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Enteromorpha compressa (L.) Greville AN EDIBLE GREEN ALGA AS A SOURCE OF ANTI-ALLERGIC PRINCIPLE (S)

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ABSTRACT

Enteromorpha compressa a marine green algal species grows extensively in North coastal Andhra Pradesh. Besides its nutritional importance it has also been identified as source of anti-anaphylactic compound(s). *E. compressa* extracts alleviated the IgE levels raised against ovalbumin and other allergens in mice. Further, *Enteromorpha* extract also significantly down regulated the serum IgE levels in different murine models irrespective of their genetic background. The results obtained in this study suggest that *E. compressa* extract has compound(s), which inhibit IgE immune response and may have potential in curing various types of allergies.

KEY WORDS

Enteromorpha, ovalbumin, anti-allergic, food allergy

INTRODUCTION

Marine algae are found to be important source(s) of useful bioactive substances since two decades. Several investigators have reported the medicinal importance of marine algae from different countries throughout the world (1–5). The vast diversity of plants and animals in tropical countries like India provides an immense scope to explore and identify the immuno active principles. Several reports are available pertaining to immunomodulatory properties of plants all over the world. Studies from India especially from Andhra Pradesh on the medicinal importance of algae are scanty. However, some information is available on the anti-microbial properties of certain marine algae (6–8). *Enteromorpha* an edible green alga produce a dizzying array of bioactive compounds that have proven to be useful in the treatment of certain bacterial & viral infections, cancer and inflammation (9-10).

Visakhapatnam is one of the developing cities with a vast seacoast in the north coastal Andhra Pradesh. *Enteromorpha compressa (L.) Greville* an edible green alga grows extensively on semi-exposed and protected boulder of Visakhapatnam seacoast. Jalaripeta, Tenneti Park, Jodugullapalem and several other areas of Visakhapatnam coast are important localities where this alga grows luxuriantly on rocky outcrops (11-13). *E. compressa* is being used as Chinese folk medicine and has been proved as safe non-toxic human health food in South East Asia (2,7,14). Some people frequently suffer from severe or moderate allergic responses after consumption of dietary proteins. Type-I or immunoglobulin E (IgE) mediated allergic reactions are known to play a major role in food allergy. An immediate reaction takes place at epidermal and mucosal surfaces, where allergic effector cells, preloaded with FCE receptor bound specific IgE antibodies are prepared for allergen contact. Dimerization of effector bound IgE with allergen is the critical event that lead to the explosive release of biological mediators that would cascade many inflammatory reactions (15). In view of its importance, immunological studies were carried out on *E. compressa* to explore its possible applications in combating immunological disorders like allergy. In our laboratory this green alga has been tested on different murine models for immunomodulatory, anti-allergic compound(s).

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MATERIALS AND METHODS

Ovalbumin (OVA) and Tween-20, were purchased from Sigma Chemical Co. (St. Louis, MO, USA). Goat anti-mouse IgE antibodies conjugated to HRPO were obtained from Nordiac immuno chemicals, Netherlands. 96 well ELISA plates were procured from Nunc Denmark. *E. compressa (L.) Greville* has been collected from different places at Visakhapatnam coast, AP, India.

Eight-week old inbred female mice viz., Balb/C (H-2^d), C57BL/6 (H-2^b), SWR/J (H-2^q) and outbred Wistar strain male rats weighing about 310 – 350g were procured from the Laboratory Animal Information Service Centre (LAISC), National Institute of Nutrition, Hyderabad, India. The animals were housed under standard conditions and fed with the feed procured from Brooke-Bond India Lipton Ltd., Bangalore. Groups of mice 4–6 were caged

separately according to their treatment. Food and water were given *ad libitum*.

Green alga *E. compressa* was harvested, washed with distilled water and blotted between folds of filter paper. For the preparation of the crude extract a known quantity of the alga was taken in a glass mortar and pestle and ground with acid washed sand in the presence of phosphate buffer saline (PBS: 0.005M phosphate and 0.15M NaCl, pH 7.4). It was then centrifuged (Remi R-10 model) at 10,000 rpm for 15 minutes at 4° C, the pellet was discarded and the supernatant was diluted with PBS to get 20% extract.

The three different allergens used in this study for immunization are OVA, crude extracts of cowpea (*Vigna sinensis*) and finger millet (*Eleusine coracana*). The seeds of cowpea and finger millet were locally obtained and their extracts were prepared in PBS (16). The protein contents of these extracts were estimated by the method of Lowry *et al* (17). The mice were immunized intraperitoneally with and without the presence of an adjuvant (alum) on days 0th, 14th and 35th with 1.0 ml of PBS containing 1.0 µg of OVA or 100 µg each of cowpea or finger millet extracts (in terms of protein concentration) per mouse and were bled on day 14th after primary and 7 days after secondary and tertiary immunizations. Similarly, the test groups of mice were injected intraperitoneally with OVA or 10% extracts of cowpea or finger millet in PBS mixed with extract of *E. compressa* (0.2 mg/ mouse) and bled as mentioned above. Both enzyme linked immunosorbent assay (ELISA) and passive cutaneous anaphylaxis (PCA) reactions have been used to assess the antisera for the presence of allergen specific antibody responses.

Passive Cutaneous Anaphylaxis (PCA)

The IgE antibody response was measured by passive cutaneous anaphylaxis (18, 19). In brief, the antisera of mice were passively transferred intradermally on the shaved dorsal side of wistar male rats in aliquots of 50 µl each in 2 or 4 fold dilutions. After 24 hours the rats were challenged intravenously (penal vein) with corresponding 1.0 mg of OVA or 5 mg of each of *V.sinensis* or *E.coracana* seed extracts in the presence of 0.5% Evans blue dye. The appearance of blue spots measuring greater than 5 mm was taken as a positive response and the reciprocal of highest dilution of antisera, which gave blue spots, was considered as an IgE antibody titer.

Enzyme Linked Immunosorbent Assay (ELISA)

IgE antibody levels raised against OVA in the test and control sera were estimated by Enzyme linked immunosorbent assay (20-21). Microtiter plates with 96 wells were coated with OVA (500 ng/ml) in carbonate buffer pH 9.6 and incubated overnight at 4°C with a proper cover. After the incubation the unbound OVA was washed off thrice with 0.01M PBS buffer containing 0.05% Tween-20 (PBS-T). The nonspecific binding sites were blocked with 3% skimmed milk powder dissolved in PBS pH 7.25 and incubated for 10-12 hours at room temperature. The wells were washed again with PBS -T and incubated with 100 µl of 1 in 20 diluted serum in triplicates for 75 minutes at 37°C. The unbound serum proteins and other constituents were washed off. To assess the total IgE levels, 100 µl of HRP-conjugated goat anti-mouse IgE antibody at a dilution of 1:1000 was added and incubated for 75 minutes at 37°C. Finally the unbound conjugates were washed off with PBS-T and 100 µl/well of freshly prepared substrate buffer pH 5.0 were added. Substrate buffer was prepared just before use by dissolving 40 µg of Orthophenylene diamine (OPD) in 10 µl of 1.5M citrate-phosphate buffer pH 5.0, and then mixing the same with 10 µl of substrate (i.e., H_2O_2). The reaction was stopped after 3 minutes by adding 50 µl of 8 N H_2SO_4. The colour developed was read at 492 nm using an automatic ELISA reader (Anthos Labtec-HT2, version 1.21E, USA). The data represented the mean absorbance values of the samples kept in triplicates. Naïve mice sera or preimmune sera have been taken as the negative control. The absorbance values of negative controls were deducted from the absorbance values given by the sera of mice immunized with OVA or OVA plus *E. compressa* formulations.

Statistical analysis: Statistical analysis of data was performed by student's t-test. The level of significance was set at $P<0.05$.

RESULTS AND DISCUSSION

A significant increase in food allergies are being reported which accounts up to 10 percent of the world population. This can be attributed to increasing varieties of food preparations containing different food additives (22, 23). Many drugs are available for treatment of allergy but many of them have undesirable side effects. Hence there is a need to explore alternative and safe therapeutic agents. In our laboratory we are investigating the use of algal extracts and potential compound(s) that would alleviate the allergic responses to certain foods, which could be used as remedial food source to control exaggerated immunological reactions. Traditionally, algae have been used as a source of food and folk medicine in the eastern and far-eastern regions of the world (14). We have experimentally induced IgE antibodies against ovalbumin (OVA) in murine system with an optimum concentration of 1.0 µg/mouse. Both passive cutaneous anaphylaxis (PCA) reactions and Enzyme linked immunosorbent assay (ELISA) have been performed to assess the total IgE antibodies raised against OVA with and without the presence of *Enteromorpha* extract (Figs.1 and 2). Experimental results showed that the administration of *E. compressa* extract (0.2 mg/mouse) to Balb/C mice along with OVA significantly lowered the anti-OVA IgE antibodies in primary, secondary and even tertiary responses ($P <$

0.05). Similar response was observed when alum was used as an adjuvant. Both positive and negative controls have been maintained for the above experiments. Dose response experiments were conducted with different formulations (0.001mg/mouse to 0.25 μg/mouse) of *Enteromorpha* crude extract with fixed concentration of OVA (1.0 mg/mouse). Results showed that *E. compressa* extract concentrations such as 0.25 mg and 0.1 mg per mouse exhibited significant suppression ($P < 0.05$) while in group tested with 0.01 mg/mouse extract along with OVA exhibited moderate suppression of anti-OVA IgE levels whereas in mice treated with 0.001mg/mouse of extract along with OVA showed very little effect. However, dose response studies with extracts of *Enteromorpha* showed that the 100 μg extract (in terms of protein concentration) per mouse is the minimum dose in crude state is sufficient to down regulate the elicitation of IgE antibodies against OVA. This effect was sustained even when the concentration increased up to 250μg extract / mouse (Figure 3).

Immune systems have evolved to counteract all pathogens or antigens and their offensive activities. However, the mode and intensity of action against them varies from person to person. Depending on the genetic constitution of an individual. Therefore it is worthwhile to examine the potentials of *Enteromorpha* and its IgE down regulatory property in different haplotypes of mice. In order to determine the effect of genetic constitution on immune induction against OVA as allergen, investigations have been carried out with different haplotypes of mice (Table 1). These experiments revealed that three different strains of mice showed different responses to OVA when administered through intraperitoneal route. However, Balb/c mice are very responsive than C57BL/6 against ovalbumin while Swiss ($H\text{-}2^{q}$) strain mice are very poorly responsive to develop IgE antibodies against OVA. Even though the genetic constitution and immune response against same OVA in three different strains of mice were different, experimental results with *Enteromorpha* showed the same potency and consistency in its action as an anti-allergic alga. These results would give immense scope for further studies on *Enteromorpha* to be used as supplementary food for allergic patients who are scared to eat staple food of interest, which causes allergic response in them. Local people of Visakhapatnam are using cowpea and finger millet as food source for making different types of curries for regular consumption. Experimental results reveal that these people often suffer from different food allergy problems (24). Reports of allergy due to vegetables in Visakhapatnam are known (16, 25). Therefore we have attempted to see the effect of *Enteromorpha* on suppressing IgE response against cowpea and finger millet. IgE antibodies against cowpea and finger millets have been raised in Balb/C mice and administration of *Enteromorpha* extract to the animals has showed significant ($P < 0.05$) suppression of allergic response (Figure 4).

It is clear from the present investigation that *Enteromorpha* extract enhance the immune function through down regulation of the plasma cell in secreting IgE antibodies against food allergens. It is likely that certain compound(s) present in the *Enteromorpha* are responsible for potent anti-allergic effects against different allergens in different haplotypes of mice irrespective of their genetic background. For better understanding of the beneficial effects of *Enteromorpha* more extensive studies have to be done concerning humoral immune function including role of cytokines in suppression of IgE antibodies and

Fig. 1: Immunomodulatory effect of *Enteromorpha* on induction of Anti-OVA IgE antibodies in Balb/c mice

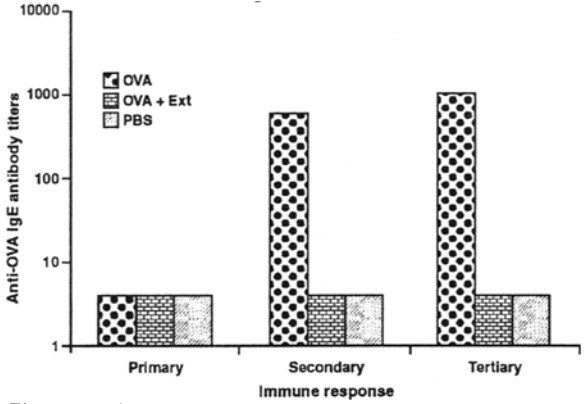

Figure 1: Three different groups of mice immunized with ovalbumin, ovalbumin plus aqueous *Enteromorpha* extract and PBS on days 0, 14 and 35. The sera collected from mice were assessed for the total IgE antibodies raised against ovalbumin by PCA and the results expressed as $\log_{10}$ Anti-OVA IgE antibody titers

Fig. 2: Effect of *Enteromorpha* on induction of OVA specific IgE response in Balb/c mice

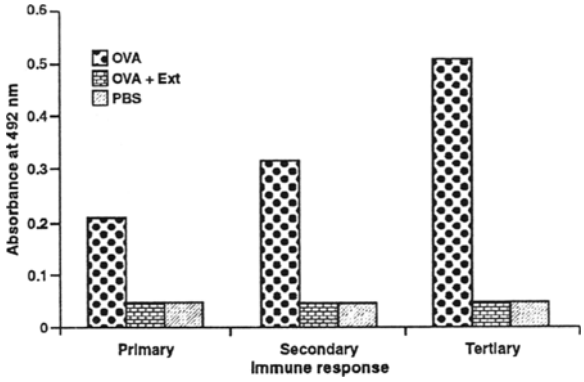

Figure 2: Mice were immunized with and without the presence of aqueous extract of *Enteromorpha* on days 0, 14 and 35. Mice were bled and total IgE antibodies raised agaist ovalbumin have been assessed by ELISA. Intensity of color developed was read at 492 nm using OPD as substrate.

Fig. 3: Effect of *Enteromorpha* formulations on Induction of anti-OVA IgE antibody response

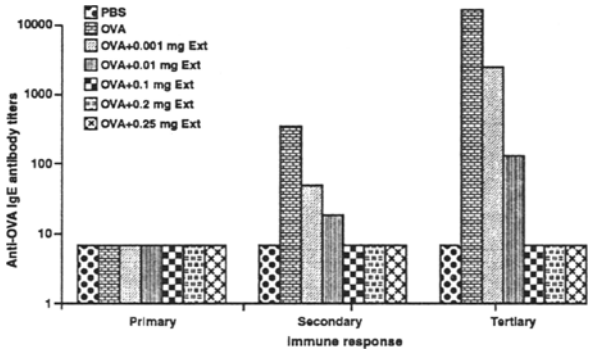

Figure 3: Different groups of Balb/c mice were immunized with different formulations of *Enteromorpha* extract plus ovalbumin (1.0 ug/mouse). Sera collected from experimental mice were used for estimation of total IgE antibodies raised against OVA and the results are expressed as $\log_{10}$ Anti-OVA IgE antibody titers.

Fig. 4: Effect of *Enteromorpha* on different food allergens specific IgE antibody response

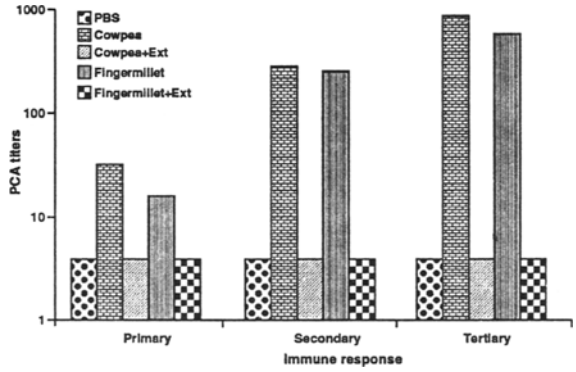

Figure 4: Balb/c mice were immunized with cowpea and finger millet formulations with and without the presenmce of Enteromorpha keeping PBS as negative control. Mice were bled and total IgE antibodies are estimated using Passive Cutaneous Anaphylaxis and the results expressed as $\log_{10}$ PCA titers.

Table 1: Genetic variation in anti-OVA IgE antibody response in mice treated with Enteromorpha extract

Haplotype	Formulations (1.0 ml PBS/ mouse)	PCA titers		
		Primary	Secondary	Tertiary
Balb/C mice ($H\text{-}2^d$)	1.0 μg (OVA) Control	4	256	1024
	1.0 μg (OVA) + 0.2 mg (E. ext)	<4	<4	<4
C57B1/6 ($H\text{-}2^b$)	1.0 μg (OVA) Control	4	64	512
	1.0 μg(OVA) + 0.2 mg (E. ext)	<4	<4	<4
SWR/J ($H\text{-}2^q$)	1.0 μg (OVA) Control	ND	<4	64
	1.0 μg (OVA) + 0.2 mg (E. ext)	<4	<4	<4

ND: Not detected; E. ext: *Enteromorpha* extract per mouse; OBA: Ovalbumin

identification of the active component(s) of *Enteromorpha*. These results indicate the importance of *Enteromorpha* as supplementary food, which would reduce the food allergy risks of patients. Further studies are under progress in our laboratory.

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