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RESEARCH ARTICLE

EVALUATION OF TOXICOLOGICAL EFFECTS OF CONTAMINATED GENTAMICIN INJECTION ON LIVER ENZYMES OF JUVENILE WISTAR RATS

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ABSTRACT

The toxicological effects of contaminated gentamicin injections obtained from patent medicine stores on the liver enzymes of wistar rats after intramuscular administration have been investigated. Six(6) adult wistar rats weighing between 100g-250g were used. These animals were kept two(2) per cage/group(A, B, and C), where group A was fed with 0.1mL water for injection, and also served as control. Groups B and C were the experimental groups and administered 58mg of gentamicin injection intramuscularly per rat/day at 6hr divided doses twice daily for 5 days. The effects of contaminated gentamicin injection on levels of serum parameters such as aspartate aminotransferase(AST), alanine aminotransferase (ALT) and alkaline phosphatase(ALP) enzymes were compared with control. The level of ALP was significantly high in the experimental groups(B and C) at $P<0.05$ than control(A). There was a significant difference at $P<0.05$ in the mean levels of ALP as compared with the control. Similarly, significant elevation in mean levels of ASP and AST enzymes were observed at $P<0.05$ after treatment with gentamicin injection as compared with control in groups B and C respectively. Estimation of protein plasma concentration revealed hepatocellular damage, followed by a drop in plasma albumin and a differential increase in the globulin fractions, especially in r-globulin as compared with control at $P<0.05$. Similarly, the total and direct bilirubin revealed a morderate deviation at $P<0.05$ from the control. Similarly, estimation of the toxic effects of gentamicin injection on rat tissues revealed significant alterations as compared to the control. Blood parameters such as erythrocytes, Platelets and MCHC were not adversely affected when compared with control at $P<0.05$. This study has revealed that contaminated gentamicin injection could adversely elevate liver enzyme levels and induced hepatocellular toxicity. These effects raise serious health concerns for patients considering its route of administration.

Keywords: Gentamicin injection, Liver enzymes, wistar rats, contamination.

Statement of originality of work

The manuscript has been read and approved by all the authors, the requirements for authorship have been met, and that each author believe that the manuscript represents honest and original work.

INTRODUCTION

The formulation of an elegant product which is commercially acceptable with desirable efficiency, stability and patient acceptability can result in the incorporation of a wide variety of ingredients in a complex physical balance (Owu *et al*; 1998, Takon and Antai, 2006). This offers considerable potential for microbial attack and even extensive growth in the product (Takon *and* Antai, 2006). Pharmaceutical products are meant to be safe and potent during manufacture, storage and use (Takon and Antai, 2012). Microbial contamination could result in deterioration of these products, which in turn may lead to loss of potency or even initiate infection in the user (Takon and Antai 2013).

According to Ezejindu *et al*; (2013), treatment of patients with contaminated material is bad in principle. The question is 'how much harm is actually done?' This depends largely on the route of administration. The metabolic versatility of microorganism is such that almost any formulation ingredients, from simple sugars to complex aromatic molecules may undergo chemical modification by a suitable organism thus leading to contamination and spoilage of the product (Brooks *et al*., 2011). Toxic metabolites may persist even after removal of microorganisms originally present or where detectable physical and chemical changes have occurred in the product. (Maisanaba *et al*.,2014). Gentamicin injection is a sterile drug with gentamicin as its active pharmaceutical ingredient (British Pharmacopoeia, 1988).

The liver acts as a vast chemical factory with diverse vital functions (Jurczuk *et al*, 2004). It is responsible for the detoxification of many drugs and exogenous toxic chemical substances (Hariharakrishnan *et al*., 2009). It excretes the breakdown products of haemoglobin, which are principal constituents of the bile (Jayaraj *et al*., 2006). These widely varying functions are performed by parenchymal cells of uniform structure which contain many complex enzyme systems (Hariharakrishnan *et al*., 2009). Liver is also responsible for the inactivation of hormone, plays essential role in metabolism of protein, carbohydrate and fat, as well as the absorption and storage of vitamins, with the synthesis of prothrombin and other factors concerned with blood clotting (Owu, 1998).

Liver is seriously affected by drug use. It is directly responsible for the discharge of toxins from the body and blood purification (Takon *et al*., 2013). However, Liver cells cannot withstand the severe toxins effects for a long time without being destroyed (Wihastuti, *et al.*, 2015). These drugs affect the liver functions, damage it or both (Brooks *et al*., 2011). Some drugs may increase the levels of liver enzymes and cause liver damage with or without symptoms. These symptoms include jaundice, abdominal pain, itching and bruise or bleeding (Uemitsu *et al*., 1984). They are often

referred to as drug-induced liver injury (Wihastuti *et al*, 2015). Drugs are not only hepatotoxic, but are capable of enzyme inductions which may alter the liver's response to other exogenous agents. In Nigeria, a humid tropical developing country, most pharmaceutical preparations are frequently stored under uncontrolled conditions, this may adversely affect the active ingredients of these drugs (Takon *et al*., 2013).

This study is aimed at evaluating the toxicological effects of contaminated Gentamicin injection on liver enzymes of Wistar rats and to suggest possible ways of controlling drug contamination and the attendant health risk it poses.

MATERIALS AND METHODS

Sample Collection and Study Area

Drug samples were collected from some patent medicine stores in Calabar – Nigeria for analysis. These drug samples were gentamicin injection with gentamicin as the active pharmaceutical ingredient.

Media and reagents:

The media and reagents used were of microbiological standard- (Oxoid products).

Methods:

Processing of drug samples for microbiological analysis involved different aseptic bacteriological techniques.

Liver Function Tests

Hepatotoxicity level was assessed using the level of certain liver enzymes in the serum. The blood samples were centrifuged at 3000 rpm for 5min and the serum recovered was used for enzyme assay.

Estimation of total and direct serum bilirubin was carried out according to the method described by Hall, (2007) and Salawu *et al*. (2009). A deviation from the normal value (5-7µmol/L), was indicative of hepatotoxicity. Similarly, estimation of serum enzyme concentration was carried out using the method described by Tulsawani and Bhattacharya (2006). Also the Plasma protein concentration was determined using the method described by Bain *et al.* (1991).

Toxicity Test:

Toxicity of the drugs was determined using the method described by Ezejindu *et al* . (2013).

A. **Animals:** A total of six (6) adult Wistar rats weighing between 100g and 250g were bred locally in the Department of Microbiology, University of Calabar. The animals were kept two (2) per cage and fed ad-libitum with standard rat chow (Royal Livestock Feeds Plc, Nigeria) and 75cl tap water supplied to each cage daily with 12h light-dark cycle exposure, with environmental temperature range from 20°C (night) to 30°C (daytime) for acclimatization. Rats were handled according to standard protocols for the use of animals to toxicological experiments as described in Guide to the Use and Care of Laboratory animals (1996).

Treatment of Experimental Animals

The Animal Care Review Committee of University of Calabar, approved and reviewed the method used in the treatment of the experimental animals.

B. Preparation of Gentamicin Injection: Samples of gentamicin injections obtained from patent medicine stores were used in the analysis. Gentamicin injection purchased from University of Calabar Pharmacy served as positive control while water for injection served as negative control. Gentamicin injection was administered at 58mg per rat per day at 6h divided doses intramuscularly, two times daily for five (5) days, using disposable 2ml sterile syringe.

C. Assessment of toxicities: Body weights and locomotor activity of animals were monitored on days 1, 3 and 5 of administration. On each day, pharmacological and toxicological signs of lethargy, morbidity, mortality, food consumption rate, haematological and blood chemistry were measured. Also animals were observed for systemic shock. Sensitive electronic locomotor meter (40fL, motron products, Sweden) was used to record animal locomotion for 30min on each day, as described by Osim *et al*., (1996).

The animals were sacrificed after 2day from the last drug administration and dissected longitudinally, after being anesthetized with Bouin's fluid vapour soaked cotton wool in a dessicator container for 5min. The liver tissues were extracted and weighed.

Five millilitres blood samples were collected from the carotid artery cannulation into blood sample bottles and stored in a refrigerator for future use. Blood samples for serum preparation were collected into three sterile bottles without anti-coagulant. Blood samples were centrifuged at 3000rpm for 5min using a centrifuge to separate serum. Separated serum samples were stored at 4^0C for future use. The remaining blood sample was mixed with 0.5ml of 3.8% sodium citrate and used for haematological analyses. The activities of serum alkaline phosphatase (ALP), alanine aminotransferase (ALT) and aspartate aminotransferase (AST) enzymes were determined using randox kit methods.

Platelet count (PC), white blood counts (WBC) and packed cell volume (PCV) were determined using Bain *et al*, (1991) method. Dissecting fluorescent lamp (Thousand and One lamps, England), was used to examine stomach and intestinal lesions after washing with 10% formaldehyde saline.

Statistical Analysis: One way analysis of variance **(**ANOVA**)** was used in this study to determine the effect of contaminated gentamicin injection on liver enzymes of Wistar rats**.** Statistical Product and Services Solutions **(**SPSS**)** Software**,** version 20 **(**IBM**,** New York**)** was used for the data analysis**.** The data for the liver function tests were presented as the mean ±

standard error of the mean (SEM). And for animal weight as standard deviation (SD) and number of rats used per group (n). Data was compared using student t-test (Graph pad prism software, UK) $P<0.05$ was regarded as significant.

RESULTS

The results of the effects of contaminated gentamicin injection on liver enzymes of Wistar rats are presented in the following figures. Fig 1 showed the effects of contaminated gentamicin on total bilirubin assay of wistar rats. The effect was significant at $P<0.05$ when compared with the control. Similarly, Fig. 2 and Fig. 3 showed significant difference at $P<0.05$ for the effects of contaminated gentamicin injection on aspartate aminotransferase (AST) enzyme and alanine amino transferase (ALT) enzymes respectively, when compared with control.

DISCUSSION

The toxicological effects of contaminated gentamicin injection on the liver enzymes of some Wistar rats have been investigated. Gentamicin injection is an antibiotic that is meant to be sterile, the presence of microorganism and consequent contamination of this drug by microorganism in this study, affected the drug content and the active pharmaceutical ingredients as compared to control. The active ingredient was below the acceptable standard range as stipulated by the British Pharmacopoeia (1988). This reduction may have been enhanced by breakdown of substrate by aerobic organisms that grew and proliferate within a close system when not properly compounded, thus, lowering the oxygen tension to permit growth of anaerobes which spoil the drug products. Jurczuk *et al*., (2004) also reported similar observation. Metabolic by-products formed during multiplication of these organisms served as substrate for other organisms, which adversely affected the expected purity, quality and potency of the active ingredients of these drug samples. This opinion is shared by Maisanaba *et al*., (2014) they observed a reduction in expected quality, identity and purity of drug samples in their experiments.

Interstitial haemorrhage was observed on the administration of contaminated expired gentamicin injection, as compared with the positive control. This result is in agreement with that obtained by Osim *et al* (1996) and Owu *et al*, (1998) they observed that administration of contaminated parenterals or nutritional fluid samples could lead to tissue damage or even death in patients.

Estimation of the toxic effect of these contaminated drug samples on liver of Wistar rats revealed significant alteration at $P<0.05$ as compared to the control. Estimation of total and direct serum bilirubin, revealed a deviation from the control at $P\leq0.05$, the results were highly

significant. Similarly, estimation of the serum enzymes revealed hepatocellular damage accompanied by raised level of a number of enzymes, in particular, r-glutamyl transpeptidase, alanine aminotransferase (glutamic pyruvic transaminase) and aspartate aminotransferase (glutamic oxaloacetic transaminase). Alkaline phosphatase enzyme was moderately raised, which was indicative of obstructive lesion. The effect of contaminated gentamicin injection was significantly different at $P<0.05$ as compared with the control, on the three different liver enzymes. This result is in agreement with that obtained by Wihastuti *et al*., (2015), where it was observed that liver enzymes were affected by used of contaminated drugs.

Similarly, determination of the plasma protein concentration revealed hepatocellular damage followed by a drop in plasma albumin and a differential increase in the globulin fractions, especially in r-globulin, as compared with control. This result is in agreement with that observed by Takon *and* Antai, (2012), where they observed that contaminated drug products adversely affect blood parameters.

The statistical database of the haematological parameters of the Wistar rats revealed effect of the contaminated gentamicin injection on the haemoglobin (HGB) of these rats. There was no significant difference on the erythrocytic indices at $P\leq0.05$ between the three groups on administration of the drug samples. This result is indicative of the similarity of the haematological parameters, with slight difference in the packed cell volume (PCV), mean corpuscular haemoglobin concentration (MCHC) $P<0.05$ and red cell distribution width (RDW) $P\leq0.05$ as compared with control. This result differs from that obtained by Jayaraj *et al.*, (2006) who observed that, depending on the volume of contaminating organisms, there was little or no significant differences in the mean haematological parameters of some group of Wistar rats treated with the contaminated drug sample. This effect raises serious health concerns, considering the risk posed by contaminated drugs on patients.

CONCLUSION

This work has shown that the use of contaminated drug product, especially those meant to be sterile due to their route of administration, is dangerous to health with adverse effects on the patients. Medicines are essential part of human life and their safety is of utmost importance in providing pharmaceutical healthcare needs of patients, therefore sterile drug products should be monitored periodically and their statuses substantiated so as to avoid contamination, which may be fatal.

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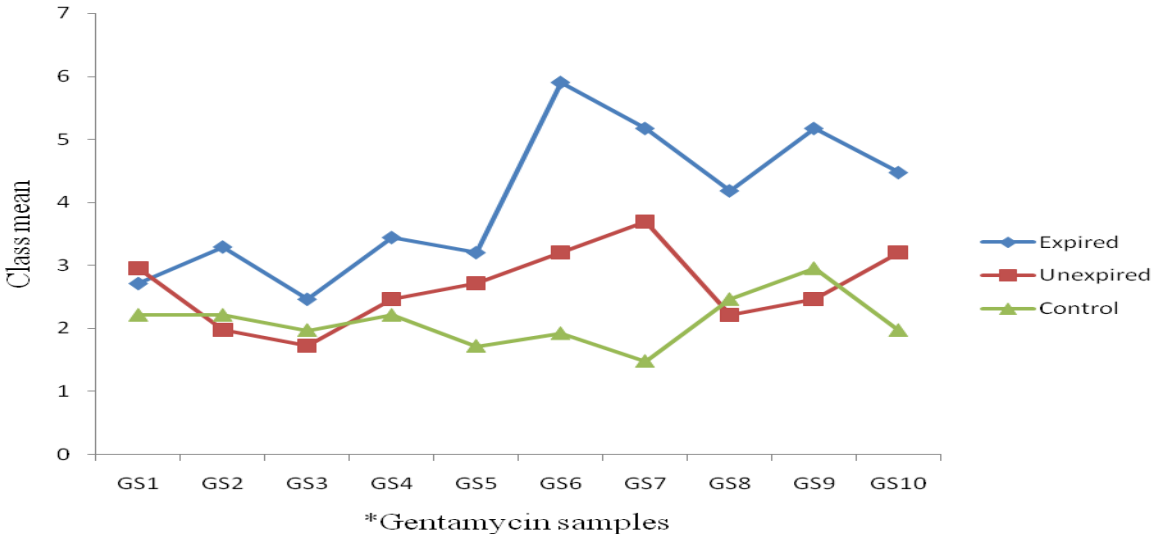

Fig 1: **Effects of contaminated gentamycin on direct bilirubin assay of wistar rats.**

***GS 1 – 10 = Gentamycin samples collected for each of expired, unexpired and control drugs.**

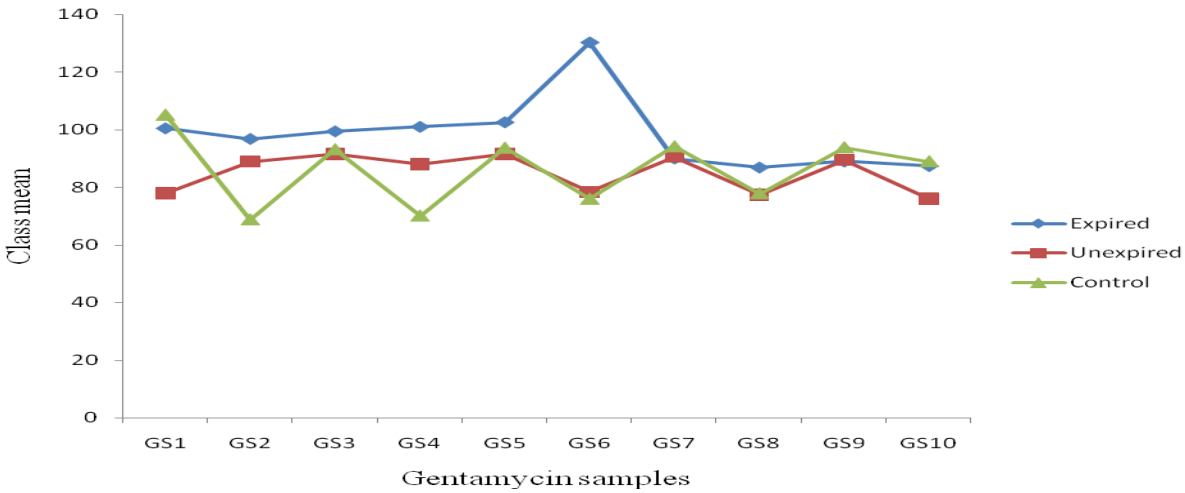

Fig 2: Effects of contaminated gentamycin on aspartate transaminase enzyme (AST)of wistar rat's liver.

***GS 1 – 10 = Gentamycin samples collected for each of expired, unexpired and control drugs.**

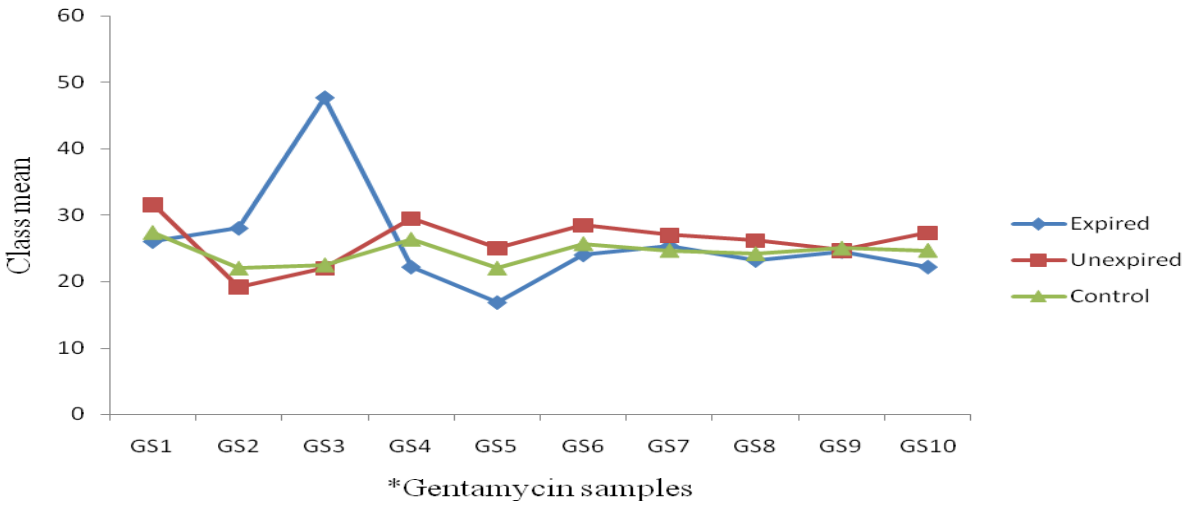

Fig 3: Effects of contaminated gentamycin on alanine aminotransferase enzyme (ALT) of wistar rat's liver.

***GS 1 – 10 = Gentamycin samples collected for each of expired, unexpired and control drugs.**

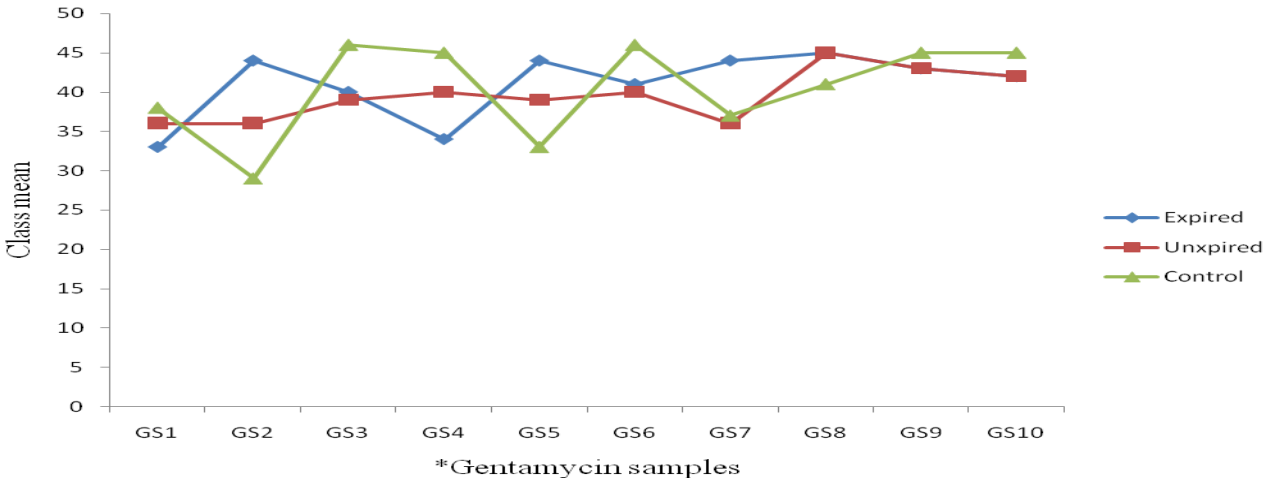

Fig 4: Effects of contaminated gentamycin on alkaline phosphatase enzyme (ALP) of wistar rat's liver.

***GS 1 – 10 = Gentamycin samples (expired, unexpired and control) drugs collected from study area.**