



Protective Effects of Fish Oil on Carbon tetrachloride Induced Hepatotoxicity in Rabbits

M. Alaraj ^{a*}, S. Qiblawi ^b

^a Department of Pharmacology, College of Medicine, University of Hail, Saudi Arabia

^b Department of Histology, College of Medicine, University of Hail, Saudi Arabia

^a Email: ibrahim_naseem@yahoo.com

^b Email: drsam_2013@yahoo.com

Abstract

The present investigation was carried out to evaluate the role of fish oil in modulating carbon tetrachloride (CCl₄) induced hepatotoxicity in rabbits. Fish oil was given orally to rabbits for 7 days and then a single dose of CCl₄ was administered (1 ml/kg body weight intraperitoneally). Serum parameters (Serum alanine aminotransferase, ALT; and serum aspartate aminotransferase, AST activities, total bilirubin and albumin concentration) were estimated for experimental and control animals. Fish oil showed significant protection against CCl₄ -induced hepatocellular damage as evident from a significant reduction (p<0.001) of elevated serum AST and ALT compared to rabbits treated with single dose of CCl₄ only in control group. Also, fish oil significantly prevented CCl₄ induced elevation of serum bilirubin and showed significant elevation in the depleted concentration of albumin in comparison with control group treated with CCl₄ only. These findings indicate that fish oil have a protective activity against CCl₄ induced hepatotoxicity.

Keywords: Fish oil; hepatotoxicity; carbon tetrachloride; rabbits.

* Corresponding author.

E-mail address: ibrahim_naseem@yahoo.com.

1. Introduction

Humans are continuously exposed to different kinds of chemically and structurally diverse present in the air, food and soil. Most of these pollutants cause free radicals- mediated lipid peroxidation, leading to disruption of bio-membranes, inhibition of cellular and antioxidant defense system and dysfunction of cells and tissues and contribute significantly to various human disease pathophysiology including hepatotoxicity. [1-4]

Carbon tetrachloride (CCl₄) has widespread use in various industries as a solvent and is readily absorbed from the gastrointestinal and respiratory tracts [5]. It may cause lipid peroxidation and is known to cause liver damage [4]. It has been shown, that the principle clinical signs of liver injury in humans who ingest carbon tetrachloride are swollen and tender of liver, elevated levels of hepatic enzyme in the serum, elevated serum bilirubin levels and appearance of jaundice, and decreased serum levels of proteins such as albumin and fibrinogen [6, 7]. CCl₄ has been widely used to induce liver injury and fibrosis in different experimental models [8].

In recent years, fish oil is gaining attention as a nutraceutical and source of potential pharmaceuticals [9]. Fish oil contains omega-3 fatty acids, one of the two main classes of fatty acids. These omega -3 polyunsaturated fatty acids, primarily eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA), have been found as agents responsible for the beneficial and protective effects of dietary fish oil [10]. Omega-3 fatty acids found in fish oil are known for their anti inflammatory properties throughout the body, in delaying the onset of autoimmune disease in animals models and the severity of arthritis in human [11]. Evidences also indicate that consumption of dietary fish oil is associated with suppressed inflammatory response in patients with rheumatoid arthritis [12] and psoriasis [13] and that it reduces mortality from cardiovascular disease [14]. Moreover, fish oil rich in omega -3 polyunsaturated fatty acids such as eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) has been shown to be protective against experimentally induced colon cancer in large number of studies [15, 16]. It has also been reported that the intake of omega-3 fatty acids suppresses the so-called free radical diseases such as cancer, aging and atherosclerosis, [17]. Furthermore, many studies showed that fish oil prevents gentamicin and cyclosporine-induced nephrotoxicity [18, 19], and cisplatin-induced hepatotoxicity [20].

Fish oil feeding also appears to have the most benefit in reducing free radical damage and increasing antioxidant enzyme activity in response to the induction of oxidative stress by cyclophosphamide injection [21]. The present investigation was carried out to evaluate the role of fish oil in modulating the carbon tetrachloride- induced hepatotoxicity in rabbits.

2. Materials and Methods

2.1. Chemicals

Carbon tetrachloride (CCl₄) was purchased from Koch Light laboratories Ltd., England. Fish oil was procured locally from Wadii Alnaheel Company, Hail, Saudi Arabia.

2.2. Animals

Male rabbits weighing 1-1.5 kg were used in the study. The animals were maintained under standard laboratory conditions of temperature and relative humidity with 12 h light-dark cycle. The animal studies were approved by the ethics committee of University of Hail.

2.3. Experimental Design

Animals were divided into the following groups of 6 rabbits each: **Normal Group:** animals received only fish oil and put on normal diet. **Control Group:** animals received CCl_4 , at the single dose 1ml /kg body weight Intraperitoneally (i.p) with olive oil, 1:1, on 7th day. **Experimental Group:** Animals received fish oil, 1.5ml/kg body weight/day, orally for 7 days. On the 7th day animals received CCl_4 1ml/kg 5ml/kg body weight/day intraperitoneally (i. p) with olive oil, 1:1. The selected dose of fish oil based on previous report [22] (Alicja et al., 1998). Animals were killed under chloroform anesthesia after 24 hour of the hepatotoxin administration of carbon tetrachloride. Blood was collected by cardiac puncture using a syringe and allowed to coagulate at room temperature for 30 minute and serum separated by centrifugation at 3000 rpm for determining the serum ALT and AST activities, total bilirubin and albumin concentration.

2.4. Assay of serum ALT and AST activities

Serum alanine aminotransferase (ALT) and serum aspartate aminotransferase (AST) were determined spectrophotometrically using commercial kit according to the instruction of the manufacturer (Sigma- Aldrich, St. Louis, MO, USA).

2.5. Assay of serum bilirubin and albumin concentrations

Serum bilirubin (TB) and albumin (Alb) concentrations were also determined spectrophotometrically

Using commercial kit and following the instruction of the manufacturer (Sigma-Aldrich, St. Louis, MO, USA).

3. Results

3.1. Serum enzyme parameters

Rabbits administered fish oil (1.5ml/kg body wt) and CCl_4 in fish oil (1: 1) treatment (experimental group) showed significant protection against CCl_4 induced hepatocellular damage as evident from a significant reduction ($p<0.001$) of elevated serum activities of aspartate aminotransferase (AST) and alanine aminotransferase (ALT) compared to rabbits treated with single dose of CCl_4 only in control group (Fig. 1 and 2).

3.2. Estimation of total bilirubin and albumin concentrations

The present study showed that animals treated with fish oil (1.5ml/kg body wt) for 7 days and a single dose of CCl₄ in fish oil (1:1) displayed a significant difference ($p < 0.001$, $p < 0.001$) in the concentration of total bilirubin and albumin respectively compared with the control animals (Fig. 3 and 4) which are treated with a single dose of CCl₄ only. There was a significant increase in the concentration of bilirubin in the serum of CCl₄-treated rabbits as compared to the experimental group. Administration of fish oil prevented CCl₄-induced elevation of serum bilirubin and showed significant elevation in the depleted concentration of albumin in comparison with control group treated with CCl₄ only.

3.2. Statistical analysis:

The SPSS version 15 was used in data analysis. Data were expressed as mean \pm SD. Mean values were compared using analysis of variance (ANOVA) followed by Duncan multiple range test. P-values less than 0.05 were considered significant.

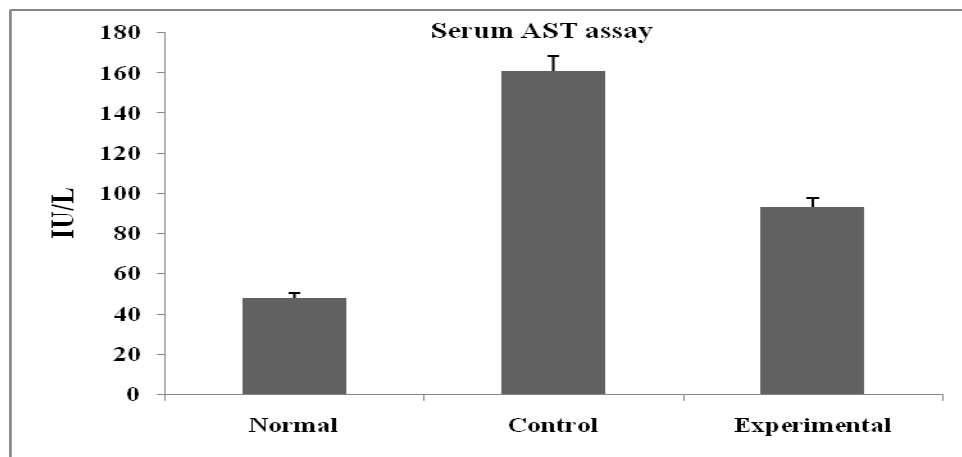


Figure 1: Influence of fish oil on serum activity of AST in rabbit subjected to carbon tetrachloride induced hepatotoxicity.

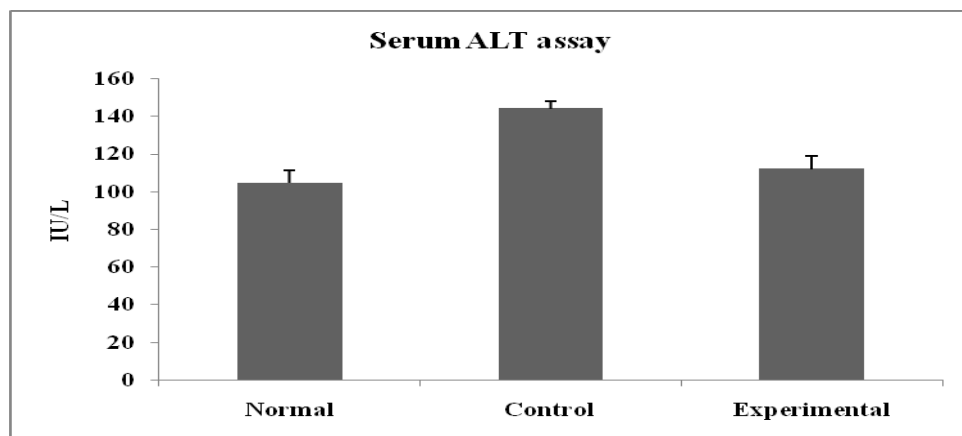


Figure 2: Influence of fish oil on serum activity of ALT in rabbit subjected to carbon tetrachloride induced hepatotoxicity.

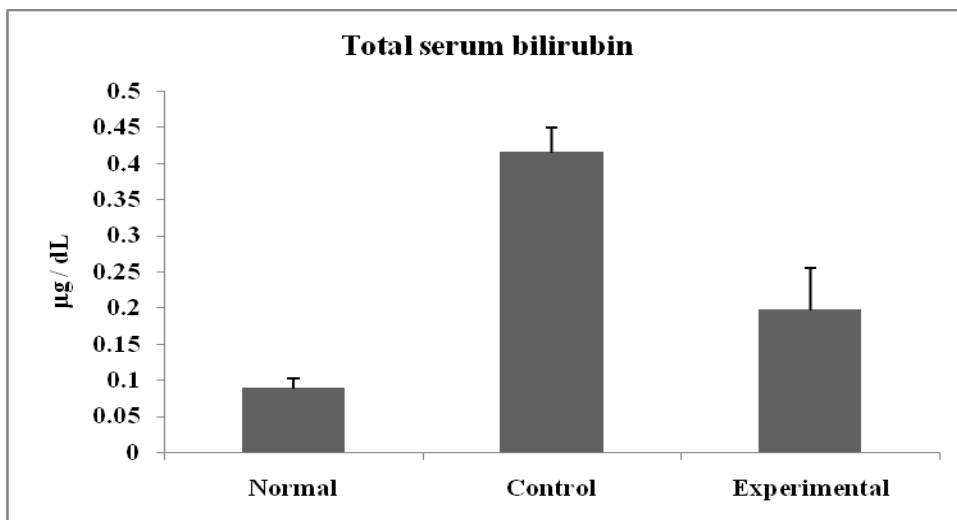


Figure 3: Influence of fish oil on total serum bilirubin in rabbits subjected to CCl_4 induced hepatotoxicity.

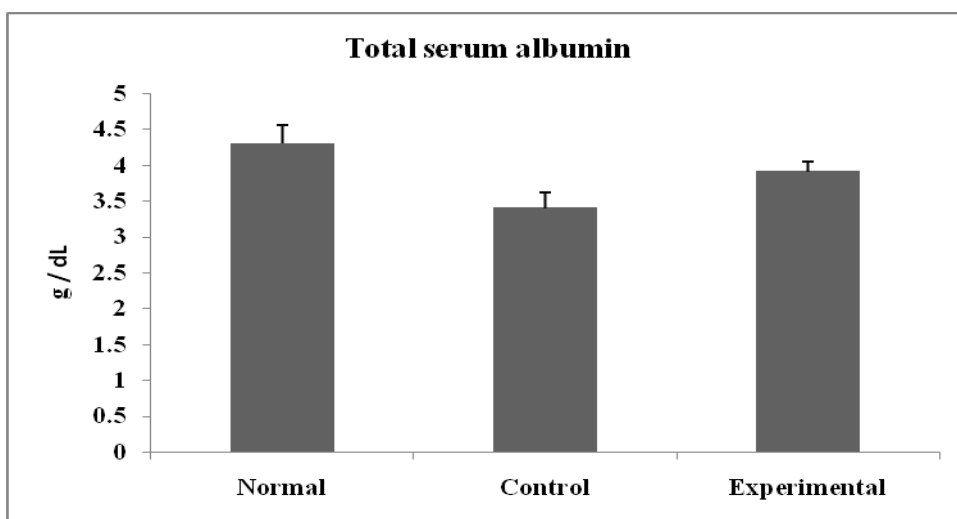


Figure 4: Influence of fish oil on total serum albumin in rabbits subjected to CCl_4 induced hepatotoxicity.

4. Discussion

CCl_4 is a well-known hepatotoxic agent and the preventive action of its liver damage has been widely used as an indicator of liver protective activity of drugs in general [23, 24]. CCl_4 is biotransformed by Cytochrome P-450 system to produce trichloromethyl free radicals. These free radicals may again react with oxygen to form trichloromethyl peroxy radicals, which may attack lipids on the membrane of endoplasmic reticulum to elicit lipid peroxidation, finally resulting in cell necrosis and consequent cell death [25, 26]. Since free radicals play such an important role in CCl_4 -induced hepatotoxicity, it seems logical that compounds that neutralize such radicals may have a hepatoprotective effect. Indeed, various natural products have been reported to protect against CCl_4 -induced hepatotoxicity [24, 27].

The present study showed that animals treated with single dose of CCl₄ showed significant hepatic damage which was noted through a substantial increase in the levels of serum enzymes AST and ALT. Serum transaminases AST and ALT have long been considered as sensitive indicator of hepatic injury [24, 28, 29]. Injury to the hepatocytes alters their transport function and membrane permeability, leading to leakage of enzymes from the cells [24], this leakage causes a decrease in levels of ALT and AST in hepatic cells but increase in levels of serum ALT and AST [29]. This may explain the increase in levels of serum AST and ALT observed in CCl₄ treated rabbits in the present study. Treatment of rabbits with fish oil prior to the challenge of CCl₄, appears to have a protective effects against hepatic injury to considerable extent which was reflected by the ability of fish oil to lower the elevated serum enzyme levels resulting from the administration of CCl₄ alone.

The present study also showed that pretreatment with fish oil for 7days prior to the CCl₄ offered hepatoprotection as evidenced by significant reduction of the rise in concentration of bilirubin compared to control group (CCl₄ only). This suggests the possibility of fish oil being able to stabilize biliary dysfunction in rabbit liver during hepatic injury with CCl₄.

Hypoalbuminemia can be deemed as useful index of severity of hepatocellular damage. The lowered levels of albumin shown in the serum of CCl₄ -treated rats reveal the severity of hepatopathy [30] . In the present study, albumin concentration was very low in rabbits treated with CCl₄ alone. Fish oil treatment 7 days before intraperitoneal administration of CCl₄ has shown significantly increased albumin level compared to control group.

5. Conclusion

The results of the present study suggest that fish oil possess protective activity against CCl₄ induced hepatotoxicity. According to these results it can be proposed that fish oil can serve as a potent hepatoprotective agent.

References

- [1] Ames, B.N. "Pollution, pesticides and cancer." *Journal of Association of Analytical Chemists International*. vol. 75, pp. 1-5, 1992.
- [2] Kehrer, J.P. "Free radicals as mediators of tissue injury and disease." *Critical Reviews in Toxicology*, vol. 23, pp 21-48, 1993.
- [3] Stohs, S.J. & Bagchi, D. "Oxidative mechanisms in the toxicity of metal ions." *Free Radical Biology and Medicine*, vol. 18, pp. 321-336, 1995.
- [4] Battu, G. R., Rao, Y. V. and Dasari, V. S. "Antihepatotoxic effect of *Elephantopus scaber* L. on carbon tetrachloride induced hepatotoxicity in rats." *Recent Research in Science and Technology*, vol. 4, pp. 21-24, 2012.

- [5] Sanzgiri, U. Y., Kim, H. J., Muralidhara, S., Dallas, C. E. & Bruckner, J. V. "Effect of route and pattern of exposure on the pharmacokinetics and acute hepatotoxicity of carbon tetrachloride." *Toxicology and Applied Pharmacology*, vol. 134, pp. 148-154, 1995.
- [6] New, P. S., Lubash, G. D., Scherr, L., et al, "Acute renal failure associated with carbon tetrachloride intoxication." *Journal of American Medical Association*, vol. 181, pp. 903-906. 1962.
- [7] Straus, B. "Aplastic anemia following exposure to carbon tetrachloride." *Journal of American Medical Association*, vol. 55, pp. 737-739, 1954.
- [8] Shaker, M. E., Houssen, M. E., Abo-Hashem, E. M. & Ibrahim, T. M. "Comparison of vitamin E, L-carnitine and melatonin in ameliorating carbon tetrachloride and diabetes induced hepatic oxidative stress." *Journal of Physiology and Biochemistry*, vol. 65, pp. 225-234, 2009.
- [9] Kaur, N., Chugh, V. & Gupta, A. K. "Essential fatty acids as functional components of foods: a review." *Journal of Food Science and Technology*, vol. 10, pp. 2289-2303, 2014.
- [10] Christopher A. J. "Diet manipulation and prevention of aging, cancer and autoimmune disease." *Current Opinion in Clinical Nutrition and Metabolic Care* vol. 8, pp. 382-387, 2005.
- [11] Chapkin, R. S., McMurray, D. N. & Jolly, C. A. "Dietary N-3 polyunsaturated fatty acids modulate T-lymphocyte activation: clinical relevance in treating diseases of chronic inflammation." In "*Nutrition and Immunology: principles and practice*" Gershwin, M. E., German, B. J. & Keen, C. L. Eds.. Totowa: Humana Press, 2000, pp. 121-134.
- [12] Kremer, J. M., Jubiz, M. A., Rynes, R. J., Bartholomew, L. E., Bigaouette, J., Timchalk, M., Beefer, D. & Lininger, L. "Fish oil fatty acid supplementation in active rheumatoid arthritis: a double-blind, controlled, cross-over study." *Annals of Internal Medicine*, vol. 106, pp. 497-503, 1987.
- [13] Bittiner, S. B., Tucker, W. F., Cartwright, I. & Bleehan, S. S. "A double blind, randomized, placebo-controlled trial of fish oil in psoriasis." *The Lancet*, vol. 331, pp. 378-380, 1988.
- [14] Dolecek, T. A. & Granditis, G. "Dietary polyunsaturated fatty acids and mortality in the multiple risk factor intervention trial (MRFIT)." *World Review of Nutrition and Dietetics*, vol. 66, pp. 205-216, 1991.
- [15] Deschner, E. E., Lytle, J. S., Wong, G., Ruperto, J. F. & Newmark, H. L. "The effect of dietary omega-3 fatty acids (fish oil) on azoxymethanol-induced focal areas of dysplasia and colon tumor incidence." *Cancer*, vol. 66, pp. 2350-2356, 1990.
- [16] Chang, W. C. L., Chapkin, R. S. & Lupton, J. R. "Predictive value of proliferation, differentiation and apoptosis as intermediate markers for colon tumorigenesis." *Carcinogenesis*, vol. 18, pp. 721-730, 1997.

- [17] Darghosian, L., Free, M., Li, J., Gebretsadik, T., Bian, A., Shintani, A., McBride, B. F., Solus, J., Milne, G., Crossley, G. H., Thompson, D., Vidaillet, H., Okafor, H., Darbar, D., Murray, K. T. and Stein, C.M. "Effect of omega-three polyunsaturated Fatty acids on inflammation, oxidative stress, and recurrence of atrial fibrillation. *American Journal of Cardiology*, vol.115, pp.196-201, 2015.
- [18] Abdel-Gayoum, A. A., Bashir, A. A. & El-Fakhri, M. M. Effect of fish oil and sunflower oil supplementations on gentamicin induced nephrotoxicity in rat. *Human and Experimental Toxicology*, vol. 14, pp. 884–888, 1995.
- [19] Priyamvada, S., Priyadarshini, M., Arivarasu, N. A., Farooq, N., Khan, S., Khan, S. A., Khan, M. W. & Yusufi, A. N. K. "Studies on the protective effect of dietary fish oil on gentamicin-induced nephrotoxicity and oxidative damage in rat kidney." *Prostaglandins, Leukotriens and Essential Fatty Acids*, vol. 78, pp. 369–381, 2008.
- [20] Naqshbandi, A., Khan, M. W., Rizwan, S., Yusufi, A. N. K. & Khan, F. "Studies on the protective effect of fish oil against cisplatin induced hepatotoxicity." *Biology and Medicine*, vol. 3, pp. 86-97, 2011.
- [21] Bhattacharya, A., Lawrence, R. A & Krishnan, A. "Effect of dietary n-3 and n-6 oils with and without food restriction on activity of antioxidant enzymes and lipid peroxidation in livers of cyclophosphamide treated autoimmune-prone NZB/W female mice." *Journal of American College of Nutrition*, vol.22, pp. 388-399.,2003.
- [22] Alicja, M., Birgit, H., Jorgen, H., Henrik, F., Bartnikowska, E., Anderson, P. S. & Bertelsen, L. S. "Comparison of the effects of fish oil and olive oil on blood lipids and aortic atherosclerosis in Watanabe heritable hyperlipidaemic rabbits." *British Journal of Nutrition*, vol. 80, pp. 565-573, 1998.
- [23] Feng, Y., Siu, K., Ye, X., Wang, N., Yuen, M., Leung, C., et al. "Hepatoprotective effects of berberine on carbon tetrachloride-induced acute hepatotoxicity in rats." *Chinese Medicine*, vol. 5, pp. 1-6, 2010.
- [24] Jain, N. K. & Singha, A. K. "Ameliorative effects of *Spinacia oleracea* L. seeds on carbon tetrachloride (CCl₄) induced hepatotoxicity: *In vitro* and *in vivo* studies." *Asian Pacific Journal of Tropical Biomedicine*; S232-S237, 2012.
- [25] Ilavenil, S., Kaleeswaran, B. & Ravikumar S. "Protective effects of lycorine against carbon tetrachloride induced hepatotoxicity in Swiss albino mice." *Fundamental and Clinical Pharmacology*, vol. 26, pp 393-401, 2011.
- [26] Nada, S. A., Omara, E. A., Abdel-Salam, O.M. & Zahran, H.G. "Mushroom insoluble polysaccharides prevent carbon tetrachloride-induced hepatotoxicity in rat." *Food and Chemical Toxicology*, vol. 48, pp. 3184-3188, 2010.

- [27] Hsiao, G., Shen, M.Y., Lin, K.H., Lan, M.H., Wu, L.Y., Chou, D.S., Lin, C.H., Su, C.H., Sheu, J.R. "Antioxidative and hepatoprotective effects of *Antrodia camphorate* extract." *Journal of Agricultural and Food Chemistry*, vol. 51, pp. 3302- 3308, 2003.
- [28] Molander, D.W., Wroblewsk, F. & La Due, J.S. "Transaminase compared with cholinesterase and alkaline phosphatase an index of hepatocellular integrity." *Clinical Research Proceedings*, vol. 3, pp. 20-24, 1955.
- [29] Raja, S., Ahamed, K. F. H., Kumar, V., Mukherjee, K., Bandyopadhyay, A. & Mukherjee, P.K. "Antioxidant effect of *Cytisus scoparius* against carbon tetrachloride treated liver injury in rats." *Journal of Ethnopharmacol*, vol. 109, pp. 41-47, 2007.
- [30] Aniya, Y., Koyoma, T., Miyagi, C., Miyahira, M., Inomata, C., Kinoshita S.T. "Free radical scavenging and hepatoprotective actions of the medicinal herb, *Crassocephalum crepidioides* from the Okinawa islands." *Biological and Pharmaceutical Bulletin*, vol.28, pp. 19-23, 2005.