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The Impact of the Beta-Amino Butyric Acid on Serum Levels of Interleukin 22; Interleukin 23; Protein phosphatase 2A in Sprague Dawley male Rats.

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ABSTRACT

This study aimed to investigate the effect of β-aminobutyric acid (BABA) on IL-22, IL-23, and PP2A levels in a rat model, BABA is a non-protein amino acid, its role in modulating a defensive response on a portion of immune indicators that are closely related to gut health. Interleukins are considered an important key by regulating interactions between innate and acquired immunity, where IL22 and IL23 significantly contribute due to their role in maintaining the integrity and intestinal barrier health leading to inflammatory processes regulating. Twenty white Sprague Dawley male rats were used, divided into four groups, orally administered twice weekly with BABA at a dose of 0, 100 ,150 and 200 mg/kg body weight. The serum was carefully collected, and concentrations of IL-22, IL-23, and PP2A levels estimated using ELISA. Analyzed statistically using one-way ANOVA at $P \leq 0.05$ significance. The results showed no significant difference in IL22 levels between the treatments and the control group, indicating that BABA administration didn't affect the immune pathway. Conversely, strong significant differences were found in IL23 levels, as they decreased at concentrations of 100 and 200, suggesting a possible role for this cytokine in modulating immunity related to exposure to the acid. Results correspondingly indicated that PP2A levels had no significant differences, which implies that PP2A remained stable and wasn't affected by the BABA during the experiment period. In conclusion, BABA may exert a partial regulatory effect on immune responses, particularly through modulation of IL-23, without significantly affecting IL-22 or PP2A pathways.

Keywords: Interleukin 22; Interleukin 23; PP2A; Amino Acid (BABA); Immunity

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1.Introduction:

Beta-aminobutyric acid (BABA) is classified as a non-protein amino compound and is known for its role in enhancing plant resistance against a wide range of biotic and abiotic stresses [1]. BABA naturally occurs at low levels in plant tissues, and its concentrations can increase five to tenfold under stress conditions [2][3]. Recent studies suggest that β-aminobutyric acid (BABA) may exert immunomodulatory effects through regulation of inflammatory pathways, and it also stimulates B cells to produce antibodies of the IgG and IgM types, leading to the activation of the complement system and supporting the functional coordination between innate and adaptive immunity[4] . The immune system is considered an integrated system of cells, molecules, and biological mechanisms that work to protect the body from infection and harm. In areas and tissues that are directly exposed to pathogens, such as bacteria, viruses, fungi, and parasites, the immune system provides active and continuous defense. This role is particularly evident on surfaces that interact with the external environment, such as the skin, respiratory tracts, and the digestive and reproductive systems. In addition, the immune system participates in mitigating the effects of cancer cells and toxins within the body [5][6]. IL-22 belongs to the IL-10 family and is one of the cytokines with a helical structure. It plays an important role in maintaining the integrity of the intestinal epithelium and the function of the mucosal barrier. IL-22 is expressed in response to microbial signals, being stimulated by cytokines secreted by myeloid cells, and is also produced by innate lymphoid cells (ILCs) and $CD4^+$ T cells, including Th17 and Th22 cells [7][8][9]. L-23 Interleukin-23 (IL-23) consists of two distinct subunits: p19, which is responsible for the functional specificity and biological activity of the cytokine[10], and p40, a subunit shared with interleukin-12 (IL-12) [11]. IL-23 is primarily secreted by antigen-presenting cells (APCs), particularly macrophages and dendritic cells, in response to microbial stimuli and various immune signals .Since its discovery, IL-23 has been identified as a key regulator of pathological inflammation, largely attributed to its role in promoting the differentiation, expansion, and activation of Th17 helper T cells[12][13][14].
(PP2A) Protein phosphatase 2A (PP2A) is a major serine/threonine phosphatase that controls the dephosphorylation of key proteins such as Akt, p53, c-Myc, and β-catenin, making it an essential component in regulating cell proliferation, signaling, and programmed cell death. PP2A is

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characterized by a multi-subunit structure that includes the catalytic subunit, the structural subunit (A/PR65), and the regulatory subunit, which is the most diverse among the subunits[15][16].This study aims to evaluate the effect of (BABA) acid on the levels of some cytokines and immune markers IL_22, IL_23, and PP2A in a laboratory rat model.

2.Materials and Methods

Twenty white Sprague Dawley male rats were used in this study, obtained from the Faculty of Veterinary Medicine – University of Tikrit. Their weights ranged between 130–160 grams, and their ages were between 6–8 weeks. The animals were housed in plastic cages covered with a metal mesh that allowed ventilation, inside the animal house designated for the Faculty of Education for Women, University of Anbar, Iraq. under controlled environmental conditions that included moderate temperature, good ventilation, and a 12-hour light/12-hour dark cycle . The animals were provided with standard feed and drinking water ad libitum and were left for 15 days to acclimate to the surrounding environmental conditions before the start of the experiment.

Experimental Design

After the acclimation period, rats were randomly divided into four equal groups (5 per group) as follows:

• Group 1 (Control): administered a normal saline solution.

• Group 2: administered BABA at a dose of 100 mg/kg body weight.

• Group 3: administered BABA at a dose of 150 mg/kg body weight.

• Group 4: administered BABA at a dose of 200 mg/kg body weight.

BABA was administered orally twice weekly for 30 days under controlled experimental conditions

Ethical Approval:

The experimental protocols and the use of animals were approved by the Scientific Research Ethics Committee at the University of Anbar according to approval letter No. (94) dated 29/09/2025.

Blood Sample Collection:

After the treatment period (30 days), the animals were anesthetized using chloroform, and then blood samples were drawn directly from the heart using sterile syringes. The blood samples were transferred to anticoagulant tubes and then used to perform immunological tests .The serum was

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separated from the blood samples by leaving them at room temperature for 20–30 minutes to allow clotting, then centrifuged at a speed of 3000 rpm for 10 minutes. The resulting serum was carefully collected, placed in clean microtubes, and stored at −20 °C until the tests were conducted.

ELISA Test:

The concentrations of Interleukin-22 (IL-22), Interleukin-23 (IL-23), and PP2A in serum were estimated using the Sandwich ELISA technique with commercial ELISA kits (Sunlong Biotech /China), following the manufacturer's instructions:

Standard solutions and samples were added to the ELISA plate, and incubation and washing steps were carried out according to the established protocol. After that, the HRP-conjugated detection antibody was added, and the color reaction was performed using the TMB substrate. The reaction was stopped by adding a stop solution, and the optical density was measured at a wavelength of 450 nm using an ELISA reader.

Concentrations of IL-22 and IL-23 in the samples were calculated based on the standard curve.

Statistical Analysis

The statistical analysis was performed using the Statistical Package for the Social Sciences (SPSS), version 25 (2019). Differences among the experimental groups were analyzed using one-way analysis of variance (ANOVA). The Least Significant Difference (LSD) test was used for multiple comparisons between group means. Differences were considered statistically significant at $P \leq 0.05$[17].

3. Results

No significant differences ($P > 0.05$) were observed in IL-22 levels between treatment and control group, under experimental circumstances as shown in figure (1).

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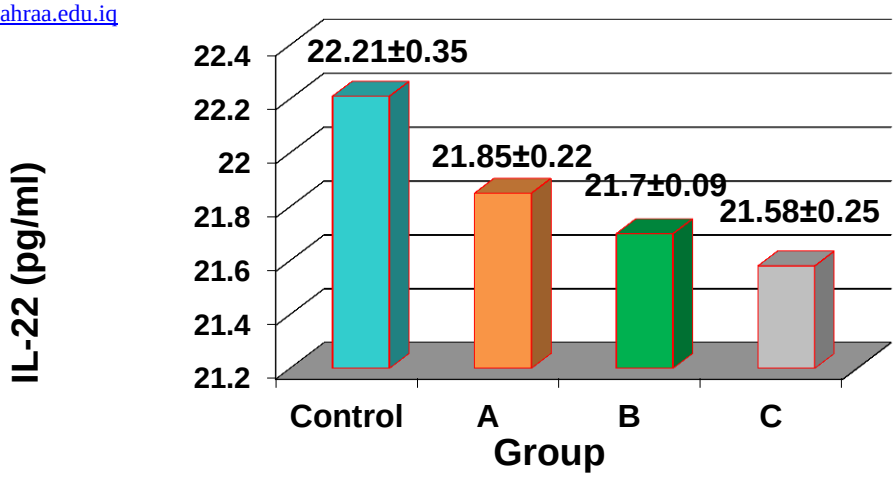

Figure 1. Effect of BABA on IL-22 levels

Moreover, figure (2) shows significant differences ($P < 0.01$) between treatments and control group, reflecting the effect of BABA on IL-23 level among different concentrations treatments.

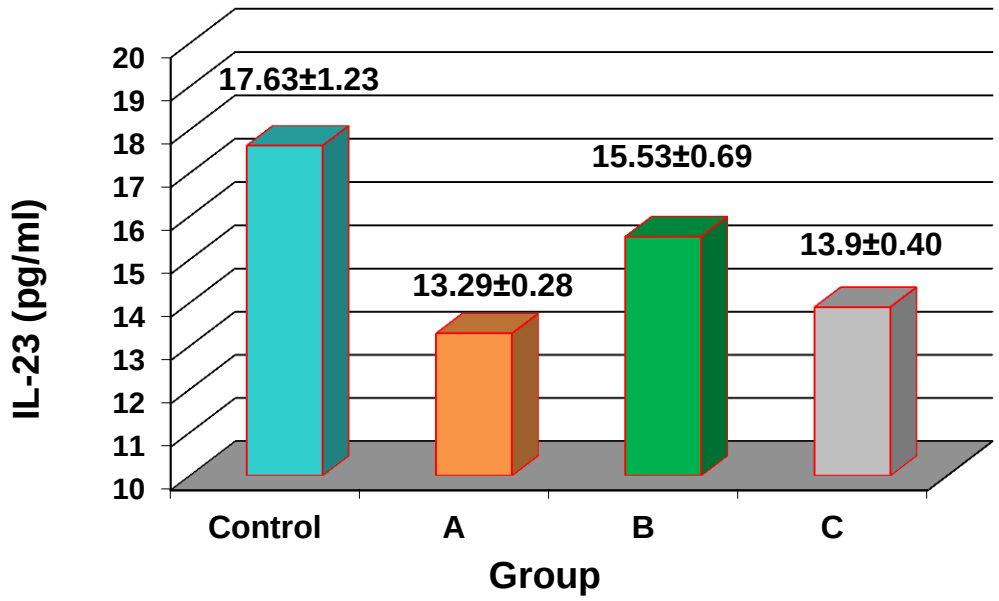

Figure 2.Effect of BABA on IL-23

Furthermore, Figure (3) illustrates no statistical significance between the treated group and the control group, indicating the absence of a significant effect of BABA on IL-23 levels in treatments under the experimental conditions.

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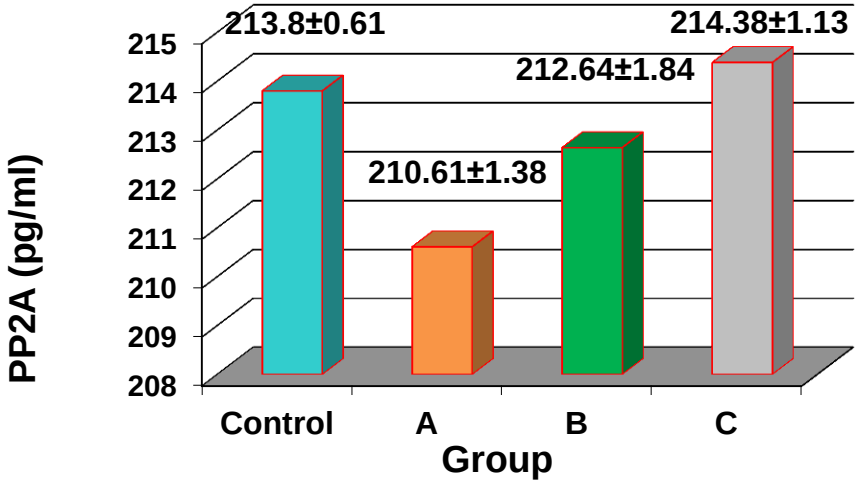

Figure 3. Effect of BABA on PP2A

4.Discussion

The current results indicated that there was no statistically significant difference in the level of interleukin-22 (IL-22) among the experimental groups. This can be explained by the fact that IL-22 is not a cytokine that responds to all biological stimuli, as its secretion is generally associated with the presence of active inflammation or direct damage to the intestinal mucosal tissue. Studies have shown that the role of IL-22 is to support the integrity of the gut barrier and regulate microbial balance, without a noticeable increase or decrease in its level, especially in cases that are not accompanied by acute inflammation [18]. In this context, a deficiency or absence of IL-22 does not necessarily lead to obvious pathological changes but may be associated with subclinical effects[7].

Furthermore, the increase in IL-22 is more evident in cases of active intestinal inflammation such as ulcerative colitis, where it is associated with the degree of inflammatory activity [19]. Therefore, the absence of a significant difference in IL-22 may indicate that the amino acid BABA did not activate the IL-22 immune pathway.

The results showed statistically significant differences in the level of interleukin 23 (IL-23) between the control group and the treated groups, and a significant decrease was observed in both groups A and C compared to the control group at the studied concentrations, indicating that the acid (BABA) has a clear effect in these treatments in regulating the immune response. IL-23 is a key inflammatory cytokine that plays a fundamental role in linking innate immunity with

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adaptive immunity by promoting cell differentiation and regulating the secretion of subsequent inflammatory cytokines, and thus it is considered a sensitive marker for changes in immune status[20][21].

The decrease in IL-23 levels may be attributed to the immunomodulatory effects of BABA, which acts as a priming molecule rather than a direct anti-inflammatory agent, to modulate the gut environment and enhance the health and integrity of the intestinal barrier, in addition to their immunoregulatory properties, as it leads to reduced inflammation severity and altered cytokine levels. Previous studies have indicated that Unlike short-chain fatty acids such as butyric acid, BABA exerts its effects primarily through modulation of immune signaling pathways in animal production due to its role in improving gut health and increasing resistance against pathogenic microorganisms[22][20]. Recent research results have shown that derivatives of butyric acid, such as tributyrin, possess multiple immune effects and are used as feed additives to enhance growth and regulate immune responses. However, it is necessary to optimize the concentrations used to avoid potential toxic effects on cells, including the induction of apoptosis[23].

In this context, additional studies have indicated that an increase in the IL-23/IL-17 axis plays an important role in the exacerbation of several inflammatory and autoimmune disorders such as lupus, confirming the pivotal role of IL-23 as a key inflammatory marker [24].

The study indicated that PP2A levels remained stable with no significant differences between the experimental groups, suggesting that the PP2A enzyme did not act as an influential variable under the applied experimental conditions. This confirms the normal, stable regulation in controlling inflammatory signaling pathways, which does not change in activity unless directly stimulated or inhibited. Studies have also confirmed that PP2A plays a crucial role in suppressing inflammatory pathways and that its activity remains relatively stable unless specifically targeted by activation or inhibition mechanisms, which is consistent with the lack of observed changes in its levels in this experiment [25].

Research has shown that PP2A acts as a major inhibitor of inflammatory responses, and that enhancing its activity represents a potential therapeutic approach in chronic inflammatory conditions, which explains the amino acid [26]. This explains why the stable level of the BABA acid did not affect the molecular pathway. Accordingly, the stability of PP2A levels in the current study indicates that the amino acid used did not influence this molecular pathway and did

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not induce any modification in the inflammatory response through a PP2A-dependent mechanism.

Conclusion

The results of the study indicated that treatment with BABA acid for 30 days did not cause significant differences in the levels of IL22 and PP2A in the serum, reflecting a lack of direct effect on these immune pathways under the experimental conditions used.On the other hand, a significant decrease in IL-23 levels was observed in some treated groups compared to the control, suggesting that the effect of BABA acid was selective and targeted towards pathways associated with IL-23/Th17. BABA demonstrated a selective immunomodulatory effect by significantly reducing IL-23 levels without affecting IL-22 or PP2A, without causing a comprehensive change in all immune pathways examined within the animal model.

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