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Immune response of Enterobacter aerogenes ghost vaccine in mice

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ABSTRACT

Background: The emergence of multidrug-resistant Enterobacter aerogenes has become a public health concern. E. aerogenes is a common cause of hospital-acquired infections such as pneumonia and urinary tract infections. **Aim**: This study focused on the use of E. aerogenes ghosts as a potential vaccine against the opportunistic pathogen E. aerogenes. **Methods**: This study used a cost-effective method to generate the E. aerogenes ghosts using certain chemicals. The safety and effectiveness of the ghosts were evaluated in a mouse model by administering three doses over a 14-day period. The mice were then challenged with a live E. aerogenes strain. **Results**: The results of the cytokine assays showed that vaccinated mice produced higher amounts of IL-10 and TNF-α than did control saline mice, indicating a good immune response. Additionally, the results of the liver function tests indicated that there was a decrease in the activities of ALT and AST in the vaccinated mice as compared to the non-vaccinated mice and low levels of TP and Alb in non-vaccinated mice, suggesting a reduced liver injury and inflammation in vaccinated mice. **Conclusion**: Overall, the study suggests that E. aerogenes ghosts may be a promising candidate for use as a vaccine against E. aerogenes infection.

1. Introduction

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The issue of multidrug resistance (MDR) poses a major challenge for the treatment of bacterial infections globally. While carbapenems represent the final line of defense against Gram-negative bacteria, they are not immune to carbapenem-resistant *Enterobacteriaceae* (CRE), which have emerged as a significant cause of morbidity and mortality in hospital-acquired and long-term care infections [1]. *Enterobacter aerogenes* belongs to the *Enterobacteriaceae* family of gram-negative bacteria and is among the most prevalent carbapenem-resistant *Enterobacteriaceae* (CRE), along with *Escherichia coli* and *Klebsiella pneumoniae* [2]. This bacterium is frequently present in both the human gastrointestinal tract and various environmental sources [3]. *E. aerogenes has been identified as a significant opportunistic pathogen for humans* [4], and it is known to be resistant to several antibiotics that are commonly used to treat infections caused by *Enterobacter* [5].

Bacterial ghosts (BGs) are the empty cell envelopes of gram-negative bacteria that are produced by controlled expression of cloned gene *E* from bacteriophage. This expression results in the formation of a lysis tunnel structure within the envelope of living bacteria [6]. BGs are completely lacking in cytoplasmic content, but they maintain all of the original bio-adhesive surface properties of the bacterial cell envelope [7]. There is a potential risk associated with the use of cloned or genetically modified elements, such as the *E* lysis gene, in the preparation of bacterial ghosts (BGs). These genetic elements could potentially interact with other elements in a way that may be harmful if introduced into the human body [8]. To address the potential risks associated with the use of cloned or genetically modified elements in the production of bacterial ghosts (BGs), alternative methods have been developed. One such method involves using critical concentrations of chemical compounds that are capable of converting viable cells into BGs, instead of relying on the E lysis gene [9]. Sodium dodecyl sulfate (SDS) and sodium hydroxide (NaOH) have been shown to be effective at disrupting the bacterial cell wall and producing bacterial ghosts (BGs). Hydrogen peroxide (H_2O_2), on the other hand, is well-known for its ability to degrade DNA and other genetic elements, making it a potential candidate for use in BG preparation [10].

This study aimed to investigate the use of *E. aerogenes* ghosts as a potential vaccine platform and its effect on the immune response and liver function in mice.

2. Materials and Methods:

2.1. Bacterial strain and culture condition

The *Enterobacter aerogenes* strain ATCC 13048 was cultivated in Nutrient Broth (NB) or on Nutrient Agar (NA) plates at 37°C [11].

2.2. Preparation of *E. aerogenes* ghosts

E. aerogenes ghosts were prepared by sponge like reduced protocol "SLRP" for preparing the BGs according to Amara et al. [9].

2.3. Evaluation of prepared *E. aerogenes* ghosts

2.3.1. Light microscope

Smears of cells of *E. aerogenes ghosts* were stained with crystal violet and compared with stained live *E. aerogenes* under light microscope [12].

2.3.2. Electron microscope

Electron microscope was used to evaluate the bacterial and ghost cells 3D structure [12].

2.3.3. Viability test

The prepared *E. aerogenes ghosts* were evaluated for the existence of any still viable cells, where samples were taken from preparation and grow on Nutrient agar and MacConkey agar plates. The plates then were incubated (in the incubator) at 37∘ C for 3 days [13].

2.4. Experimental animals

One hundred twenty male Swiss-Albino mice, each weighing 25 ± 3 g, were housed in the animal house at the Faculty of Science at Zagazig University in Egypt. Food and water *ad libitum* were available. All animal experiments were approved by the ethical committee – Zagazig University. Number ZU-IACUC/1/F/390/2022.

2.5. Vaccination and challenge protocol

Mice were equally divided into four groups as follows: -

Group-I: NC (Negative control) non-vaccinated control group not inoculated with bacterial ghosts.

Group-II: PC (Positive control) non-vaccinated group challenged with viable *E. aerogenes* bacteria.

Group-III: EAG (EA bacterial ghost) vaccinated group. Each mouse received 200 µl (10^7 CFU) of *E. aerogenes ghosts* intraperitoneally in sterile saline [14].

Group-IV: EAGA (EA bacterial ghost + adjuvant) vaccinated group. Each mouse received intraperitoneally 100 µl (10^7 CFU) of *E. aerogenes ghosts* in sterile saline + 100 µl Freund's adjuvant [15].

The *E. aerogenes ghosts* were administered in an initial dose on day 0, followed by booster doses on days 14 and 28 [15].

After 14 days from the final vaccination, all mice "except the NC group" were IP injected with 100 µl (10^8 CFU) viable *E. aerogenes* strain in sterile saline [16].

2.6. Evaluation of Cytokines

Seven days after the last vaccination, the mouse spleens were homogenized using 2 ml of RPMI 1640 supplemented with 10% fetal calf serum, and the erythrocytes were lysed using Gey's solution. The resulting splenocytes were seeded at a density of 2×10^5 cells per well and stimulated with concanavalin A (ConA) at a concentration of 3 mg/ml. The cells were then cultured in duplicate in 96-well plates at 37°C in a 5% CO_2 atmosphere in RPMI medium [14]. Supernatants were harvested on day 3 for evaluation of interleukin-10 (IL-10) and tumor necrosis factor alpha (TNF-α). Cytokines were detected using an ELISA kit (Bioneovan Co., China) according to the manufacturer's instructions.

2.7. Liver functions' tests investigation

Some liver functions' tests including, serum alanine transaminase (ALT) [17], serum aspartate transaminase (AST) [18], total protein (TP) [19] and albumin (Alb) [20] were assayed in the serum of all mice groups 7 days after the challenge.

2.8. Statistical analysis

Statistical analysis was done using one-way ANOVA with SPSS software (version 14.0). The data were expressed as the mean ± standard error of the mean [21].

3. Results:

3.1. Preparation of *E. aerogenes* ghosts

The main step is based on determining the minimum inhibition concentration (MIC) and the minimum growth concentration (MGC) of H_2O_2, NaOH, and SDS. In case of H_2O_2, the MIC and the MGC were 0.3 mg/ml and 0.03 mg/ml, respectively. In case of NaOH, the MIC and the MGC were 1 mg/ml

and 0.1 mg/ml, respectively. In case of SDS, the MIC and the MGC were 0.2 mg/ml and 0.02 mg/ ml, respectively. In case of $CaCO_3$, the used amount of +1 value was 1.05 µg/ml while -1 values were 0.35 µg/ ml.

3.2. Evaluation of prepared *E. aerogenes* ghosts

3.2.1. Light microscope

Cells of prepared *E. aerogenes* ghosts showed high quality after staining by crystal violet and examined under light microscope (as shown in Figure 1).

3.2.2. Electron microscope

Electron microscope examination showed the correct 3D structure of empty *E. aerogenes* ghost cells (as shown in Figure 2).

3.2.3. Viability test

None of the prepared *E. aerogenes* ghost cells showed any growth upon cultivation on Nutrient agar and MacConkey agar plates for three days at 37∘ C.

3.3. Evaluation of cytokines

High levels of IL-10 and TNF-α were detected in the splenocytes of mice immunized with *E. aerogenes* ghosts or *E. aerogenes* ghosts combined with freund's adjuvant compared with the control group (as shown in Figure 3,4). The levels of IL-10 and TNF-α from animals immunized with *E. aerogenes* ghosts combined with freund's adjuvant was slightly higher than from those immunized with *E. aerogenes* ghosts only.

3.4. Liver functions' tests investigation

The results of the liver function tests indicated that there was a decrease in the activities of ALT and AST in the vaccinated mice as compared to the non-vaccinated mice (Figure 5,6). Additionally, the non-vaccinated mice displayed lower levels of TP and Alb (Figure 7,8).

4. Discussion

Enterobacter aerogenes is a gram-negative, facultative anaerobic, rod-shaped bacterium that is commonly found in the environment, such as in soil and water [22]. It is also considered an opportunistic pathogen, meaning that it can cause infections in individuals with weakened immune systems or in healthcare settings. Infections caused by *E. aerogenes* can include urinary tract infections, wound infections, and pneumonia [23]. Treatment for *E. aerogenes* infections typically involves the use of antibiotics, such as cephalosporins, carbapenems, and aminoglycosides [24]. However, some strains of *E. aerogenes* have been found to be resistant to certain antibiotics, making treatment more difficult [25].

Bacterial ghosts have been proposed as a potential platform for the development of new vaccines [26]. One of the main advantages of using bacterial ghosts as a vaccine delivery system is that they can be engineered to display a wide range of foreign antigens on their surface, which can then be recognized and targeted by the immune system [27]. Additionally, the use of bacterial ghosts as vaccines can reduce the risk of infection by live bacteria, which is a concern with traditional bacterial vaccines [28]. Several studies have been conducted to evaluate the potential of bacterial ghosts as vaccines. Researchers have shown that bacterial ghosts displaying various antigens can effectively induce an immune response in animal models, including the production of antibodies and T-cell activation [29]. Some studies have also reported that bacterial ghost-based vaccines are effective in protecting animals against infectious diseases [30].

The study reported that when the prepared *E. aerogenes ghost* cells were cultured on nutrient agar and MacConkey agar plates for three days at 37°C, no growth was observed. This suggests that the *E. aerogenes ghost*

cells were unable to reproduce or metabolize when placed in these conditions. The quality of the prepared *E. aerogenes ghosts* was then evaluated using light and electron microscope. The results of this examination showed that the cells maintained their three-dimensional shape, which is an indication that the process used to create the *E. aerogenes ghosts* was successful. This suggests that the *E. aerogenes ghost* cells were not able to survive or grow under normal culture conditions, which is expected as they are a form of bacterial cells that had their cytoplasmic content removed. The preservation of shape as seen through light and electron microscope confirms the cells remained intact (Figure 1,2). These results agreed with [31] which used the same method to prepare bacterial ghosts of *Pseudomonas aeruginosa*.

To determine the types of cytokines expressed by spleen cells from vaccinated mice, the concentrations of IL-10 (Figure 3) and TNF-α (Figure 4) in mouse splenocytes were assessed. Mice vaccinated with *E. aerogenes ghosts* produced higher amounts of IL-10 and TNF-α than did control saline mice. IL-10 and TNF-α are two important cytokines that play a role in the immune response. Elevated levels of IL-10 are associated with a reduction in inflammation and can be protective in certain conditions, while TNF-α is a pro-inflammatory cytokine and its elevation is associated with increased inflammation. Measuring the levels of these cytokines in mice can provide valuable information about the immune response and this data agreed with [32] which studied the cytokine expression induced by *Salmonella typhimurium* ghost vaccine.

ALT and AST are enzymes that are found primarily in the liver, and their activities in the blood can be used to detect liver injury. Elevated activities of ALT and AST can indicate liver damage and inflammation. TP and albumin Alb are proteins that are produced by the liver. The levels of these proteins in the blood can be used to evaluate the overall health of the liver and to detect liver disease. Low levels of these proteins can indicate liver damage. The results of the liver function tests indicated that there was a decrease in the activities of ALT and AST in the vaccinated mice as compared to the non-vaccinated mice (Figure 5,6). Additionally, the non-vaccinated mice displayed lower levels of TP and Alb (Figure 7,8). These results suggest that the vaccination led to a reduction in liver injury and inflammation and agreed with [33] which studied the protection induced by *Klebsiella pneumoniae* ghost vaccine in mice.

5. Conclusion

In conclusion, this research focused on the use of *E. aerogenes* ghosts as a potential vaccine platform and its effect on the immune response and liver function in mice. The cytokine assay results indicated that mice vaccinated with *E. aerogenes* ghosts had higher levels of IL-10 and TNF-α compared to control mice given saline, signifying a positive immune response. Furthermore, liver function tests showed a decline in ALT and AST activities in vaccinated mice compared to non-vaccinated mice and lower levels of TP and Alb in non-vaccinated mice, pointing to a reduced liver injury and inflammation in vaccinated mice. These findings suggest that *E. aerogenes* ghosts have the potential to be an effective vaccine against *E. aerogenes* infections.

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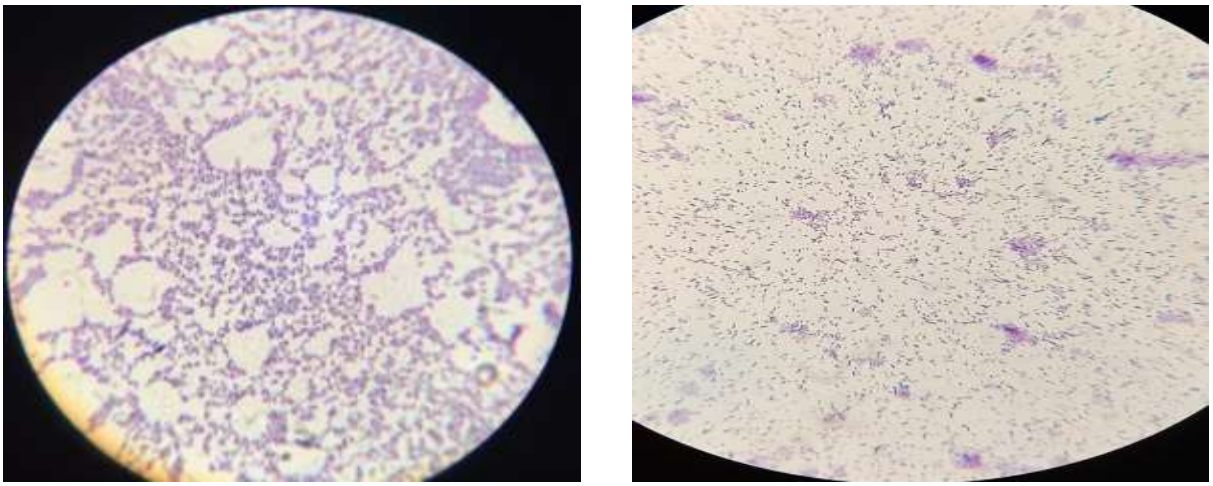
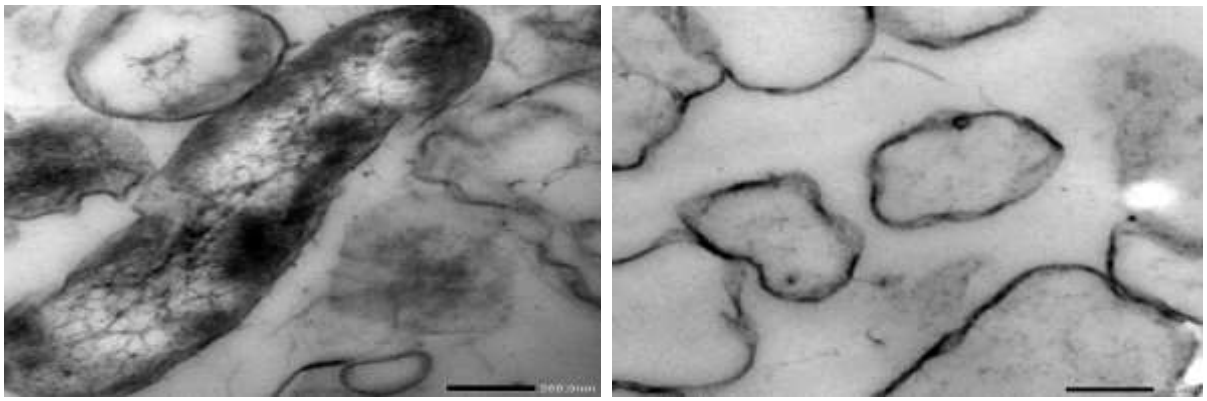


(A) **(B)**

Figure 1: Light microscope of live *E. aerogenes* (A) and *E. aerogenes* ghosts (B)



(A) **(B)**

Figure 2: Electron microscope of live *E. aerogenes* (A) and *E. aerogenes* ghosts (B)

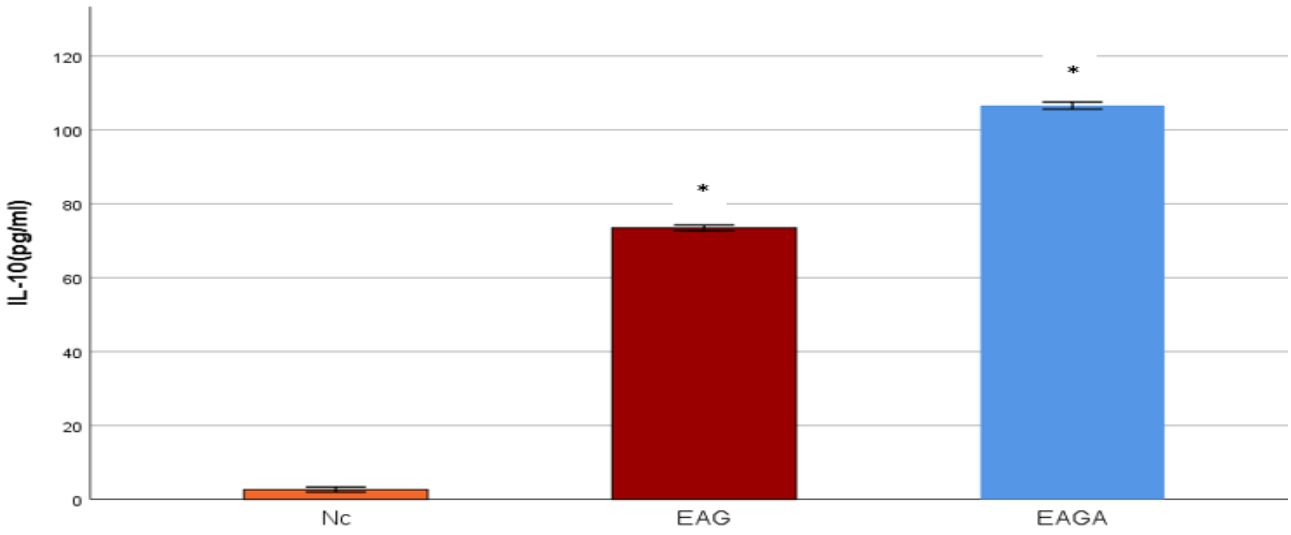


Figure 3: IL-10 levels in all studied groups

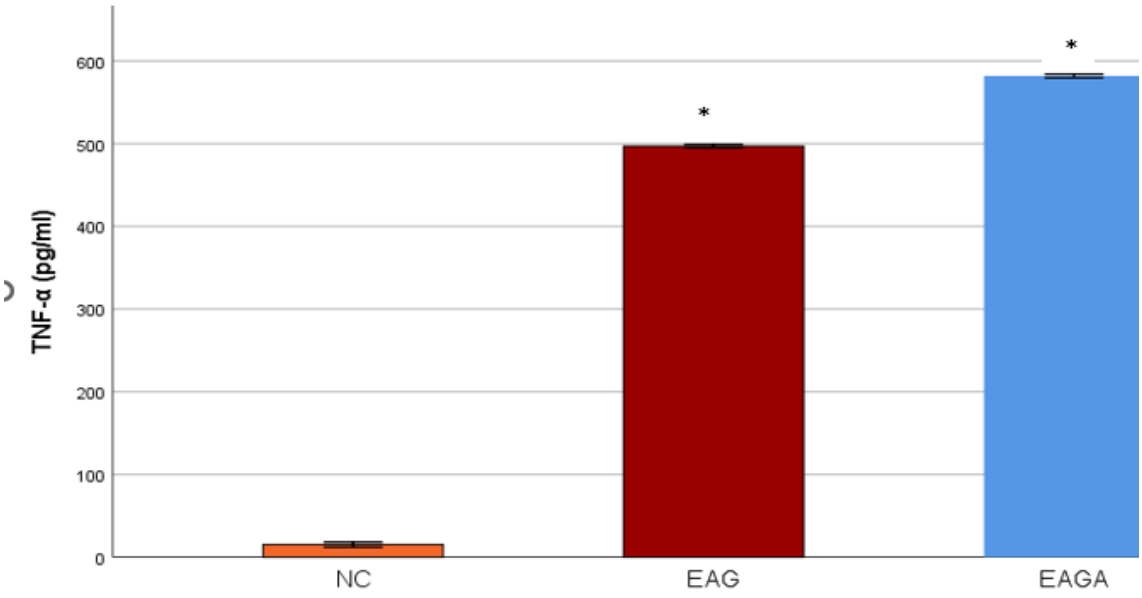


Figure 4: TNF-α levels in all studied groups

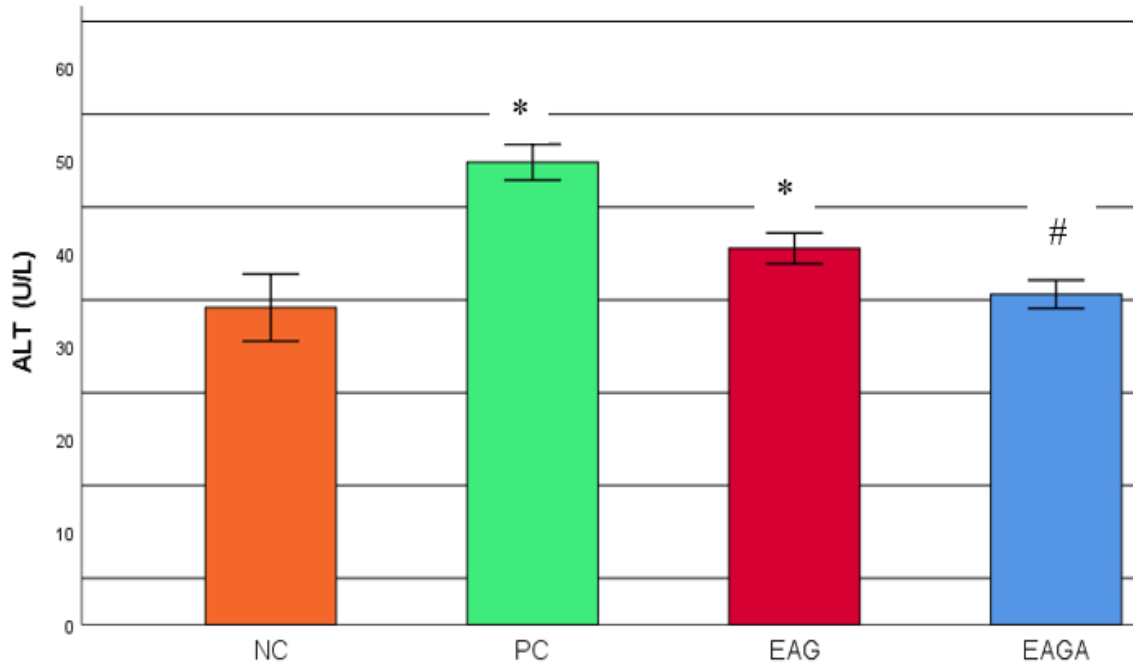


Figure 5: ALT activities in serum of all studied groups

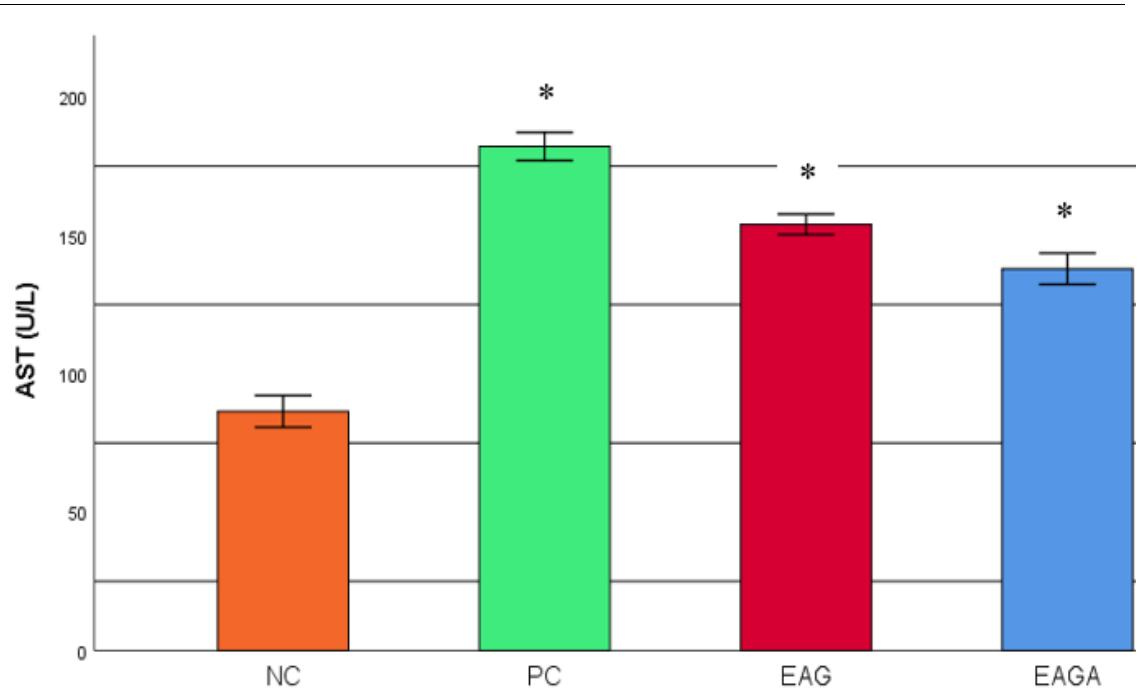


Figure 6: AST activities in serum of all studied groups

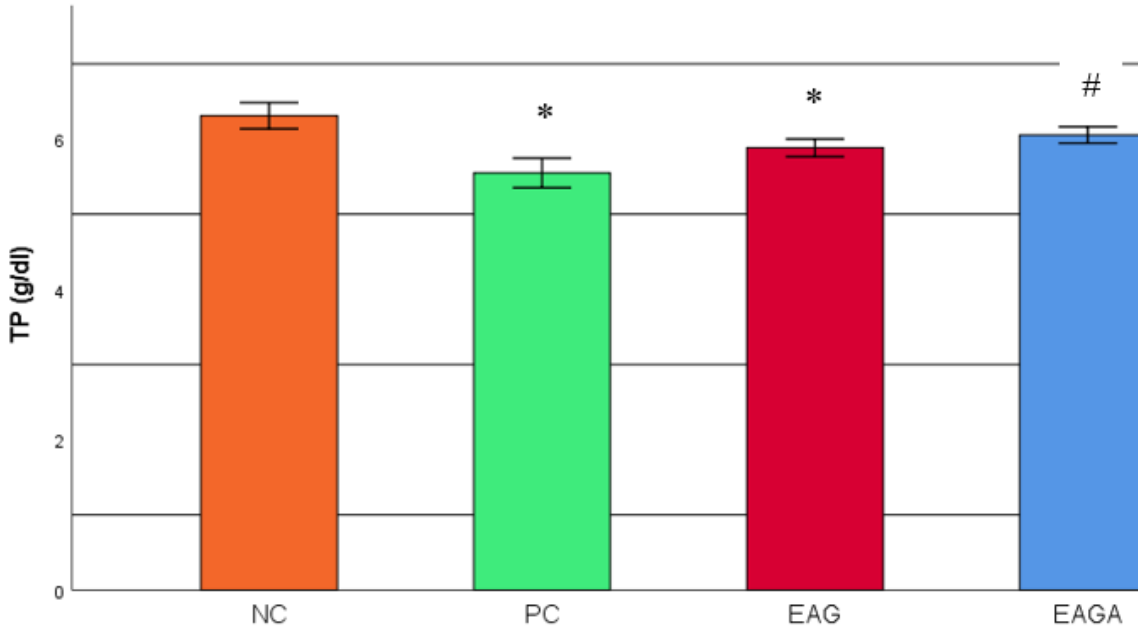


Figure 7: TP levels in serum of all studied groups

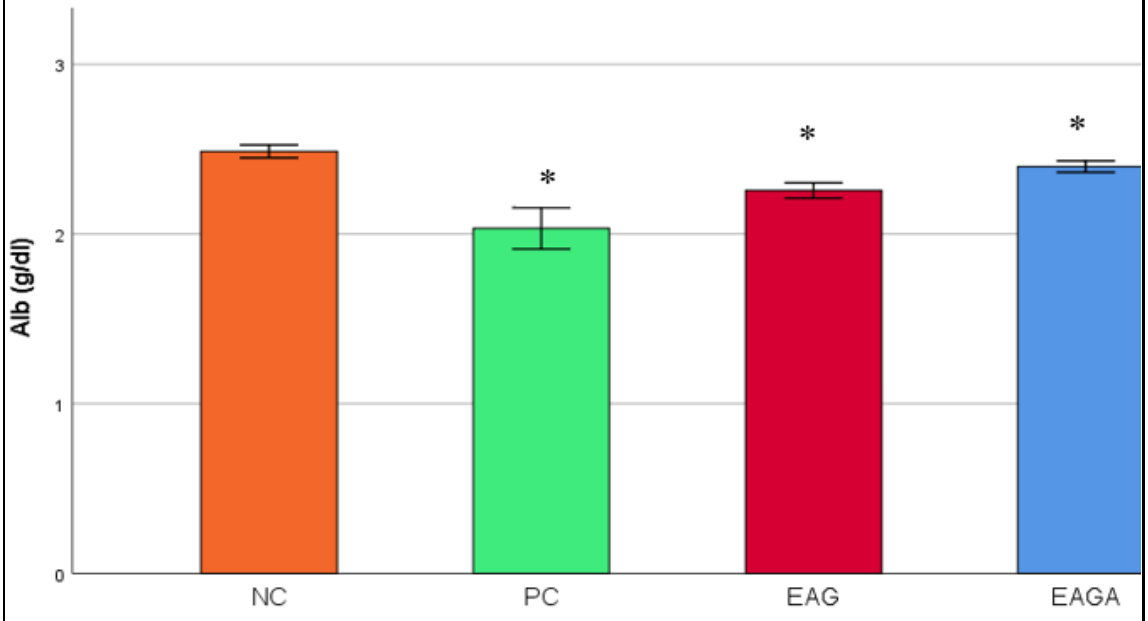


Figure8: Alb levels in serum of all studied groups