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Histological and Biochemical Effects of Chronic Chloramphenicol Administration on the Liver of Albino Wistar Rats.

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ABSTRACT

Chloramphenicol (CAP) is a broad-spectrum antibiotic affecting both Gram- positive and Gram- negative organisms. It is used in many parts of the world for the treatment of life-treating infections including typhoid fever and meningitis. It is a commonly abused drug in Nigeria. The effect of chronic administration of Chloramphenicol on the histology of the liver was investigated in rats. fifteen mature albino wistar rats weighing between 180g-302g were divided into three groups of five rats each. Group A was the control group and was administered with 1 ml of distil water, Group B was administered with 7.14 mg/kg body weight of chloramphenicol, Group C was administered with 14.29 mg/kg body weight of chloramphenicol. Drug administration was done twice daily for 4 weeks. On the 29th day, the rats were sacrificed using chloroform inhalation method, the livers were harvested and processed. The tissues sections were stained using haematoxylin and eosin staining technique. Blood samples were collected for liver function test; aspartate aminotransaminase (AST), alanine aminotransaminase (ALT), alkaline phosphotase ALP. Histological results revealed moderate to severe cellular distortion, with nuclear clumping, vascular congestion and degeneration, vacuolization, enlargement of the central vein, dilatation of the sinusoids and nuclei pyknosis in the treated groups, when compared with the control (Group A), biochemical analysis revealed increased levels of Alanine aminotransaminase (ALT) when compared with the control group. These results suggest that chronic administration of chloramphenicol is toxic to the liver and should be taken with caution.

Key words: Chloramphenicol, liver, biochemical, histological

INTRODUCTION

Chloramphenicol (CAP) is a broad-spectrum antibacterial drug that is widely used for topical application in ophthalmology and dermatology[1]. The drug has gained wide acceptance in the third world countries particularly, because it is cheap and effective[2]. Despite CAP's known haemotoxicity, it is used in the treatment of ampicillin-resistant *Haemophilius influenzae* infections[3], vancomycin-resistant *Enterococcus faecuium bacteremia*[4,5], and it is effective in the management of anaerobic infections of the central nervous system[6].

Choramphenicol is used extensively in non-industrialized countries for life-threatening infections because it is cheap and effective despite it known haematoxicity and linkage to fatal aplastic anaemia[7]. Because of its rare but severe side effects, this otherwise excellent drug is used only when there is no alternate antibiotic, and thus the benefits far overweigh the risks[8].

Chloramphenicol is a bacteriostatic antimicrobial drug that became available in 1949 and as it is both cheap and easy to manufacture, it is frequently an antibiotic of choice in the Third World. Due to resistance and safety concerns, it is no longer a first line agent for any infection in developed nations, although it is sometimes used tropically for eye infections. Nevertheless, the global problem of advancing bacterial resistance to newer drugs has led to renewed interest in its use[9]. Chloramphenicol represents the product of years of antibiotic development. Due to its pH, it shines above most antibiotics in terms of ability to penetrate into infected tissues and tissues with biological barriers. It can easily pass deeply through purulent material to the organisms hiding within, through cell membranes to attack parasites living within and into organs where other antibiotics cannot[10]. Chloramphenicol acts on the protein manufacturing system of bacteria yet it does not affect mammalian, reptilian or avian ribosomes. Highly susceptible bacteria are killed outright while others are merely rendered unable to divide and the host's immune system then destroys them upon discovery[10].

Despite the therapeutic use of Chloramphericol, some important adverse effects include; depression of bone

marrow, grey baby syndrome, diarrhea, flaccidity, toxicity, collapse, cyanosis, shock and abdominal distension[11]. Haemotoxic effects includes a reversible, dose-related reticulocytopenia and anaemia developing during treatment. Second, a non-dose-related aplastic anaemia, developing weeks after treatment, is often irreversible and fatal[12]. In most developing countries, the general approach to cushioning anticipated anaemic effect of CAP in man or animal, is by concurrent administration of multivitamins and haematinics. It is expected that increased delivery of newly formed red blood cells into the circulatory systems, as products from increased rate of haemopoiesis caused by multivatimin–haematinics will serve to reverse the state of anaemia precipitated by CAP[13].

Metabolism of CAP appears to play a pivotal role in its toxicity and reactive oxygen species (ROS) have been implicated[14]. The drug is known to undergo biotransformation by hepatic glutathione *S*-transferases (GSTs) to aldehyde derivatives, which in turn may generate free radicals due to oxidation by xanthine and aldehyde oxidases[14, 15]. CAP enabled the oxidative stress response of neutrophils and increased ROS production[16]. It has also been shown to inhibit drug metabolizing enzymes, affect antioxidant enzymes and hepatic esterases and amidases[2,17]. There has been increase in the use of this drug due to self medication, cheap cost of procurement and over the counter purchase of the drug for the treatment of typhoid fever and other bacterial infection in Nigeria. The objective of this study therefore was to investigate the effect of chronic administration of this drug on the histology of liver and liver enzymes in Wistar rats.

MATERIALS AND METHODS

Twenty (15) mature albino Wistar rats weighing between 180g-302g were divided into three groups of five rats each. Group A was the control group and was administered with 1 ml of distil water, Group B was administered with 7.14 mg/kg of chloramphenicol, Group C was administered with 14.29 mg/kg of chloramphenicol. Drug administration was done twice daily for 4 weeks. On the 29th day, the rats were sacrificed using chloroform inhalation method, the livers were harvested and the tissues sections were stained using haematoxylin and eosin staining technique. Blood samples from each rat were collected using syringes and needles and separated into sample bottles and allowed to stand for 30 minutes for clotting to take place and then centrifuged. The serum extracted into fresh test tubes and stored in a refrigerator for analysis of aspartate aminotransaminase test (AST), alanine aminotransaminase test (ALT), alkaline phosphotase ALP.

Measurement of alkaline phosphatase

This was by the optimized standard method recommended by the deutsche Geseiischage fur Klinishche Chemic GSCC (1972). P-nitrophenyl phosphate is hydrolysed to phosphate and p-nitrophenol in the presence of ALP. A calculated amount of sample 0.01 ml in a test tube was mixed with reagent (0.5 ml) containing the substrate p-nitrophenyl phosphate and brought to room temperature. The solution was mixed, initial absorbance read after 1 minute. The reaction was allowed to stand for 3 minutes and the absorbance read again at 405 nm. Alkaline phosphates activity was calculated from.

UL = 2760 x ΔAnm/minute micro

UL = Unit of aklaine phosphatase affi nity

ΔA = Change in absorbance

Measurement of alanine and aspartate transferase

The measurement of AST and ALT activities in the serum were done using endpoint colorimetric-diagnostic kit (Randox; Labouratories UK) based on Reitman and Frankel (1952)10 method.9,10 The pyruvate produced by transamination reaction between L-alanine andketoglutarate reacts with 2, 4, dinitrophenyl hydrazine to give a coloured hydrazone, which represents alanine aminotransferase activity. The oxaloacetate hydrazone formed with 2, 4 dinitrophenyl hydrazine is used to measure
aspartate aminotransfarase (AST). Both AST and ALT were read at 540 nm wavelength.

All results were analyzed using one way Analysis of Variance (ANOVA) and post hoc test.

RESULTS

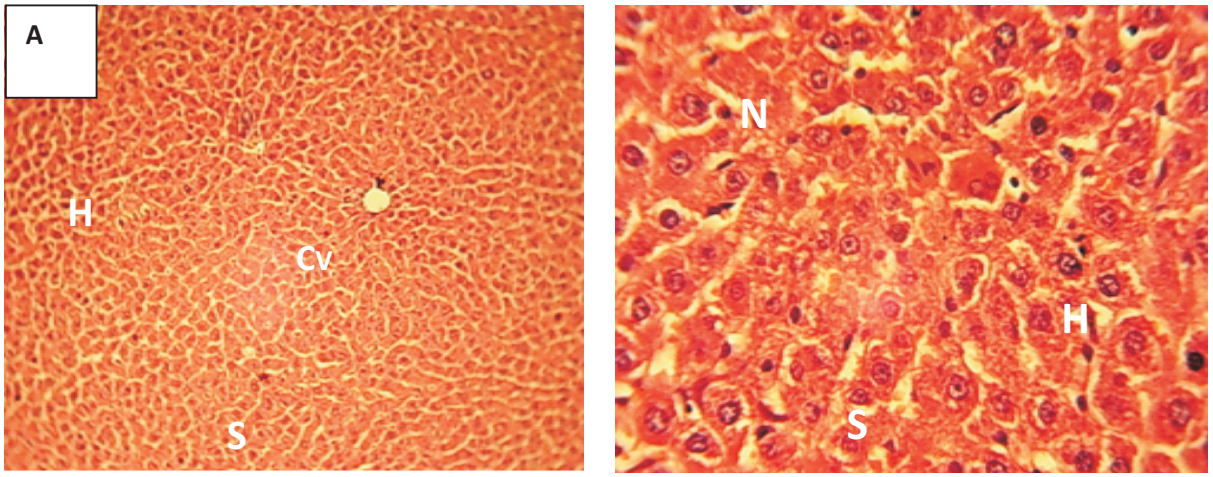

Plate 1: Photomicrograph of the histology of the liver of group A showing the normal liver architecture; the central vein (V), hepatocytes plates (H) and sinusoidal spaces (S) are well shown. The nuclei (N) are also normal H & E, × 100 and X 400

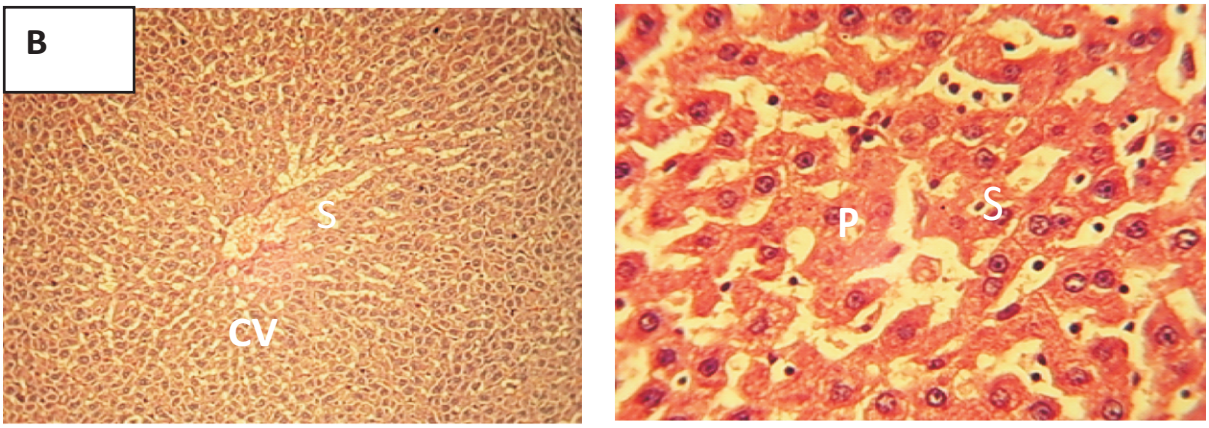

Plate 2: Photomicrograph of the histology of the liver of group A treated with 7.14 mg/kgl showing mild distortion of liver cellular architecture; the central vein (V) are dilated , hepatocytes plates (H) are shrunken with cellular degeneration (CD) and sinusoidal spaces (S) dilation with pyknotic nuclei (P) and karyorrhexis H & E, × 100.

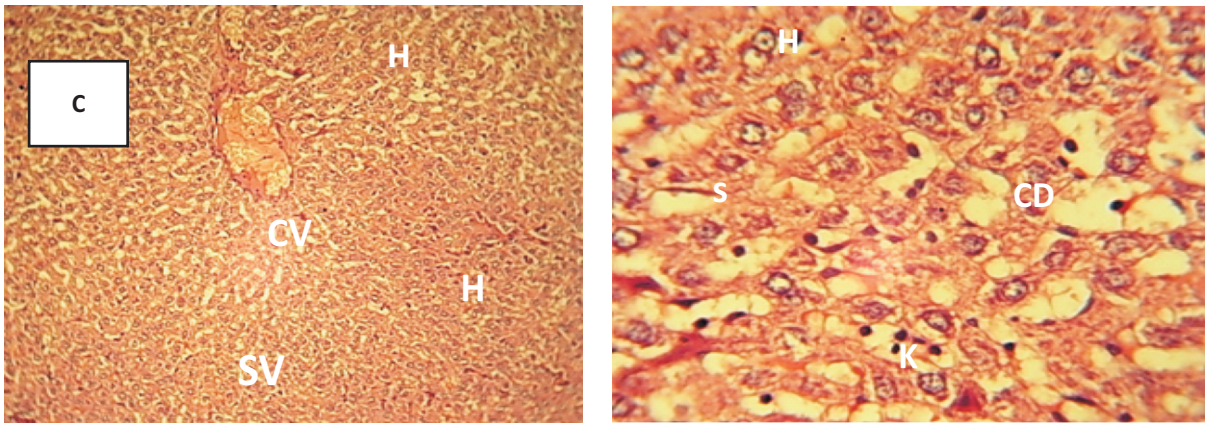

Plate 3: Photomicrograph of the histology of the liver of group C treated with 14.29 mg/kg showing moderate distortion of liver cellular architecture; the central vein (V) are dilated, , hepatocytes plates (H) are shrunken with cellular degeneration (CD) and sinusoidal spaces (S) are dilated with fragmented pyknotic nucleus and karyorrhexis (K)when compared with the control group A. H & E, × 100.

Table 1: Levels of AST, ALT and ALP in the Serum of Wistar Rats

Group(s)	AST Mean ± SEM	ALT Mean ± SEM	ALP Mean ± SEM
1	100.28 ± 6.82	21.80 ± 1.07	58.2 0 ± 2.67
2	88.20 ± 5.89 ns	21.20 ± 0.89 ns	57.60. ± 2.93 ns
3	77.00 ± 10.01ns	36.40 ± 2.62*	61.20 ± 1.80 ns

Values are mean ± of 5 rats in a group
ns = non- significant difference from control group (P>0.05)
* = significant difference from control group (P<0.1)

DISCUSSION

Chloramphenicol (CAP) is a broad-spectrum antibiotic affecting both Gram- positive and Gram- negative organisms. It is used in many parts of the world for the treatment of life-treating infections including typhoid fever and meningitis[18].

The liver plays a major role in the metabolism of antibiotic drugs and this makes it vulnerable to various kinds of injuries including; neurotoxicity, bone marrow depression, leukemia, aplastic anemia and gray baby syndrome [19]

The results obtained from this study revealed that oral administration of Chloramphenicol had toxic effects on the liver, with moderate to severe distortion of liver cellular architecture; dilatation of the central vein and sinusoidal spaces with pyknotic nuclei changes when compared with the control group this findings corroborated with the work of earlier researchers who reported a significant reduction in epididymal sperm count and sperm motility with a discernable increase in sperm abnormalities in rats administered with chloramphenicol[20,21] . In *vitro* experiments, had shown that chloramphenicol had adverse effects on bone marrow cells. Chloramphenicol caused dose-related inhibition of erythroid and granulocytic colony forming units obtained from LAF_1 mice. The lowest concentration used (5 µg/ml) caused some degree of inhibition of erythroid cells, while the highest concentration (60 µg/ml) produced complete inhibition[22, 23]. Chloramphenicol toxicities may be due to its ability to inhibited DNA synthesis. *It may also be* associated with the oxidative stress through generation of free radicals and reactive oxygen species (ROS).[20] Saba *et al.,* found that prolonged administration of Chloramphenicol could cause liver and kidney damage[24] and Ahmadizadeh et al., reported that prololong administration of Chloramphenicol can cause liver and intestinal damage[25].

ALT and AST are liberated into the blood whenever liver cells are damaged and increased plasma enzymes activity is a sensitive index of hepatic damage.[26 ,27] Neither of these enzymes is specific to the liver but ALT occurs in much higher concentration in the liver than elsewhere[27]. Therefore, the increased serum ALT activity in this study more specifically reflects hepatic damage. [26, 27]This agreed with the histological findings which revealed liver damage.

In conclusion, chronic administration of Chloramphenicol leads to toxic changes in the histology and biochemical indices in the liver of Wistar rats. The cellular distortions were dose dependent. Therefore chloramphenicol should not be sold over the counter without medical prescription.

REFERENCES

1. *Popadic S, Popadic D, Ramic Z, Stojkovic MM, Trajkovic V, Milinkovic M. Chloramphenicol induces in vitro growth arrest and apoptosis of human keratinocytes. Cell Bio Toxicol., 2006;22:371–9.*
2. *Farombi EO, Adaramoye OA, Emerole GO. Influence of chloramphenicol on rat hepatic microsomal components and biomarkers of oxidative stress: protective role of antioxidants. Pharmacol Toxicol., 2002; 91:129–34.*
3. *Holt DE, Harvey D, Hurley R. Chloramphenicol toxicity. Adv Drug React Toxicol Rev., 1993; 12:83–95.*
4. *Lautenbach E, Schuster MG, Bilker WB, Brennan PJ. The role of chloramphenicol in the treatment of bloodstream infection due to vancomycin-resistant Enterococcus. Clin Infect Dis., 1998; 27:1259–65.*
5. *Ricaurte JC, Boucher HW, Turett GS, Moellering RC, Labombardi VJ, Kislak JW. Chloramphenicol treatment for vancomycin-resistant Enterococcus faecium bacteremia. Clin Microbiol Infect., 2001; 7:17–21.*
6. *Pari L, Velmurugan K. Protective and antioxidant role of caffeic acid against chloramphenicol-induced hepatotoxicity in rats. In: EddouksM (ed.). Advances in Phytotherapy Research. Research Signpost, Kerala, India. 2009; 79–89.*
7. Saba, A. B., Ola-Davies, O. E., Oyeyemi, M. O. and Ajala, O. O. The Toxic Effects of Prolonged Administration of Chloramphenicol on the Liver and Kidney of Rats. Afr. J. Biomed. Res., 2000; 3: 133-7.
8 Mark G. and Bill Trattler. Clinical Microbiology, 3rd ed. Med Master, Inc. Miami. 2001.

9 Falagas, M. E., Grammatikos, A. P., Michalopoulos, A. Potential of Old Generation Antibiotics to Address Current need for New Antibiotics. Expert Review of Anti-infective Therapy. 2008; 6(5): 593-600.

10. Marvistavet.Com. Body-Chloramphenicol.html. 2012 (Retrieved June 1 2015)

11 Range, M. P., P. K. Moore, M. M. Dale. Pharmacology (5th ed). United Kingdom. 2006

12 *Turton JA, Andrews CM, Havard AC, Williams TC. Studies on the haemotoxicity of chloramphenicol succinate in the Dunkin Hartley guinea pig. Int J Exp Pathol., 2002; 83:225–38.*

13 *Saba AB, Oyagbemi AA. The effect of multivitamin-haematinic complex on chloramphenicol-induced anaemia in rabbits. Int J Pharm., 2007; 3:411–5.*

14 *Holt DE, Ryder TA, Fairbairn A, Hurley R, Harvey D. The myelotoxicity of chloramphenicol: in vitro and in vivo studies: I. In vitro effects on cells in culture. Hum Exp Toxico., 1997; 16:570–6.*

15 *Holt DE, Hurley R, Harvey D. Metabolism of chloramphenicol by glutathione S-transferase in human fetal and neonatal liver. Biol Neonate. 1995; 67:230–9.*

16 *Paez PL, Becerra MC, Albesa I. Chloramphenicol-induced oxidative stress in human neutrophils. Basic Clin Pharmacol Toxicol 2008;103:349–53.*

17. *Farombi EO, Nwankwo JO, Wara SH, Odutola B, Emerole GO. Chloramphenicol and ampicillin-induced changes in rat hepatic esterase and amidase activities. Biosci Rep; 2000; 20:13–9.*

18 Ruef C, Blaser J. Miscellaneous antibacterial drugs: aminoglycosides, chloramphenicol and thiamphenicol, fluoroquinolones, glycopeptides. In: Dukes MNG, Aronson JK, editors. Meyler's side effects of drugs. The Netherlands: Elsevier Sciences. 2000; pp. 848–851.

19 Kshirsagar, A, YVetal, Y. Ashok, P. Bhosle, P. Ingawale , D. *Drug Induced Hepatotoxicity: A Comprehensive Review*. The Internet Journal of Pharmacology. 2008; 7(1). https://ispub.com/IJPHARM/7/1/3723 (Retrieved June 1 2015)

20 Oyagbemi, A. A., Adedara, I. A., Saba, A. B. and Farombi, E. O. Role of Oxidative Stress in Reproductive Toxicity Induced by Co-administration of Chloramphenicol and Multivitamin–Haematinics Complex in Rats. Basic & Clinical Pharmacology & Toxicology, 2010; 107: 703–708

21 *Oyeyemi MO, Adeniji DA. Morphological characteristics and haematological studies in wistar rats subjected to prolonged treatment of chloramphenicol. Int J Morphol., 2009; 27:7–11.*

22 Yunis, A.A. Differential *in vitro* sensitivity of marrow erythroid and granulocytic colony forming cells to chloramphenicol. *Am. J. Haemtol. 1977;* 2: 355-363.

23 Hara, H., Kohsaki, M., Noguchi, K., & Nagai, K. Effect of chloramphenicol on colony formation from erythrocytic precursors. *Am. J. Haematol. 1978;* 5: 123-130.

24 Saba AB, Ola-davies O, Oyeyem MO, Ajala O. The toxic effects prolonged administration of chloramphenicol on the liver and kidney of rats. Afr J Biomed Res ., 2000; 3:133–137.

25 Ahmadizadeh, M. Esmailpoor, M. and Goodarzi, Z. Effect of Phenobarbital on Chloramphonicol-Induced Toxicity in Rat Liver and Small Intestine, Iran J Basic Med Sci. Dec., 2013; 16(12): 1282–1285.

26. Edwards CRW, Bouchier IAD, Haslett C and Chilvers EE. Diabetes Mellitus in Davidson's Principle and Practice of Medicine (10th Edition). Churchhill Livingstone, London. 2008; 724–774.

27. Crook, M. A. Clinical Biochemistry and metabolic Medicine. 8TH ed. Edwaed Arnold publishers, London. 2012; 254-255