IMMUNOLOGICAL AND ANTIOXIDANTS VARIATION AFFECTED BY BETA-AMINO BUTYRIC ACID IN SPRAGUE DAWLEY MALES RATS INFECTED WITH METHICILLIN RESISTANCE STAPHYLOCOCCUS AUREUS (MRSA)

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ABSTRACT

β-amino butyric acid (BABA) is one of the rare free compounds found in nature. It has been known for its ability to stimulate plant resistance against various pathogens such as viruses, bacteria, fungi and worms. This study aimed to identify the effect of BABA on some hematological, biochemical and immunological parameters of male Sprague Dawley rats experimentally infected with S. aureus bacteria. Methicillin resistance S. aureus isolated from different clinical patients, identified and their resistance against antibiotics was measured, then an isolate was chosen for later experiments and infection. Our study included (35) animals divided into seven groups (five rats each), four groups were injected with two concentrations of BABA (100 or 200mg/kg), one of the other two groups was injected with bacteria and the other considered as control. At the end of the 5 weeks, experiments conducted measuring interleukins(IL1α and β, IL6), antioxidants (SOD and catalase), Immunoglobulins (IgG, IgM), Complement (C3 and C4). Results showed a significant increase in the level of IL1α, β and IL6 compared to the control group. the level of complement proteins C3 and C4, Groups varied in their response. In addition, groups recorded a significant decrease in C4 level. IgM of two groups injected with BABA recorded a significant increase. Measuring IgG, groups had a significant difference compared to the control group. Moreover, ALT, and AST, the results showed a significant difference in the group in measuring ALT enzyme level compared to the control group. There was also a significant increase in the level of AST enzyme in most of groups. However, most of groups showed a significant decrease in SOD compared to the control group. Hence, Catalase recorded a different results from significant decrease to a non-significantly differences.

Keywords: BABA, MRSA, Rats, Antioxidants, Immunology

I. INTRODUCTION

The sudden emergence of bacterial resistance to antibiotics around the world determined many of antibiotics effectiveness that saved millions of people previously and caused a paradigm shift in medicine. Since bacteria are one of the most important pathogens, they can be considered the main cause of important diseases spread globally that threaten human life. Mostly due to their ability to produce many virulence factors that enable them to penetrate body tissues and generate infections and resistance against antibiotics such as Methicillin resistant staphylococcus aureus (MRSA) leading to be a source of threat to patients, especially patients with inflammation of wounds and burns, as it can enter the bloodstream causing sepsisemia, and the situation increases equally in Immunodeficiency patients (Jalil et al., 2017). It characterized by their ability to secrete many extracellular substances that have a role in the processes of invasion of the body and reproduction within tissues, including lipase nuclease, catalase, protease, β-lactamase, etc. (Brooks et al., 2013). Researchers developed chemicals other than antibiotics, which in turn stimulate the body's resistance to bacterial infection Such as organic acids (Panda et al., 2009). It includes many non-proteinogenic amino acids, an example of which is Beta-Amino Butyric acid (BABA), where studies have shown that it increases the stimulation of immunity in plants treated with, as it increases plants resistance against fungi, viruses, bacteria and nematodes (Cohen, 2002). In addition, recent
studies have demonstrated that histidine may plays a role in increasing IgG production in rats treated with BABA amino acid (Salih, 2019). Another recent study showed an increase in the level of interleukins (IL) and IL-10 in male rats treated with BABA (Al-Esawy, 2020).

II. MATERIAL AND METHODS

Preparation of reagents and solutions: β-aminobutyric acid was provided from supplier (Shanghai Adamas Reagent/China). Two concentration of BABA had used (20 mg/ml & 40 mg/ml).

Samples collection: Clinical samples were collected (wounds, otitis media, vaginal swabs, burns, abscess, and urine) from patients at consulting clinics in Ramadi Teaching Hospital, ranging (6-60 years) old from both sexes in the period from October to November 2020. Samples then cultured on blood agar plates, and incubated at 37 °C for 24 hours. After the end of the incubation period bacterial microscopic, biochemical and culture tests performed. Diagnosis of S. aureus confirmed using Vitek® compact device (BioMerieux). Bacterial isolates preserved by inoculating tubes containing 20 ml of glycerol to 80 ml of the brain-heart infusion broth 37 °C for 24 hours, and transferred to the 4 °C until use, or left at room temperature for 30 minutes, then kept at -20 C until use.

Bacterial resistance: Disc diffusion based on Kirby Bauer method was used. Plates were prepared using Muller-Hinton agar, inoculated with (810 x 1.5) CFU/ml of the bacterial suspension. Antibiotics (Amoxicillin, Cefebime, Cefoxitin, Methicillin) discs then the plates incubated at 37° C for 16-18hours. (Rostami et al., 2013).

Animals: In this experiment, (35) male Sprague Dawley rats were used, aged from (10-12) weeks, with weights (300 ± 50 g). A standard diet used to feed animals. Animals divided into seven groups (five animals each). Groups were: group A (control) left without infection with bacteria and without using BABA. Group B (BABAIP/100 mg): Animals injected only with the first concentration of amino acid solution (20 ml / mg) for five weeks. Group C (BABA IP/200mg): Animals dosed only with the second concentration of amino acid solution (40 ml/ mg) for five weeks. Group D (IP/100mg+S. aureus): Animals injected with the first concentration of amino acid solution of 20 mg/ml for five weeks and infected with the bacterial isolate S. aureus. Group E (S. aureus+IP/200mg): Animals injected with a second concentration of amino acid solution 40 mg/ml for five weeks and infected with the bacterial isolate S. aureus. Group F (S. aureus): Animals infected with S. aureus. Group G (S. aureus+5mg/kg Local solution): Animals infected with bacteria and BABA was applied (5 mg /ml) externally on the injury area for five weeks.

Rats infected with S. aureus, after the second week of intraperitoneal injection with BABA. Animals were (12-16) weeks old, weighted between (250 – 400 gm), the process of infecting the animals included the following steps: Bacterial isolate activated on saline mannitol medium then incubated at 37 °C for 24 hours. Each dilution on the nutrient agar medium, rats anesthetized with chloroform, and thendorsal part of head shaved then awound was made. Moreover, sterilizing wound using forceps and cotton, then using sterile medical scissors to cut a biopsy of skin with a diameter of approximately 5 mm for animal groups.

After the end of the experiment period, Rats weighted, then they were kindly anesthetized with chloroform, and blood samples were taken from the posterior vena cava according to the (Perret-Gentil method2010), and necessary hematological, immunological and biochemical tests were performed.

Liver function tests: Determination of the activity of the ALP, ALT and LDHL in the blood serum according to the kit (Alp 2L:ACN8683, ALT: ACN 684& LDHL:CAN 672 respectively) using CObas c311 (Roche diagnostics, Germany).

Immunological tests: The levels of IL-1α, IL-1β and IL-6 estimated in serum of rats using enzyme-linked immunosorbent assay (ELISA) according to the instructions in PicoKin kit (IL-6: Cat. No. EK0412: IL-1 alpha: Cat. No. EK0390 and IL-1 beta: Cat. No. EK0393) supplied by Boster Biological Technology, USA, However, Complement C3 and C4 levels in serum measured using Kit (cat. No. ACN20320) using CObas c311 (Roche diagnostics, Germany).

Statistical analysis of test results: Results were statistically analyzed using One way ANOVA analysis using the statistical program SPSS (ver. 22) and the value of the arithmetic mean and standard deviation was calculated.
Deviation and LSD value from multiple comparisons table at significance level of 0.05. Lowercase letters indicates significant differences (0.05≥P) within the experimental period.

III. RESULTS AND DISCUSSION

Identification of Staphylococcus aureus

The culture and morphological characteristics, of S. aureus showed growth on blood agar mediums as colonies surrounded by a transparent halo of type β-hemolysis, however it showed the growth on mannitol salt agar as slightly raised soft yellow colonies. This is consistent with what stated by Non-motile on a semi-solid agar medium microscopically microscopic cells appeared as Gram-positive clusters (Ariyantyet al., 2011; Watkins et al., 2012; Dhakal, 2012).

Also, The biochemical tests cleared a positive result in the tests for catalase and coagulase, methyl red, citrate utilization, urease test, fermentation of mannitol, lactose, maltose, glucose, sucrose, was able to reduce nitrate NO3 to nitrite NO2, produce acetone and hydrolyze it for gelatin sugars. On the other hand, it was negative in Indole test, have no ability to produce agreed with (Kateete et al., 2010; Todar, 2011), these results confirmed by VITEK2®.

The effect of BABA on the level of interleukins

The results shown in Figure (1) showed that the total G, F, E, D) (D) (76.14 + 3.81) and E (72.71 + 3.17), F (104.43 + 6.18) and G (88.71 + 4.99). It recorded a significant increase (P<0.05) in the level of (IL 1α) in the serum compared to the control group and the two groups (B,C) that were not exposed to infection with S. aureus. The pathogen, even in the absence of antibodies, is the primary source of interleukin generation (Abraham & Malaviya, 1997). The increase in interleukin in the infected groups is a result of infection was previously mentioned by (Tanget al., 2018), as the high level of interleukin while infected with S. aureus. However, mast cells are the active cells that are responsible for the healing process that activated at the same time with infection with the pathogen, (Nikita et al., 2013).

The decrease in the level of interleukin compared to the group treated with S.aureus bacteria can be explained by the presence of the BABA, which reduced the effect of the bacteria on the animal leading to a decrease in the level of interleukin near the control group result, this is consistent with what was mentioned (Al-Issawi, 2020).

Figure (1) shows the level of IL-1α (Blue) and IL-1β (Orange) in the serum of male rats treated with BABA and infected with S.aureus for a day.

The effect of BABA on the level of IL-6

The results in Figure (2) showed a significant increase (P<0.05) in the level of IL6) in the serum of rats for group F (307.14 + 10.77) G (310.57 + 4.44), as well as the two groups recorded 206.43 + 7.8 (E) and (252.14 + 6.03) D significant difference compared to the control group (A). The group C (176.57 + 9.24) recorded a significant increase compared to groups D, E (206.43 + 7.8) (E) (252.14 + 6.03, D). This may due to the ability of BABA to reduce bacterial infection, which led to a decrease in interleukin levels. However, the concentration of interleukin reached near the control group, was found that the higher concentration was more effective. Results consistent with what was mentioned by (Roach et al., 2008), which stated that GABA inhibits the release of interleukin IL 6
with a decrease in the level of interleukin IL 6 due to the inhibition in translation Gene translation of interleukin IL-6 by GABA.

Figure (2) shows the level of IL-6 in the serum of male rats treated with BABA and infected with S.aureus for 30 days.

**Effect of BABA on the level of complement proteins C3 and C4**

The results of the statistical analysis (P<0.05) as shown in Figure (3) cleared that the two groups C (0.504 ± 0.1436) and D (0.558 ± 0.1814) had significant differences in the level of C3 complement protein with a significant decrease in the level of C3 For group B (0.252 ± 0.0766), while aggregates E (0.708 ± 0.0349), F (0.75 ± 0.1068), G (0.714 ± 0.0666), it may be noted that there are no significant differences between C3 concentrations in the group treated with bacteria compared to the control group except in the concentration. The first, which is BABA 100 led to a decrease in the value of C3 in the treatments BABA 100, BABA 200 and S.aureus + (IP/100 mg). This can be explained by the lack of effective dose of BABA on the humoral immunity. No significant differences were found in the C3 level, lower values indicate C3 activation (Hussain et al., 2008).

Figure (3) shows the level of C3 in the serum of rats treated with BABA and infected with S.aureus for 30 days.

As per for C4 complement protein, statistical analysis results showed (P<0.05) as shown in Figure (4). The group F(0.098±0.0327) showed a significant difference in the level of C4 compared to the control group, while the groups B(0.054±0.0114), C(0.06±0.01225), D(0.062±0.00447), E(0.058±0.00837), G (0.052 ± 0.01789) recorded a significant decrease in the level of C4 complement protein. These results was not consistent with (Abbas & Lichman, 2011), that the activation of natural immunity, which in turn leads to the activation of cells to produce antibodies and activate the classical pathway.

Besides, This increase may be due to bacterial infection, and it agrees with what was mentioned (Al-Issawi, 2020) who impute the cause due to bacterial infection, which indicates that BABA in all concentrations (BABA 100 and BABA 200) lead to a decrease the level of complement protein C3 and C4,
Figure (4) shows the level of C4 in the serum of male rats treated with BABA and infected with S.aureus for 30 days.

**Effect on IgM, IgG**

The results of the statistical analysis of the level of IgM antibody as shown in Figure (5) showed that the two groups B(0.446±0.0709), C(0.466±0.0611) recorded a significant increase in the level of IgM compared to the control group, while the other groups did not record any difference.

Significantly, this is consistent with what was mentioned (Al-Issawi, 2020) that BABA increases the production of IgM, the high level of IgM in the two groups (BABA 100), (BABA 200) can be explained as a result of the effect of the amino acid BABA on the cells producing IgM antibodies as their levels are normal or close to normal. It also agrees with what was mentioned by (Tang & Chen, 2016) with a GABA analogue that increases the production of IgM antibody in chickens under heat stress.

Figure (5) shows the level of IgM in the serum of male rats treated with BABA and infected with S.aureus for 30 days.

Equally, for the level of IgG, the results of the statistical analysis as in Figure (6) showed that there was a significant increase in the concentration of IgG in the groups compared to the control group. The highest mean was reached in the group E (4.612 ± 0.753), which contains the highest concentration of amino acid compared with the control group. The results of the current study showed an increase in the level of IgG in rats injected with BABA. The reason for the elevated level of IgG for the two BABA treated groups may be due to the ability of amino acid to generate a humoral immune response (Zhang, 2012). However, the results of this study confirmed the findings of (Jin, 2013), that the activation of cytokine secretion is a result of the amino acid to allow the cell to communicate with other cells, and this is consistent with the results of (Saleh, 2019) that BABA can elevate IgG antibodies.
Figure (6) shows the level of IgG in the serum of male rats treated with BABA and infected with S.aureus for 30 days.

The activity of the aminotransferase enzyme ALT

The statistical analysis results Figure (7) showed a significant increase (P≤0.5) in ALT level of the group D 72.4±8.56, while there was no significant difference for the other groups compared to the control group. The reason may be due to the ability of the amino acid to reduce the enzyme with (BABA200), while it did not contribute to reducing the concentration of the enzyme at the lowest concentration (BABA100), and this is agreed with (Saleh, 2019; Al Kubaisi, 2020).

Figure (7) shows the level of ALT in the serum of male rats treated with BABA and infected with S.aureus for 30 days.

The activity of the enzyme AST

Figure (8) shows that there was a significant difference in the activity of AST enzyme increased in the groups B(135.2±14.1), C(125.6±13.54), D(133.2±17.02), E(127.6±15.45). While the activity of AST enzyme decreased within group G (105.2 ± 11.45) compared to the control group. However, group F did not show any significant difference (110.4 ± 8.88), and this explains the lack of effect of bacteria on AST enzyme and this differs with what was mentioned by (Saleh, 2019). That amino acid has a positive role on the enzyme AST on regulating liver functions and regulating the secretion of enzymes and hormones. As well as inconsistent with previous studies related to the effect of GABA, which works to improve liver function by reducing fats (Jin et al., 2013; Kimura et al., 2002).
Figure (8) shows the AST level in the serum of male rats treated with BABA and infected with S.aureus for 30 days.

**Effect of BABA on the activity of SOD and Catalase**

Figure (9) showing a significant decrease among groups compared to the control group, the two groups B(6.659±0.762) and C(6.058±0.587) treated with amino acid only showed a significant difference.

Figure (9) shows the level of SOD in the serum of male rats treated with BABA and infected with S. aureus.

Moreover, Results Figure (10) showed that group D (291.31 ± 9.3) E (310.59 ± 7.63) F (205.48 ± 7.53) and G (315.03 ± 6.63) had a significant difference. The level of catalase decreased in the two groups F(205.48±7.53)D(291.31+9.3) compared to the control group A.

Figure (10) shows the level of catalase in the serum of male rats treated with BABA and infected with S. aureus.

The results of SOD and Catalase showed a flawless decrease in the level of SOD in the uninfected groups injected with BABA (C,B), this indicates an increase in oxidative stress as a result of the animals being exposed to BABA. The non-BABA treated group infected with bacteria showed a decrease in SOD and catalase level. This
may explained by the effect of S. aureus infection on the rat corresponds with (Tang et al., 2018). Whereas was mentioned that S. aureus can cause severe inflammation and high oxidative stress on the liver and this is evident from the high inflammatory factors and low Antioxidant enzymes and oxidative metabolism increase. In place of these two groups infected with bacteria (D, E) and treated with BABA, the results showed a clear increase in the enzyme, SOD catalase, which indicates that BABA has a role in preventing oxidative stress in the case of bacterial infection and where the level of SOD, catalase increases with increasing BABA concentration. This indicates that there is a stimulation of an action in the animal body in the presence of virulence factors of S. aureus.

IV. CONCLUSIONS:

The non-proteinogenic amino acid BABA has a positive role supporting resistance against S. aureus, stimulating the innate immunity in the body by stimulating B cells to produce IgG and IgM, reducing oxidative stress in the case of infection with S. aureus. BABA with a dose of 200 mg/kg had positive effects in most of the conducted experiments.

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