Hematological regulation using β-aminobutyric acid in staphylococcus aureus infected rats

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Abstract

Background: Several studies have directed the use of different chemicals as an antibiotics alternative that kills bacteria or may lead to stimulating the body's resistance against infections, such as organic acids that include non-protein amino acids especially β-aminobutyric acid (BABA), which has proven in this study its ability to increase the systemic resistance of male rats against *Staphylococcus aureus*. As well as increasing the numbers of white blood cells and lymphocytes and their positive effect on improving the immune complement system. The aim of study was to know the effect of β-aminobutyric Acid on the response immune system of animals that was infected with s. aureus bacteria, Aim of the study: The current study aimed to identify the hematological variations of animals treated with BABA, which were experimentally infected with *Staphylococcus aureus*. These parameters included (PCV, Hb, RBC, and WBC).

Materials used in this experiment mannitol salt field, as well as blood agar and MacConkey aga9r were used for the development of Staphylococcus aureus bacteria, as well as amino acid β-aminobutyric Acid with different concentrations Prepare the first solution of amino acid concentration (20 mg/ml).The other amino acid was prepared at a concentration of (40 mg/ml)Then the required doses for each animal were prepared according to the weight of each animal Results: The effect of BABA on the level of (RBC – WBC – PLT –LYM –GRA) The results showed that there was no effect of the amino acid BABA on the level of (RBC-MCV-PLT) in groups infected with bacteria and treated with BABAThe results showed that there was no effect of the amino acid BABA on the level of (WBC) in the groups infected with bacteria and treated with BABA, while the percentage of (LYM-GRA) increased.

Keywords: BABA (Beta –Amino Butyric Acid) / **RBC** (Red Blood cell) / **WBC** (White Blood Cell) / **Hb** (Hemoglobin) / **PCV** (Packed Cell Volume) / **PLT** (Platelets).

Introduction:

The body of animals, including humans, contains many cells that play an important role in transporting nutrients and gas exchange, called red blood corpuscles (RBCs), which are biconcave disc cells produced by the bone marrow that carry hemoglobin (1,2) .It is a protein containing iron, carried on red blood corpuscles, and its concentration in the blood varies according to age and gender, and its percentage increases with the increase in the production of red blood cells (Polycythemia, and its percentage decreases in the case of anemia and malnutrition) (3,4).

The blood also contains white blood cells (WBCs), which are essential elements in the humoral and acquired immune response. They arise from cells located in the bone marrow, which later differentiate into different immune cells according to the body's requirements. White blood cells are classified into three types (5). The main ones are granulocytes, which can also be called polymorphic nuclei (neutrophils, eosinophils, and basophils), lymphocytes, which include (B and T) cells, and finally, mononuclear cells, and this division depends on the phenotypic shape of the cells when stained with Leishman's stain. These cells perform multiple immune functions and are complementary to each other that contributing to enhancing the immune response against foreign invaders and pathogens (6).

The blood also contains platelets, which are small cells without nuclei that move in the form of disc fragments in the blood circulation, and their average life is (8-9) days. They are produced by megakaryocytes in the bone marrow and are released into the bloodstream and help prevent blood loss through adhesion processes. The assembly and when activated form a barrier that prevents the flow of blood from inside the blood vessels to the outside. (7,8,9).

All this prompted researchers to develop chemicals other than antibiotics, which in turn stimulate the body's resistance to bacterial infection. An example of these materials to replace antibiotics are organic acids (10).

It includes many non-protein amino acids, such as Beta-Amino Butyric acid (BABA), as studies have shown that it increases immunity in plants treated with it, as it increases plant resistance against fungi, viruses, bacteria and nematodes (11).

Also, recent studies have demonstrated that the amino acid has a role in increasing the number of white blood cells and lymphocytes and increasing the production of IgG in rats treated with the amino acid BABA (12).

Another recent study also showed an increase in the level of interleukins (IL) as IL 10 in male rats that treated with BABA (13).

Working methods

Hematological tests

Preparation of the amino acid solution (Preparation solution BABA)

The amino acid solution was prepared by dissolving (400) mg of amino acid in (20) ml of physiological solution (N.S Normal saline) to obtain the first concentration (20 mg/ml), and then the other or second amino acid solution was prepared by dissolving (800 mg) of the acid amino acid in (20 ml) of physiological salt solution ((N.S Normal saline) to obtain the second concentration (40 mg/ml), and then the required doses were prepared for each animal according to the weight of each animal, which is the dose (100 mg/kg) of the solution with the first concentration and a dose (200 mg/kg) of an acid solution of second concentration (Al-Esawy, 2020).

Design of experiment:

Distribution of animals groups:

The animals were divided into seven groups, and each group consisted of five animals. The groups were distributed as follows:

- First group (control group) A: The rats were left without infection with bacteria and without using the amino acid.

- Second group B: the animals were dosed only with the first concentration of amino acid solution 20 mg/ml for five weeks.
- Third group C: The animals were dosed only with the amino acid solution of the second concentration, 40 mg/ml for five weeks.
- Fourth group D: Animals were dosed with the first concentration of amino acid solution 20 mg/ml for five weeks and were infected with the bacterial isolate S. aureus, and the infection took place in the second week of dosing.
- Five group E: The animals were dosed with a second concentration of amino acid solution at 40 mg/ml for five weeks and were infected with the bacterial isolate S. aureus. The infection was in the second week of dosing.
- Sixth group F: The animals were infected with the bacterial isolate S. aureus and were not dosed with amino acids.
- Seven group G: The animals were infected with bacteria and the acid was given to them in the form of a solution at a concentration of 5 mg/ml and externally on the area of infection and without dosing for five weeks.

3- S. aureus: infection of animals with bacteria

The laboratory animals were infected with S. aureus bacteria, after the second week of intraperitoneal injection with BABA amino acid. The animals were 10-12 weeks old, with a weight ranging from (250 to 350). The process of infecting the animals included the following steps:

- The bacterial isolate was activated by taking a small part of the bacterial colony and planting it on plates of saline mannitol medium by the planning method, and the dishes were incubated at 37°C for 24 hours. After bacterial growth, a series of decadal dilutions were carried out using physiological saline solution, and 1 ml of each dilution was cultured on the agar nutrient medium, in order to obtain separate colonies for counting the colonies in the suspension.
- The rats were anaesthetized with chloroform, and then shaved the lower part of the head from the back.
- A full-thickness wound was inflicted on the rats in an area behind the head from below, taking into account the integrity of the skin muscles, by sterilizing the place using forceps and cotton, Then use sterile medical scissors to cut a skin biopsy with a diameter of approximately 5 mm for animal groups (infected control group) - (200 + BABA infection) - (100 BABA + infection) - (infected + exogenous BABA).
- Placing 200 µl of the original suspension (2 * 106) per milliliter using the micropipette as shown in Figure (2,2). The wounds of four animal groups were infected with bacteria, which included the (infected control group) - (BABA + infection 200) - (injury + BABA 100) - (injury + exogenous BABA) , while the wounds of the group (the control group) and (BABA 100 group) and (BABA 200 group) were not contaminated with bacteria.

Feeding, movement, and wound healing were observed. After five weeks of injection, the rats were dissected and the necessary hematological, immunological and biochemical tests were performed.

4- Killing animals and dissection

After the end of the experiment period, the weight of the rats was measured, then they were anaesthetized with chloroform and sedated, and blood samples were taken from the posterior vena cava according to the method (Perret-Gentil, 2010), a portion of the drawn blood was placed in a test tube containing an anticoagulant substance EDTA, while the remaining blood was used to obtain blood serum by separating it in a refrigerated centrifuge at a speed of 3500 r/min, then keeping the serum at a temperature of less than 20°C until the necessary tests were performed.

Use a Complete blood count device C.B.C for this purpose, according to the manufacturer's (Cell-Dyn Emerald) instructions

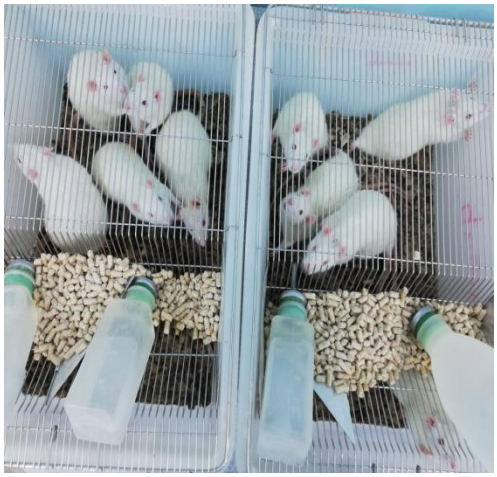
Blood tests include:

1- Total white blood cells (WBCs)/ml (lymphocytes (LYM) count and their percentage/ml, granulocytes (GRAN) and percentage/ml, as well as MID monocytes) and their percentage/ml (3).

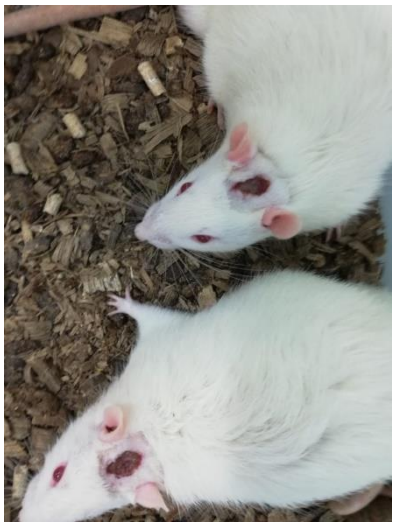
2- Total number of RBCs (red blood cells)/ml, including the following tests:

A. Measurement of hemoglobin concentration (HGB = HB g/dL)
B. Measurement Packed cell volume (PCV)
C. Measurement Mean cellular volume MCV (fL) and its value extracted from the following equation: (5).
D. MCV(fL) = (PCV X 10) /RBC
E. Mean cellular hemoglobin (MCH) pg is measured, and its value is extracted from the following equation: (Hillman & Ault, 2002) (5).
F. MCH (pg/cell) = (Hb X 10) /RBCs
G. Measurement Mean Hemoglobin concentration in RBCs (MCHC g/dL) was measured, and its value was extracted from the following equation: (Hillman & Ault, 2002) (5).
H. MCHC (g/dL) = (Hb X 100) / PCV
I. Red cell distribution width RWD, in which the distribution ratio of red blood cells / RDW ml is measured.

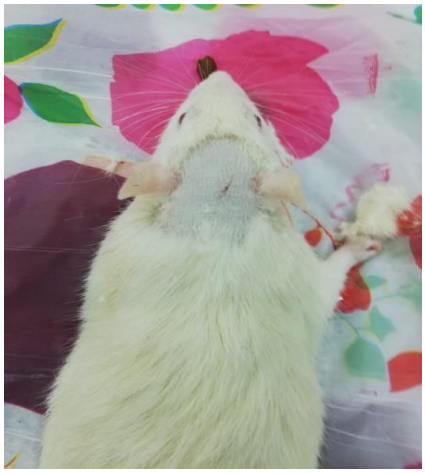
3- Platelets Blood platelets (PLT (10^3/ 3-L) µ it includes Mean platelets volume (FL) ((Mean platelets volume MPV).



1- Before bacterial infected and use amino acid



2- During infected bacterial infected and use amino acid



3- After infected with bacteria and use amino acid

4- Killing animals and dissection

5- Analysis of test results

Complete Randomized Design (CRD) was used, and the results were statistically analyzed using one-way ANOVA. Using the statistical program SPSS version 22 and calculating the value of the arithmetic mean and standard deviation Std. Deviation, and extracting the LSD value from the multiple comparisons table at the significance level of 0.05. (Duncan *et al.*, 1977).

Results

Red blood cells (RBCs)

The statistical analysis results (P≤0.05) are shown in (Fig. 1) showed significant varies in the number of red blood cells, as the two groups (B)IP/100mg) BABA) and (G) (*S. aureus* +S (5mg) recorded a significant increase of (4.234 ± 1.057) and 4.776 ± 0.802) respectively compared to the control group (A and a significant decrease of totals C, D, E, and F amounted to (1.538 ± 0.2077) and (1.722 ± 0.382) and (1.576 ± 0.379 and 2.026 ± 0.344)) While group G recorded a significant decrease in the mean MCV cell volume (53.72 ± 1.359) and the average MCH cell hemoglobin 17.76 ± 0.602)), on the other hand, the C group recorded a significant increase (91.26 ± 9.43) in the mean MCV cell volume and a decrease in the mean The group E (30.2±6.34) and F (30.12±7.23) exhibited a substantial rise in the mean of MCH cell hemoglobin compared to the control group (A).

As for the concentration of hemoglobin Hb, different significant differences were recorded, as the F group recorded a significant increase (17.7±1) compared to the control group (A).

However, for platelets (PLTS), Results C(285.6±36.1), D(279.8±24.3), E(297.6±89.2), F(307.6±49.4), G(368±57.4), showed a significant decrease, while group B(397.2±50.1) showed no significant differences compared to the control group (A), and no significant differences were recorded in the volume of HCT cells, as well as the distribution of red blood cells RDW compared to the control group (A).

Effect of BABA on White Blood Cells:

The results of the statistical analysis (P≤0.05) showed a significant difference in the number and types of white blood cells, as there were significant differences in the total number of white blood cells (WBC) for the six groups treated with BABA, whereas group F recorded a significant increase (16.4±3.18) in the percentage of total white blood cells, while group (E) and (C) recorded a significant decrease of (10.4±2.53) and (8.54±1.75), respectively compared to the control group (A).

Also, In comparison to the control group, all groups showed a significant difference in the totals which are recorded (49.78+1.893) D, (32.35+3.22) E, (C28.98+2.32) F, (49.31+2.55) G, (significantly increased in the percentage of LYM lymphocytes compared to the control group (A)), Also, the groups D (53.68 + 7.23), E (46.76 + 4.09), F (62.8 + 6.91), and G (49.1 + 5.85) sh.owed a significant increase for GRA granulosa cells compared to the control group A, and the groups recorded (16.7 + 1.657) D (13.64 +. 1.579), F (14.88 + 1.514), G significantly increased the percentage of MID (MON) cells compared to the control group, while the groups (12.92 + 1.675) B (13.08 + 0.766) (C) and no significant difference was recorded compared to the control group A.

Discussion

The current study's findings revealed that there is no negative effect of BABAon blood components RBC, WBC, or PLT, while the percentage of WBCs decreased in groups treated with amino acid and infected with bacteria in groups treated with a concentration of 100 mg/kg and the decrease increased with increasing concentration to 200 mg/kg this indicates the role of histidine in resistance to toxins secreted by *S. aureus* bacteria. This is consistent with what was mentioned by (10,1) that there were no negative effects of BABA on blood variables RBC, WBC, and PLT, and the positive role of BABA in maintaining the normal ratios of blood components.

Schedule (1)

Explain the level of blood cells in the serum of rat males that treated with BABA and infected with bacteria for duration of 30 days

Parameters / Groups	WBC Mean+SD	RBC Mean+SD	PLT Mean+SD
control	BC 12.61±3.02	B 3.832±0.676	A 391.2±69.3
(IP /100mg) BABA	ABC 13.26±3.17	AB 4.234±1.057	A 397.2±50.1
(IP 200/mg) BABA	D 8.54±1.75	C 1.538±0.2077	C 285.6±36.1
S.aureus+(IP /100mg) BABA	AB 13.78±1.698	C 1.722±0.382	C 279.8±24.3
S.aureus+(IP 200/mg) BABA	CD 10.4±2.53	C 1.576±0.379	BC 297.6±89.2
S.aureus	A 16.4±3.18	C 2.026±0.344	BC 307.6±49.4
S.aureus+S (5mg)	AB 14.614±0.573	A 4.776±0.802	AB 368±57.4
LSD	**3.16931**	**0.79972**	**74.0912**
P-Value	**0.001**	**0.0002**	**0.005**

Schedule (2)

Explain the level of (MCV, MCH, HGB) in the serum of rat males that treated with BABA and infected with bacteria for duration of 30 days

Parameters / Groups	MCV Mean+SD	MCH Mean+SD	HGB Mean+SD
control	A 89.64±14.58	A 28.5±4.03	D 13±1.768
(IP /100mg) BABA	B 77.74±8.94	A 26.8±4.15	BCD 14.78±2.098
(IP 200/mg) BABA	A 91.26±9.43	A 25.02±4.06	ABC 15.74±2.131
S.aureus+(IP /100mg) BABA	AB 86.96±3.12	A 28.52±4.18	AB 6.36±1.435
S.aureus+(IP 200/mg) BABA	AB 84.22±2.73	A 30.2±6.34	AB 16.06±1.165
S.aureus	AB 87.3±2.23	A 30.12±7.23	A 17.7±1.082
S.aureus+S (5mg)	C 53.72±1.359	B 17.76±0.602	CD 13.82±0.729
LSD	**9.85852**	**6.19702**	**2.02948**
P-Value	**0.0001**	**0.004**	**0.001**

Schedule(3)

Explain the level of (MPV, RDW,HCT) in the serum of rat males that treated with BABA and infected with bacteria for duration of 30 days

Parameters / Groups	HCT Mean+SD	RDW Mean+SD	MPV Mean+SD
control	A 20.8+1.444	AB 26.36+0.94	A 8.3+0.863
(IP /100mg) BABA	AB 20.52+1.064	AB 26.18+0.687	A 8.14+0.68
(IP 200/mg) BABA	AB 20+1.194	B 24.96+1.448	A 8.52+0.701
S.aureus+(IP /100mg) BABA	B 18.92+1.169	A 28.08+1.613	A 8.42+0.746
S.aureus+(IP 200/mg) BABA	AB 19.24+1.932	A 28+2.131	A 8.58+1.062
S.aureus	B 18.88+1.521	A 27.96+3.92	A 8.66+0.844
S.aureus+S (5mg)	AB 20.46+1.282	AB 27.2+2.59	A 8+0.742
LSD	1.811988	2.797001	**1.052281**
P-Value	**0.0065**	**0.0021**	**0.848**

Schedule (4)

Explain the level of white blood cells in the serum of rat males that treated with BABA and infected with bacteria for duration of 30 days

Parameters / Groups	GRA Mean+SD	MON Mean+SD	LYM Mean+SD
control	D 38.12+2.5	C 12.896+1.155	CD 25.78+2.56
(IP /100mg) BABA	D 38+4.38	C 13.08+0.766	D 25.24+3.4
(IP 200/mg) BABA	D 35.72+3.59	C 12.92+1.675	D 25.38+1.85
S.aureus+(IP /100mg) BABA	B 53.68+7.23	BC 13.64+1.579	B 32.35+3.22
S.aureus+(IP 200/mg) BABA	C 46.76+4.09	C 12.16+0.856	B C28.98+2.32
S.aureus	A 62.8+6.91	BC 14.88+1.514	A 49.31+2.55
S.aureus+S (5mg)	BC 49.1+5.85	A 16.7+1.657	A 49.78+1.893
LSD	6.735398	1.764611	3.369691
P-Value	**0.0002**	**0.0021**	**0.0005**

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تنظيم المقاييس الدموية باستخدام الحامض الاميني بيتا امينو بيوتريك في الجرذان المصابة ببكتريا العنقوديات الذهبية

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الخلاصة

خلفية عن الموضوع: العديد من الدراسات توجهت نحو استخدام مختلف المواد الكيمائية كبديل للمضادات الحيوية والتي تقتل البكتريا او التي تؤدي الى تحفيز مناعة الجسم ضد الاصابات البكتيرية ومثال على ذلك الاحماض الامينية والتي تتضمن الحامض الاميني الغير بروتيني وخاصة حامض الامينوبيوترك نوع بيتا والذي اثبت خلال مقدرته على زيادة المقاومة المناعية لذكور الجرذان ضد العنقودية الذهبية بالاضافة الى زيادة اعداد كريات الدم البيض والخلايا اللمفية وكذلك كان له تأثيرايجابي في تحسين الجهاز المناعي المكمل

الهدف من الدراسة: هو التعرف على تأثير الحامض الاميني بيتاامينوبيوترك في الاستجابة المناعية للحيوانات المصابة تجريبيا ببكتريا العنقوديات الذهبية وكذلك دراسة المتغيرات الدموية للحيوانات المعاملة بالحامض الاميني بيتا امينوبيوترك وتشمل (WBC ,RBC , Hb , PCV)

المواد و طرق العمل: استخدمت في هذه التجربة وسط المانتول اكار ووسط اغار الدم ووسط المانكوكي لتنمية بكتريا العنقودية الذهبية وكذلك استخدم الحامض الاميني بتراكيز مختلفة حيث حضر المحلول الاول للحامض الاميني بتركيز (20ملغم / مل) وحضر محلول الحامض الاميني الاخر بتركيز (40 ملغم / مل) ومن ثم حضرت الجرعات المطلوبة لكل حيوان حسب وزن كل حيوان

النتائج: التي تم الحصول عليها من خلال هذه التجربة لايوجد تأثير للحامض الأميني البيوتريك من نوع بيتا في المجاميع المصابة بالبكتريا والمعاملة بالحامض الاميني على مستوى كريات الدم الحمر وكريات الدم البيض والخلايا اللمفاوية والصفيحات الدموية ومتوسط حجم كريات الدم الحمراء(RBC -WBC –MCV-PLT) في حين ارتفعت النسبة في مستوى الخلايا اللمفاوية والخلايا الحبيبية (GRA –LYM) .

الكلمات المفتاحية: الحامض الاميني بيوتابيوترك اسد / كريات الدم الحمر / كريات الدم البيض / هيموغلوبين الدم / حجم الخلايا المضغوطة / الصفيحات الدموية / متوسط هيموغلوبين الدم / خلايا لمفية / خلايا حبيبية / متوسط حجم الكرية