Mal J Nutr 5:55-59, 1999

The potential of the coelomic fluid in sea cucumber as an antioxidant

Hawa I, Zulaikah M, Jamaludin M, Zainal Abidin AA, Kaswandi MA and Ridzwan BH

Department of Biomedical Science, Faculty of Allied Health Sciences,
Universiti Kebangsaan Malaysia, Jalan Raja Muda A. Aziz, 50300 K. Lumpur, Malaysia.

ABSTRACT

In a biological system the product of free-radical induced-lipid peroxidation are conjugated dienes (CDs), lipid peroxides and malondialdehyde (MDA). Its control depends on antioxidant activities. Antioxidant defend the body system by controlling the damage caused by the free radicals. This paper describes lipid peroxidation process and antioxidant activities present in three species of holothuroid obtained from the coastal waters of Terengganu, Malaysia. Among the three species studied, the coelomic fluid of *Bohadschia mamorata vitiensis* contained the highest level of protein (7.24 $\pm$ 0.04)g/L. The coelomic fluid of *Stichopus badionotus* Selenka contained the highest level of superoxide dismutase (SOD) (9.46 $\pm$ 0.99)X 10^5 IU/g protein, MDA (16.46 $\pm$ 1.28)nmol/g protein and total antioxidant activities (AOA) (58.81 $\pm$ 5.70) %. In *Stichopus variegatus* Semper the coelomic fluid only demonstrated the highest level of CDs (94.19 $\pm$ 11.34) RU/g protein compared with the other two species. This preliminary study indicated that some form of antioxidant activities are present in the coelomic fluid of holothuroids.

INTRODUCTION

In the south-east Asia regions sea cucumbers are taken as a food supplement (Ridzwan and Che Bashaah 1985; Ridzwan 1993). Holothuroid and its product could cure certain ailments (Shimada 1969; Sit 1998). Fishermen often consume the liquid portion or the coelomic fluid of sea cucumbers in an attempt to remain healthy for days while out at sea. It is believed that if consumed regularly sea cucumbers could reduce hypertension, asthma, heal internal wounds and cancer. This bentoic organism has a vascular system filled with fluid believed to contain bioactive substances important for wound healing (Perchenik 1969).

Certain extracts of sea cucumbers had also been described to contain vitamin E, an antioxidant (Madhavan 1998). Therefore, the purpose of this study is to further investigate whether other antioxidant activities are present in the coelomic fluid of three species of holothuroids that inhabited the Malaysian coastal waters. The species investigated are *Bohadschia mamorata vitiensis, Stichopus badionotus* Selenka and S*tichopus variegatus* Semper.

MATERIALS AND METHODS

Samples were collected from the coastal waters of Terengganu, Malaysia. An incision measuring 3-5 cm were made on the ventral side taking care not to injure the internal organs. The coelomic fluid were collected in separate beakers, later transferred into test tubes, frozen until analysed. The protein component was measured by using the Biuret calorimetric method (Wooton and Freeman 1982), while the MDA levels determined using the standard procedures (Hunter and Jamaludin 1986). The activity of SOD was determined by a modified Flohe and Otting (1984) method, whereas total antioxidant activity was measured according to Stock *et al.* (19874). Conjugated dienes was determined following the Lunec and Dormandy (1978) method.

RESULTS

The results of the antioxidant activities in the coelomic fluid of the three species of holothuroids is shown in Table 1. The coelomic fluid of *Bohadschia mamorata vitiensis* contained the highest concentration of crude protein (7.24 $\pm$ 0.04 g/L) followed by *Stichopus variegatus* Semper (2.05 $\pm$ 0.40 g/L) and *Stichopus badionotus* Selenka (1.80 $\pm$ 0.24 g/L).

Among the three species studied the highest level of SOD activity was detected in the coelomic fluid of *Stichopus badionotus* Selenka (9.40 $\pm$ 0.99)X 10^5 IU/g protein followed by *Stichopus variegatus* Semper (8.20 $\pm$ 0.91) X 10^5 IU/g protein and *Bohadschia mamorata vitiensis* (5.30 $\pm$ 0.18) X 10^5 IU/g protein.

The coelomic fluid also contained CDs, the product of primary lipid peroxidation. It was the highest in *Stichopus variegatus* (94.19 $\pm$ 11.34) RU/g protein followed by *Bohadschia mamorata vitiensis* (78.71 $\pm$ 1.56) RU/g protein and *Stichopus badionotus* Selenka (70.00 $\pm$ 4.55) RU/g protein. *Stichopus badionatotus* contained the highest level of (16.46 $\pm$1.29) nmol/g protein, followed by *Stichopus variegatus* (9.89 $\pm$ 0.04) nmol/g protein and *Bohadschia mamorata vitiensis* (7.41 $\pm$ 0.86) nmol/g protein. Total antioxidant activity was found to be the highest in *Stichopus badionotus* Selenka (58.81 $\pm$ 5.70) %, followed by *Bohadschia mamorata vitiensis* (52.20 $\pm$ 5.70) % and *Stichopus variegatus* Semper (47.71 $\pm$ 8.95) %.

TABLE 1. The Composition of Sea cucumber Coelomic Fluid

Species	Product	Amount
Stichopus badionotus Selenka	Crude protein	1.80 ± 0.24 g/L
	Superoxide dismutase	9.40 ± 0.99 X 10^5 IU/g protein
	Malondialdehyde	16.46 ± 1.29 nmol/g protein
	Conjugated dienes	70.00 ± 4.55 RU/g protein
	Total antioxidant activity	8.81 ± 5.7 %
Stichopus variegatus Semper	Crude protein	2.05 ± 0.40 g/L
	Superoxide dismutase	8.20 ± 0.91 X 10^5IU/g protein
	Malondialdehyde	9.89 + 0.04 nmol/g protein
	Conjugated dienes	94.19 ± 1134 RU/g protein
	Total antioxidant activity	47.71 ± 8.95 %
Bohadschia mamorata vitiensis	Crude protein	7.24 ± 0.04 g/L
	Superoxide dismutase	5.30 ± 0.18 X 10^5IU/g protein
	Malondialdehyde	7.41 ± 0.86 nmol/g protein
	Conjugated dienes	78.71 ± 1.56 RU/g protein
	Total antioxidant activity	52.20 ± 5.70 %

DISCUSSION

Sea cucumbers, as an alternative source of natural dietary antioxidants probably plays an important role to counter the detrimental effects of oxygen free radicals formed from normal metabolism and external factors (pollution, radiation). This is implicated in the development of today's common illness such as cancer (gene damage), premature ageing, cardiovascular and other degenerative diseases. Indeed, our study revealed the presence of a mild concentration of total protein in the coelomic fluid of all the three species of holothuroid. The findings, however, are similar to the results reported by Canicatti (1989, 1990) but on *Holothuria polii* and *Marthasterias glacialis*. Furthermore, our study also showed a remarkable increase in the activity of antioxidant enzymes, namely, SOD. This is in agreement with our study on lipid peroxidation of polyunsaturated fatty acids of ox brain homogenates as a bioassay system for auto oxidation. A similar result was obtained when using plasma serum incubated in tissue homogenate inhibit lipid auto oxidation process (Barber 1961). According to Dormandy (1969) all animal tissues have a natural defence system to inhibit the process of oxidation towards polyunsaturated fatty acid either by enzymatic or non-enzymatic reaction. The main role of antioxidants *invivo* is in the prevention of the peroxidation of PUFAs and the stabilization of the biological membranes.

However, the role of this antioxidant in holothuroids remain to be elucidated. Most probably, the increased production of oxygen derived free radicals during hypoxic alteration due to external changes

occurred at a highly accelerated rate, that is, when molecular oxygen was reintroduced to the previously hypoxic tissues of holothuroids. Thus, hypoxia enhanced the damages induced by oxygen metabolites complicated with the reintroduction of molecular oxygen when the antioxidant defense system of holothuroid was not effective or overloaded. Thus, our findings are similar to previous reports on human plasma (Barber 1961; Vidlakova *et al*. 1972; Slater 1972).

CONCLUSIONS

These results indicated that the coelomic fluid of holothuroids from Terengganu coastal areas contained antioxidant substances. These substances could possibly be produced during the hypoxic re-oxygenation, during which a high level of protection against the oxygen induced damage of "stunned holothuroid" after being exposed to the polluted habitat. It also appears that all animals have some antioxidant activities but the strength varies with species. Therefore, holothuroids could provide a good source of external antioxidant for human in the future.

ACKNOWLEDGEMENTS

The study is funded by IRPA grant 09-02-02-0021. "Identification and evaluation of structure, bioactive agent and consumption of local sea cucumbers".

REFERENCES

Barber, A.A. 1961. Inhibition of lipid peroxide formation by vertebrate blood serum. *Arch. Biochem. Biophysics*. **96**:30-43.

Canicatti, C. 1989. Tthe lytic system of *Holothuria polii (Echinodermata*) : A review. *Boll. Zool*. **55**:139-144.

Canicatti, C. 1990. Protease activity in *Holothuria polii* coelomic fluid and coelomocyte lysate. *Comp. Biochem. Physiol*. **96B(1)**:145-148.

Dormandy, T.L. 1969. Biological rancidification. *Lancet.* **27**(2) 2722.

Flohe, L. and Otting, F. 1984. Oxygen radicals in biological system.(Part A : Oxygen radical and antioxidants). *Method. Enzymology* **105**: 93-97.

Hunter, M.I.S. and Jamaludin, M. 1986. Plasma antioxidants and lipid peroxidation product in Duchenne muscular dystrophy. *Clin. Chim. Act*. **155**:123-132.

Lunec, J. and Dormandy, T.L. 1978. Flourescent lipid-peroxidation products in synovial fluid. *Clin. Sci*. **56**:53-59.

Madhavan, R. K.1998. Mengesan kehadiran vitamin E dan unsur-unsur logam dalam dalam tiga spesies timun laut. *Tesis Sarjana Muda Sains Bioperubatan* (Kepujian). K. Lumpur : Fakulti Sains Kesihatan Bersekutu, Universiti Kebangsaan Malaysia.

Perchenik, J.A. 1996. The Echinoderms. In Dubuque W.M.C *Biology of Invertebrates*. 3rd ed. 445-474. London : Brown Publisher.

Ridzwan, B.H. 1993. Ekinoderma yang boleh dimakan: holothuroid: **Dlm.** *Sumber Makanan persisiran laut Sabah*. 149-188. Kuala Lumpur : Dewan Bahasa dan Pustaka.

Ridzwan, B.H. and Che Bashah,C.C. 1985. Tinjauan awal taburan dan penilaian holothurian di beberapa kawasan perairan Sabah sebagai sumber sumber pemakanan. *Sumber*, **1**:143-155. Fakulti Sains Sumber Alam, Universiti Kebangsaan Malaysia.

Shimada, S. 1969. Antifungal steroid glycoside from sea cucumber. *Science*. **163**:1462.

Sit, N.W. 1998. Aktiviti antifungus timun laut terhadap yis. *Tesis Sarjana Muda Sains Bioperubatan* (Kepujian). K. Lumpur : Fakulti Sains Kesihatan Bersekutu, Universiti Kebangsaan Malaysia.

Slater, T.F.1972. **In**: *Free radical mechanism in tissue injury*. London. Pion

Stock., J. Gutteridge, J.M.C. Sharp, R. J. Dormandy, T.L.1974. The inhibition of lipid autoxidation by serum and its relation to serum protein and alpha-tocopherol. *Clin. Sci. Mol. Medic*. **47**:223-233.

Vidlakova, M. Erazimova, J. Horka, J. and Placer, Z. 1972. Relationship of serum antioxidant activity to tocopherol and the serum inhibition of lipid peroxidation. *Clin. Chem. Acta*. **36**:61-66.

Wootoon, I.D.P. and Freeman, H. 1982. *Proteins, microanalysis in medical biochemistry* 6th edition. New York: Churchill Livingstone.