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The effect of Beta-amino butyric acid (BABA) on *Staphylococcus aureus* bacteria and some interleukins

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ABSTRACT

β-amino butyric acid (BABA) is one of the rare free compounds found in nature. It is recognized for its ability to enhance plant immunity against a wide range of diseases, including viruses, bacteria, fungus, and worms. This research aimed the effects of BABA on Staphylococcus aureus isolated from Sprague-Dawley male rats' feces and on IL-4, IL-10, and IL-27. The study included 25 Sprague-Dawley male rats, divided into five groups, each group contain 5 rats, The first group for control, while the second, third ,fourth, and fifth groups for rats dosed orally with different concentration of BABA as (0.250 ,0.315,0.358,0.360) mg/kg respectively, all rats dosed twice in week and for four weeks. The result show one isolate from the control group was found at each stages (T1, T2, T3, T6, T7) and none at (T5 and T8). In the second group, one isolate was in each stage T2, T3, T7, and none in T1, T5, and T8. In the third group, one isolate was at each stage (T2, T3, T4, and T7) and none in other stages. Groups four and five showed no Staphylococcus aureus growth. The study show significant increase of IL-27 in fifth group (98.60 \pm 1.57) in comparison with control (70.60 \pm 0.75). Furthermore significant increase IL-10 in fourth and fifth groups (1082.83 ±23.33, 1104.36 ±43.44) respectively in comparison with control (504.06 \pm 7.12). While IL-4 decrease in the fourth and fifth groups (8.04 \pm 0.78 , 14.07 ± 0.16) respectively in comparison with control (34.89 ± 1.51). The study concluded the most effective dose of BABA in inhibiting *Staphylococcus aureus* growth is 0.358,0.360 mg/kg dosage. In addition 0.358,0.360 mg/kg increase IL-27 and IL-10, while decrease IL-4.

Introduction

Bacteria is one of the most important pathological causes, as it can be considered the main cause of the events of important diseases that are scattered globally, whose effect ranges from minor diseases to diseases that threaten human life [1]. Where the intestines of mammals harbor many diverse microorganisms that play an essential role in maintaining the physiological balance of the digestive system. These symbiotic microorganisms are important for the normal development of the host immune system, as diet is one of the main drivers in the formation of microorganisms in the intestine throughout life, and intestinal bacteria help maintain immune balance, metabolism, and protection from pathogens [2,4].

Beta-aminobutyric acid: It is a non-protein amino acid that has been shown to protect plants from pathogens by strengthening the various defense mechanisms found in plants. Also, it works to increase drought resistance and salinity severity in some plants[5]. It was also found that the amino acid BABA is used to stimulate the resistance of grapes to mold diseases [6]. The amino acid BABA has also been found to be used to stimulate grape resistance to mold disease[6]. The effect of BABA on the animal cell, recent messages on this topic have recently appeared, in one research, the experiment was conducted on healthy male Sprague Dawley mice, and it was found to change blood parameters and immunity. Research has shown that BABA plays a beneficial function in speeding up the healing of wounds and reducing inflammation. Additionally, BABA stimulates B cells to generate Igg and IgM antibodies, which in turn activate the complementary system and facilitate communication between innate immunity and acquired immunity[7]. It

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enhances the synthesis of interleukin-10, hence playing a regulatory role in the immune system [8].

One type of cytokines is a group of proteins with different structures and functions that contribute to communication between white blood cells by binding to receptors on the surface of cells and are very important in activating and inhibiting the immune system and cell division. Immune cell interleukins are synthesized by T lymphocytes, CD4, monocytes, nucleotides, and macrophages [9]. Interleukins also have a crucial impact on the progression of stroke. Interleukins are signaling molecules that facilitate communication between lymphocytes, which are white blood cells or immune cells. It belongs to the same class as the blood cell growth factor and is classified as a cytokine. Interleukins and blood cell growth factors are both types of cytokines that work together to carry out essential activities in blood formation and immunological control. Interleukins are essential for transmitting information activating, and regulating immune cells. They are responsible for activating, reproducing, and differentiating T and B cells and initiating the inflammatory response. [10]. This study aimed to study the effect of beta-amino butyric acid (BABA) with different doses on interleukins IL-4, IL-10, IL-27, and Staphylococcus aureus bacteria that isolated from Sprague Dawley male rats.

Materials and methods Sample collection

The experiment used 25 Sprague-Dawley male rats prepared by the National Center for Drug Control at the age of 10-12 weeks and weights ranging between (160-210) g. The animals were placed in special cages for rats (plastic with a metal cover clasped for ventilation) and then placed in the animal house of the College of Education for Girls - Anbar University, and the appropriate environmental conditions were created for the animals from ventilation and temperature ranging between (28-25) m and appropriate lighting periods of up to 12 hours/day and feeding them with a diet dedicated to certain proportions according to the animal's need. The rats were divided into five groups, and each group contained five rats. The first group were for control. The second group were for rats orally treated with BABA (0.250) mg/kg. The third group were for rats orally treated with BABA (0.315) mg/kg. The fourth group were for rats orally treated with BABA (0.358) mg/kg. The fifth group were for rats orally treated with BABA(0.360), all rats dosed twice in week and for four weeks .

Assessment IL-4, IL-10, IL-27 ELISA test

The serum concentrations of IL-4, IL-10, IL-27 were assessed using the enzyme-linked immunosorbent assay (ELISA) and according to the instructions in the ELISA kit (Sunlong,China). The ELISA plates were precoated with IL-4, IL-10, IL-27 antibodies. The samples were added to the plates, and the color development in the substrate solution correlated with the level of IL-4, IL-10, IL-27. The process was stopped by adding a stop solution, and absorbance was measured at 450 nm.

Isolation and identification of Staphylococcus aureus

All samples that collected from feces of Dawley rats that treated with different concentration of BABA and dosed for 8 stage, were streaked on blood agar, mannitol salt agar, Congored agar and nutrient agar [11]. Gram Stain and biochemical (catalase, oxidase and coagulase) tests.

Preparation of the non-protein B-amino butyric acid (BABA) solution

The amino acid solution was prepared by dissolving it in 5 ml of distilled water for each of the five groups and according to the concentrations indicative of each of them, and then the required doses for each animal were prepared by weight and dosed twice a week for four weeks using a special dosing tube [12].

Ethical approval

Ethical approval for the use of animals in this research was given by Anbar University Health Research Ethics Committee (protocol number 80 in 14/2/2023), and all procedures were in strict compliance with set ethical standards. Twenty five male Sprague-Dawley male rats weighing between 160 and 210 g were purchased From the animal house at Tikrit University, Saladin Governorate, and used for this experiment. Animals were housed in clean, well-ventilated cages for standard 12-h light and dark cycles at room temperature

in the animal house in the College of Education for Girls - Anbar University. Animals were fed standard rat chow and clean water ad libitum and allowed to acclimatize for 10 days prior to the start of the experiment.

Statistical analysis

The System Analysis Statistical SAS (2018) program was used to detect the effect of variation factors on the study's parameters. Using the Least Significant Difference - LSD (Analysis of Variance - ANOVA) For significant comparison between averages [13].

Results and discussion

Isolation and diagnosis of Staphylococcus aureus

Taking samples from the feces of the rats used in the experiment before starting the dosing phase and after dosing the second, third, fourth, fifth groups with (0.250), 0.315, 0.358, 0.360) mg/kg respectively, twice in week for 4 weeks. After each of the eight stages (T) of dosing and dilution, the bacteria were isolated and diagnosed and grown on culture media specific for bacteria. Then biochemical tests were conducted. The results obtained showed that 19 isolates were of Staphylococcus aureus bacteria . The results, in Figure (1), showed the number of *Staphylococcus aureus* that isolated from control group were one isolates at stages (T1,T2, T3, T6, T7), and 3 isolates in (T4), while no isolates at stage (T5, T8). Furthermore in the second group that dosed with 0.250 mg/kg, were one isolates in (T2,T3,T7) and two isolates in (T4,T6) while no Isolates at T1,T5 and T8.As for third group that dosed with BABA at 0.315 mg/kg were one isolates at stage (T2, T3, T4, and T7) while no isolates at other stages. On the other hand no growth of Staphylococcus aureus in the group four and five when dosed at (0.358, 0.360)mg/kg respectively.

The BABA had an effective role in affecting the bacteria *Staphylococcus aureus*, as it led to the disappearance of the bacteria at two concentrations 0.358gm and 0.360gm. The dosing stages also had a role in the disappearance of the bacteria . This agrees with what was indicated by [14], that the BABA act as an antibacterial by the ability of the amino acid to increase the resistance of animals and strengthen their immune system and thus reduce the proliferation of bacteria. The

study done by Jasim *et al.*, [15], shown in their research that BABA has a beneficial impact on bacterial resistance against staphylococcus bacteria when exposed to acid. The treatment with BABA led to a reduction in the proliferation of living organisms [16], also BABA can act as an anti-bacterial [17]. In addition of BABA led to a decrease in the number of bacteria[18].

Immunological tests

Serum levels of interleukins (IL-4, IL-10, IL-27) were measured by sandwich Enzyme-Linked Immuno-Sorbent Assay (ELISA). The results of the statistical analysis in Table (1) showed that there were significant differences at the probability level (P≤0.01) between the study groups for (IL-4). The statistical analysis results in Table (4-2) showed significant differences. At the probability level (P≤0.01) among the study groups for (IL-4). The average of the Control treatment was 34.89 with a standard error of 1.51, the average of the treatment with concentration (0.250) was 16.67 with a standard error of 0.98, and the average of the treatment with concentration (0.315) was 11.57 with an error. Standard 0.31, the average of the treatment with concentration (0.358) was 8.04, with a standard error of 0.78, and the average of the treatment with concentration (0.360) was 14.07, with a standard error of 0.16, under the probability level of 0.0001 at a significance level of 2.693. While there are no significant differences between the average of the treatment with concentration (0.250) 16.67, with a standard error of 0.98, and the average of the treatment with concentration (0.360)14.07, with a standard error of 0.16, under the probability level of 0.0001 at a significance level of 2.693 ** .

While there are no significant differences between the average of the treatment with concentration (0.250)16.67, with a standard error of 0.98, and the average of the treatment with concentration (0.360) 14.07, with a standard error of 0.16, under the probability level of 0.0001 at a significance level of 2.693**.

There are also no significant differences between the average of the treatment with a concentration (0.315) of 11.57, with a standard error of 0.31, and the average of the treatment with a concentration of (0.360) of 14.07, with a standard error of 0.16, under the probability level of 0.0001 at a significance level of 2.693 **. IL-4 results showed a noticeable and continuous decrease at each level. Concentrations, but the greatest decrease was at the concentration of $0.358 \text{gm} (8.04 \pm 0.78)$, This decrease may be attributed to the amino acid (BABA). This result disagrees with the results of [8], as the percentages were high due to infection with P. aeruginosa, where mast cells are the main store of IL-4, as proven by studies, so mast cells will be provoked by pathogenic bacteria even if antibodies are not formed, and thus they are the first source of IL-4 formation [8].





Among the study totals for (IL-10), the results of the statistical analysis in Table (1) showed significant differences at the level of probability (P \leq 0.01), where the average control transaction was 504.06 with a standard error of 7.12, the average transaction with concentration (0.250) 871.92 with a standard error of 8.42, and the average treatment with concentration (0.315) 947.50 and a standard error of 110.56, The average transaction with concentration (0.358) is 1082.83 with a standard error of 23.33, and the average transaction with concentration (0.360) is 1104.36 and a standard error of 43.44, below the probability level of 0.0001 at a significant level of 69.587** .

While there were no significant differences between the average transaction with concentration (0.358) 1082.83 with a standard error of 23.33, and the average transaction with concentration (0.360) 1104.36 with a standard error of 43.44, below the probability level of 0.0001 at a significant level of 69.587 **. For the 10-IL,

BABA had a positive role in increasing its levels in all concentrations, where the increase was noticeable and gradual according to the increase in concentration; the highest results were recorded in the highest concentration, 0.360gm (1104.36 ±43.44). This study agree with [7], that show increase IL10 value in animal groups treated with BABA.

BABA, increase the production of IL10 significantly in case of bacterial inflammation induced in mice, that cause immunological changes [19].

IL-10 has a regulatory role in the immune system The results suggest that metabolism regulates the expression of coli cytokines, especially IL-10, possibly by effects on gut bacteria [20]. IL-10 is produced mainly by leukocytes (such as T cells and B cells) and epithelial cells. IL-10 plays an important role in maintaining the balance of the intestinal mucosa and in suppressing inflammatory responses of innate and adaptive immune cells [21]. It also plays an important role in wound healing and tissue repair. IL-10 may also play a role in autoimmune diseases by inhibiting the production of cytokines produced by T helper 1 (Th1) instead of producing Recombinant IL-10, which requires the production of It costs a lot of money and effort, so it is possible to benefit from the amino acid (BABA) as a stimulant for the production of IL-10 internally, but it requires extensive studies so far [15].

Among the study groups for (IL-27), the results of the statistical analysis in Table (4-2) showed that there were significant differences, at the probability level (P \leq 0.01), where the average of the control treatment was 70.60, with a standard error of 0.75, and the average of the concentration treatment ((0.250) is 50.02, with a standard error of 0.33, and the average of the treatment with concentration (0.315) is 49.95, with a standard error of 0.87, The average of the treatment with concentration (0.358) is 43.22, with a standard error of 0.77, and the average of the treatment with concentration (0.360) is 98.60, with a standard error of 1.57, under the probability level of 0.0001 at a significance level of 2.866 **.

While there are no significant differences, the average of the treatment with a concentration (0.250) is 50.02, with a standard error of 0.33, and the average of the treatment with a concentration (0.315) is 49.95, with

a standard error of 0.87, below the probability level of 0.0001 at a significance level of 2.866 **.

In the IL-27 test, there were clear significant differences between the control and the treatment with the highest concentration of $0.360 \text{gm} (1.57\pm98.60)$, The rest of the concentrations led to a decrease in the level of IL-27. The reason for the decrease in residual values may be due to a lack of amino acid In a study that proved that amino acid deficiency can cause systemic inflammation, the effects of TRP deficiency (tryptophan from amino acids, which are protein building blocks).

Tryptophan is also called the essential amino acid because the body cannot synthesize it but must be obtained from food (versus a diet rich in TRP on the systemic immune profile, as a diet low in TRP led to a significant decrease in IL-27 [22].

| Figure(2): Shows | the co | ompar | ison | betwee | n groups for |
|------------------|--------|-------|------|--------|--------------|
| differences | in (II | 4 Π | 10 | and H | 27) |

| differences in $(12-4, 12-10, and 12-27)$. | | | | | | |
|---|----------------|-----------------------------|---------------|--|--|--|
| Group - | Mean ± SE | | | | | |
| | IL-4 | IL-10 | IL-27 | | | |
| Control | 34.89 ±1.51 a | $504.06 \pm 7.12 \text{ d}$ | 70.60 ±0.75 b | | | |
| 0.2509gm | 16.67 ±0.98 b | 871.92 ±8.42 c | 50.02 ±0.33 c | | | |
| 0.315gm | 11.57 ±0.31 c | 947.50 ±10.56 b | 49.95 ±0.87 c | | | |
| 0.358gm | 8.04 ±0.78 d | 1082.83 ±23.33 a | 43.22 ±0.77 d | | | |
| 0.360gm | 14.07 ±0.16 bc | 1104.36 ±43.44 a | 98.60 ±1.57 a | | | |
| LSD value | 2.693 ** | 69.587 ** | 2.866 ** | | | |
| P-value | 0.0001 | 0.0001 | 0.0001 | | | |
| Means having with the different letters in same column differed | | | | | | |
| significantly, ** (P≤0.01). | | | | | | |



Figure(3): Comparison between difference groups in IL- 10



Conclusion

The results obtained in this study showed that the non-protein amino acid BABA affects IL-4, IL-10, and IL-27. The tests also showed a significant increase in IL-10 levels, a decrease in IL-4 levels, and a variation in IL-4 levels. IL-27. It also had an effect on S. aureus, a non-proteinogenic amino acid (BABA) that can act as an antibacterial by increasing the systemic immunity of the organism.

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Conflicts of interest

There are no conflicts of interest.

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تأثير حمض بيتا أمينوبوتيريك (BABA) على بكتيريا المكورات العنقودية الذهبية وبعض الإنترلوكينات

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