Biological and Immunological Effects of Penicillic Acid on Mice liver and Intestine

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Abstract
The aim of the present study was to investigates the effect of penicillic acid on some morphological and biochemical changes induced in mice as a result of penicillic acid mycotoxicosis.
The results showed that the intraperitonal injection of penicllic acid to mice caused pathological signs of marked increase in body weight, dyspnea, shivering, bristling up of hair, hair falling, anomalies of eyes and irritation around neck. A biochemical study on liver function was conducted by measuring GOT and GPT enzyme levels.
The results indicated an increase in GOT and GPT enzyme levels in treated mice compared with control mice which indicating failure in liver function. A radial immunodiffusion plate kit was used to assay immunoglobulin IgG, IgM, and IgA levels, results report an increase of these immunoglobulin level in treated mice which indicating immune response to this toxin.in addation ,treatment of mice with penicillic acid showed an increase in total and differential count of leukocyte.

Keywords: penicillic acid, GPT, GOT, Immunoglobulin.

Introduction

Penicillic acid is a mycotoxin produced by Aspergillus and Penicillium species, particularly by a typical strain of *Penicillium roqueforti*, This mycotoxin is responsible for economic losses and poses health hazards to humans and farm animals.
About twenty known penicillic acids (PA) are produced by Aspergillus and Penicillium and by a typicaal strain of *Penicillium roqueforti* was cultured under different conditions in shaker flasks to determine the highest yielding strains and their requirements in order to maintain maximum toxin production.Blunt *et al* (2018).
There are two strains of *Penicillium roqueforti:* chemo type I and chemo type II., Chemo type I strains were considered optimal producers of secondary (toxic) metabolites, including PR toxin, mycophenolic acid, roquefortine B, C and D, marcfortine A, B and C, fumigaclavine A and B, eremofortine A–E, festuclavine ,and agroclavine. In contrast, chemo type II strains were considered to produce patulin, mycophenolic acid, penicillic acid, roquefortine C, and botryodiplodin.El-Sayed and Amira(2023).
In Raulin–Thom medium, the ability of cultures to utilize eight different carbon sources for mycotoxin synthesis was determined at four different incubation temperatures: 15, 20, 25 and 28°C. Of the 20 cultures, *Penicillium roqueforti* was superior, yielding up to 4 mg of PA per ml when mannitol as the carbon source .Hermsen *et al*(2015).
Penicillic acid (PA) produced by the seed-borne fungus Aspergillus persii EML-HPB1-11 showed antibacterial activity against various plant pathogenic bacteria. The compound effectively inhibited the growth of 12 plant pathogenic bacteria and successfully controlled bacterial spot disease on peach leaf.Nguyen *etal*(2016)

The accumulation of PA in mold was measured following incubation under air, 20% CO_2, 20% O_2, 60% N_2, 40% CO_2, 20% O_2, 40% N_2 and 60% CO_2, 20% O_2, 20% N_2. Although reduced temperature initially leads to Inhibit PA production, at the end of the incubation period, the largest quantity of PA was observed in air-incubated cultures at the lowest tested temperature 15°, Atmospheres enriched with 60% CO_2 reduced the accumulation of penicillic acid to below detectable levels at 10 and 15°C after a four-week incubation period.van den Berg *et al*(2017).
The toxicity of penicillic acid in dogs has been studied experimentally by Egbuna et al(2021),They record that most sensitive organs were the lungs and the gastrointestinal tract, Clinical signs of toxicosis included hematemesis, diarrhea, lethargy, pulmonary hemorrhage, and pulmonary edema.
the filtrate of P. italicum from a natural medium (rotted orange) as a solid state fermentation was more weighted and gave many effective metabolites compared to what was produced by liquid fermentation on a synthetic medium, and both liquid and solid fermentation filtrates demonstrated efficacy against harmful bacteria.Hussain A.F(2024).

When penicillic acid was administered intravenously at doses greater than 10 mg/kg body weight, only a slight decrease in appetence was observed without any other clinical signs. Urusov,*et al*(2015).
The present study investigated the administration of penicillic acid and examines some morphological and biochemical changes induced in mice as a result of penicillic acid mycotoxicosis.

Materials And Methods

In this study, mature male mice weighted 19.6–20.2 gm were used, Those mice were divided into three groups (six mice per each group), The first group was treated daily intraperitoneally with 0.1 ml of distilled water for 35 days (5 weeks) and was considered as control group.
The second group was treated daily intraperitoneally with 0.1 ml of the toxin (2.5 mg/kg body weight) for 35 days (5 weeks) and was considered the low-dose group. The third group was treated daily intraperitoneally with 0.1 ml of the toxin (5 mg/kg body weight) for 35 days (5 weeks) and was considered as high-dose group.
For blood samples collection, mice were anesthesia and blood by cardiac puncture method using insulin syringe coated internally with heparin as an anticoagulant, blood was pulled from mice at a volume of approximately 0.71-1.0 ml for hematological test.

White Blood Cell (WBC) Counts

White blood cell counts were calculated based on the number of cells counted in a defined area and the dilution factor, using the following formula :
Number of cells (cells/mm3 blood) = (Number of cells counted in four large squares X dilution factor) / Volume. Brundha *et al*(2019).
The activity of the GOT enzyme was evaluated in mouse serum using an enzymatic colorimetric kit method produced by Randox company, according to the method of Reitman and Frankel (1957), This assay is based on the calculation of oxaloacetate formed from L-Aspartate according to the following reaction.
α-Oxoglutarate +L-aspartate ----(GOT)--- L- glutamate + oxaloacetate
The activity of GPT enzyme was measured in blood serum using a colorimetric method with an enzyme kit produced by Randox company, according to Reitman through calculating the free pyruvate produced from the substrate L-Alanine, as shown in the following reaction,(Reitman and Frankel,1957).
α-Oxoglutarate +L-Alanine--- (GPT)---- L- glutamate + pyruvate
Absorbance was measured at a wavelength of 546nm using a spectrophotometer.
A radial immunodiffusion plate kit was used to assay IgG, IgM, and IgA levels, The procedure was performed by immunoprecipitation in agarose gel between an antigen and its homologous antibody, this test was carried out by uniformly incorporating one of the two immune reactants (usually the antibody) throughout
a layer of agarose gel, followed by introducing the other reactant (usually the antigen) into wells punched in the gel. The antigen diffused radially from the well into the surrounding gel-antibody mixture, forming a visible ring of precipitation at the site where the antigen-antibody reaction occurred, as described by Domenico Rizzo *et al* (2021).

Results

Effect of penicillic acid on GOT and GPT enzymes.

The effect of penicillic acid on glutamate oxaloacetate transaminase (GOT) enzyme levels is presented in (Figure 1). The results showed that treatment with 2.5 mg/kg of penicillic acid caused a significant increase ($P < 0.05$) in GOT activity, reaching 344.7 U/L, compared with the control value of 284.233 U/L.
A further significant increase ($P < 0.05$) in GOT activity was observed after treatment with 5 mg/kg of Penicillic acid, reaching 398.85 U/L, compared with the control group (284.233 U/L).

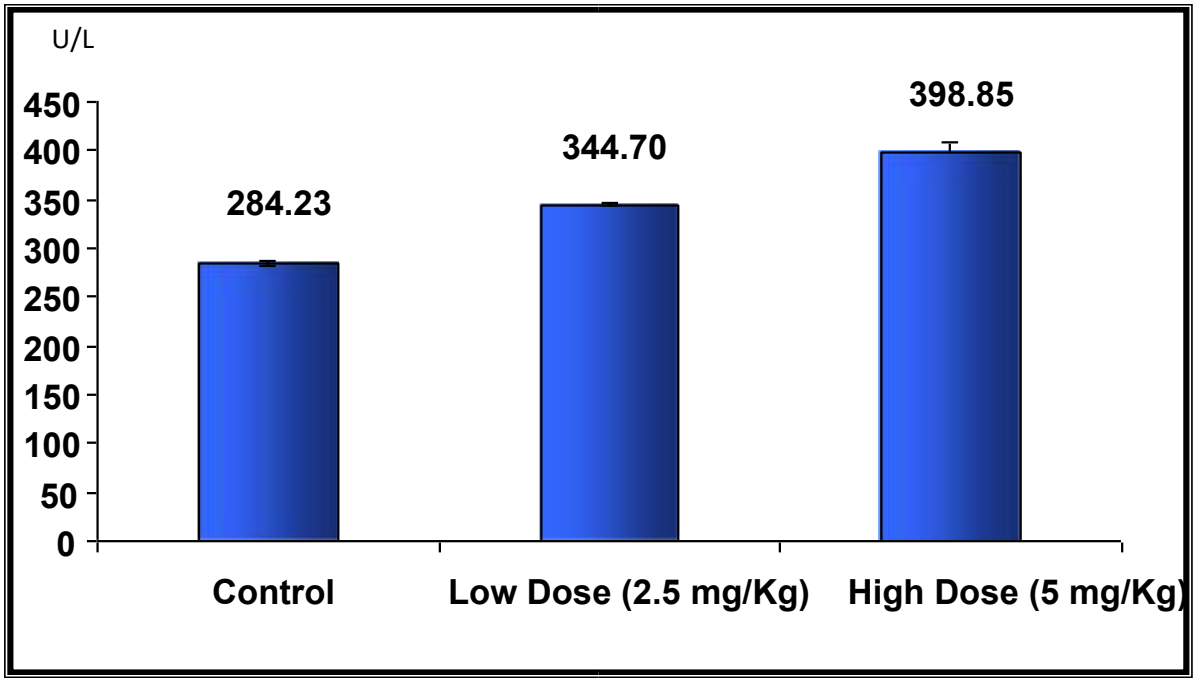

Figure 1: Effect of penicillic acid on **GOT** enzyme activity showing a significant increase.

Effect of penicillic acid on Glutamate Pyruvate Transaminase (GPT)

A significant increase in glutamate pyruvate transaminase (GPT) activity was observed following treatment with 2.5 mg/kg of penicillic acid, GPT levels increased to 124.03 U/L compared with the control value of 95.58 U/L (Figure 2).

Furthermore, treatment with 5 mg/kg of penicillic acid resulted in a significant increase ($P < 0.05$) in GPT activity, reaching 139.08 U/L compared with the control group .

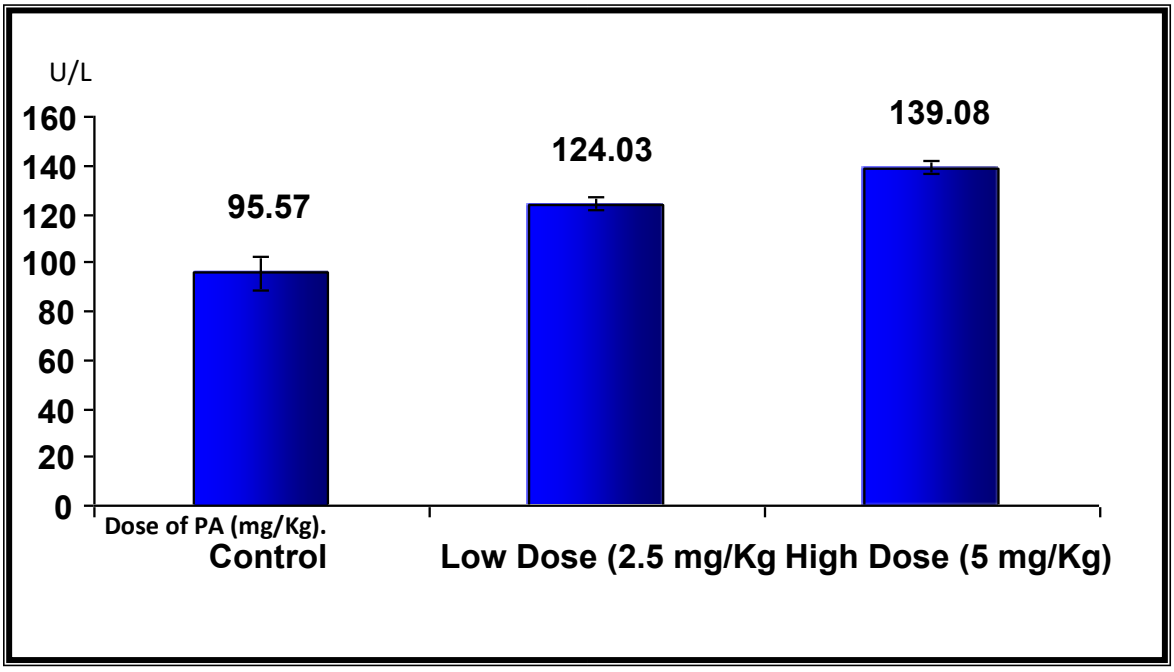

Figure 2: Effect of penicillic acid on the **GPT** enzyme showing a significant increase.

Effect on the Total and Differential counts of Mouse Leukocytes.

Treatment with penicillic acid at a dose of 2.5 mg/kg caused a significant increase ($P < 0.05$) in the total leukocyte count, reaching 14466.67 cells/cu.mm of blood, compared with the control group (7266.66 cells/cu.mm of blood).

Similarly, administration of 5 mg/kg of penicillic acid (PA) also showed a significant increase ($P < 0.05$) in the total leukocyte count, which reached 18566.67 cells/cu.mm of blood when compared with the same control group (Figure 3).

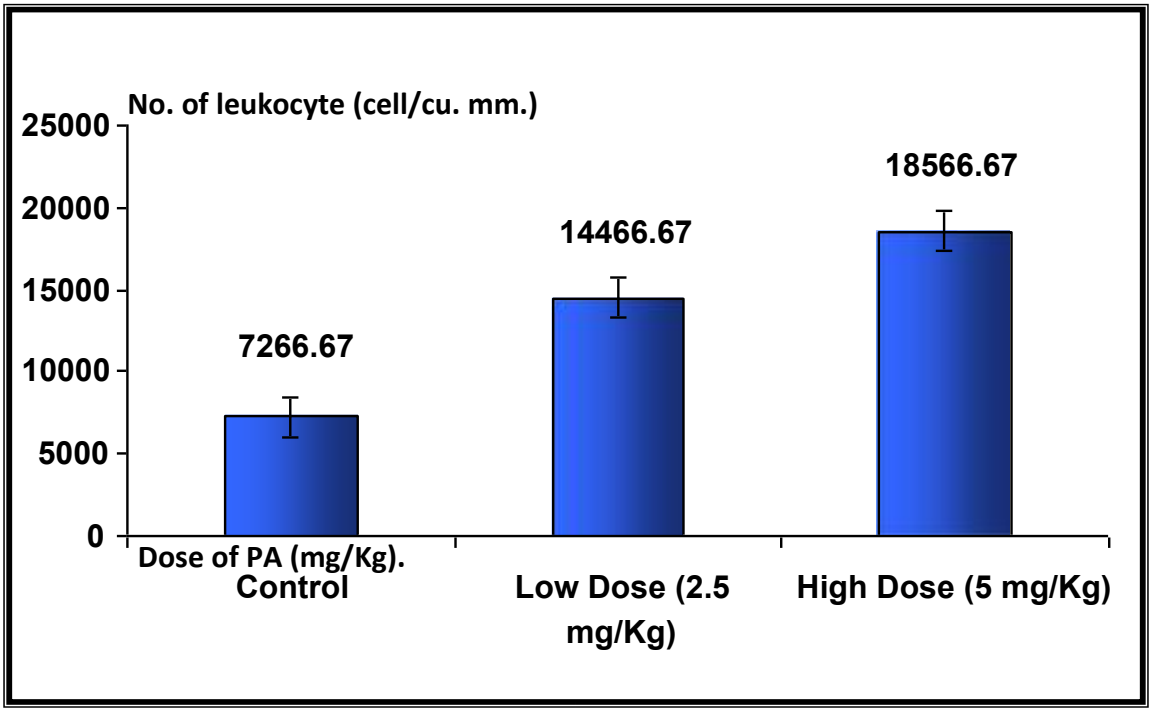

Figure.3: Effect of penicillic acid on leukocyte count.

Mer et al (2020) reported a marked increase in leukocyte counts following penicillic acid treatment.
A significant increase ($P < 0.05$) in the numbers of lymphocytes and neutrophils was observed after treatment with 2.5 mg/kg of penicillic acid, reaching 10614.67 and 2365.33 cells/cu.mm. blood, respectively, compared with the control group 4017.33 and 2279.33 cells/cu.mm . blood respectively.
Furthermore, treatment with 5 mg/kg of the same toxin resulted in a significant increase ($P < 0.05$) in these cell counts, reaching 12766 and 3426.67 cells/cu.mm . blood respectively, when compared with the same control group (Figures .4 and.5).

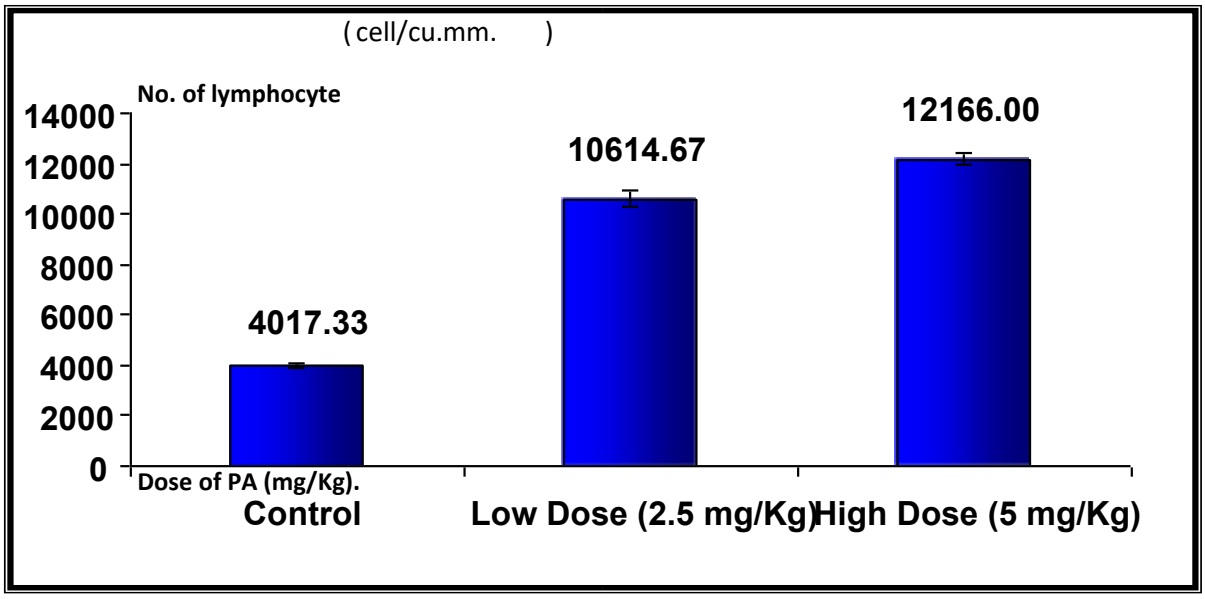

Figure 4. Effect of penicillic acid on lymphocyte.

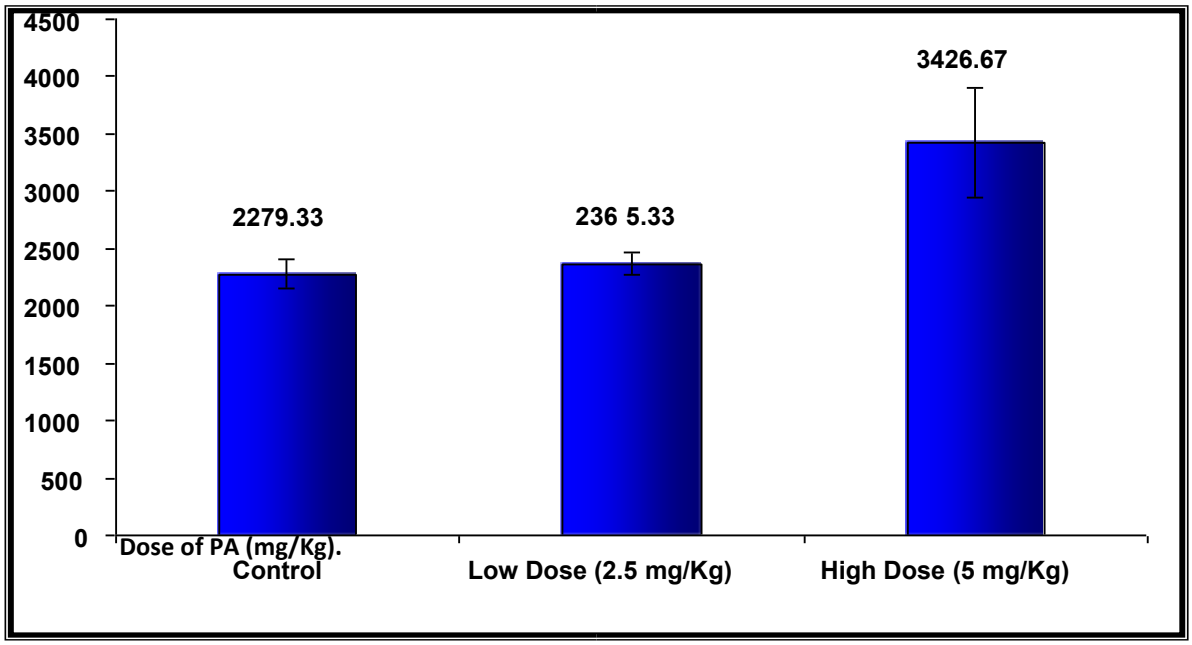

Figure 5. Effect of penicillic acid on neutrophil

Also, penicillic acid at a dose of 2.5 mg/kg showed a significant increase ($P < 0.05$) in the number of monocytes, reaching 1160 cells/cu.mm . blood compared with the control group (920 cells/cu.mm).
Similarly, PA at a dose of 5 mg/kg caused a significant increase ($P < 0.05$) in the number of monocytes, reaching 1749 cells/cu.mm. blood compared with the control group.

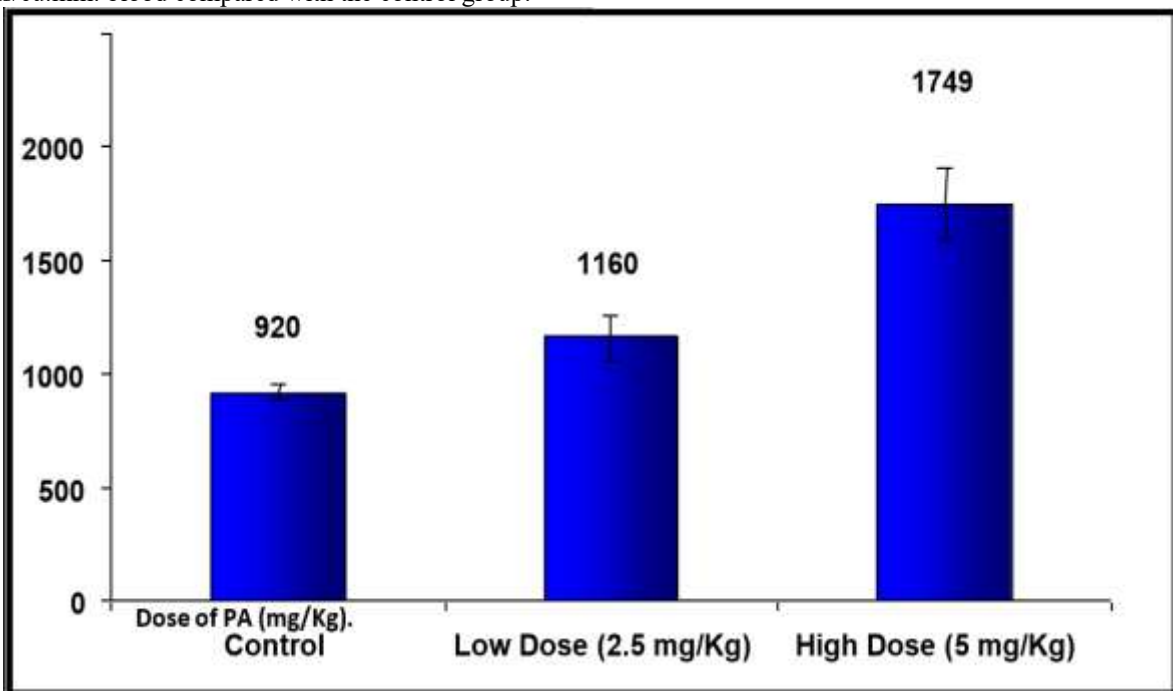

Figure 6: Effect of penicillic acid on monocyte count and its significant increase.

While examining the effect of this toxin on eosinophils, PA at a dose of 2.5 mg/kg caused a significant increase ($P < 0.05$) in the number of eosinophils, reaching 239.33 cells/cu.mm. blood in comparison with the control group (24.66 cells/cu.mm).
In addition, PA at a dose of 5 mg/kg caused a significant increase ($P < 0.05$) in the number of eosinophils which increased to 309.33 cells/cu.mm.blood compared
with the control group (24.66 cells/cu.mm. blood), as shown in Figure 7.

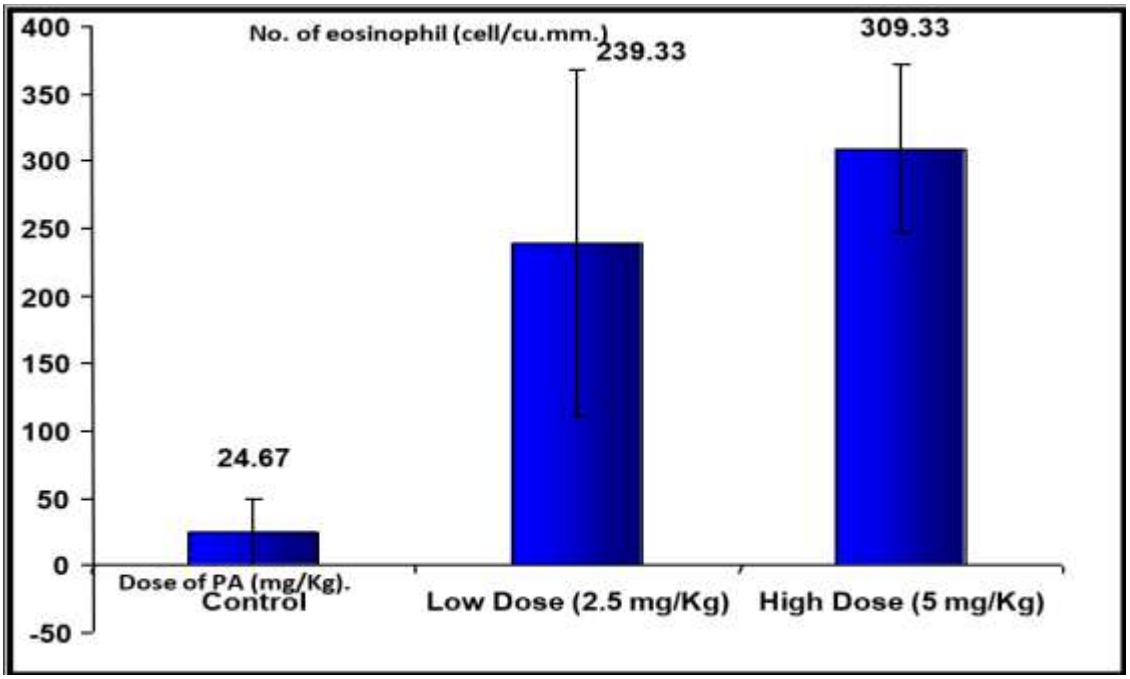

Figure 7: Effect of penicillic acid on eosinophil count and its significant increase.

Different results were recorded for the basophil count after treatment with the toxin, A non-significant increase was observed after treatment with 2.5 mg/kg of the toxin, whereas the basophil count increased from 25.33 cells/cu.mm . blood to 249 cells/cu.mm . blood when a concentration of 5 mg/kg of the toxin was used. This increase was statistically significant ($P < 0.05$), as shown in Figure 8.

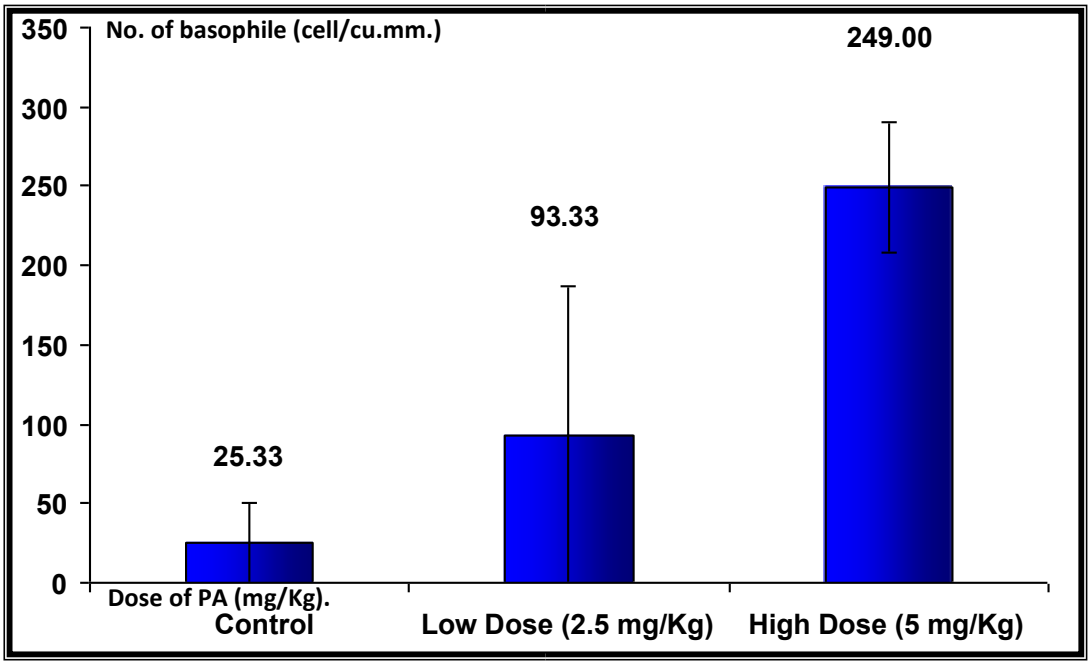

Figure 8: Effect of penicillic acid on basophil count and its significant increase.

Toxicity of penicillic acid

An increase in body weight was observed in treated mice compared with the body weight of control animals, this increase occurred gradually during the treatment period and became more apparent in the last week. In general, the increase in body weight was dose-dependent.

Body weight increased in mice treated with the high dose of penicillic acid (5 mg/kg) from 22.3 ±0-39 g to 27.21 ± 0.35 g.

Similarly, in mice treated with the low dose (2.5 mg/kg), body weight increased from 21.25 ± 0.17 g to 24.28 ± 0.12 g ,this increase was statistically significant ($P < 0.05$), as shown in Figure 9.

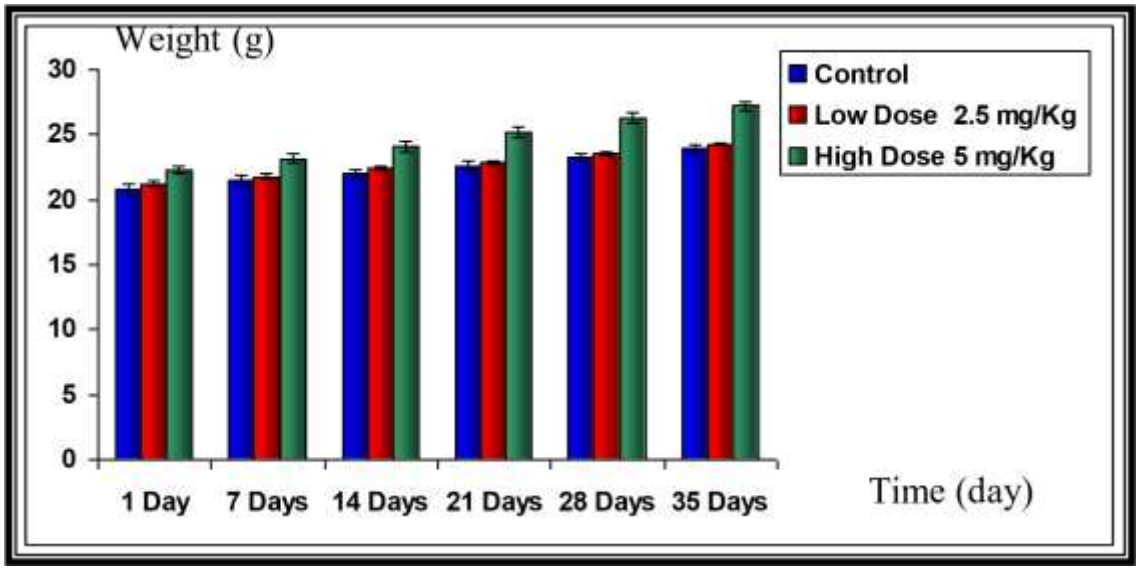

Figure 9: changes in the mean body weight of mice treated with penicillic acid compared with the control group.

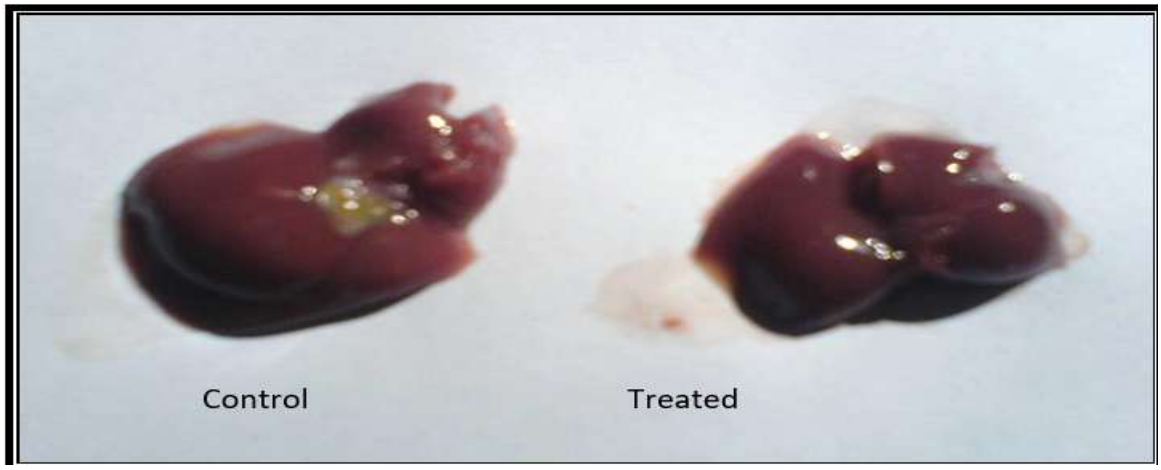

Figure 10: Showing the relative increase in liver size after treatment**.**

Figure 11: shows the relative increase in spleen size after penicillic acid treatment.

Effect of penicillic acid on Immunoglobulins (IgA, IgG, and IgM)

1. IgA

The effect of penicillic acid on immunoglobulin A (IgA) levels was recorded in Figures 12 and 13. The results showed that administration of 2.5 mg/kg of penicillic acid caused a significant increase ($P < 0.05$) in IgA levels, reaching 240.43 mg/dl, compared with the control group (153.87 mg/dl).

A further significant increase ($P < 0.05$) in IgA levels was observed after treatment with 5 mg/kg of this toxin, reaching 322.30 mg/dl compared with the control group.

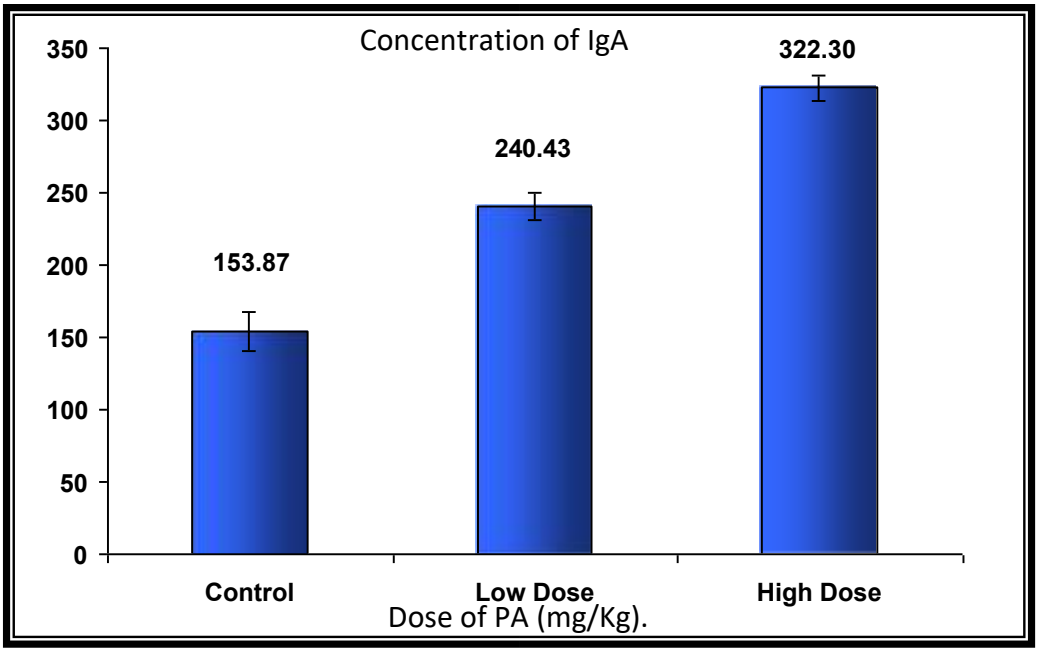

Figure 12: Effect of penicillic acid on immunoglobulin A (IgA) concentration and its significant increase.

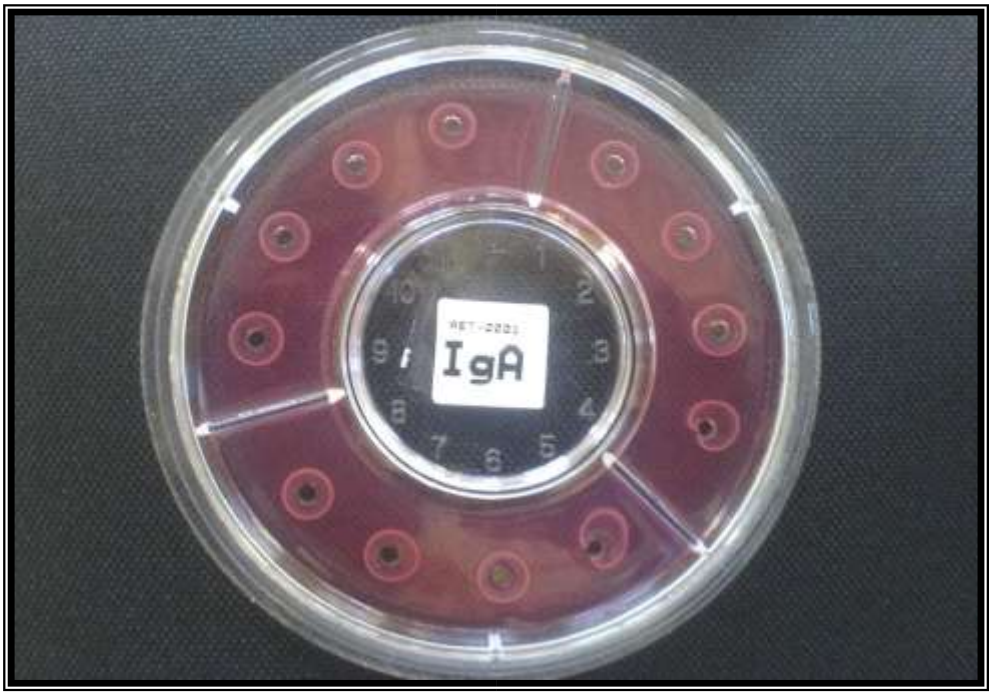

Figure 13. Kit of immunoglobulin A (IgA**)**

2. IgG

The results showed that penicillic acid significantly affected immunoglobulin G (IgG) levels. Administration of penicillic acid at a dose of 2.5 mg/kg caused a significant increase ($P < 0.05$) in IgG levels, reaching 1540.43 mg/dl, compared with the control group (1141.97 mg/dl).

In addition, treatment with 5 mg/kg of penicillic acid resulted in a further significant increase ($P < 0.05$) in IgG levels, reaching 1955.22 mg/dl, compared with the same control group, as shown in Figures 14 and 3-15.

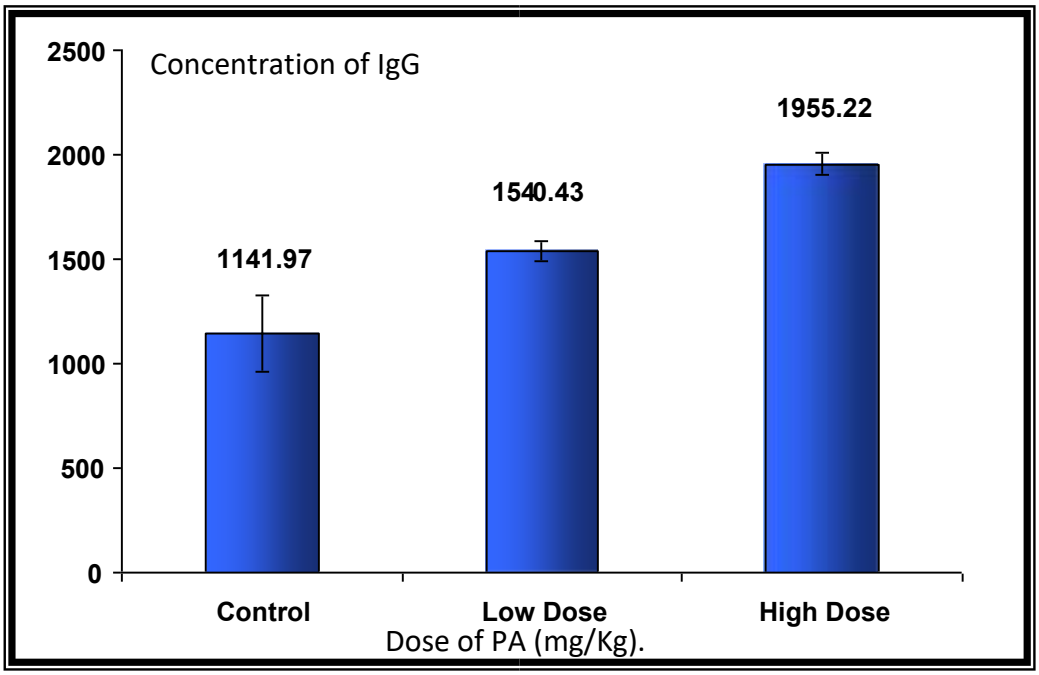

Figure 14 Effect of penicillic acid on immunoglobulin IgG and its significant increase

Figure 15 Kit of immunoglobulin G (IgG).

3. IgM

Treatment with penicillic acid at a dose of 2.5 mg/kg caused a significant increase ($P < 0.05$) in immunoglobulin M (IgM) levels, reaching 86.07 mg/dl, compared with the control group (23.87 mg/dl).

While at a higher dose of 5 mg/kg, penicillic acid produced a further increase in IgM levels, reaching 146.93 mg/dl, when compared to the control group, as shown in Figures 16 and 17.

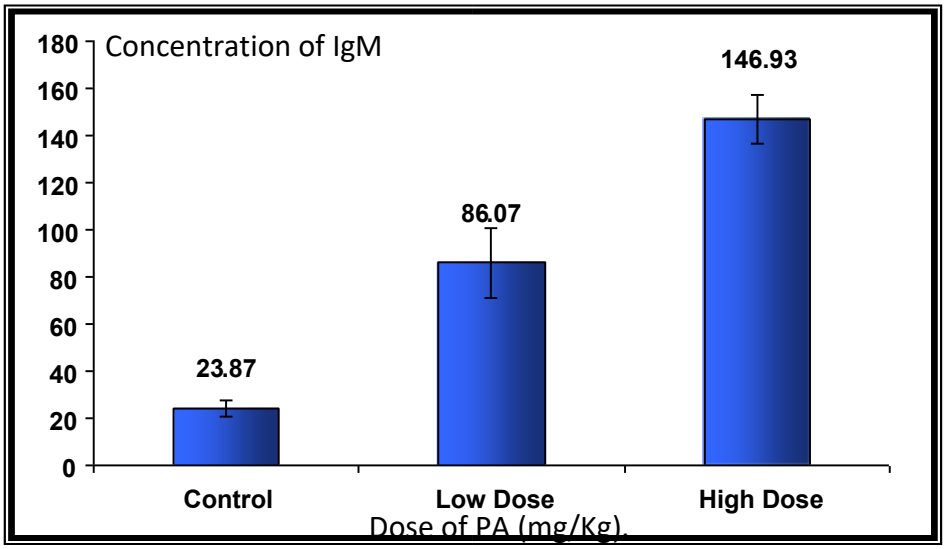

Figure 16 Effect of penicillic acid on immunoglobulin IgM and its significant increase.

Figure 17. Kit of immunoglobulin M (IgM).

Discussion

The increase in blood levels of glutamate oxaloacetate transaminase (GOT) and glutamate pyruvate transaminase (GPT) enzymes in treated mice may be attributed to the ability of penicillic acid (PA) to induce hepatotoxicity by causing hepatic lesions, which in turn lead to elevated enzyme levels.Wolter and Jean,(1997).

The increase in these enzyme levels may also be due to the cytotoxic effect of PA on liver cells. This cytotoxicity increases the permeability of the liver cell membrane, resulting in the release of large quantities of these enzymes into the blood serum. This phenomenon explains the elevated enzyme levels observed in blood serum and the corresponding decrease in liver tissue after exposure to these toxic agents Egler *et al*(2016).

In general, the results indicate that increasing doses of PA lead to an increase in both lymphocytes and neutrophils, these findings are agreed with those reported by Domer *et al*. (2021), who found that PA and roquefortine induced lymphocyte and neutrophil proliferation,and dose response curve for each mycotoxins were generated and the concentration of 8 mg/kg were administered and resulted in an approximately 50% increase in cell proliferation.

IgA levels were observed to increase in a dose-dependent manner. The increase in IgA levels with increasing toxin doses may be attributed to elevated IgA immune complex formation. In this regard, Schena and Nistor (2018) showed that low exposure to penicillic acid was less effective in increasing IgA levels in mice than high exposure. Additionally, the effect of penicillic acid on IgA levels may be related to antibody synthesis occurring during exposure to PA.

Vandenbroucke *et al*. (2011) reported that many mycotoxins affect humoral immunity, particularly immunoglobulin levels. These results are supported by the findings of Pabst and Slack (2020), who demonstrated that in mice, one of the most pronounced effects of this toxin is a significant elevation in serum immunoglobulin A (IgA).

Differences in total plasma IgG concentrations were observed among the PA-treated groups. In general, a significant increase in IgG levels was detected in treated groups, which may be attributed to the fact that IgG is the predominant immunoglobulin involved in toxin neutralizationemke et al(2016) pointed that repeated exposure to PA induced the concentration of maternal antibodies. Munoz and Jamieson (2019) reported an increase in serum IgG levels in mice treated with different doses of penicillic acid.

These results are came in agreement with those of Lemke *et al.* (2016), who observed increases in serum IgA and IgM concentrations following treatment with penicillic acid. Furthermore, Risselada *et al*. (2013) found that penicillic acid treatment stimulates IgM production in mice, leading to elevated circulating IgM concentrations, but that differ to the result obtained by Magill . *et al.* (2014) who concluded that IgM and IgA were unaffected by penicillic acid treatment serum IgM.

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Conclusions

- ✓ Liver and intestine are target organs that can affected by (PA).
- ✓ Total and differential count of mice leukocyte are affected after (PA) treatment.
- ✓ *(PA) is consider as the one of the potent toxin that lead to elevated* concentrated of immunoglobulin.
- ✓ Mouse organs function affected by (PA) toxicity and this can be revealed by biochemical test and microscopically examination.

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