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# AMOXICILLIN EXPOSURE DISRUPTS HISTOLOGICAL STRUCTURES, HEMATOBIOCHEMICAL PROFILE AND ANTIOXIDANT STATUS OF MALE DOMESTIC RABBIT (*ORYCTOLAGUS CUNICULUS*)

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**Abstract**

Hemato-biochemical and histopathological assessments are important methods to find any physiological malfunctioning of animal body including human for disease diagnosis, treatment and toxicity assessment of any toxicant. Present study was conducted for the evaluation of variation in hematobiochemical parameters and histopathological lesions in different visceral organs of rabbits after amoxicillin treatment. For this purpose, a total of 16 (n=16) male rabbits *(Oryctolagus cuniculus)* were purchased from local market and were equally divided into four groups on random basis. After acclimatization period, the rabbits were exposed with different doses of amoxicillin (100, 150 and 200 mg/kg body weight) for 21 days. Hematobiochemical examination showed that total white blood cells, granulocytes, neutrophils, serum globulin, triglycerides, cholesterol, urea and uric acid increased significantly. Whereas, lymphocytes, monocytes, RBCs, hemoglobin, blood sugar, albumin and plasma fibrinogen significantly decreased in amoxicillin treated male rabbit groups ($P < 0.05$). In present study antioxidant parameter like malondialdehyde concentration and liver enzyme like alanine aminotransferase, aspartate transaminase, alkaline phosphatase, lactate dehydrogenase increased significantly. Whereas, other antioxidant parameters like peroxidases, catalases, reduced glutathione and superoxide dismutase decreased significantly in amoxicillin treated groups. Histopathological examination showed congestion, pulmonary edema, leukocyte infiltration, lungs inflammation, interstitial pneumonia, emphysema, interstitial pneumonia and emphysema in lungs; splenic atrophy, red pulp pigments, fibrosis, neoplastic, splenic necrosis, lymphoid necrosis, red pulp hyperplasia, white pulp hyperplasia and splenomegaly in spleen; vacuolation, atrophic seminiferous tubules, necrotic lumen cells, aggregated spermatozoa, vacuolated seminiferous epithelium and decreased spermatozoa in testis; degenerative epithelium, decreased villus length, villus atrophy, congestion, vacuolation and sloughing of epithelial cells in the intestine of amoxicillin treated male rabbits. In conclusion current study indicates amoxicillin exposure causes disruption in hematobiochemical profile and induces histopathological lesions in amoxicillin treated male rabbits.

**Key words**: Amoxicillin; Rabbit; Histopathology; Hematology; Biochemistry

## 1. Introduction

Antibiotics prevents bacterial infections in human and animals to maintain their health. Apart their role in disease prevention antibiotics are also used as growth promoter in commercial animals to meet global food demand for increasing human population (Van et al. 2020). Commonly prescribed antibiotics to uphold the health of human and livestock are tetracycline,

sulfonamides, aminoglycosides and amoxicillin. Amoxicillin, a bactericidal amino-penicillin widely applied in veterinary animals to control bacterial infection for example Escherichia coli, Staphylococcus, and Streptococcus species (Alhaji et al. 2018).

Apart from countless benefits, antibiotics are associated with adverse side effects that's why European Union introduced a general prohibition for their use as growth promoter. Beside these prohibitory guidelines livestock producers still use various doses of different antibiotics to treat infections in livestock animals (Commission 2010). It is also a common practice among producers and breeders that they treat entire stock, flock of animals instead of disease affected. Veterinary practitioners recommend antibiotics in viral infections despite the fact that antibiotics do not respond to viral diseases. Due to these practices antibiotic residues enters human body indirectly through animal-derived food source and are the basis for emerging problems of public health like antibiotic resistance and antibiotic pollution (Prajwal et al. 2017).

Since antibiotic application in livestock leaves their residues in food items of animal origin like milk, meat, and eggs with wide ranging side effects in these livestock animals. Sometimes these antibiotic residues exceeds maximum residue limits approved by WHO for safe human consumption (animal derived food) leads to emerging threat of antibiotic pollution (Hoelzer et al. 2017). The antibiotics remains in animal foodstuffs are due to following reasons: farmer and breeders treat whole stock of animals at farm instead of affected ones, application of extra-label doses, contamination of animal feedstuff with the manure of antibiotic treated animals, practice of unauthorized antibiotics, hospital sewage, disposal of medicines down the drain and surface runoff from treated animals manure that results in ground water contamination (Kivits et al. 2018).

Due to these above mentioned reasons and practices antibiotics are concentrating into the environment through these several means that involves a cycle of bioaccumulation in food chain and disturbs public health. Due to these practices antibiotic pollution is novel and emerging threat for all living organism including human. These antibiotic residues are associated with adverse effects like sensitivity reactions, liver injury, hepatitis, nephrotoxicity, teratogenicity, carcinogenicity and reduces life expectancy (Vishnuraj et al. 2016).

That's why since from antibiotic discovery research studies are conducted to reveal pharmacodynamics properties and adverse effects associated with these antimicrobial agents. Although antibiotics including amoxicillin are generally considered safe in their application. But latest research prove that these antimicrobial agents are linked with side effects. For example, gentamicin induces mutagenicity, nephropathy and metabolic dysfunction; chloramphenicol have toxic effects e.g. hepatotoxicity, bone marrow toxicity, reproductive dysfunction and carcinogenicity (Jeena et al. 2020, Tadesse & Tadesse 2017).

In published literature previous studies highlights that antibiotics including amoxicillin from multiple sources interferes with public health and induces adverse effects such as hematobiochemical, physiological and reproductive disorders in exposed animals. For example chloramphenicol induces hepatotoxicity, reproductive ailments and bone marrow poisonousness; sulphamethazine and oxytetracycline induces adverse effects on immune system and histopathological lesions in various organs whereas, penicillin causes carcinogenicity and allergic

reactions (Abdelmoaty 2015). Amoxicillin induces adverse physiological reactions, hepatotoxicity, renal toxicity, renal dysfunction, lethargy, insomnia, mild hepatitis, confusion, vomiting and anxiety (Elmajdoub et al. 2014). Acute and cholestatic type liver injury was found among amoxicillin users (Ferreira et al. 2020).

In previous studies when rabbits were exposed with amoxicillin resultantly liver function enzymes like ALT, AST, ALP, LDH and malondialdehyde concentrations (MDA) significantly increased. Whereas, other antioxidant enzymes like catalase, superoxide dismutase, reduced glutathione, and peroxidase reduced significantly in amoxicillin treated rabbits. Furthermore, Hussain et al. also observed histopathological lesions such as cytoplasmic vacuolation; necrotic hepatocyte; karyorrhexis; disorganized hepatic cord in liver; necrotic renal tubular epithelial cell; wider Bowman's space; nuclear hypertrophy; hemorrhage and degenerative renal glomerulus; vacuolization, necrotic cardiac cells and neutrophilic myocarditis was observed in the heart of amoxicillin treated male and female rabbits (Hussain et al. 2022).

In the light of these previous studies, it is revealed that there is a little scientific information with reference to amoxicillin induced toxicity on hematobiochemical profile, liver function enzymes, antioxidant status and pathology of different visceral organs of animals hence there is need of work to fill this research gap. Therefore, we hypothesized that Amoxicillin may have adverse effects on hematobiochemical values, antioxidant parameters and histological structures of male rabbit, *Oryctolagus cuniculus* after exposure with variable doses of amoxicillin.

## 2. Materials and Methods

### 2.1. Study animals, experimental design and laboratory conditions

16 male domestic rabbits about 1.2 years old (average weight 1.25 Kg) were purchased and collected from local market in Bahawalpur, Punjab (Pakistan). After purchase the rabbits were transported and shifted in the laboratory of Department of Zoology, the Islamia University Bahawalpur. Prior to animal shifting laboratory condition such as light, water, fresh air availability, cleanliness and laboratory temperature was maintained at 25-27 °C but also throughout the study as per standard protocols of animal laboratory (Care & Animals 1986). After shifting, rabbits were subjected to the acclimatization period to laboratory conditions for 7 days. Throughout acclimatization period, drinking water was changed on daily basis with ad libitum and fed twice daily on green fodder Barseem (*Trifolium alexandrinum*) in morning and evening arrangements. After the completion of acclimatization period rabbits were randomly divided into 4 group; 3 experimental groups (A, B, C) and 1 control having 4 rabbits each. Amoxicillin (GlaxoSmithKline) was purchased from a registered pharmaceutical shop in Bahawalpur. Variable doses of amoxicillin 100, 150 and 200mg/Kg body weight of rabbit were fed orally to the animals of experimental group A, B, C, respectively on daily basis for 21 days. In previous studies laboratory animals were exposed with higher amoxicillin doses as compared to our variable amoxicillin doses in current experimental trial for rabbit (Pujadas et al. 1986). Amoxicillin exposure was carried out to investigate hematobiochemical profile and histopathological lesions in lungs, spleen, testis and intestine of the experimental rabbits to access its potential toxic effects. During whole experiment control group was run in parallel without any antibiotic treatment. The ethics of animals handling

provided by the Institutional Bioethics Committee (IBC) of The Islamia University of Bahawalpur, Pakistan were strictly followed.

**2.2. Clinical parameters, body and organ weight estimation**

Each group was examined twice a day to observe various clinical signs in test animals induced by amoxicillin exposure during treatment period. The various clinical signs of each animal were scored on the basis of intensity of clinical signs showed by them using standard methods. Symbol for clinical sign to assign score includes normal (-), mild (+), moderate (++), severe (+++), very severe (++++). Each animal of all groups were weighed before and after experiment to estimate their gross body weight and also their organs were also weighed individually too.

**2.3. Hemato-biochemical analysis**

After the completion of experiment blood sampling was performed for hematological and serum biochemical analysis. For this purpose, 5 mL blood was collected from the marginal ear vein of each male rabbit. Blood parameters like hemoglobin, hematocrit, RBC count, mean corpuscular hemoglobin, mean corpuscular volume, leucocyte count such as lymphocyte, monocyte, granulocyte, neutrophils were assessed by using automated hematological analyzer. After hematological examination the serum was used to evaluate urea, uric acid, creatine, calcium, cholesterol, triglyceride, serum globulin, serum albumin, total protein, and blood sugar were estimated (EIA method) using commercial diagnostic kit (BioMed Diagnostics, GmbH, Germany) and Chem Analyzer model (BTS, BioSystem, Spain). Finally, the serum was used to determine AST, ALT, ALP, and LDH concentration according to protocol of using commercial kit through the same automated serum biochemical analyzer (Esievo 2017).

**2.4. Histotechniques and microscopy**

At the end of experimental period after blood sampling tissue samples from organs like lungs, spleen testis and intestine were surgically removed and preserved in 10 % paraformaldehyde solution from all experimental groups of rabbits. Dehydration was performed in ascending grades of available ethyl alcohol with different grades for example 30%, 50%,70%, 90% and 100% after that tissues were cleared to cut into 5µ thick sections with the help of rotary microtome. Following mounting these sections on clean glass slides, staining with eosin and hematoxylin (Kittel et al. 2004). Finally, tissues sections were evaluated under a light microscope at low and high magnification powers to observe any morphological changes in histological structure of the tissue under consideration. The clinical histopathological signs of each sample organ from all individually test animal were scored on the basis of histopathological lesions they showed. Symbol for histopathological lesions in various organ to assign score includes normal (-), mild (+), moderate (++), severe (+++), very severe (++++).

**2.5. Statistical analysis**

Hematobiochemical, antioxidant and liver enzyme values were collected and arranged from amoxicillin treated and control group rabbit for statistical evaluation through SPSS. Research data was examined and analyzed by applying one-way ANOVA and Tukey's test was applied as post-hoc test while significance level was set at $p < 0.05$. Whole data is represented as mean value ± SD for each parameter.

## 3. Results

### 3.1. Relative comparison of body and organ weight in male rabbits

In this study the relative weight of lungs and spleen increased significantly whereas, Gross body weight, weight of liver, kidney, heart and testis decreased significantly in amoxicillin treated male rabbit groups when compared with control ($P < 0.05$) (Table-1).

**Table-1.** Relative comparison of body and organ weight of male rabbits in different groups.

| **Body and organs weight** | **Groups** | | | |
|---|---|---|---|---|
| | Control (0.0 mg / Kg) | A (100 mg / Kg) | B (150 mg / Kg) | C (200 mg / Kg) |
| Body weight (g) | 1525.1 ± 20.3 | 1419.3 ± 19.1* | 1365.4 ± 21.7* | 1245.5 ± 14.8* |
| Liver (x $10^3$ ppm) | 38.4±1.45 | 36.17±1.34* | 33.27±1.34* | 30.16±1.46* |
| Kidney (x $10^3$ ppm) | 6.78±1.23 | 5.34±1.43* | 4.19±1.18* | 3.73±1.34* |
| Heart (x $10^3$ ppm) | 5.78±1.34 | 4.36±1.34* | 3.15±1.11* | 2.89±0.45* |
| Lungs (x $10^3$ ppm) | 6.78±1.34 | 8.73±1.63* | 9.79±1.48* | 11.74±1.75* |
| Spleen (x $10^3$ ppm) | 280.69±39.37 | 290.46±28.43* | 305.34±25.33* | 328.19±19.17* |
| Testis (x $10^3$ ppm) | 290.37±21.17 | 278.13±19.11* | 245.43±23.28* | 224.46±22.44* |

**Note:** The data in this table is represented as Mean ± SD. Asterisk mark bearing values shows significant difference with reference to control group values ($p < 0.05$).

### 3.2. Hematological studies in male rabbits

During present study hematological parameters such as total WBCs, granulocytes and neutrophils showed significantly increasing trend. Whereas, lymphocytes, monocytes, RBCs, hemoglobin, mean corpuscular hemoglobin, mean corpuscular hemoglobin concentration and mean corpuscular volume showed significantly decreasing trend in amoxicillin treated male rabbit groups as compared to control group ($P < 0.05$) (Table-2).

**Table-2.** Relative comparison of hematological parameters of male rabbits in different groups.

| **Hematological parameters** | **Groups** | | | |
|---|---|---|---|---|
| | Control (0.0 mg / Kg) | A (100 mg / Kg) | B (150 mg / Kg) | C (200 mg / Kg) |
| Total White blood cells ($10^3$/µL) | 6.68 ± 1.56 | 6.76 ± 1.58 | 7.13 ± 1.32* | 8.56 ± 1.65* |
| Lymphocytes ($10^3$ / µL) | 4.78 ± 0.85 | 3.67 ± 0.73* | 3.45 ± 0.43 * | 2.54 ± 0.49 * |
| Monocytes ($10^3$ / µL) | 1.57 ± 0.32 | 1.47 ± 1.33 | 1.37 ± 0.28* | 1.29 ± 0.11 * |
| Granulocytes ($10^3$ /µL) | 3.68 ± 0.49 | 3.57 ± 0.47 | 4.45 ± 0.89* | 5.32 ± 0.78 * |
| Neutrophils ($10^3$ / µL) | 1.89 ± 0.56 | 2.34 ± 0.56* | 3.33 ± 0.44* | 2.98 ± 1.33 * |

| | | | | |
|---|---|---|---|---|
| Red blood cells ($10^6$ / μL) | 6.76 ± 0.76 | 6.17 ± 0.51* | 5.78 ± 0.73* | 4.69 ± 0.48 * |
| Hemoglobin (g/dL) | 12.53 ± 0.87 | 11.34 ± 0.86* | 10.43 ± 0.76* | 9.43 ± 0.53 * |
| Mean corpuscular hemoglobin (pg) | 27.31 ± 1.23 | 24.56 ± 0.61 * | 21.56 ± 0.63* | 20.56 ± 0.71 * |
| Mean corpuscular hemoglobin concentration (g/dL) | 39.89 ± 1.23 | 36.45 ± 0.79 * | 34.42 ± 0.59 * | 31.23 ± 0.57* |
| Mean corpuscular volume (fL) | 65.54 ± 1.57 | 64.43 ± 1.35 | 62.78 ± 1.35* | 61.56 ± 1.38* |

**Note:** The data in this table is represented as Mean ± SD. Asterisk mark bearing values shows significant difference with reference to control group values ($p < 0.05$).

### 3.3. Studies of biochemical parameters in male rabbit

In present study biochemical parameters such as blood sugar, albumin, plasma fibrinogen and total protein decreased significantly. Whereas, serum globulin, high density lipoprotein, low density lipoprotein, triglycerides, cholesterol, urea, uric acid and creatine increased significantly in amoxicillin treated male rabbit groups when compared with control ($P < 0.05$) (Table-3).

**Table-3.** Relative comparison of biochemical parameters of male rabbits in different groups.

| **Serum biochemical parameters** | **Groups** | | | |
|---|---|---|---|---|
| | Control (0.0 mg/ Kg) | A (100 mg / Kg) | B (150 mg / Kg) | C (200 mg / Kg) |
| Blood Sugar | 120.34±2.35 | 117.23 ± 2.40 * | 1113.23 ±2.67* | 111.33 ± 2.59 * |
| Albumin | 5.85 ± 0.39 | 5.11 ± 0.48* | 4.81 ± 0.46* | 4.56 ± 0.43* |
| Serum globulin | 2.89 ± 0.32 | 3.43 ± 0.34 | 3.86 ± 0.37* | 4.34 ± 0.58* |
| Plasma fibrinogen | 578.3 ± 248.3 | 515.4 ± 235.7* | 453.2 ± 218.4* | 410.3 ± 208.5* |
| Total protein | 7.45 ± 0.39 | 7.21 ± 0.34 | 5.76 ± 0.47* | 4.16 ± 0.22 * |
| HD Lipoprotein (mg/dl) | 46.43 ± 1.42 | 49.48 ± 1.57* | 54.66 ± 2.37* | 63.36 ± 2.29* |
| LD Lipoprotein (mg/dl) | 15.23 ± 1.38 | 17.21 ± 1.43* | 22.67 ± 1.89* | 26.64 ± 1.73* |
| Cholesterol | 121.48±2.53 | 131.14 ± 2.63* | 134.68 ± 2.35 * | 140.35 ± 1.48* |
| Triglyceride | 142.46±5.34 | 148.53 ± 3.47* | 151.23 ± 3.54* | 157.64 ± 4.28* |
| Uric acid | 2.11 ± 0.26 | 2.50 ± 0.36 | 2.98 ± 0.37 * | 3.68 ± 0.23 * |
| Urea | 37.48 ± 3.18 | 39.31 ± 3.56* | 41.45 ± 3.67 * | 43.65 ± 3.74* |
| Creatine | 1.15 ± 0.18 | 1.23 ± 0.45 | 1.89 ± 0.46 * | 2.23 ± 0.39 * |

**Note:** The data in this table is represented as Mean ± SD. Asterisk mark bearing values shows significant difference with reference to control group values ($p < 0.05$).

### 3.4. Antioxidant and liver enzymes analyses in male rabbits

In this present study antioxidant parameter like malondialdehyde concentration increased significantly. Whereas, other antioxidant enzyme like peroxidases, catalases, reduced glutathione and superoxide dismutase decreased significantly in amoxicillin treated groups as compared to

control ($P < 0.05$). During this study liver enzyme like alanine aminotransferase, aspartate transaminase, alkaline phosphatase, lactate dehydrogenase and bilirubin were increased significantly in amoxicillin treated male rabbit groups ($P < 0.05$) (Table-4).

**Table-4.** Relative comparison of antioxidant and liver enzymes of male rabbits in different groups.

| **Antioxidant parameters** | **Groups** | | | |
|---|---|---|---|---|
| | Control (0.0 mg/ Kg) | A (100 mg/ Kg) | B (150 mg / Kg) | C (200 mg / Kg) |
| Malondialdehyde concentrations | 31.35 ± 2.36 | 33.58 ± 2.56* | 37.37 ± 2.51* | 43.38 ± 3.75* |
| Peroxidase (U/mg p) | 4.26 ± 0.36 | 3.58 ± 0.47* | 2.94 ± 0.64* | 2.17 ± 0.69* |
| Catalase (U/mg p) | 27.37 ± 2.56 | 24.49 ± 2.86* | 19.34 ± 2.67* | 16.41 ± 2.28* |
| Reduced glutathione (U/mg p) | 36.67 ± 2.21 | 31.39 ± 2.47* | 28.46 ± 2.79* | 23.45 ± 2.43* |
| Superoxide dismutase (U/mg p) | 34.42 ± 2.56 | 29.68 ± 2.87* | 26.57 ± 2.89* | 23.45 ± 2.70* |
| **Liver enzymes** | | | | |
| Alanine aminotransferase (IU) | 131.56 ± 3.71 | 133.38 ± 3.56* | 138.24 ± 4.45* | 141.45 ± 4.78* |
| Aspartate transaminase (IU) | 132.46±18.26 | 136.19±26.67* | 151.37±23.4* | 163.56±34.65* |
| Alkaline phosphatase (IU) | 241.27 ± 6.89 | 243.55 ± 5.76 | 250.74 ± 7.43* | 257.82 ± 5.58* |
| Lactate dehydrogenase (IU) | 475.43±130.11 | 503.73±148.54* | 580.37±229.53* | 649.67±247.65* |
| Bilirubin (mg / dL) | 0.61 ± 0.02 | 0.69 ± 0.04* | 0.78 ± 0.06* | 0.85 ± 0.05* |

**Note:** The data in this table is represented as Mean ± SD. Asterisk mark bearing values shows significant difference with reference to control group values ($p < 0.05$).

### 3.5. Histopathological studies of different organs in male rabbit

In this present histopathological study, we observed congestion, serous exudation, pulmonary edema, leukocyte infiltration, lungs inflammation, interstitial pneumonia, emphysema, interstitial pneumonia and emphysema in lungs; splenic atrophy, red pulp pigments, fibrosis, neoplastic, congestion, splenic necrosis, lymphoid necrosis, red pulp hyperplasia, white pulp hyperplasia and splenomegaly in spleen; vacuolation, atrophic seminiferous tubules, arrested spermatogenesis, necrotic cells in lumen, aggregated spermatozoa in lumen, seminiferous epithelium vacuolization, decreased spermatozoa and vascular congestion in testes; degenerative epithelium, decreased villus height, decreased villus length, villus width, villus atrophy, congestion, vacuolation and sloughing of epithelial cells in the intestine of amoxicillin treated rabbit groups (Table-5).

**Table-5.** Severity of different histopathological changes in organs of male rabbits exposed to variable doses of amoxicillin.

| **Histopathological lesions** | **Groups / Treatments** | | |
|---|---|---|---|
| | A (100 mg / Kg) | B (150 mg / Kg) | C (200 mg / Kg) |
| **Lungs** | | | |
| Congestion | + | ++ | +++ |

| | | | |
|---|---|---|---|
| Serous exudation | + | + | ++ |
| Pulmonary edema | + | + | + |
| Leukocyte infiltration | ++ | ++ | +++ |
| Lungs inflammation | + | ++ | +++ |
| Interstitial pneumonia | + | + | ++ |
| Emphysema | ++ | ++ | +++ |
| Interstitial pneumonia | + | + | ++ |
| Emphysema | ++ | +++ | +++ |
| **Spleen** | | | |
| Splenic atrophy | + | ++ | +++ |
| Red pulp pigments | ++ | +++ | ++++ |
| Fibrosis | + | ++ | ++ |
| Neoplastic | - | + | ++ |
| Congestion | + | + | ++ |
| Splenic necrosis | ++ | ++ | +++ |
| Lymphoid necrosis | + | + | ++ |
| Red pulp hyperplasia | + | ++ | +++ |
| White pulp hyperplasia | + | + | ++ |
| Splenomegaly | + | ++ | +++ |
| **Testes** | | | |
| Vacuolation | + | ++ | +++ |
| Atrophic seminiferous tubules | + | + | +++ |
| Arrested spermatogenesis | + | ++ | ++ |
| Necrotic cells in lumen | - | + | ++ |
| Aggregated spermatozoa in lumen | + | ++ | ++ |
| Seminiferous epithelium vacuolization | ++ | +++ | ++++ |
| Decreased spermatozoa | + | ++ | ++ |
| Vascular congestion | + | ++ | +++ |
| **Intestine** | | | |
| Degenerative epithelium | + | ++ | +++ |
| Decreased Villus height | ++ | +++ | ++++ |
| Decreased Villus length | ++ | ++ | +++ |
| Villus width | + | +++ | ++++ |
| Villus atrophy | + | ++ | +++ |
| Congestion | + | + | ++ |
| Vacuolation | - | + | ++ |
| Sloughing of epithelial cells | + | + | + |

Normal (-), Mild (+), Moderate (++), Severe (+++), Very severe (++++)

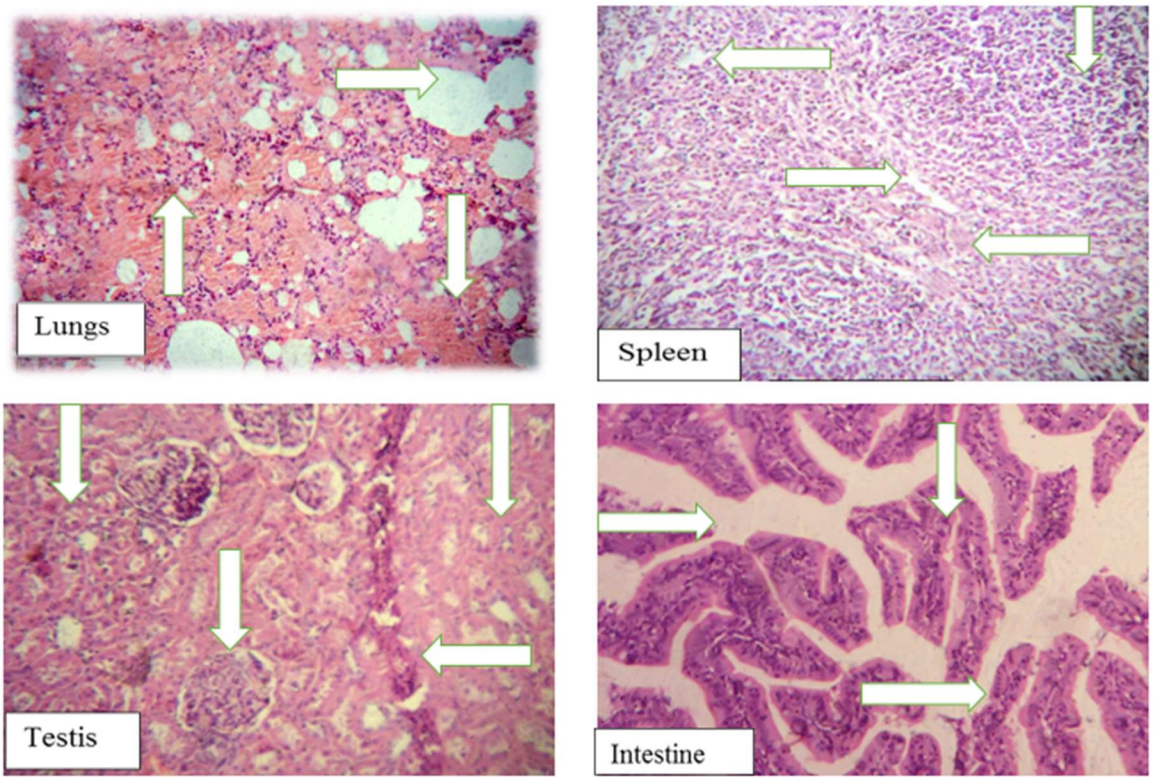


**Figure-1.** Photomicrographs denoting histopathological lesions in lungs, spleen, testis and intestine of amoxicillin treated male rabbits (dose @ 200 mg / Kg body weight) (400X, H&E stain).

## 4. Discussion

### 4.1. Hematological parameters of male rabbits

During our present study total white blood cells, granulocytes and neutrophils increased significantly in amoxicillin treated male rabbit groups in dose dependent manner when compared with compared with control group ($P < 0.05$) (Table-2). These clinical condition of increased total white blood cell, granulocytes and neutrophils are respectively referred to as leukocytosis, granulocytosis and neutrophilia. These clinical conditions indicate inflammation, injury of hematopoietic stem cells of WBCs, granulocytes, neutrophils or immune disorder due to amoxicillin treatment. These results can also be related to damage induced by amoxicillin to several important body organs as result granulocytes and neutrophils number increased to counter the damage. Similar to our current finding is reported in previous literature when rabbit were orally treated with neomycin (Aggag et al. 2022).

In current study we investigated significant decreased lymphocytes quantity in amoxicillin treated male rabbit groups as compared to control ($P < 0.05$) (Table-2). A condition that is called as lymphopenia which might be due to decreased production from lymphocyte hematopoietic stem cells or autoimmune disorder due to toxicity induced by amoxicillin exposure. Similar to our significant decreased lymphocyte number is reported in rabbit when they were orally exposed with neomycin at the dose rate of 50mg / 5ml water (Aggag et al. 2022).

In present study we investigated decreased monocytes production in amoxicillin treated male rabbit groups when compared with control rabbits ($P < 0.05$) (Table-2). This condition is referred to as monocytopenia caused by aplastic anemia probably due amoxicillin exposure. As a result of amoxicillin treatment monocyte were decreased which might be due to their decreased production from hematopoietic stem cells or their early bursting (breakdown).

In our recent study of hematological parameters, we observed significant decreased values of RBCs in amoxicillin treated male rabbit groups as compared to control ($P < 0.05$) (Table-2). This condition is called as anemia which might be due to hemolytic condition or their decreased production from their stem cells following amoxicillin exposure. In current study we observed decreased hemoglobin concentration in amoxicillin treated male rabbit groups as compared to control ($P < 0.05$) (Table-2). This indicates anemic condition which is due to decreased production of RBCs or their hemolysis results in decreased hemoglobin level due to amoxicillin exposure. It is evident from the current analysis that RBCs quantity and hemoglobin concentration are related to each other because RBCs contained hemoglobin on their surface (Aggag et al. 2022).

In our current study we investigated significant decreased values of mean corpuscular hemoglobin and mean corpuscular hemoglobin concentration in amoxicillin treated male rabbit groups as compared to control group ($P < 0.05$) (Table-2). This condition is called as hypochromic anemia which indicates iron deficiency in amoxicillin treated rabbit because of poorer iron absorption by the intestine from food content to meet the metabolic needs of body. It also might be due to breakdown of RBCs and hemoglobin consequently reduced MCH and MCHC values are reported. These finding are similar to previous study when male and female rabbits were treated with amoxicillin at various doses (Hussain et al. 2022).

**4.2. Serum-biochemical parameters of male rabbits**

In present study biochemical parameters such as blood sugar decreased significantly in amoxicillin treated male rabbit groups when compared with control group ($P < 0.05$) (Table-3). This condition is called as hypoglycemic condition which might be due to toxicity induced by amoxicillin treatment results in poor food intake or poorer absorption of food content i.e. glucose from gut due severe atrophy intestine. Similar to our result of decreased blood sugar level are reported in literature when common carp was treated with gentamicin (Aggag et al. 2022).

In current study we investigated significant decreased values of albumin in amoxicillin treated male rabbit groups when compared with control group ($P < 0.05$) (Table-3). Contrary to this result, significant increased albumin was found when they were treated rabbits with antibiotic monensin (Hashem et al. 2019). In present research study we observed significant decline in plasma fibrinogen and total protein in amoxicillin treated male rabbits ($P < 0.05$) (Table-3). These results are consistent with previous study when rabbit were treated with amoxicillin (Hussain et al. 2022). These results indicate liver damage because of toxicity induced by amoxicillin treatment. Due to amoxicillin toxicity liver is unable to maintain homeostatic range of plasma albumin, plasma fibrinogen and total proteins required for normal body functions.

In this study we observed significant increase in serum globulin, high density lipoprotein, low density lipoprotein, triglycerides and cholesterol in amoxicillin treated male rabbit groups

when compared with control group rabbit ($P < 0.05$) (Table-3). Similar to our result of increased serum globulin are observed when rabbits were treated with different concentration of chick egg lysozyme (LZM) (El-Deep et al. 2020).

In our present study we observed increased values of high density lipoproteins, low density lipoproteins, triglycerides and cholesterol. These values are contrary to El-Deep et al. as they investigated when they treated rabbits with LZM (El-Deep et al. 2020). These results indicate liver damage because of amoxicillin treatment. That's why liver is unable to maintain homeostatic range of serum globulin, HD lipoproteins, LD lipoproteins, triglycerides and cholesterol required for body metabolism.

In our current study we investigated that urea, uric acid and creatine increased significantly in amoxicillin treated male rabbit groups ($P < 0.05$) (Table-3). Similar to our findings are reported in literature when rabbits were given subcutaneous injection of amoxicillin for 14 days (Olaniyan & Fowowe 2020). Significant increased results of these renal parameters shows that amoxicillin induced toxicity causes renal damage in terms of renal injury and nephrotoxicity that's why kidney is unable to perform its functions like clearing, cleansing and removing nitrogenous waste products from blood that's why the level of urea, uric acid and creatine deviates from their homeostatic range required by the body to function normal.

**4.3. Antioxidants and liver enzymes of male rabbits**

In this present study we observed increased malondialdehyde concentration in amoxicillin treated male rabbit groups when compared with control group ($P < 0.05$) (Table-4). Malondialdehyde concentration is an oxidative stress parameter which is produce during peroxidation of polyunsaturated fatty acids. Its overproduction occurs due to the presence of excess free radicals in the body. Increased MDA level causes oxidative damage to vital organs such as brain. Contrary MDA result are observed when *Nigella sativa* seed were fed to rabbit (El-Gindy et al. 2020). In present investigation we observed significant decrease values of peroxidases, catalases, reduced glutathione (GSH) and superoxide dismutase (SOD) in amoxicillin treated male rabbits ($P < 0.05$). These results are contrary to Singh et al. when they feed rabbits with melon seed oil as supplementary diet and found no significant difference in SOD, peroxidases, GSH and catalase (Singh et al. 2021). Similarly, contrary to our results significant increased total antioxidant capacity was observed in monensin treated rabbits (Hashem et al. 2019).

AST, ALT, ALP and LDH are used to measure hepatitis, hepatic injury induced by toxicants such as drugs and are also used in the estimation of liver health (Olaniyan & Ateni 2018). During this study we investigated liver enzyme like alanine aminotransferase, aspartate transaminase, alkaline phosphatase, lactate dehydrogenase and bilirubin were increased significantly in amoxicillin treated male rabbits ($P < 0.05$) (Table-4). These results indicate possible liver injury and leakage of liver enzymes form injured hepatic cells due to amoxicillin treatment as AST, ALT, ALP and LDH level in blood serum increased beyond normal range required for liver to perform its normal function. Increased bilirubin level which is due to increased hemoglobin breakdown in liver indicates amoxicillin toxicity in male rabbits. Similar to our

findings of increased liver enzyme values are reported by Olaniyan & Adepoju when they exposed rabbits with amoxicillin and raw cucumber juice as supplementation (Olaniyan & Ateni 2018).

**4.4. Histopathological studies in male rabbits**

In present toxico-pathological study, we observed congestion, serous exudation, pulmonary edema, leukocyte infiltration, lungs inflammation, interstitial pneumonia, emphysema, interstitial pneumonia and emphysema in the lungs of amoxicillin treated rabbits (Table-5 and Figure-1, lungs). These lungs histopathological lesions are in accordance with Patel et al. when they exposed rats with various doses of methotrexate and found mild to moderate congestion, emphysema, alveolar hemorrhages, alveolar distension and alveolar dilation (Patel et al. 2014). Contrary findings are investigated in previous research when rat and mice were exposed with variable doses of indomethacin and found histopathological lesions such as congestion and hemorrhage in blood vessels of lungs (Olusegun Taiwo & Lawal Conteh 2008).

In recent pathological study we investigated splenic atrophy, red pulp pigments, fibrosis, neoplastic, congestion, splenic necrosis, lymphoid necrosis, red pulp hyperplasia, white pulp hyperplasia and splenomegaly in the spleen of amoxicillin treated rabbit groups (Table-5 and Figure-1, spleen). Our results are somehow related with Patel et al. when they exposed rats with various doses of methotrexate and found mild congestion, enlarged spleen and presence of hemosiderin pigments in spleen (Patel et al. 2014). Contrary findings are investigated in previous study when rat and mice were exposed with variable doses of indomethacin and found histopathological lesions such as splenic fat, congestion and hemorrhage in blood vessels of the spleen (Olusegun Taiwo & Lawal Conteh 2008).

In recent histopathological investigation we observed vacuolation, atrophic seminiferous tubules, arrested spermatogenesis, necrotic cells in lumen, aggregated spermatozoa in lumen, seminiferous epithelium vacuolization, decreased spermatozoa and vascular congestion in the testes of amoxicillin treated rabbit groups (Table-5 and Figure-1, Testis). These testicular pathological results are mostly contrary with Patel et al. when they exposed rats with various doses of methotrexate and found swollen, bulging, mild atrophic seminiferous tubules and moderate sloughed germ cells in testes (Patel et al. 2014). Our findings are somehow related to as reported by Taiwo et al. when they exposed rat and mice with variable doses of indomethacin and found histopathological lesions such as testicular degeneration, congestion and hemorrhage of blood vessels of the testes (Olusegun Taiwo & Lawal Conteh 2008).

In present histopathological analysis we observed degenerative epithelium, decreased villus height, decreased villus length, villus width, villus atrophy, congestion, vacuolation and sloughing of epithelial cells in the intestine of amoxicillin treated rabbit groups (Table-5 and Figure-1, intestine). Similar to our findings are investigated by Patel et al. in methotrexate treated rats and found mild mucosal congestion, desquamation, denudated villus epithelium, erosions, necrosis and neutrophilic infiltration in the intestine of treated rats (Patel et al. 2014). Similar findings are reported by Taiwo et al. when they exposed rat and mice with variable doses of indomethacin and found histopathological lesions such as congestion, hemorrhage, glandular

degeneration, necrosis, erosions, ulcerations in the intestines of treated animals (Olusegun Taiwo & Lawal Conteh 2008).

**Conclusion**

Conclusively, amoxicillin induces metabolic dysfunction and suppress immunity due to its toxico-pathological effects on vital organs like lungs, spleen, testes and intestine of the male rabbits. Therefore, amoxicillin monitoring is required to reduce its adverse effects on public health.

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