Brea, USA) in the Laboratory of Nanotech Herbal Indonesia, Bogor, West Java, Indonesia. The zeta potential of NBSE was analyzed using a Beckman Coulter Zeta Potential analyzer. Measurements were adapted from previous studies with slight modifications [10].

Ethical approval

This study used 24 male rats of the strain Wistar from the fishery and animal husbandry Karanganyar, Central Java, Indonesia (028/SKKH/III/2019). The ethical approval of this study was ethically approved by the Health Research Ethics Committee of the Jakarta Veterans National Development University with No: 231/VI/2021/KEPK, Jakarta, Indonesia.

Animal treatments

The *in vivo* assay was done according to the protocol by Makhlouf *et al.* (2021) with modification [11]. Twenty-four male Wistar rats were divided into six groups of four rats each. One group of normal control groups was given a standard diet as a baseline. Rats were induced with a high-fat diet for 35 d. The HFD rats were divided into five equal subgroups of four animals each: an untreated group as a negative control; a group treated with vitamin E as a positive control; and three NBSE treatment groups that had oral administration of 50, 100, or 200 mg/kg BW, respectively, for 14 d. Blood sampling was performed on days 0, 14, 35, and 49 to see antioxidant activity against antioxidant parameters such as MDA, SOD, and catalase parameters.

Malonaldehyde plasma measurement with thiobarbituric acid method

Plasma MDA levels were measured according to the TBARs method. 200 µl of sample solution (plasma) was added with 1 ml of trichloroacetic acid (TCA) at 20% and 2 ml of thiobarbituric acid (TBA) at 0.67%. The solution is mixed homogeneously and heated in a water bath for 30 m. After chilling, it was centrifuged at 3000 rpm for 10 m. The pink filtrate was measured at a 532 nm wavelength using a UV-VIS spectrophotometer. MDA levels are calculated using a standard TEP curve [12, 13].

Superoxide dismutase (SOD) measurement

SOD levels were examined in red blood cells according to a modification of the Misra and Fridovich methods. As much as 250 µl hemolysate red blood cells were added to 400 µl mixed 96% (3:5) chloroform-ethanol solution. The mixture was mixed for one minute, then centrifuged at a speed of 3000 rpm for 10 m. Clear light yellow filtrate was taken at 10 µl then added 90 µl of distilled water, and added 2775 µl of 0.0518 M carbonate buffer, and 125 µl of 0.01 M epinephrine solution. The mixture is homogeneous and put into a cuvette to measure its absorption after minutes 1, 2, 3, and 4 at a wavelength of 480 nm and a temperature of 30 °C using a UV-Vis spectrophotometer. Then the same way is done also for distilled water (blank) with readings absorption after minutes 1, 2, 3, and 4 using a UV-Vis spectrophotometer [14].

Catalase measurement

Catalase levels were measured according to the Beer and Sizer method. 250 µl rat red blood cells were added to 250 µl aqua dest, then homogenized. A centrifuge with a speed of 3000 rpm for 5 m. A total of 100 µl centrifuge supernatant was mixed with 1.0 ml of H_2O_2 (0.059 M) and 1.9 ml of phosphate buffer (0.05 M) pH 7. The solution is measured for absorption at a wavelength of 240 nm for 4 m and a decrease is recorded every 1 minute. Catalase activity is defined as micromol peroxide H_2O2 consumed per minute per ml of the sample at room temperature (25 °C) [11, 15].

RESULTS AND DISCUSSION

Plant determination

The sample used in this study was brown seaweed, which was determined at the Oceanographic Research Center-Indonesian Institute of Sciences, North Jakarta, Indonesia. The result of the determination on the sample shows that the brown seaweed used was *Sargassum polycystum* with the following classification (fig. 1):

Fig. 1: The brown seaweed (*Sargassum polycystum*) samples, Filum: Ochrophyta, Class: Phaeophyceae, Ordo: Fucales, Famili: Sargassaceae, Genus: Sargasum, Species: *Sargassum polycystum*

Brown seaweed (*Sargassum polycystum*) taken from Garut, West Java, Indonesia is a marine species. It can be found in large communities on rocks in relatively calm water in the lower interstitial zone [16]. Upright branches have numerous spines on their stems. The leaves are spear-shaped to oval, and the vesicles are spherical. The height of the plant is 1-2 m. It is a dark brown plant with a height of 20-30 cm, and the base forms a thick disk-shaped mount. The upper part is abundantly branched. Due to the existence of short processes, the axis of the plant becomes coarse. It narrows toward the top, leaving a length of about 2 cm and a width of 0.5 rams. The edges of the leaves are serrated and the tips are rounded. Midribs are more or less noticeable. The vesicles are small, spherical and 1-2 mm wide. The blood vessels are slightly thin with no spines. It grows on coastal and sub-coastal rocks, stones, and dead coral during all months of the year [16].

Brown seaweed extract nanoemulsion

The liquid of the nanoemulsion of brown seaweed extract appeared brown and homogenous, with an average size of about 891.2±44.3 d nm (fig. 2). The nanoemulsion characterization is shown in table 1 below. The size was measured with a particle size analyzer (PSA) at Nanotech Herbal Indonesia and the polydisperse index was 0.382±0.03. The results of our nanoemulsion are similar to the nanoemulsion of Okra extract (NOE) emulsion studied by Djamil *et al.*; it has a polydispersity index of 0.512 [8, 17]. The particle size distribution of NBSE, which reflects the polydispersity of the emulsion, ranged from 0 to 1 [18]. The index value<0.5 indicates a relatively homogeneous dispersion. Brown seaweed was made in the form of nanoparticles with a chitosan polymer carrier which was crosslinked with sodium tripolyphosphate using the ionic gelation method. This ionic gelation method is used because the application of the method is easy, does not require organic solvents, and has good biocompatibility [19].

Table 1: Measurement condition of nanoemulsion

Parameter	Result
Temperature	25 °C
Diluent name	Water
Refractive index	1.3300±0.0066
Viscocity	1.000±0.005
Scattering intensity	14496
Color	Light brown transparent
Phase	Liquid

The data was presented in mean±SD, n=3

The test preparation is made in the form of nanoparticles because of the characteristics of nanoparticles, such as the ability to penetrate intercellular spaces or cell walls, higher so that it is expected to increase bioavailability and provide better pharmacological effects, as well as the flexibility to be combined with various other technologies so that it opens up the wide potential to be developed for various purposes [17]. The zeta potential value of NBSE was 11.7±0.2 mV with a positive charge. The zeta potential measurement provides insight into the surface charge of the dispersed nanoemulsion globules, which imparts stability to the formulated NBSE. A positive charge of zeta potential was desirable as it confirms the presence of a chitosan layer on the surface of the droplet and promotes intestinal absorption of the encapsulated BSE [20]. So, the concentration of NBSE that we used in our previous research was thought to be the best for the formulation because it had the right balance of particle size and zeta potential, which is a measure of how stable it is.

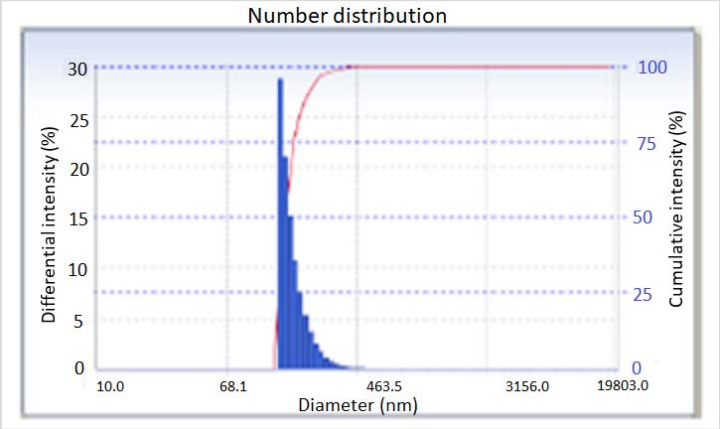

Fig. 2: The particle size distribution

MDA analysis

Analysis was carried out on the results of changes in MDA levels starting from day 0 until day 35 of the induction high-fat feed and then administration of seaweed extract nanoparticles for 14 d after induction. From the change in MDA levels, one can calculate the average MDA level and percent inhibition of MDA activity, or the ability of the antioxidant power of each group, as can be seen in fig. 3 and 4.

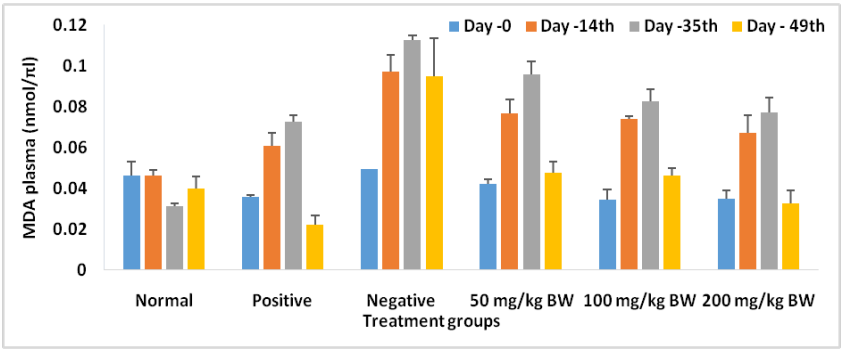

Fig. 3: Bar chart of average MDA levels; data were given in average±SD, n=4

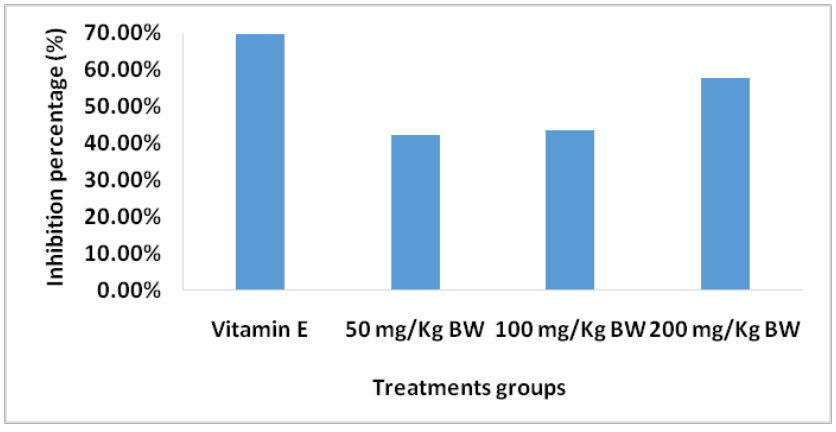

Fig. 4: Inhibition percentage chart of brown seaweed extract nanoparticles

Based on the results of the percent inhibition in fig. 3 and 4, it can be seen that NBSE contains antioxidants. This effect is caused by the pharmacological activity of secondary metabolites in brown seaweed, namely phenols and flavonoids [22]. Phenols and flavonoids in brown seaweed as antioxidants with lipid peroxidation are used as radical scavengers because they require free radicals to conjugate double bonds and H atoms as donors from phenolic hydroxyl (-OH) groups [23]. The greatest inhibitory ability is by Dose III, which is equal to 57.90%, while the first dose is 42.28%, dose II is 43.64%, and positive control is 69.66%. Analysis of variations in the percentage of MDA levels was used to see whether there were significant differences in brown seaweed extract nanoparticles towards a high-fat diet as a negative control and vitamin E as a positive control. Statistical test results obtained from the positive control group and dosages I, II, and III show the difference obtained ($p>0.05$) with the negative control so that it can be related to the group that has antioxidant effects. Dose III with positive control did not show a significant difference ($p<0.05$). Increasing doses I and II showed significant differences, whereas doses II and III did not show significant differences. This is an increased effect that does not provide an increased effect of antioxidants.

Treatment of dyslipidemia rats with the *Sargassum polycystum* methanol extract significantly improved plasma lipid profile, significantly reduced serum MDA, NO, leptin, and TNF-α levels, and demonstrated serum adiponectin levels [16]. In a similar study of the Sargassum genus, a diet containing 4% *Sargassum latifolium* extract reduced MDA levels and significantly improved the antioxidant defense system in the blood of non-stressed sheep [24].

SOD analysis

Analysis was carried out on the results of changes in SOD activity from day 0 to day 35 in the induction of high-fat feed and the administration of seaweed extract nanoparticles for 14 d after induction. SOD is an enzymatic antioxidant that acts as a defense against oxidative stress conditions by protecting body cells and preventing damage. The high activity of SOD will be illustrated by the low lipid oxidation product [14, 25]. Based on changes in SOD activity, it can be calculated the percentage of SOD activity and the average SOD activity of each group, or the ability of the antioxidant power of each group, which can be seen in fig. 5 and 6.

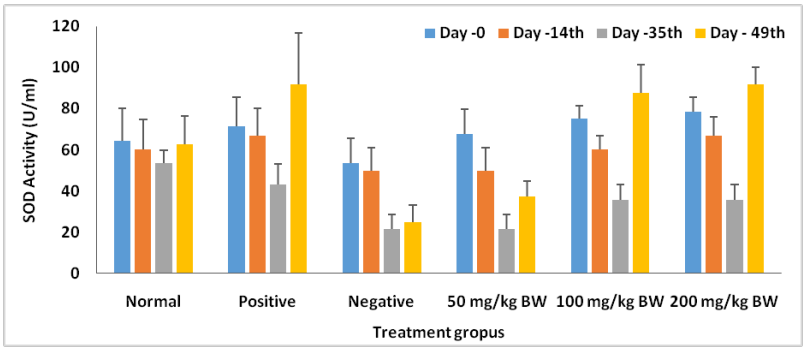

Fig. 5: Bar chart of average SOD levels, Data was given in mean±SD, n=4

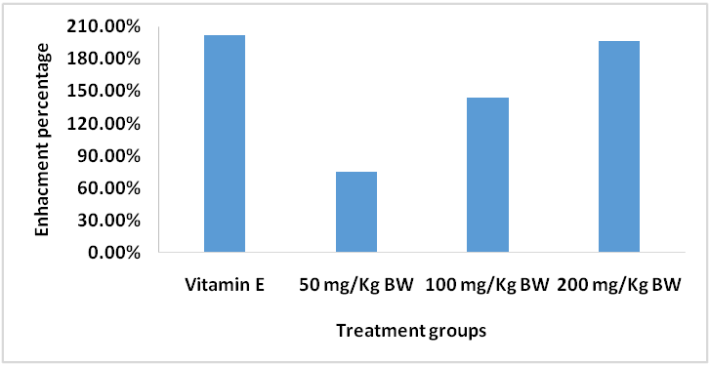

Fig. 6: Enhancement percentage chart of brown seaweed extract nanoparticles

Based on the results of the average activity in fig. 5 and 6, it can be seen in NBSE has an antioxidant effect. This effect is caused by the pharmacological activity of secondary metabolites in brown seaweed, namely phenols and flavonoids [7]. The study reported by M. Johnson *et al.*, (2019) that extract of *Sargassum polycystum* produces high total phenols, flavonoids, and alkaloids, that may be responsible for the antioxidant activities [26]. Phenols and flavonoids in brown seaweed as antioxidants with lipid peroxidation are used as radical scavengers because they need free radicals associated with conjugated double bonds and H atoms as donors from the phenolic hydroxyl (-OH) group [6].

The ability of NBSE to increase is greatest by Dose III, which is 196.71%, while the first dose is 75.03%, dose II is 144.28% and positive control is 202.35%. Analysis of variations in average activity was used to see if there were significant differences in nanoparticle extracts of brown seaweed on a high-fat diet as a negative control and vitamin E as a positive control. Statistical test results obtained from the positive control group and dosages II and III show a significant difference obtained ($p>0.05$) from with negative control, so it can be concluded that brown seaweed nanoparticles at this dose have antioxidant effects. While dose I do not yet have activity as an antioxidant. Doses II and III with positive controls showed no significant differences ($p<0.05$). Increasing doses I and II showed significant differences, whereas doses II and III did not show significant differences. This increased effect does not provide an increased effect of antioxidants.

The result was similar to a study by Ellamie *et al.* (2020) that use brown seaweed (*Sargassum latifolium*) for dietary supplementation in male Barki sheep (*Ovis aries*), It has improved significantly the activities of SOD and catalase as the blood antioxidant defense system [24]. SOD and catalase are important antioxidant enzymes to

maintain the structure and function of cell membranes and prevent lipid peroxidation [27].

Catalase analysis

Analysis was carried out on the results of changes in catalase activity from day 0 to day 35 in the induction of high-fat feed and the administration of seaweed extract nanoparticles for 14 d after induction. Catalase is an enzyme-containing heme that can catalyze hydrogen peroxide dismutase (H_2O_2) into water and oxygen [28, 29]. Based on changes in catalase activity, it can be calculated the percentage of catalase activity and the average catalase activity of each gr8.

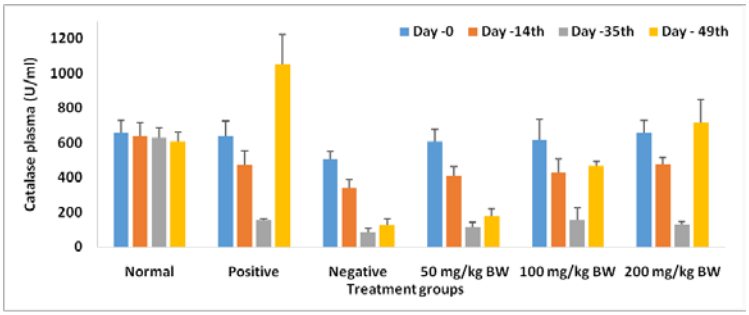

Fig. 7: Bar chart of average catalase levels, data was given in mean±SD, n=4

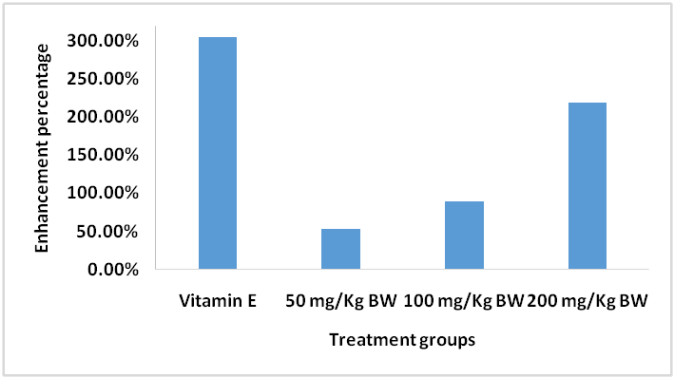

Fig. 8: Enhancement percentage chart of brown seaweed extract nanoparticles

The ability to increase is greatest with Dose III, which is 218.73%, while the first dose was 52.85%, dose II was 88.77%, and positive control was 305.17%. Analysis of variations in average activity was used to see if there were significant differences in nanoparticle extracts of brown seaweed on a high-fat diet as a negative control and vitamin E as a positive control. Statistical test results obtained from the positive control group and doses II and III showed significant differences ($p>0.05$) from negative controls, so it can be concluded that brown seaweed nanoparticles at these doses have antioxidant effects. While I do not yet have activity as an antioxidant, Dose III with positive control did not show a significant difference (p 0.05). Increasing doses I and II did not show a significant difference, whereas, at doses I and III, doses II and III showed significant differences. The result was similar to a study conducted by Fatma *et al.* that determined the effects of Sargassum latifolium from Egypt on increasing the catalase antioxidant enzymes [30]. The other Sargassum genus, Sargassum horneri, brown seaweed taken from Korea, also possessed antioxidative activity because its potency mitigated the increase in catalase activity [31].

CONCLUSION

The nanoparticle has enhanced the activity of BSE. The NBSE has antioxidant activity and could prevent oxidative stress on HFD rats as seen from the MDA, SOD, and catalase parameters. It has good antihyperglycaemic compounds that could be turned into commercially profitable modern formulations of traditional medicine.

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AUTHORS CONTRIBUTIONS

All the authors have contributed equally.

CONFLICT OF INTERESTS

The author declares no conflict of interest.

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