



Evaluation of Histopathological Effects of Contaminated Unexpired Gentamicin Injections on the Kidney Tissues of Juvenile Wistar Rats

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Abstract

The histo-pathological effects of contaminated gentamicin injections on the kidney tissues of juvenile wistar rats have been investigated using standard microbiological and histo-pathological techniques. Freeze-dried and heat fixed thin sections of wistar rats' kidney were observed under the microscope using x10, x40 and x100 magnifications. The results revealed marked necrosis and erosion of proximal borders especially the focal epithelium of proximal convoluted tubules (PCT) of the kidney, with the retention of the cyto-architecture. Plasmolysis was observed in the epithelial cells of the distal convoluted tubules (DCT) when treated with contaminated unexpired gentamicin injection sample as compared with control. Glomerular hypoplasia and fibrosis of the Bowman's space occurred in the kidney when compared with the control. Also medulla interstitial oedema leading to focal tubular fibrosis as a result of microbial activity was observed when compared with control. Interstitial hemorrhage which led to complete tubular necrosis with loss of defined tubular cyto-architecture was observed. This effect made the tissue fibrous, resulting in a non-functional or shrunken kidney as compared to control. Results obtained from this work have raised serious health concerns, considering the risk posed by contaminated drugs on patients.

Keywords: Histo-pathological, kidney tissues, Gentamicin, contamination, unexpired.

Introduction

Compounding of an elegant, safe, stable, potent and efficacious pharmaceutical product which is acceptable to the patients, requires the use of a wide range of ingredients in a complex form^[11]. This aspect has created conditions conducive to the survival and even extensive replication of contaminant microorganisms that might enter the product during manufacture or use by patient^[3] and^[9]. The terrestrial range of microorganisms

show such metabolic versatility that almost any formulation ingredients, from simple sugars to complex aromatic molecules or organic substances may undergo chemical modification by suitable organisms, thus, leading to spoilage of the product^[1]. Similarly, poor packaging materials and preservatives have been reported to be easily degradable by these organisms. Also, microorganisms have been reported as a source of threat to these pharmaceutical products, which

may be contaminated by these organisms, if not properly compounded and handled, leading to the deterioration of these products^[11]. This contamination may result in loss in potency of the pharmaceutical product and/or even initiate infections in the user^[2]. and^[10]. These contaminants include true pathogens to a collection of opportunistic pathogens (*Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Salmonella* sp, *Clostridium tetani*, *Serratia* sp, *Klebsiella* sp and coliforms)^[11].

According to^[1], in-hospitable environments such as tablets, disinfectants, antiseptics, powders, and other products have been reported to be at risk to contaminating microbes. Similarly, products with nutritious components, such as creams and lotions with carbohydrates, amino acids, vitamins and often appreciable volume of water are also susceptible to deterioration^[2]. It has been observed that, the physical and chemical status of a pharmaceutical formulation influences considerably the type and extent of microbial spoilage it is at risk^[4]. Sterile and self-sterilizing products have been shown to have low levels of contaminants without multiplication or with minimal multiplication during intended shelf-life of the product^[7]. Similarly, toxic metabolites have been observed to persist even after removal of microorganisms that were originally present^[6]. It has been observed that medicament-borne diseases could spread for some time, before it is properly diagnosed^[12].

According to^[2], treatment of patients with contaminated materials is bad in principle. The question is, 'how much harm is actually done?' This largely depends on the route of administration. This study was aimed at evaluating the histopathological effects of contaminated unexpired gentamicin injection on the kidney tissues of juvenile Wistar rats.

Materials and Methods

Collection and transportation of samples: Three (3) unexpired drug samples (Gentamicin injections) of same make and active ingredient

were obtained from patent medicine stores in Calabar, Nigeria. These samples were transported aseptically in sterile bags to the Department of Microbiology laboratory in the University of Calabar for analysis.

Media and reagents used: Media and reagents used in this study were of international standard, media were mostly Oxoid products (Oxoid, Basingstoke, UK).

Methods

The samples were processed aseptically using standard microbiological and histopathological techniques. Ethical conditions were strictly observed.

Toxicity test: Toxicity of the drugs was determined using the method described by^[6] and^[5].

A) Animals used: Adult wistar rats weighing between 100g and 250g were bred locally in Microbiology Department, University of Calabar. There were three(3) animals per cage and these animals were fed with standard rat chow (Sunshine livestock Feeds Plc Nigeria) and 65cL tap water supplied to each cage daily, with 12h light-dark cycle exposure. Environmental temperature range was 20⁰C (night) to 30⁰ C (day). Standard protocols for the use of animals for toxicological experiments were observed.

B) Preparation and administration of drugs: Drug samples (contaminated unexpired gentamicin injections), standard gentamicin injection (positive control) and water for injection (negative control) were used for the study. The active ingredient was gentamicin 80mg. Gentamicin was administered at 58mg per kilogramme rat body weight, intramuscularly two times daily at 6h divided doses for 5days using a 2mL sterile syringe.

C) Assessment of toxicities: Animals body weight and locomotor activity were monitored on days 1, 3 and 5 of administration of injection. On each day the animals were carefully observed for general pharmacological and toxicological signs of lethargy, morbidity, mortality, food consumption rate, haematological and blood

chemistry measurements as described by^[8] and^[13]. Animal locomotion was recorded for 30minutes each day of the monitoring by a sensitive electronic locomotor metre (40fc, Motron Products, Sweden). Diet consumption and systemic shocks were also monitored. On the 5th day, animals were anaesthetized with Bouin's fluid-soaked cotton wool inside a closed desiccator for 5minutes. The animal became drowsy and sober. They were later sacrificed after 2 days from the last drug administration and dissected longitudinally. Five milliliters blood samples were collected from the carotid artery cannulation into blood sample bottles. Blood samples in five bottles were mixed with 0.5mL of 3.8% Sodium citrate and used for haematological analysis. The stomach and intestines were washed with 10% formaldehyde saline and examined for lesions using a magnifying glass attached to a dissecting fluorescent lamp (Thousand and one lamps, England).

Histopathological analysis: Histo-pathological analysis was carried out on wistar rats' kidneys treated with contaminated unexpired Gentamicin

injection according to the method described by^[13]. This organ (kidney) was freeze-dried and heat fixed in wax. After which it was cut into thin sections by a microtome machine. These thin sections were viewed under the microscope using X10, X40 and X100 lenses. The images observed were made into slides and snapped, using specialized software known as Motic Image Plus 2.0. Kidney slides were made up of kidney medullar, cortex, glomerular and convoluted tubules.

Results

Table 1 Results of the mean viable counts of microbial isolates from unexpired contaminated gentamicin injections from patent medicine stores. From this table, the mean heterotrophic aerobic microbial count showed a range from $0.63 \pm 0.76 \times 10^4$ cfu/mL to $2.50 \pm 0.08 \times 10^4$ cfu/mL, the mean coliform count ranged from $0.54 \pm 0.27 \times 10^4$ cfu/mL to $1.58 \pm 0.55 \times 10^4$ cfu/mL and the mean yeasts count ranged from 0 to $0.14 \pm 0.10 \times 10^4$ cfu/mL respectively.

| Sampling point | Drug Status | Total No. of Samples Analysed | Microorganisms (X10 ⁴ CFU/ml/mg) | | |
|-----------------------|-------------|-------------------------------|---|----------------|-------------|
| | | | heterotrophic Count | Coliform Count | Yeast Count |
| Patent Medicine Store | Unexpired | 5 | 2.50±0.08 | 1.158±0.55 | 0.14±0.10 |
| | | | 0.63±0.76 | 0.54±0.27 | 0 |

Table 2: Biochemical characterization and identification of isolates from contaminated unexpired gentamicin injections obtained from patent medicine stores is presented in Table 2. The results obtained from this study showed the presence of the following bacterial and fungal isolates: *Pseudomonas aeruginosa*, *Bacillus subtilis*, *Escherichia coli*, *Staphylococcus aureus*, *Candida albicans* and *Aspergillus niger* respectively.

2a

| Sample code | Cell Morphology | Colonial Pigmentation | Gram Stain | Catalase | Coagulase | Oxidase | Citrate utilization | Mannitol | Glucose | Lactose | Urease | ONPG | Spore Formation | Voges Proskauer | Methyl red | Starch hydrolysis | Casein hydrolysis | Motility | Nitrate reductase | Indole production | Most probable organism/inference |
|-------------|-------------------|---------------------------|------------|----------|-----------|---------|---------------------|----------|---------|---------|--------|------|-----------------|-----------------|------------|-------------------|-------------------|----------|-------------------|-------------------|----------------------------------|
| 01 | Short rods | Yellowish green | - | + | | + | + | - | - | - | - | - | - | - | + | - | | + | + | - | Pseudomonas Aeruginosa |
| 02 | Long rods | White | + | + | | + | - | | | | | | + | + | | + | + | | | - | Bacillus subtilis |
| 03 | Short rods | Red colonies or Mackonkey | - | + | | - | + | + | + | + | - | + | - | - | + | - | | + | + | + | Escherichia coli |
| 04 | Cocci in clusters | Yellow or White Cream | + | + | + | + | + | AG | AG | AG | AG | | | | + | | | | + | | Staphylococcus aureus |

2b

| Sample Code | Colonial Pigmentation | Substratum Colour | Nature of somatic hyphae | Nature of spores | Nature of production hyphae | Special vegetative structure | Budding | Vesicle shape | Germ tube Formation | Somatic nature | Type of productive spore | Growth on liquid (YEDP) Medium | Most Probable Organism/ Inference |
|-------------|-----------------------|-------------------|--------------------------|------------------|-----------------------------|------------------------------|---------|---------------|---------------------|----------------|--------------------------|--------------------------------|-----------------------------------|
| 06 | Creamy | - | Pseudo hyphae | Chlamydo spore | | | + | Oval | + | - | Chlamydo phore | Film | Candida albicans |
| 07 | Black | Brown | Deprate | Conida | Conidiophore | Footcell | | Globose | - | filament | Conidia | - | Aspergillus niger |

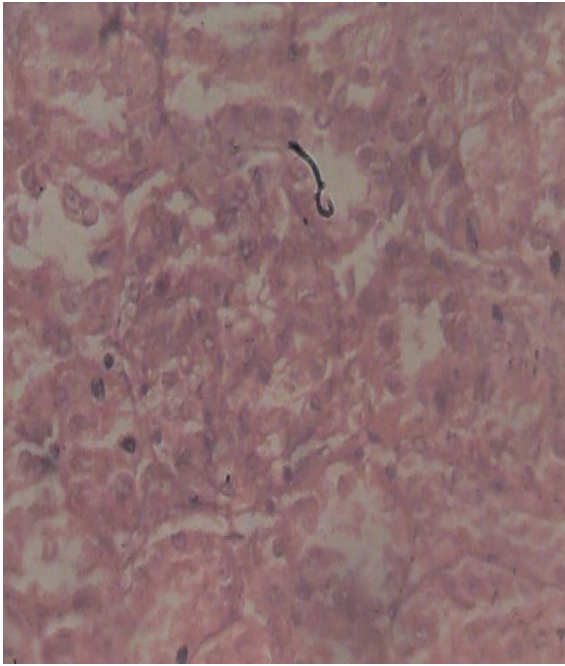


Plate 1: Histopathological slide results of thin section of kidney cortex treated with unexpired contaminated gentamicin injection viewed under x40 lens of the microscope. The results revealed glomerular tissue hypoplasia with focal areas of necrosis. There were capsular fibrosis and proximal convoluted tubular necrosis. There were also interstitial haemorrhage which caused expansion of blood vessels and also segmental glomerular necrosis. Bowman's capsular fibrosis. No inflammatory cells seen.

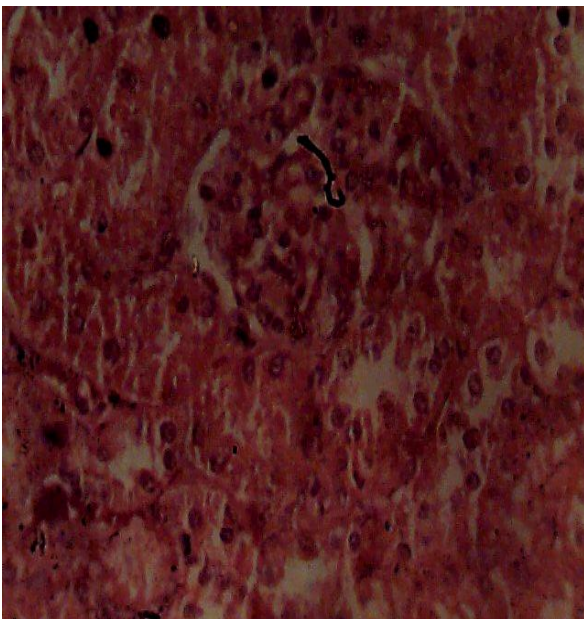


Plate 2: Histopathological slide result of thin sections of kidney medulla treated with unexpired contaminated gentamicin injection

viewed under x40 lens of light microscope. Tubular epithelial necrosis and bigger, thicker and swollen cells leading to interstitial oedema were observed. Also pan tubular necrosis with swollen interstitial were present. Renal tissues showing pan tubular necrosis with loss of defined tubular cyto-architecture were observed. Diffused necrosis with all tubules being damaged leading to the kidney becoming fibrous was observed.

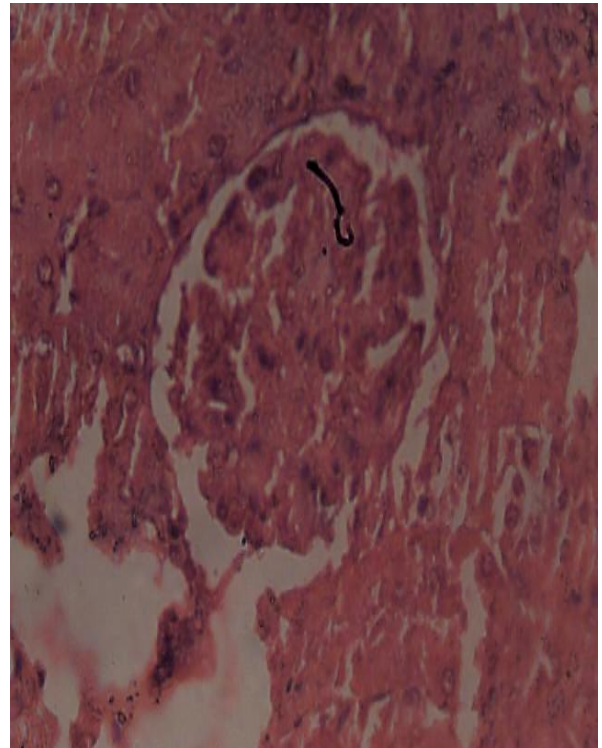


Plate 3: Histopathological slide results of thin section of kidney proximal borders treated with unexpired contaminated gentamicin injection viewed under x40 lens of light microscope. Results showed erosion of the proximal borders of the kidney. The proximal convoluted tubes (PCT) showed brush border erosion. In the cortex, the proximal convoluted tubules had focal epithelial (areas) necrosis though the outlines or cyto-architecture of the tubes were retained. In the distal convoluted tubes (DCT), plasmolysis (i e, epithelial cell shrinkage) were observed. Similarly, in the renal corpuscles, glomerular hypoplasia (or few cells) as compared with normal or control were present. There were fibrosis of urinary space, where fibrous tissue had gone into the bowman's space to replace it.

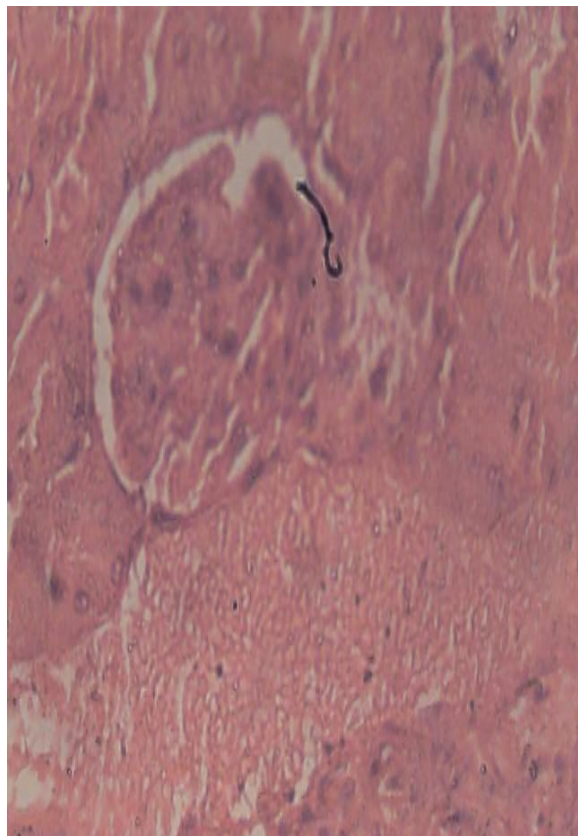


Plate 4: Histopathological slide results of thin sections of kidney medullar treated with normal unexpired gentamicin injection viewed under x40 lens of light microscope. Results showed normal medullary features in the renal tissues. The medullary tubes and tubules were normal, with the retention of the cyto-architecture. The glomerular was normal, with regular shaped- cells, Cortex, convoluted tubules (proximal and distal) and the bowman's space did not show any abnormalities. This plate served as control.

Discussion and Summary

The histopathological effects on wistar rats' kidney treated with contaminated unexpired gentamicin injection have been investigated using standard microbiological and histopathological techniques. Results of Mean viable counts of microbial isolates from contaminated unexpired gentamicin injections as shown in Table 1 were: mean heterotrophic count- $2.50 \pm 0.08 \times 10^4$ cfu/ml as compared with control, mean coliform count $1.58 \pm 0.55 \times 10^4$ cfu/mL and mean mold/ yeasts counts $0.14 \pm 0.14 \times 10^4$ cfu/mL on the nutrient agar for bacteria and sabouraud dextrose agar for

fungi/yeasts respectively. These results agreed with that reported by^[4] and later^[13] where it was observed that sterile drugs meant to be without any microorganisms were contaminated and even degraded when not properly compounded and stored, these results suggest a breakdown in manufacturing practice and storage conditions. The microorganisms isolated were identified and reported in Table 2 as *Pseudomonas aeruginosa*, *Bacillus subtilis*, *Escherichia coli*, *Staphylococcus aureus*, *Aspergillus niger* and *Candida albicans*. These organisms have been implicated in drug spoilage and contamination as reported by^[9] and^[11], where, it was observed that, most of the organisms utilize the active ingredients of these drugs as carbon source and food substrate. The results of the histopathological effects on wistar rats' kidney on treatment with contaminated unexpired gentamicin were presented in Plates 1-3. Plate 4 served as control. The results revealed marked necrosis of proximal border, especially, the focal epithelium of the proximal convoluted tubules of the kidney, though the cyto-architecture was retained. These results agreed with that obtained by^[7], where it was observed that toxicological impacts of drug contaminants may adversely affect the organs of the patient on usage. Similarly, plasmolysis was observed in the epithelial cells of the distal convoluted tubules. This was also reported by^[12] in India. Glomerular hypoplasia and fibrosis of the Bowman's space occurred in the kidney when compared to the control. Also medulla interstitial oedema leading to focal tubular fibrosis were observed, this may be as a result of microbial activity. This result agreed with that obtained by^[4], who observed that administration of contaminated parenteral products could lead to tissue damage or adversely affect organs or even cause death of patients who use these products. Similarly, interstitial haemorrhages were observed on administration of contaminated unexpired gentamicin injection as compared with the control. This effect led to complete tubular necrosis with loss of defined tubular cyto-architecture, making the tissues

fibrous, resulting in non-functional or shrunken kidney as compared to controls. These effects raise serious health concerns, considering the risk posed by contaminated drugs on patients.

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