ISSN 0975-6299 Vol 2/Issue 2/Apr-Jun 2011

International Journal of Pharma and Bio Sciences

RESEARCH ARTICLE BIO TECHENOLOGY

IMPACT OF RED VELVET MITE, *TROMBIDIUM GRANDISSIMUM* KOCH IN IMMUNE AND MICROBIAL SYSTEM OF SWISS ALBINO MICE

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ABSTRACT

Attempts were made to study the effect of haemolymph of *Trombidium grandissimum* on gut bacterial population in rodents and its impact on immune system. Total heterotrophic bacterial population densities in gut increased considerably, it will lead's to enhance immune system. Increment in B-Lymphocyte was much pronounced in test samples exposed mice in the first week and second week also had more or less similar impact on B-cell estimations. The present study, clearly confirm the increment in B-cell number in mouse exposed to test samples. The increase in total heterotrophic bacterial population (THBP) in different regions of the gut due to samples treatment indicated the increase in beneficial probiotic microbes. The increment of beneficial gut micro flora had interfered with the digestive ability, food consumption energetic and overall health of the animal. Feeding and changes in the energy budget also questions the functioning of immune system and execution of immune response. Changes in the energy budget were one of the reasons for modulation to the immunological parameters and immunity. The immunostimulant potential in the haemolymph of Red velvet mite suggests that the mite possess bioactive compounds and this has to be explored in future.

KEYWORDS

Gut bacterial population; haemolymph; lymphoidal tissue; immune response.

INTRODUCTION

Red velvet mite, *Trombidium grandissimum* Koch is one of the beautiful mites. The animal known to natives as birbahoti and which is described as the rain insect, the red velvet insect, the lady cow, in the Cyclopedia of India, and as Bucella carniola in Platts dictionary, is a red mite about half an inch long and from a quarter to three-eighths of an inch in its widest part. Insects had been proved to be very important sources of drugs for modern medicine since they have immunological, analgesic, anti-bacterial, diuretic, anti-rheumatic and anesthetic properties (Yamakawa 1998). Beattie *et al.,* (1986) had stated that the arthropods that live on close proximity to each other such as wasps, bees, mole crickets, scarablarvae, cicadanymphs, and centipedes are subject to microbial attacks and epidemic diseases. To limit disease activity they incorporate antimicrobial compounds into their nests.

The human colonic micro biota comprises more than 500 distinct bacterial species and has an important role in human nutrition and health, by promoting nutrient supply, preventing pathogen colonization and shaping and monitoring normal mucosal immunity (Svenson *et al.,* 1994). Evidence that host nutrition is supplemented by the metabolic capabilities of the resident microflora is derived from studies on mono-associated and conventionalized mice. The genome sequences of several commensal bacterial species provide insights into how bacteria have evolved to perform their various metabolic functions within the host gut (De Ridder *et al.,* 1985). For instance, the gut micro flora of man provides certain essential vitamins, which they synthesize within the intestine (Domech *et al.,* 1983). They too lodge populations of some species of microbes, which are symbiotic and complement the native enzymes (Ranjitsingh *et al.,* 2003). All these associations are mutually beneficial and such relationships should have existed from early evolutionary times. Haemolymph treatment may modulate the beneficial microbe and change the energetics make the immune system strong. This is an indirect assault on the immune response and hence it needs an immediate attention. It explains the alterations in the microenvironment and how it is reflected in health status of the mice. In order to have an insight into the distribution of beneficial heterotrophic bacterial population in normal and haemolymph treated mice, the present study was undertaken. As any modulation in the beneficial microbial load in the gut could reflect on the health status of the host, it is imperative to study whether haemolymph can change the functioning of immune system indirectly by modifying food and energetic of the animal.

MATERIALS AND METHODS

Sample: In the present study red velvet mites were selected as a test sample. The test samples were collected from soils during rainy season and transported to the laboratory. The animals were fed insects (termites) and cow dung, larva diet and water. For the experiments, the mites were grouped into several groups each group with six individuals. Each group was reared in a plastic tray with moisture conditions.

Animals and Treatment: For the experimental study, Swiss albino mice (BALB/c) were chosen as candidate species. The mice were obtained from Tetrox biosuppliers, Madurai, Tamilnadu, India and reared in laboratory under standard conditions. The mice were grouped into several groups and each group with six

individuals. Test samples were given through the drinking water bottle everyday. Using standard toxicological test LD_{50} doses for the two velvet samples were found out. From the LD_{50} doses two sublethal concentrations were derived for each velvet sample, for further experiments. The sublethal doses were $1/10^{th}$ and $1/20^{th}$ of 96 hrs LD_{50} doses of haemolymph, 30 and 15 ppm respectively. The sublethal doses of velvet samples were dissolved in water and were given to the animal through feeding bottle. The velvet sample doses were renewed everyday. Along with water, standard pellet feed was also given everyday in *ad libitum.*

Experimental system: Blood samples of mice were collected on the third, fourth and fifth weeks following velvet sample exposure by cardiac puncture, after anesthetizing the mice with chloroform. The serum was separated for each group separately and kept at -20^0 C, till analyzed. Heparin was used in collecting whole blood and leucocyte rich plasma for lymphocyte subset enumeration.

Immunological assays: In the present study, the normal mice and velvet sample exposed mice immunological changes analyzed by following assays.

Antibody titration: The total amount of antibody production was carried out by Log_2 titre plate method. 50 µl of physiological saline was added into all the wells of a clean microtitre plate. Then 50 µl of the antiserum is taken in a pipette man and is serially diluted 50 µl in the wells from the first row till the 11^{th} well of the microtitre plate leaving the 12^{th} well as a negative control. 25 µl of 1% SRBC in saline was added to all the wells of the microtitre plate. The microtitre plates were hand shaken for effective mixing of reagents and incubated for an hour at 37^o C and for one more hour at 10^o C. The highest dilution of the serum samples, which shows detectable agglutination, is recorded and expressed in Log_2 titre of antibody.

B and T cell E - rosette assay: 5 - 10 ml of blood was collected from test samples exposed animals and it was introduced into sterile conical flask / beaker containing (4 - 5) sterile glass beads. It was then continuously swirled until no sounds were heard from the beads. This indicates that all the fibrins have adhered to the beads. This blood was considered as defibrinated blood. This defibrinated blood was taken and diluted with equal volume of physiological saline. 3 ml of the lymphoprep solution was taken in a centrifuge tube. The tube was kept in slanting position and 9 ml of diluted blood was slowly added along the sides of the centrifuge tube using Pasteur pipette. Care was taken so that the FICON layer of the lymphoprep solution present in the centrifuge tube was not disturbed. The content of the centrifuge tube was then centrifuged at 1600 rpm for 20 min. The interphase (containing lymphocytes) was removed using pipette. The cells were washed with 1 ml saline and excess FICON was removed. The sample was again washed with 1 ml of saline after centrifugation the supernatant was decanted by inverting the tube over a filter paper after all saline was drained; the pellet was then resuspended in 300 µl of RPMI 1640 medium. The lymphocytes were separated using nylon wool column by hot/cold saline. Separated lymphocytes identified with help of erythrocyte antigens and enumerated under microscope.

Total heterotrophic bacterial population (THBP): The total heterotrophic bacterial population was enumerated by pour plate technique using nutrient agar medium. The foregut, midgut and hindgut samples were homogenized individually using a known volume of sterilized distilled water to make serial dilutions. After serial dilution with precaution, one ml of aliquots of appropriate dilutions of the sample was pipetted out into sterile petridishes and 15 to 20 ml of sterile nutrient agar medium were poured. The medium and the inoculums were thoroughly mixed using turntable and the medium was allowed to solidify. Duplicate plates were also maintained. The numbers of bacterial colonies were counted after 48 hours of incubation. The bacterial populations were

expressed as number of colony forming units (CFU) per gram samples analyzed.

RESULT AND DISCUSSION

Immunological reflection in health system was analyzed in normal and treated animals by two different methods like, humoral and cellular immune response.

***Qualitative and quantitative analysis of antibody*:** To optimize the serum dilution with saline, control serum were loaded over a range of dilution from 1:16, and a dilution of 1:254 appeared to be highest antibody titre (Table 1).

Table 1
Antibody titre value against SRBC (2.5 x 10^5 cells/mL) antigen in normal and treated animals

S. No.	Test sample	Antibody titre
1.	Normal group	4 $\log_2$ 2
2.	Haemolymph 1/10^{th} conc. treated	8 $\log_2$ 2
3.	Haemolymph 1/20^{th} conc. treated	6 $\log_2$ 2

B and T cell e- rosette formation assay: B-Lymphocytes counts using rosette forming assay revealed increment significance in test samples exposed mice than control (Table 2). Increment in B-Lymphocyte was much pronounced in test samples exposed mice in the first week and second week also had more or less similar impact on B-cell estimations. The present study, clearly confirm the increment in B-cell number in mouse exposed to test samples. So the impact of test samples on the synthesis, proliferation and activation of lymphocytes was documented. Gebel *et al.,* (1997) reported the differentiation of B-cell counts affected by samples.

Here, it is remarkably noted that increment in B-cell production in test animals due to administration of haemolymph. The increment of this type of immune responses confirms the potential of test samples to be toxin in health system. B-cell proliferations modification depends on the exposure of antigens. But in the present study nucleotide (DNA) produce moderate B-cell proliferations (Gapel *et al.,* 1997).

The impact of test samples on T-cell production in mice is given in Table 2. T cell is a vital component in cell mediated immune response, gets enhanced due to exposure of antigens. It is found to be enhancing to T cell production so induction in cell mediate immunity has confirmed pathogenic potential of test samples. Several workers like Manjula *et al.,* (2009) and Lighty George *et al*., (2010) had reported that the immunoenhance drugs inhibit cell proliferation and T-cell cytotoxicity. It also induces apoptosis in activated as well as resting cells. T-cell population which has reduced T cell counts the inhibition of T-cell activation, proliferation, immunity exclusion and co-operation with other cells had affected the overall immunity in mice.

Table- 2

Enumeration of B and T cells using rosette forming assay in normal and test samples exposed mice

S. No.	Test samples	Lymphocyte estimation at different weeks					
		% of B cell			% of T cell		
		I	II	III	I	II	III
1.	Normal	21.7	22.1	23.8	57.6	58.2	58.6
2.	Haemolymph 1/10th conc. Treated	22.6	23.6	26.8	57.8	59.2	62.8
3.	Haemolymph 1/20th conc. Treated	21.8	23.2	25.2	57.6	58.3	59.6

Gut microbial study: Total heterotrophic bacterial population count in the different regions of the gut included symbiotic as well as pathogenic microbes. Symbiotic microbes have probiotic role and promote digestive ability by producing microbial enzymes. The increase in total heterotrophic bacterial population (THBP) in different regions of the gut due to samples treatment indicated the increase in beneficial probiotic microbes. The increment of beneficial gut micro flora had interfered with the digestive ability, food consumption energetic and overall health of the animal. The THBP in different regions of control and haemolymph treated mice are given in Table 3.

Table – 3

Bacterial density of the digestive tract of normal and haemolymph administered Swiss albino mice

S. No.	Source sample	Bacterial density (CFU/g)		
		Normal	1/10 conc.	1/20 conc.
1.	Foregut	7.2×10^5	8.5×10^3	9.1×10^4
2.	Midgut	6.7×10^6	9.4×10^4	4.1×10^5
3.	Hindgut	9.1×10^8	7.4×10^6	5.4×10^7

Further, the presence of digested food in stomach, intestine and rectum succeed to provide suitable medium for the growth of microflora in the alimentary tract. This had lead to the increment in THBP density in the animals treated with foreign substances like plant extracts, antibody etc., (Dhasarathan, 2010). The increments of THBP in the gut of the host indirectly affect the feeding and energy budget in host animal (Tilak *et al.,* 1991). Feeding and changes in the energy budget also questions the functioning of immune system and execution of immune response. Changes in the energy budget were one of the reasons for modulation to the immunological parameters and immunity.

The microbes will not only utilize the nutrients thus provided, but also produce materials that could be made use by the host organism. For instance, the gut micro flora of man provides certain essential vitamins, which they synthesize within the intestine (Svenson *et al.,* 1994). There are cases where the micro flora enables the host organism to digest and utilize food materials that cannot otherwise be digested for want of the required enzyme (Ahamed *et al.,* 1978). The classic example is the association of termites and the *Trichonympha*, which is a flagellate. The bacteria that live in their four-chambered stomach extensively enrich the ruminants and these are responsible for the

lysis of cellulose. Thus, the bacteria and other microbes play a complementary role in digesting foodstuffs, which cannot be tackled by the native enzymes of the host organism (De Ridder *et al.,* 1985). Symbiotic microbes are mostly common in herbivorous forms as the type of food is broad spectrum of vegetable matter (Domech *et al.,* 1983). The carnivores on the other hand, do not face such a problem, since they take more or less uniform type of material as food. It does not mean that the carnivores are completely devoid of gut micro flora. They too lodge populations of some species of microbes, which are symbiotic and complement the native enzymes (Koeman, 1991). All these associations are mutually beneficial and such relationships should have existed from early evolutionary times. Haemolymph treatment may modulate the beneficial microbe and change the energetics make the immune system strong. This is an indirect assault on the immune response and hence it needs an immediate attention to prepare natural immunostimulant drugs.

Based on the knowledge that the immune system responds much more rapidly, if it encounters an invading organism that it has already battled and defeated theory, that introducing antigen into the body is just enough to elicit an immune response without causing increase would protect the body from contracting the disease at this time, have risk in procedure of vaccination. Therefore vaccination also means “active immunization” referring to the procedure where administration of an antigen results in protective immunity to the disease associated with that antigen. The immunostimulant potential in the haemolymph of Red velvet mite suggests that the mite possess bioactive compounds and this has to be explored in future.

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