The effect of Beta-amino Butyric Acid in Levels of Interleukin4 & Interleukin 10, Complement Proteins C3 & C4 and Immunoglobin IgM in Males Rats Sprague Dawley Infected with *Pseudomonas aeruginosa*

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Abstract

B–amino butyric acid (BABA) non protein amino acid has effect on some immunological parameters of male rats Sprague Dawley species infected with *pseudomonas aeruginosa.* this study include 25 animal divided into five groups A, B, C, D and E each group contain five animals, A, B and C groups injected with three concentration of amino acid solution (25 mg\ml, 50 mg\ml and 75 mg\ml) with dose (0.2 g\ kg, 0.4g\kg and 0.6 g\kg) respectively, intra peritoneal weekly for six weeks, group D (first control group) and E (second control group) injected with normal saline. At the fifth week the four groups A, B, C and D exposed to *pseudomonas aeruginosa,* while group E did not exposed to infections after the termination of period of treatment, The blood samples were collected to do the immunological tests, the statistical analysis $P<0.05$ results showed BABA did not enhance sensitivity through measuring interleukin IL4, and significant increase of IL10 and complement protein C4, while BABA didn't have a negative effect on the complement protein C3 activation and increase the level of immunoglobulin IgM.

Keywords: *Beta-aminobutyric acid, Sprague Dawley rats, Pseudomonas aeruginosa, Interleukin IL10, IL4, Complement proteins C3 & C4, Immunoglobin IgM.*

Introduction

The bacteria considered important causes of disease, that include the most of diseases that infect humans in the word and the most important prefix and present and futurism epidemiological diseases[(1)], *Pseudomonas aeruginosa* considered important bacterial species due to its content of virulence factor that increase its pathogenicity[(2)]. It's considered dangerous for patients especially patients with wounds & burn inflammation, that it can invade blood supply and cause Septicemia especially patients with Immunodeficiency[(3)]. *P. aeruginosa* characterized by its resistance to antibiotics and disinfectant, and this cause big problem in whole the word and the main cause of this resistance is random use of antibiotics without restriction[(4)], due to continues antibiotic residue lead to development of bacterial resistance to antibiotics[(5)], this what make researchers to new applications by using chemical materials other than antibiotics[(6)], that enhance body resistance to bacterial infections, the nominated materials to replace antibiotics is organic acids[(5)], Which are include many acids like Non-protein amino acids that present in the nature, more than 1000 non protein amino acid produced by plants and microorganism and other sources[(7)], these components did not have specific functions in the nature, but they noticed have many physiological functions *In vivo* studies[(8)], For the example, Gamma-amino butyric acid (GABA) work as stimulator and regulator of immune response that it boosted the Innate immunity and Adaptive immunity, that work on improvement of body resistance against bacteria by activation of Phagocytosis, and matureness of Macrophages and improve its response against microorganism[(9)], and activation of T cells and B cells, increase antibodies like IgG and IgM[(10)], GABA increase of Anti-inflammatory cytokines like interleukin IL10 and suppression of Pro-inflammatory cytokines and work on stimulation of Apoptosis of cells that damaged by pathogenic factors[(11)], also the Non-protein amino acids β-amino butyric acid (BABA) was improved recently that make hematological and immunological changes, that increase

red blood cells, white blood cells, hemoglobin, packed cells volume and lymphocyte, also cause increase in immunoglobulin IgG[12].

Due to rarity of studies about the effect of this acid in animal aspect with its availability in plant aspect, and due to BABA known with its ability to stimulate plant resistance against wide range of causative agent like viruses, bacteria, fungi and worms[13], also have significant effect in plant resistance to insects[14].

So this study aimed to identification of ability Non-protein amino acids BABA to induction of rats resistance to *P. aeruginosa.*

Materials and Method

Preparation of amino acid solution

Amino acid solution prepared by dissolving 0.5g, 1g and 1.5g from acid in 20ml of normal saline to obtain the first concentration C1 (25mg\ml) and second concentration C2 (50mg\ml) and third concentration C3 (75mg\ml) respectively with continues mixing until acid dissolving then the wanted dose prepared according to animal body weight, with percentage of 0.2g\kg from first concentration C1 and 0.4g\kg from second concentration and 0.6 g\kg from third concentration.

Preparation of the lab animals

Experimental animals consist of 25 of Sprague Dawley male rats with age 10 to 12 weeks and weight ranged between (225-300) g, divided into five groups each groups five animals divided as following:

1- Group A first concentration group C1(25mg\ml)

2- Group B second concentration group C2 (50mg\ml)

3- Group C third concentration group C3 (75mg\ml)

The groups above injected with amino acid intrapersonal[15] weekly for six weeks.

4- Group D first control groups: injected with normal saline intrapersonal.

The four groups above exposed to infection with *P. aeruginosa* Bactria.

5- Group E second control group this group injected with normal saline intrapersonal and did not exposed to infection with *P. aeruginosa*.

Animal infection with *P. aeruginosa*

The animal infected after the fifth weeks of amino acid BABA injection at age (15-17) weeks with weight ranged between (265-380) g. the isolation activated with by obtain part of bacterial cultivation to inoculate nutrient broth tube and incubate with 37 C for 24 hours. After appear of the bacterial growth decimal dilution done by using normal saline and 1 ml of each diluent was obtained and inoculates on nutrient agar to count the colonies in the suspension. Rats anesthetized with Chloroform. Than shaved the area down the head from the back[16]. Wounded the skin deeply without damage the subcutaneous muscles, after sterilization by using forceps and Scissor, skin biopsy obtained in about 6mm diameter of five animal groups (A, B, C,D and E), by using Micropipette 200µm of suspension with turbidity (2×10^6) putted on the wound to contaminate it[17] in four animal groups that include group A, group B, group C and first control group D, while second control group E did not contaminated with bacteria.

The animals examined for its nutrition and activity and wound healing after expend of seven days of infections the physiological and immunological tests were done.

Serological tests

Immunological tests were carried out on the serum of the rats to detect the level of IL-4, IL-10, C3, C4 and IgM in all groups. Blood was obtained from posterior vena cava[15].

3 ml of blood was withdrawn in plastic tubes free of anticoagulant to obtain the serum using centrifuge 3500 rpm for ten minutes. The serum samples were kept at -20°C until the time of testing using (Elisa kit/ Elabscience Biotechnology Inc. /USA) and (Genus Kit/ Genrui Biotech Inc./China)

Statistical Analysis

The statistical analysis done by using One-way ANOVA test with statistical analysis program SPSS 22 edition and the mean calculated and stander deviation, finding LSD value from Multiple comparisons table at level of significance 0.05.

Results and Discussion

Effect of BABA on level of interleukin IL4

The results of statistical analysis as in table 1 which show the significant differences in LSD values between groups, and figure 1, show that A, B, C and D (three concentration group and first control group) that exposed to bacterial infection was recorded significant increase in level of interleukin IL4 compare with second control group that did not exposed to bacterial infection.

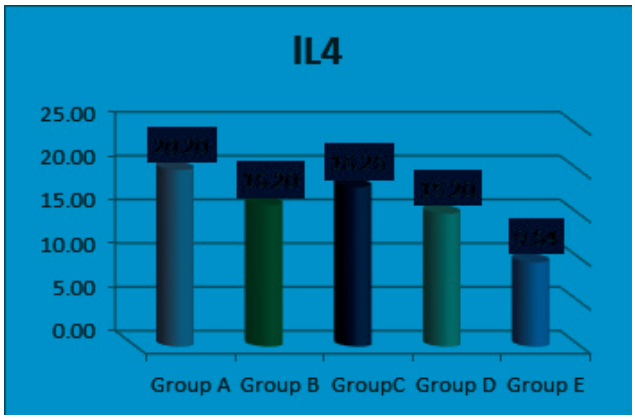

Figure 1 show the means of interleukin IL4 values

From the above the level of IL4 that increased in infected groups as the results of infection with bacteria not resulted from BABA amino acid that appeared the mast cells are activated to interact with wound repairing and wound healing at the same time with infection by microorganism, that have vigilant factors that disturb wound healing mechanism as Exotoxins of *P. aeruginosa* this cells stimulated presented in the subcutaneous tissue that migrate to the wound site and secrete its content due to expose to bacteria products, the studies conclude that the mast cells activated by its exposer to bacterial toxin or lipopolysaccharide (LPS) bacterial wall content, also fined that the mast cells that present in peritoneal layer execrate Histamine as the results of *P. aeruginosa* bacteria in rats, and it's an important source of early response cytokines like IL4 that necessary to begin the immune response and inflammation of the host ageist invaders[18], and considered main resource of IL4[19], that the mast cells activated by bacteria that cause diseases also in the case of absence of antibodies and information and in this case be the source to generate IL4 and other regulating cytokines to do Non-opsonization reactions[18], the mast cells response to many stimulators independently without interaction of IgE and release its component, in this situation did not considered hypersensitivity response because IgE not unclouded in the interaction, and this explain support absence of signs of hypersensitivity in animals.

Effect of BABA on level of IL10

The results of statistical analysis $P<0.05$ as in table 1 and figure 2 show the A group record significant increase in level of IL10 in compare with E and D control groups, also group C record showed significant increase in level of IL10 in compare with E second control group.

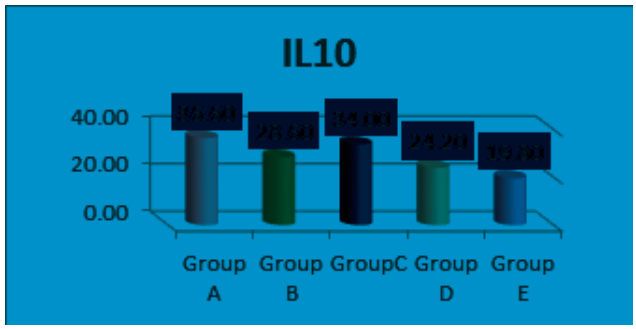

Figure 2 show the means interluken 10 value

By notest the figure 2 showed that the mean of IL10 value elevated in animal groups treated with BABA amino acid in compare with E and D control groups but just group A recorded significant increase in compare with control groups E and D, and group C recorded significant increase in compare with E second control group, while group B did not recorded any significant increase for unclrear causes, and this may explane the level of IL10 in group A and the dose 0.2 g\kg of BABA amino acid and this dose needed for production of IL10 with significant level and this agree with what mentioned by[11], that the GABA which analogous with BABA, increase the production of IL10 significantly in case of colon inflammation induced in mice, that GABA work as stimulator for antiinflammatory cytokines and inhibit Pro-inflammatory cytokines and enhance Apoptosis for cells that damaged with diseases factors.

Effect of BABA levels of complement proteins C4 and C3 and immune globulin IgM

Results of Statistical Analysis $P<0.05$ showed as appeared in table 1 and figure 3 that the five group A, B, C, D and E did not show any significant increase in level of complement protein C3, with non-significant sharp decrease of C3 mean values in group A, also decrease the value of C3 in second control group E, and the decreased values indicate the activation of complement protein[20].

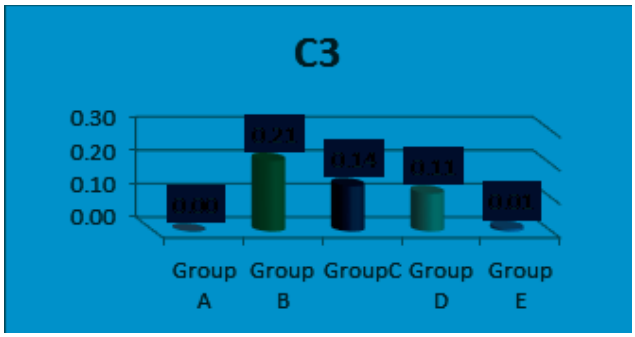

Figure 3 show the means of complement protein C3 values

While the complement protein C4 the results of statistical analysis P<0.05 were showed as in table 1 and figure 4 that the group A recorded significant increase in level of C4 in compare with group B, while other groups did not recorded any significant differences, and this increase may cause by bacterial infections or may be due to the 0.2 g\kg dose of BABA acid is the optimal dose to increase complement proteins C4 significantly, as appear in figure 4 that showed means of complement proteins C4 values.

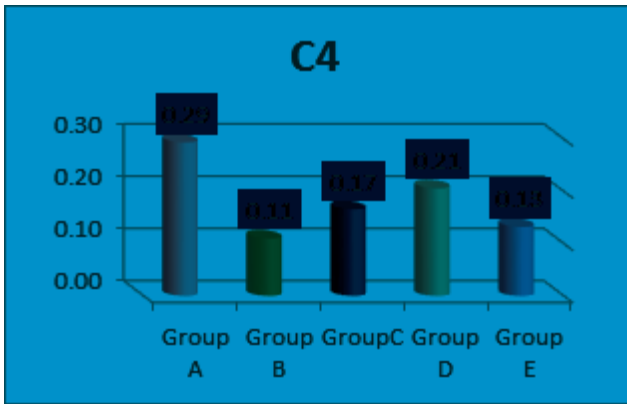

Figure 4 show the means of complement protein C4 values

From the compares between figure 3 and figure 4 noticed there are negative correlation between C3 and C4 values and this difference may return to activation of complement C3 to activate Alternative pathway or Lectin pathway as an innate immune response to infection, and lead to activation B cells to production of antibodies to activation of Classical pathway[21]. And this indicates that the BABA amino acid did not have negative effect to prevent complement C3 activation process. And have positive effect in activation of classical pathway of complement system that depends on C4 protein.

While measurement of immunoglobulin IgM, the results of statistical analysis (P<0.05) as in table 1 and figure 5, that the group A was recorded significant increase in level of IgM in compare with group B, C and E, that the 0.2 g\kg lowest dose suitable to production of IgM and this results did not agree with[21] mentioned that the increase dose increase production of immunoglobulin IgG and this may returned to interactions with bacterial infections or may results category of antibody and molecular compound of each one.

Figure 5 show the means of immunoglobulin IgM values

By compare of figure 4 and figure 5 noticed that presence of clear positive proportions between complement protein values C4 and immunoglobulin IgM, and this may lead to activation classic pathway of complement system, the interaction of B cells that produce immunoglobulin IgM that stimulate activate of classic pathway of complement system, and activation

Table 1 show the LSD values

Dependent Variable		IL4	IL10	C3	C4	IgM
Group A	Group B	.146	.166	.113	.048*	.003*
	Group C	.471	.646	.300	.158	.006
	Group D	.073	.038*	.383	.326	.105
	Group E	.001*	.007*	.951	.079	.008*
Group B	Group A	.146	.166	.113	.048*	.003*
	Group C	.445	.344	.559	.529	.737
	Group D	.709	.439	.452	.285	.103
	Group E	.022*	.130	.126	.804	.748

Cont... **Table 1 show the LSD values**

Group C	Group A	.471	.646	.300	.158	.006*
	Group B	.445	.344	.559	.529	.737
	Group D	.260	.094	.885	.652	.187
	Group E	.004*	.019*	.328	.701	.989
Group D	Group A	.073	.038*	.383	.326	.105
	Group B	.709	.439	.452	.285	.103
	Group C	.260	.094	.865	.652	.187
	Group E	.048*	.439	.417	.407	.183
Group E	Group A	.001*	.007*	.951	.079	.006*
	Group B	.022*	.130	.126	.804	.748
	Group C	.004*	.019*	.328	.701	.989
	Group D	.048*	.439	.417	.407	.103

*means difference is significant in LSD values between groups at the 0.05 level

Conclusions

Current study insure that the non-protein amino acid BABA have clear positive effect in accelerate of wound healing process and decrease inflammation, the serological tests P<0.05 ensure that the BABA did not generate hypersensitivity in animal of experiment. BABA have positive effect in production of IL10 then it has role in regulation of immune response, BABA did not stop activation of complement system but lead to stimulation of B cells to production of IgM antibody and inter action between Innate immunity and Adaptive immunity.

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