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In Vitro Effect of Ethylacetate Extract of *Stylissa Carteri,* A Marine Sponge From Andaman on Proliferation of Human PBMC and Skin Keratinocytes From Psoriatic Patients

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ABSTRACT

In the present study we focused on effect of ethyl acetate fraction purified from *stylissa carteri a* marine sponge collected from Andaman on human Blood PBMC and skin keratinocytes from psoriasis patients. The ethyl acetate fraction of *stylissa carteri, a* marine sponge collected from Andaman and nicobar islands, was found to exhibit decreased human skin keratinocyte count and human PBMC count in Blood. We also observed the elevation of keratinocyte count as well as PBMC count in various in *vitro* mitogens such as Con A, PHA, PMA induced cell proliferative responses of psoriatic humans. The ethyl acetate fraction of stylissa carteri, which then inhibits the immune mediated skin destruction, which occurs in the pathogenesis of psoriatic patients. However, the ethyl acetate fraction of stylissa carteri In vito treatment of psoriatic humans skin cells and PBMC reduced considerably the increase of cell proliferative response comparative to the normal levels. Human PBMC are proven to be valuable research programmed by the study of pathogenic mechanism of this diseases as well as for testing new therapies

Keywords: Psoriasis, mitogen, keratinocytes, PBMC, Stylissa carteri, Cellular proliferation

Abbreviations: Con A: Conconavalin A. PHA: Phytohemagglutinin, PMA: Phorbol myristic acid, PBMC: Peripheral blood mononuclear cells

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INTRODUCTION

Psoriasis is disease which affects the skin and joints. It commonly causes red scaly patches to appear on the skin. The scaly patches caused by psoriasis, called psoriatic plaques, are areas of inflammation and excessive skin production. Skin rapidly accumulates at these sites and takes a silvery white appearance. Plaques frequently occur on the skin of the elbows and knees, but can affect any area including scalp and genitals. Psoriasis is an inflammatory skin disease in which skin cells replicate at an extremely rapid rate. New skin cells are produced about eight times faster than normal--over several days instead of a month--but the rate at which old cells slough off is unchanged. This causes cells to build up on the skin's surface, forming thick patches, or plaques, of red sores (lesions) covered with flaky, silvery-white dead skin cells (scales). The first considers psoriasis as primarily a disorder of exclusive growth and reproduction of skin cells[1]. The problem is simply seen as a fault of the epidermis and its keratinocytes. The epidermis is a thin non vascular layer consisting mainly of stratifying epithelial keratinocytes. These cells undergo continuous and rapid proliferation and are thought to be continually regenerated from a pool of multipotent stem cells. As keratinocytes leave the basal layer and move upward, they undergo terminal differentiation forming a continually shedding protective barrier at the surface of the skin. Several other cell types can also be isolated from skin, including endothelial cells and epidermal melanocytes. In addition, keratinocytes can be isolated from plucked hair. The hair follicle is a complex epidermal appendage embedded deeply in the dermis. Outer root sheath (ORS) Cells surrounded the hair follicle essentially as a stratified epithelium of keratinocytes that is contiguous with the epidermis making them easy to isolate. The hair follicles contain their own stem cells, which in addition to Generating ORS keratinocytes have the capacity to contribute to other cell types as well as to epidermal keratinocytes. The present study was done on ethyl acetate fraction of stylissa carter, I marine sponge collected from Andaman Nicobar island.

Marine ecosystems (>70% of the planet's surface) comprise a continuous resource of immeasurable biological activities and immense chemical entities[2.] The most interesting phyla with respect to pharmacologically active marine compounds include bacteria, fungi, algae, sponges, soft corals, tunicates, molluscs or bryozoans[3]. Among the marine invertebrates, the sponges are a potential source of bioactive substances. Many promising lead compounds have been reported from marine sources having anti-inflammatory activity. Sponges have an evolutionary history of about 570 million years and so far, 486 species have been described in india4. Compounds isolated from marine organisms such as manoalide, pseudopterosins, topsentins and scytonemin have all been studied extensively, while

debromohymenialdisine was investigated by both Smith Kline Beecham and Osteoarthritis Sciences Inc [5] for the treatment of rheumatoid arthritis and osteoarthritis respectively. Indole alkaloids from marine sources have been reported to have anti-inflammatory potentials; these include: Plakohypaphorine D from sponge , Manzamines A–F from sponge , aplysinopsin-type compound from sponge Hyrtios erecta Manzamine from sponge ,Carteramine A from sponge [6] , with anti-inflammatory activities. New alkaloids from the indopacific sponge Stylissa carteri yielded two new bromopyrrole alkaloids: debromostevensine (1) and debromohymenin (2).[7]. These alkaloids shows biological effects on cell lines. The present studies are focused on effect of in marine sponge(*stylissa carteri*) on human PBMC and skin keratinocytes from psoriasis patients[8, 9].

MATERIALS AND METHOD

Materials:

Laminar air flow chamber, Tissue culture dishes, Petri plates, incubator, falcon tubes Cell counter), Pipettes, centrifuge, light microscope, scalpel, scissors

Isolation of keratinocytes from psoriasis patients:

Keratinocytes can be cultured based on the traditional feeder dependent method developed by Rheinwald and Green or by more recent method using defined serum –free, low calcium media that do not require feeder cells. Place skin tissues in cold HBSS (EpiLife medium) containing antibiotics and antimycotics and keep at 4^0C until use. Samples should be processed as early as possible, ideally the same day or the next morning owing to gradual loss of yield (particularly after 24 h). If it is a small biopsy sample (< 5 mm), it may be advantageous to culture the cells using explants outgrowth Place the tissues in an uncoated 100-mm bacterial Petri dish and keep moist with some medium. Remove subcutaneous fat and loose connective tissues (hypodermis) using fine tweezers and a scalpel. Open up or flatten the skin and place the epidermis side down. Use the edge of the scalpel to scrape away tissues until only the thin epidermis and the dense dermis remain**.** With foreskin samples, cut the piece into strips about 3–4 mm width. It is advisable to perform this part of the procedure late in the afternoon if overnight incubation is performed. Place the pieces with the dermal side down in a 60-mm dish containing 5–6 ml of HBSS (or EpiLife) with antibiotics and dispase. Cover and store the pieces in a sterile place at 4 °C for 12–16 hr (overnight). Take the overnight digested tissue pieces and grab the edge of the dermal part of the tissue with one tweezer and the thin epidermal part with another set of thin tweezers and slowly peel off the epidermis. Immediately transfer the epidermis (almost transparent) into another dish with either HBSS or EpiLife medium. If desired, preserve the dermis (thicker and gooey) for fibroblast isolation. Cut the epidermis using a scalpel into small pieces of 1 mm2. Place the

minced tissue pieces in a Falcon tube containing TrypL E Select. For a whole piece of foreskin, use a 50-ml Falcon tube and about 20 ml TrypL E Select. For smaller samples, use a smaller-sized tube and a lesser amount of reagent. Incubate at 37 °C for 40–45 min when using a whole foreskin (20–25 min for a small biopsy). Mix the sample gently every 5 min. The solution should become turbid. Add 20–30 ml of the medium containing a minimum of 10% serum (vol/vol) (e.g., DMEM or RM +) and pipette the solution vigorously up and down for 10–15 times. Pass the solution through a 70-µm mesh filter into a new Falcon tube to remove undigested pieces of tissue. Centrifuge at 200*g for 5 min.* Remove the supernatant and resuspend in 5 ml EpiLife medium. Count the number of cells (e.g., with a hemocytometer). Coat the culture plates with Coating Matrix (type I collagen) by adding ~5 ml of a coating solution to each 100-mm tissue culture plates and incubate for 30 min at 22 °C. The coated dishes may be stored at 4 °C for several days[10, 11].

Isolation of PBMC from whole blood in psoriatic patients:

Histopaque method:

To a 15-mL conical centrifuge tube, add 3 mL of Histopaque-1077 and bring to room temperature. Carefully layer 3 mL of whole blood onto the Histopaque. Centrifuge at 400 ´ *g for exactly 30 minutes at* room temperature. Centrifugation at lower temperatures, such as 4 °C, may result in cell clumping and poor recovery. After centrifugation, carefully aspirate the upper layer with a Pasteur pipette to within 0.5 cm of the opaque interface containing mononuclear cells. Discard upper layer. Carefully transfer the opaque interface with a Pasteur pipette into a clean conical centrifuge tube. Wash the cells by adding 10 mL of isotonic phosphate buffered saline solution or appropriate cell culture medium, and mix by gently drawing in and out of a Pasteur pipette. *Centrifuge at 250 ´ g for 10 minutes. Aspirate the supernatant and discard. Resuspend cell pellet with 5 mL of isotonic* phosphate buffered saline solution or appropriate cell culture medium, and mix by gently drawing in and out of a Pasteur pipette. Centrifuge at 250 ´ *g for 10 minutes.* Repeat steps 8, 9 and 10, discard supernatant and resuspend cell pellet in 0.5 mL of isotonic phosphate buffered saline solution or appropriate cell culture medium. Taken eppendop tube and added 1:1 dilution (trypan blue+cell suspension) mixed thoroughly and keep on the one drop of the sample on the neuber counting chamber, observe under the microscope to count the cells to each square. Medium preparation from 1x stock (RPMI w/10%FBS). Check the medium formulation of, RPMI the add serum and antibiotics from stock concentrates to obtain final concentration of 10% FBS and 1% pen step (100 U/mL penicillin and 100 micro grams streptomycin). To determine the ability of 100 µ litre, RPMI media transferred into each well in the 96 well culture plate[11],[12]. To inoculate the PBMC seed 1000-2000 cells per well in plate. And added

to the marine compound extracts in 100 µ litres. Keep at the culture plate in to the CO_2 incubator at 37^0c for 48 hours after incubation the isolated PBMC cells observed and number of cells was counted by the microscope[13].

Extraction and isolation of marine compound:

The marine sponge samples were allowed to thaw, cut into small pieces, and the placed in a 1000ml graduated cylinder containing a know amount of seawater in order to measure their volume. The samples, drained of excess water, were extracted in a 1:1 (v/v) dichloromethane: methanol (DCM : MeOH) solution for 24 hrs. at room temperature. The organic extracts were filtered, and the solvent was removed by rotary evaporation under vacuum at 37^0c. The concentrated samples was again dissolved in ethyl acetate and was keep in a separating funnel for separation. The aqueous layer was left and the remaining ethyl acetate soluble sample was taken and again concentrated using a rotary evaporator. The solvents were than evaporated to dryness under reduced pressure with a rotary evaporator, and the resulting extract was assayed for antimicrobial activity. The extract was subjected to column chromatography for separation of pure compound by gradient elution method. The crude extracts was chromatographed over a column of silica gel using eluents of increasing polarity of solvent mixture hexane, hexane ethyl acetate, pure ethyl acetate, methanol and chloroform.

$$\text{No. of cells} = \frac{\text{Cells counted X Dilution factor}}{\text{No. of squares counted}} \times 10^4$$

RESULTS AND DISCUSSION

Table 1: *In vitro* effect of Ethyl extract of *Stylissa carteri* on blood PBMC count in Normal and Psoriatic Disease Patients

Sample	Count/minute
Normal	374320
Diseased	392260
Con A	418750
PHA	427500
PMA	435000
Ethyl acetate extract	340000

Table 2: *In vitro* effect of Ethyl extract of *Stylissa carteri* on skin keratinocytes from Psoriatic patients

Sample	Count/minute
Normal	141100
Diseased	162500
Con A	185000
extraction	143750

PHA	196250
PMA	205000

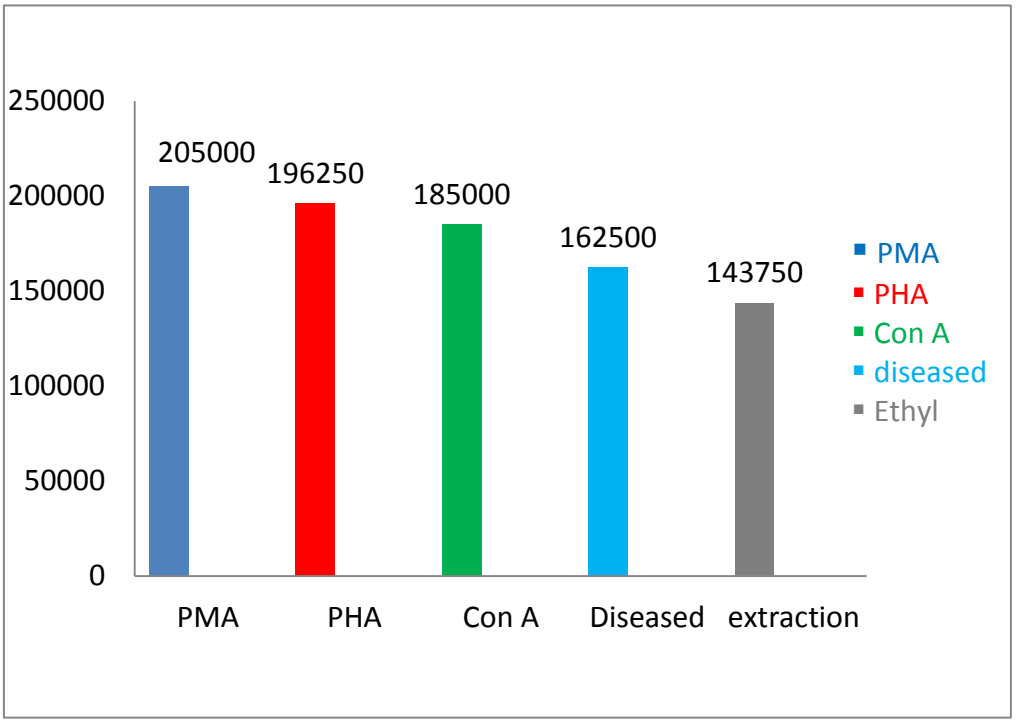

Figure: Effect of Ethyl acetate extract on skin keratinocytes from psoriatic patients

In the present study, the *in vitro* effect of ethyl acetate extract in marine sponge (stylissa carteri) on human PBMC and skin keratinocytes from psoriatic patients was determined. The ethyl acetate fraction of *stylissa carteri, a* marine sponge collected from Andaman and nicobar islands, was found to exhibit decreased human skin keratinocyte count and human PBMC count in Blood. We also observed the elevation of keratinocyte count as well as PBMC count in various *in vitro* mitogens such as Con A, PHA, PMA induced cell proliferative responses of psoriatic humans. The ethyl acetate fraction of stylissa carteri, which then inhibits the immune mediated skin destruction, which occurs in the pathogenesis of psoriatic patients. However, the ethyl acetate fraction of stylissa carteri In vito treatment of psoriatic humans skin cells and PBMC reduced considerably the increase of cell proliferative response comparative to the normal levels. Human PBMC are proven to be valuable research programmed by the study of pathogenic mechanism of this diseases as well as for testing new therapies. human PBMC models of keratinocytes from psoriatic patients[14]. The immune genetically of allogenic cultured human epidermal keratinocytes has been studied in several models with contradictory results. we studied human T-cell activation in vitro assay by incubating for 4 and 24hrs, Checkup fluent sheets with human peripheral blood mononuclear cells[15,16]; parallel HEK cultured were incubated with interferon to induce the expression of major histocompatibility complex molecules before their interaction with PBMC[17].

CONCLUSION

The Ethyl acetate fraction of marine sponge *stylisa carteri collected from Andaman* reduced the human skin keatinocyt count and Blood PBMC count psoriatic patients. The cellular proliferative responses were elevated in Psoriatic diseased condition. The decline of responses were observed in in vitro treated cells. This fraction was further subjected for purification which might be effective against Psoriasis.

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REFERENCES

1. Parisi R, Symmons DP, Griffiths CE, Ashcroft DM. Global epidemiology of psoriasis: a systematic review of incidence and prevalence. J Invest Dermatol. 2012
2. Braekman, J.C.; Daloze, D. Chemical defence in sponges. Pure Appl. Chem. 1986, 58, 357–364.
3. Faulkner, D. J. 2000. Highlights of marine natural products chemistry (1972-1999). Nat. Prod. Rep., 17: 1-6
4. Chellaram, C and J.K.P. Edward, 2009. Anti-inflammatory potential of coral reef associated gastropod, Drupa margariticola. Indian J.
5. Mayer, A.M.S and V.KB. Lehmann, 2001. Marine pharmacology in 1999: antitumour and cytotoxic compounds. Anticancer Res., 21: 2489-2500.
6. Kobayashi H, Kitamura K, Nagai K, Nakao Y, Fusetani N, van Soest RWM, Matsunaga S (2007a). Carteramine A, an inhibitor of neutrophil chemotaxis, from the marine sponge Stylissa carteri. Tetrahedron Lett., 48: 2127-2129.
7. Eder C, Proksch P, Wray V, Steube K, Bringmann G, van Soest RW, Sudarsono, Ferdinandus E, Pattisina LA, Wiryowidagdo S, Moka W,New alkaloids from the indopacific sponge Stylissa carteri. 1999 Jan;62(1):184-7.
8. 8 Braekman, J.C.: Daloze, d. chemical defense in sponges. Pure appl. Chem. 1986,58,357-364.
9. chellaram, c and J.K.P. edward,2009. anti inflammatory potential of coral reef associated gastropod, drupe margariticola. Indian j.
10. wells, j. a simple technique for establishing culture of epithelial cells. Br. J. dermatol.107, 481-482 (1982).
11. Fuchs, E. scratching the surface of the skin development. *Nature 445*, 834-842 (2007)
12. Amos, D.B., and pool, P., HLA typing' in manual of clinical immunology, rose, N.R., and Friedman.

13. Thorsby, E., and Bratile, A., "A rapid methods for preparation of pure lymphocyte suspensions. Histocompatibilty testing, terasaki, P.i., ed., ., 665-666.
14. Hudson, l. and hay, F.C.(eds) 1989 *practical immunology*. Blackwell scientific publication, oxford, UK.
15. karplus, T.E., ulevitch, R.J., and Wilson, C.B. (1987) A new method for reduction of end toxin contamination from protein solution. J. *immunol*. *Methods* 105, 211-220.
16. 16.Kaplan, J., Nolan, D, and ree, A. 1982. Altered lymphocyte markers and blastrogenic sponge associated with 24hrs delay in processing of blood samples. J.immunol. Methods, 50:187-191.
17. 17.Niku, S.D., Hoon, D.S.B Cochran, A.J.., and mortan D.L. 1987. Isolation of lymphocytes from clotted blood. J. immune. Methods 105:9-14.