

Ethnobotanical Leaflets 12: 1206-12. 2008.

Immunomodulatory Effect of Coconut Protein on Cyclophosphamide Induced Immune Suppressed *Swiss Albino* Mice

¹A. Geo Vigila and ²X. Baskaran

¹Department of Biochemistry,

² Department of plant biology and plant biotechnology,
St. Joseph's College, Trichirappalli - 620 002,
Tamil Nadu, India

Issued 15 December 2008

Abstract

The immunomodulatory properties of coconut protein were investigated in Swiss albino mice by immune suppressed with cyclophosphamide (CP). CP is a commonly used anti-cancer drug which causes toxicity by its reactive metabolites such as acrolein and phosphoramidate mustard. In this study, the animals were grouped into four of 6 mice per group. Assessment of immunomodulatory activity was carried out by testing the RBC, WBC, Platelet and differential counts. Orally administered coconut protein in CP treated animals showed the increased levels of RBC, WBC and Platelet counts. The results of treated groups were RBC (1.84 mm^3), WBC (5720.84 mm^3) and platelet (3.79 mm^3). The results showed Neutrophil (18.3%), Monocytes (9.58%), Eosinophil (7.54%), B lymphocytes (0.705%), T-lymphocytes (3.15%) and Hb (3.834g/dl) respectively. The treated mice show an increased number of cells revealing the immune stimulated condition. The results of this experiment showed that coconut protein has strong immunomodulatory activity.

Key words: Cyclophosphamide, coconut protein, immunomodulatory, RBC (Red blood cells, WBC (White blood cells).

INTRODUCTION

The term immunomodulatory means regulation of the immune system by suppression and stimulation of the cells and organs of the immune system (Bafuna and Mishra, 2005). Immunostimulation in a drug-induced immunosuppression model and immunosuppression in an experimental hyper-reactivity model by the same preparation can be said to be true immunomodulation (Patwardhan *et al.*, 1990). Certain agents have been shown to possess activity to normalize or modulate pathophysiological processes and are hence called immunomodulatory agents (Wagner, 1984). A number of plant products are being investigated for immune response modifying activity (Upadhyay, 1997). Cyclophosphamide acts on both cyclic and intermitotic cells, resulting in general depletion of immune-competent cells. Cyclophosphamide (CP) is an alkylating agent widely used in anti-neoplastic therapy (Baumann and Preiss, 2001; Fleming, 1997). It is effective against a variety of cancers such as lymphoma, myeloma and chronic lymphocytic leukemia (Baumann and Preiss, 2001). CP-induced immunosuppression is reported to

prompt various types of infection (Angulo *et al.*, 2002; Wijesundera, 1980; Williams and Luzio, 1979).

Coconut palm (*Cocos nucifera* L.) is an economic plant which is cultivated in most tropical countries. A coconut fruit is composed of about 38.5% shell, 51.7% kernel and 9.8% water (Dendy & Timmins, 1973). Hypolipidemic effect of coconut protein is due to the high content of L-arginine (Mini *et al.*, 2004), feeding coconut kernel along with coconut oil in human volunteers has been found to reduce serum total and LDL cholesterol when compared to feeding coconut oil alone (Padmakumaran Nair *et al.*, 1998). In coconut, a large globulin was originally described in 1930 and named cocosin (Sjogren *et al.*, 1930). The coconut globulin cocosin (Osborne *et al.*, 1916) is a legume class reserve protein from coconut and belongs to the fourth group of proteins, having a molecular weight of about 208 k Da. Components such as polysaccharides, lectins, proteins and peptides present in plants have been shown to stimulate the immune system (Tzianabos, 2000; Bafna and Mishra, 2005). Use of plant as a source of immunomodulators is still in infancy in modern medicine (Bafna and Mishra, 2005). The aim of the present study was to investigate immunomodulatory activities of coconut protein in immune suppressed systems with Cyclophosphamide.

MATERIALS AND METHODS

Separation of Coconut Protein

The nuts were freed from shells and skins and ground to a fine paste and suspended in 10% NaCl solution. Toluene was added to remove fat. The mixture was stirred at room temperature for 24 h. The solution was freed from oil and fat by filtering and centrifuging. Saturated ammonium sulfate was added to 60% saturation and the precipitate was filtered and washed with 50% saturated ammonium sulfate solution. The resulting protein precipitate was dissolved in 0.033 M sodium phosphate buffer, pH 6.7, containing 7% NaCl. The protein solution was then dialyzed against the same buffer for 6 days (Balasundaresan *et al.*, 2002).

Preparation of feed

The animals were fed with a synthetic diet:

Coconut protein	-	120 g
Sago	-	315 g
Groundnut oil	-	35 mL
Vitamins	-	30 g

Sources of Laboratory Animals

In the present study, healthy adult male albino mice were used as experimental animals. The animals were

purchased from Perundhurai Medical College, Erode. Adult male mice of 12 weeks old, weighing about 100 – 150g were selected. They were housed in polypropylene cages. Husk was renewed every 24 hours. The animals were fed with a standard diet (Sai Durga feeds and Foods, Bangalore, India) and water for normal animals. The mice were maintained at the local animal house conditions.

Before starting the experiments the animals were acclimatized to the laboratory condition for 7 days. After acclimatization of the laboratory condition, animals were pretreated with coconut protein for 30 days. CP (20 mg/kg/day) was given orally for 10 days for the animal of Group-II and III at the concentration of 20 mg/kg/day from the 21st day for 10days.

Evaluation of the Immunomodulatory Activity of Coconut protein on Experimental Animals

Twenty four *Swiss albino mice* were used to assess the effect of coconut protein on the immune system. The mice were divided into 4 groups of six mice per cage. The first group was given normal diet and water and this (Group-I) served as control. The second group served as test group (Group-II) fed with normal diet and induced with the CP. The third group served as experimental animals (Group-III) and was given with coconut protein and CP. The fourth group served as treated control (Group-IV) and was given only coconut protein containing coconut protein, vitamins, fats and carbohydrates.

Haematological Test

At the end of the treatment, mice were sacrificed under chloroform anesthesia. The blood was collected through cardiac puncture and blood was collected immediately. Haematological parameters were studied for White Blood Cell (WBC), Red Blood Cell (RBC), platelet count, eosinophil, neutrophil and lymphocytes.

The WBC count was done by Turke's method, RBC by Hayem's method, platelet by Rees and Ecker's method and haemoglobin by Sahli's method. The differential counts were studied using Leishman's stain and viewed under the microscope. The results were shown in Table 1 & 2.

Statistical Analysis

The data were expressed as the mean \pm standard deviation of the means (S.D) and statistical analysis was carried out employing student's t-test.

RESULTS AND DISCUSSION

Modification of immune functions by pharmacological agents is emerging as a major area of therapeutics. The RBC, platelet count of Group III animals shows increased levels when compared with Group II animals. There were slight differences between Group I and Group IV animals. The value of WBC dropped for the animals treated with CP when compared with Group III. CP of 20 mg/kg is found to be the efficient potentiating dose of hypersensitivity as compared with CP 200 mg/kg (Askenase *et al.*, 1975). Animals treated with CP of 20 mg/kg showed maximum potentiation of hypersensitivity (because CP damaged short-lived suppressor T cells in immune regulatory systems) (Askenase *et al.*, 1975; Mitsuoka *et al.*, 1976).

Table 1: The results of RBC, WBC, and Platelet count of normal and pretreated groups.

Parameters	Group-I	Group-II	Group-III	Group-IV
RBC cells count/ mm ³	1.29±0.075	1.49±0.15	1.84±0.28	1.54±0.043
WBC cells count/mm ³	4562±62.07	4670.94±162.4	5720.84±53.5	5518.2±303
Platelet Lakhs/ mm ³	3.16±0.34	2.70±0.03	3.79±0.056	3.37±0.96

Bone marrow is a site of continued proliferation and turnover of blood cells, and is a source of cells involved in immune reactivity (Pelczar *et al.*, 1990). A high degree of cell proliferation renders bone marrow a sensitive target, particularly to cytotoxic drugs. In fact, bone marrow is the organ most affected during any immunosuppression therapy with this class of drug. Loss of stem cells and inability of bone marrow to regenerate new blood cells will result in thrombocytopenia and leucopenia. The results indicate modulation of bone marrow activity, namely suppression when used CP alone and stimulation to counteract with CP-induced pretreated coconut protein groups.

Table 2: The results of differential counts of normal and pretreated groups.

Parameters	Group-I	Group-II	Group-III	Group-IV
Neutrophil (%)	13.62 ± 0.24	11.28 ± 0.29	18.3 ± 0.21	12.58 ± 0.23
Monocytes (%)	6.32 ± 0.34	5.5 ± 0.29	9.58 ± 0.21	7.38 ± 0.37
Eosinophil (%)	6.54 ± 0.27	5.59 ± 0.29	7.54 ± 0.24	6.58 ± 0.27
B- lymphocytes (%)	0.425 ± 0.78	0.211 ± 0.014	0.705 ± 0.0005	0.32 ± 0.008
T-lymphocytes (%)	2.425 ± 0.81	1.886 ± 0.033	3.15 ± 0.014	0.74 ± 0.008
Hb g / dl	1.70 ± 0.05	2.78± 0.11	3.834 ± 0.07	3.58 ± 0.03

T-lymphocyte and other key cells of the immune system are known to activate production of antibody polymorphonuclear granulocyte to destroy the antigen (Prescott, 1999). The number of neutrophil count increases significantly during treatment of protein. T and B-lymphocytes of Group III animals increased when compared with Group I. Dose of CP 20 mg/kg, reported as a B-cell depleting dose (Askenase *et al.*, 1975). An increased level in lymphocyte has been found in experimental groups. Hemoglobin also increases in Group III and Group IV when compared with group II animals. Immunosuppression involves an act that reduces the activation or efficacy of the immune system. Bone marrow is the flexible tissue found in the hollow interior of bones. In adults, marrow in large bones produces new blood cells. When it is in suppressed condition WBC count level gets reduced.

Coconut protein has the ability to stimulate the immune system by activating the bone marrow cells. By activating the cells, the treated animal parameters showed similar normal condition. The increased levels of the immune cells showed a positive result. The result obtained in the present study has showed the immunomodulatory activity of coconut protein *in vivo*.

CONCLUSION

Cyclophosphamide is a potent suppressor of immune function, at high doses resulting in a sustained decrease in both the number and function of T and B cells (Cupps *et al.*, 1982). It is an effective therapy in certain autoimmune diseases. Plant based immune stimulation may also contribute to the therapy of the autoimmune diseases. Coconut protein has counteractivity to cyclophosphamide-induced myelosuppression and thrombocytopenia. Thus, Coconut protein has immunostimulatory activity with regard to IgE mediated and cell-mediated hyper-reactivity. Further studies are required to examine the exact mechanism of CP function.

REFERENCES

- Angulo, I., Jimenez-Diaz, M.B., Garcia-Bustos, J.F., Gargallo, D., De las Heras, F.G., Munoz-Fernandez, M.A. and M. Fresno, 2002. *Candida albicans* infection enhances immunosuppression induced by cyclophosphamide by selective priming of suppressive myeloid progenitors for NO production. *Cell Immunol.*, 218: 46-58.s
- Askenase, P.W., Hayden, B.J and R.K. Gershan, 1975. Augmentation of delayed type hypersensitivity by doses of cyclophosphamide which do not affect antibody responses. *J. Exp. Med.*, 141: 697-704.
- Bafna, A.R. and S.H. Mishra, 2005. Immunomodulatory activity of methanol extract of roots of *Cissampelos pareira* Linn. Pharmacy Department. The M.S.University of Baroda. India.
- Balasundaresan, D., R. Sugadev and M.N. Ponnuswamy, 2002. Purification and crystallization of coconut globulin cocosin from *Cocos nucifera*. *Biochimica et Biophysica Acta.*, 1601: 121-122.
- Baumann, F. and R. Preiss, 2001. Cyclophosphamide and related anticancer drugs. *J. Chromatogr. B. Biomed. Sci. Appl.*, 764:173-192.
- Carr, H.J, Plumb G.W, Parker M.L and N. Lambert, 1990. Characterization and crystallization of an 11S seed storage globulin from coconut (*Cocos nucifera*). *Food Chem.*, 38:11-20.
- Cupps, T.R, Edgar, L.C and A.S. Fauci, 1982. Suppression of human B lymphocyte function by cyclophosphamide. *J. Immunol.*, 128: 2453-7.
- Dendy, D.A.V., and W.H. Timmins, 1973. Development of a wet coconut process designed to extract protein and oil from fresh coconut. London: Tropical Products Institute.
- Fleming, R.E., 1997. An overview of cyclophosphamide and ifosfamide pharmacology. *Pharmacotherapy.*, 17: 1465-1545.
- Kwon, K.S., Bae, D., Park, K.H., and K.C. Rhee, 1996. Aqueous extraction and membrane techniques improve

coconut protein concentrate functionality. *Journal of Food Science*, 61: 753-756.

- Mini, S. and T.Raja Mohan, 2004. Influence of coconut kernel protein on lipid metabolism in alcohol fed rats. *Indian journal of experimental biology.*, 42(1): 53-57.
- Mitsuoka, A., Mitsuo, B. and S. Morikowa, 1976. Enhancement of delayed hypersensitivity by depletion of suppressor T cells with cyclophosphamide in mice. *Nature*, 262: 77-79.
- Osborne T.B., 1916. Vegetable Proteins, Longmans, Green and Co., London, 57: 78.
- Padmakumaran Nair, K.G., Rajamohan, T. and P.A. Kurup, 1998. *Coconut kernel protein* (2):133-144(12).
- Patwardhan, B., Kalbag, D., Patki, P.S. and B.A. Nagsampagi, 1990. Search of immunomodulatory agents: a review. *Indian Drugs* 28 (2): 56-63.
- Pelczar, M. J., Chan, E.C.S. and N. R. Krieg, 1990. Microbiology, 5th ed. Tata Mcgraw-Hill, New Delhi, 703-715.
- Prescott, L.M., J.P. Harley and D.A. Klein, 1999. Microbiology; 5th edn. The McGraw-Hill Companies, Inc. USA. 704-733 and 818-819.
- Sjogren, B and R. Spsychalski, 1930. The molecular weight of cocosin. *J. Am. Chem. Soc.*, 52:4400-4.
- Tzianabos, A.O., 2000. Polysaccharide Immunomodulators as therapeutic agents: Structural aspects and biologic functions. *Clin. Microbid. Rev.*, 13:523-533.
- Upadhyay, S.N. 1997. Plant products as immune response modulators. Proceedings of the International Ayurveda Conference- 97. Sanjay Gandhi Post graduate Institute of Medical Sciences, Lucknow, p. 10.
- Wagner, H. and A. Proksch, 1984. Immunostimulatory drugs of fungi and higher plants. In: Economic and Medicinal Plant Research, Academic Press, New York.113-153.
- Wijesundera, M. de. S., 1980. Hepatic amoebiasis in immunodepressed mice. *Trans. R. Soc. Trop. Med. Hyg.*, 74: 216-220.
- Williams, D.L. and Di Luzio, N.R., 1979. Glucan-induced modification of experimental *Staphylococcus aureus* infection in normal, leukemic and immunosuppressed mice. *Adv. Exp. Med. Biol.*, 121: 291-306.