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ABSTRACT

Introduction: Oxidative stress appear to play a significant role in the pathology of periodontitis. Catalase is an antioxidant enzyme that involved in host response to periodontal disease. Stichopus hermannii extract has been known to have medical properties, and regarding to its antioxidant component, it could be explored as an auxiliary antioxidant strategy in periodontal therapy. Objective: The aim of this study was to examine the antioxidant potency of Stichopus hermannii extract to periodontitis by measuring the catalase activity in salivary submandibular and parotid glands of wistar rat. Methods: The study is an experimental laboratory research with post test only control group design. Twenty-four male wistar rats were divided into 4 groups, each consisted of 6 rats. Control groups were given 0.2% CMC Na, were normal group and periodontitis group. Periodontitis were induced by Porphyromonas gingivalis ATCC 33277. Treatment groups were periodontitis rats which given Stichopus hermannii extract per oral 135mg/kg BW once daily (PG group) and applied 0.1ml of 3% extract gel topically on gingival sulcus (TG group) once daily, in 14 consecutive days. The level of catalase were examined from salivary submandibular and parotid glands, measured its absorbance by spectrophotometer in 240 nm. Data were analyzed by Mann-Whitney test. Result: Catalase activity were decreased in periodontitis induced by P. gingivalis (p<0.05). Stichopus hermannii extract peroral and topical increased the catalase activity but less than in normal condition (p>0.05). Conclusion: Stichopus hermannii extract were not significantly increased the catalase activity (p>0.05) in periodontitis.

Keywords: Stichopus hermannii, catalase, periodontitis, Porphyromonas gingivalis

INTRODUCTION

Periodontal disease is an inflammatory disorder that leads to tissue damage and bone loss as a result of complex interactions between pathogenic bacteria and the host's immune response. Periodontal diseases (gingivitis and periodontitis) are among the most widespread chronic conditions affecting populations worldwide. Studies have demonstrated that periodontal disease affects between 10% and 15% of the world's population, representing the greatest cause of tooth loss. Periodontal disease is initiated by the colonization of bacterial pathogens, such as Porphyromonas gingivalis, Actinobacillus actinomycetemcomitans and Bacteroides forsythus, of which P. gingivalis is of most significance. The strong evidence linking ROS to the pathological destruction of the connective tissue during periodontal disease rests on the presence of neutrophils infiltration as the main event in the host's response to bacterial invasion. Free radicals and reactive oxygen species (ROS) are essential to many normal biologic processes. At low concentrations, these free radicals stimulate the growth of fibroblasts and epithelial cells in culture, but at higher concentrations it may result in tissue injury. Even not being considered a potent ROS, is capable of crossing the nuclear membrane and also damaging the DNA. Quantitatively, the main source of superoxide anion (O₂⁻) and other ROS responsible for initiation reactions is the respiratory chain. However, its presence in the periodontal tissue results first and foremost from the activation of phagocytes (neutrophils and
Catalase Activity of Sea Cucumber Extract (Stichopus hermani) to Periodontitis induced by Porphyromonas gingivalis

Macrophages), such as antibacterial agents. It has been suggested that superoxide anion is involved in bone reabsorption. 1,4,9 Tissue injury due to free radical production has been suggested to be enhanced in individuals with periodontal disease due to a lack of adequate antioxidant defense. 8 Total antioxidant capacity concentration was found to be reduced in serum and plasma of periodontitis patients. 9 Changes in gingival environment could impair apoptosis and promote enhanced release of ROS by phagocytes with decrease catalase and SOD activity could promote accumulation of ROS and eased further tissue destruction. 2 Several biologically important compounds have been reported to have antioxidant functions. These include vitamin C (ascorbic acid), vitamin E (a-tocopherol), vitamin A, s-carotene, metallothionein, polyamines, melatonin, nicotinamide adenine dinucleotide phosphate (NADPH), adenosine, co-enzyme Q-10, urate, ubiquinol, polyphenols, flavonoids, phytoestrogens, cysteine, homocysteine, taurine, methionine, s-adenosyl-L-methionine, resveratrol, nitroso-arginine, reduced glutathione (GSH), glutathione peroxidase (GPX), superoxide dismutase (SOD), catalase (CAT), nitric oxide synthase (NOS), heme oxygenase-1 (HO-1) and eicosanoid peroxidase (EPO). 10,11

Periodontal diseases are associated with an imbalance between oxidants and antioxidants in favor of the former due to both an increase in free radical production and a deficit in the total antioxidant activity of saliva. 2 Role of antioxidant as an adjunct in periodontal therapy could be considered to attain the better result of therapy. Indonesia is a maritime country which richly possess many kinds of marine compounds. Marine biota is the source of structurally unique natural products which later known have biomedical properties. 12,13 Sea cucumbers are important components of the marine ecosystem that have long been used for food and folk medicine in the communities of Asia and Middle East. 10,12,13,14 Sea cucumbers have an impressive profile of valuable nutrients such as Vitamin A, Vitamin B1 (thiamine), Vitamin B2 (riboflavin), Vitamin B3 (niacin), and minerals, especially calcium, magnesium, iron and zinc. Therapeutic properties and medicinal benefits of sea cucumbers can be related to the content of a wide array of bioactives especially triterpene glycosides (saponins), chondroitin sulfates, glycosaminoglycan (GAGs), sulfated polysaccharides, sterols (glycosides and saponins), phenolic, steroidal, betas, ketones, peptides, glycoprotein, glycoscyclolipids and essential fatty acids. Generally, most species of sea cucumber share the same bioactive compound mentioned above but in different level contain. 12,13 Sea cucumber also known to have antioxidant bioactive component and omega 3 which have the role in preventing destructive process and bone resorption. 12,13

Some species of sea cucumber have been known to have antioxidant properties. Aqueous and organic extracts from Holothuria scabra, Holothuria leucospiota and Stichopus chloronotus had its antioxidant and antiproliferative properties. 15 Other species, Holothuria edulis lessonii and Stichopus horrens sulenka reported to have antioxidant properties regarding to its phenolic content [16]. Methanolic extract of Stichopus hermani was reported to have its potency of antioxidant activity with IC50 value of 65.68 ppm 17 Regarding to its bioactive content, sea cucumber could be potential source of adjacent antioxidant therapy in periodontitis. In our previous studies, methanolic extract of Stichopus hermani have show antibacterial properties against Porphyromonas gingivalis in vitro. 18 Stichopus hermani extract showed no cytotoxicity to fibroblast gingiva stem cell on the concentration of ≤ 2.5% [19] and could increase salivary SOD activities in periodontitis induced by mixed periodontopathogenesis bacteria. 20 Concerning about the antioxidant adjunct periodontal therapy in periodontal disease, the attempt to have natural antioxidant will be a valuable matter. The aim of this study was to examine the antioxidant potency of Stichopus hermani extract to periodontitis by measuring the catalase activity in salivary submandibular and parotis glands of wisar rat.

METHODS

The study is an experimental laboratories research with post test only control group design.

Sample collecting and extract preparation. Sea cucumber Stichopus hermani were collected from Sumenep coast, immediately rinsed under running tap water, dissected to remove all visceral organs then cut into small pieces, packed and to be kept at ~80 °C until further process of extraction. The small pieces bodywall cut of sea cucumber were then allowed to dry in freeze dryer machine at ~85°C with 5 mtor pressure. Freeze dried sea cucumber were crushed by a blender into powder and performed maceration by immersed with 90% ethanol for 24 hours, filtered, repeated for 3 times resulted in clear filtrate. Filtrate then being evaporated with rotary evaporator at 50°C until resulted in thick extract.

Periodontitis Induction

Periodontitis Induction were started by the administration of 0.1% chlorhexidine topically and 20 mg Kanamycin and Ampicillin in rat in the drinking water as oral preconditioning once daily for 4 consecutive days. Bacterial suspension of P. gingivalis ATCC 33277 containing 10^8 CFU/ml in 2 ml PBS were inoculated by peroral administration with nasogastric tube. P. gingivalis suspension also swabbed in buccal labial-palatal gingiva along molar to molar region and anal regio. The administration of P. gingivalis were done 3
Table 1. Mean of catalase activity of Sticophus hermani extract to periodontitis induced by Porphyromonas gingivalis

<table>
<thead>
<tr>
<th></th>
<th>Catalase activity (U/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Submandibular</td>
</tr>
<tr>
<td>Normal</td>
<td>439.39 ± 54.35</td>
</tr>
<tr>
<td>Periodontitis</td>
<td>334.83 ± 19.30</td>
</tr>
<tr>
<td>PO</td>
<td>317.56 ± 11.24</td>
</tr>
<tr>
<td>TO</td>
<td>370.56 ± 118.88</td>
</tr>
</tbody>
</table>

Fig 1. Catalase activity measured an salivary submandibular and parotid gland of periodontitis Wistar rat given extract of Sticophus hermani peroral (PO) and topical on salicus gingiva (TO)

Table 2. Summary of Mann-Whitney test result on catalase activity of Sticophus hermani extract to periodontitis induced by Porphyromonas gingivalis

<table>
<thead>
<tr>
<th></th>
<th>Normal</th>
<th>Periodontitis</th>
<th>PO group</th>
<th>TO group</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Sub Mand</td>
<td>Par</td>
<td>Sub Mand</td>
<td>Par</td>
</tr>
<tr>
<td>Normal</td>
<td>0.064*</td>
<td>0.394</td>
<td>0.485</td>
<td>0.937</td>
</tr>
<tr>
<td>Periodontitis</td>
<td>0.589</td>
<td>0.937</td>
<td>0.818</td>
<td>0.398</td>
</tr>
<tr>
<td>PO group</td>
<td>0.689</td>
<td>0.937</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

times in 4 days. Periodontitis condition was achieved histopathologically on 3 weeks after the bacterial induction and ready for the next step of extract treatment

Extract administration

Twenty-four male Wistar rats age 8-10 weeks, weight 150-200 gram were divided into 4 groups, each consisted of 6 rats. Control groups were given 0.2% CMC Na, consisted of normal group and periodontitis group. Treatment groups were periodontitis rats which given Sticophus hermani extract per oral 135mg/ kg BW once daily (PO group) and applied 0.1ml of 3% extract gel topically on gingival sulcus (TO group) once daily, in 14 consecutive days.

Catalase test

Catalase activity were examined from salivary submandibular and parotid glands. A hundred microliters of each glands were crushed and mixed to 900 µl buffer 50mM at pH7 until homogen. A hundred µl of the mixture were taken and added 900 µl buffer phosphate and 1 ml H2O2 60 mM, mixed, then heat 5 minutes then measured its absorbance by spectrophotometer in 240 nm.

RESULT

Catalase activity was reduced on the periodontitis group on both samples of submandibular and parotid glands compare to normal control condition. After the per oral and topical treatment of Sticophus hermani extract to the periodontitis rats, the catalase activity were increased both on samples of submandibular and parotid glands as shown in Table 1 and Fig. 1.

Further statistical analysis by Mann-Whitney test is shown in Table 1. Result showed that catalase activity decreased on periodontitis condition (p<0.05). Treatment with Sticophus hermani extract increased the catalase activity in both submandibular and parotid sample (p<0.05) but less than in normal condition as shown in Table 2.

DISCUSSION

Oxidative stress have been known to play significant role in the pathology of periodontal disease, it could be result directly from excessive ROS activity or antioxidant deficiency or indirectly by creating pro-inflammatory condition which lead to tissue destruction. Many studies have suggested that imbalance in the level of free radicals, ROS, and antioxidant play an important role in initiation and progression of periodontal disease. It has been considered that antioxidant could be applied as adjunct periodontal therapy, it could be a breakthrough in overcome periodontal disease. While studies about the role of antioxidant as adjunct periodontal therapy are expanding, attempts to search the natural antioxidants are developed as well.

The role of antioxidant in periodontitis have been studied from many source of samples as saliva, serum, GCF, gingiva and periodontal tissue. Periodontal disease is associated with reduced salivary antioxidant status and increased oxidative damage within the oral cavity, regarding to the salivary source, in this study, the antioxidant potency of a natural source sea cucumber extract were examined on salivary submandibular and parotid glands.

An antioxidant is any substance that, when present at low concentrations compared to those of an oxidizable substrate, significantly delays or prevents oxidation of that substrate. Several biologically...
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ACKNOWLEDGEMENT
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