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Original Article

Effects of Chronic Administration of Lamivudine on the Histochemistry of the Liver in Wistar Rats

Peter A. I[1], Uwah A. F[2], Ndem J[2], Udoh B. E[3], Udoh D. K[4].

[1]Department of Anatomy University of Uyo, Nigeria
[2]Department of Biochemistry University of Uyo, Nigeria
[3]Department of Medical Microbiology and Parasitology, Olabisi Onabanjo University, Ogun state, Nigeria
4Chemical Pathology Unit, Med Lab Services, University of Uyo Teaching Hospital, Uyo, Nigeria

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ABSTRACT

Lamivudine (3TC) is a drugs used in the management of Human Immunodeficiency Virus (HIV) and Acquired Immunodeficiency Syndrome (AIDS) infection in combination with other drugs. The objective of this research study was to investigate the effects of this drug on the histology of the liver of Wistar rats. Twenty male Wistar rats were divided into 2 groups of 10 rats each. Group A served as the control and were administered with 1 ml of distill water, while group B was treated with 4.28mg/kg of lamivudine daily for 30 days, after which the rats where sacrificed using chloroform inhalation method and the liver was harvested, processed and stained using PAS crocian blue, carcino- embryonic antigen (CEA) and cytokeratin-7 (CK7) Immunochemistry methods. Stained slides were viewed using light microscope. Serum from each rat was extracted into fresh test tubes and used for analysis of aspartate aminotransaminase test (AST), alanine aminotransaminase test (ALT), alkaline phosphotase (ALP). Findings showed that the liver of Wistar rats administered with lamivudine, showed distortions with various degree of vacoulations, dilatation of sinusoidal spaces and nuclei necrotic changes. There were increased expression of CEA and CK7 in the groups treated with lamivudine than the control. This also agreed with the biochemical changes which showed significant levels of increase in AST and ALP in the groups administered with lamivudine. This suggests that lamivudine is harmful to the liver and should be taken with caution.

Key words: Lamivudine, liver, Human Immunodeficiency Virus, histochemistry.

INTRODUCTION

Lamivudine (3TC) is a levorotary pyrimidone-1,3-oxathiolane derivative and has the molecular formula C8H11N3O3S [1]. Lamivudine is an effective and well-tolerated agent for treating chronic hepatitis B infection and acquired immunodeficiency syndrome [1, 2]. It is an antiretroviral drug in the therapeutic category of nucleoside reverse transcriptase inhibitor [2]. Lamivudine is very useful in preventing HIV and hepatitis B from multiplying by way of its active form, lamivudine triphosphate (3TCTP) which is generated via intracellular triple phosphorylation process. This drugs disrupt an HIV protein or enzyme called reverse transcriptase, which is involved in making new viruses [3, 4].

Lamivudine is an antiviral drug that reduces the amount of HIV in the body. It prevents damage to the immune system, and reduces the risk of developing AIDS-related illnesses [5]. 3TC is also active against hepatitis B virus [5]. In 1996, 3TC was licensed in Europe as a treatment for HIV when used in combination with other anti-HIV drugs. It was discovered by BioChem Pharma and is manufactured by GlaxoSmithKline under the trade name *Epivir*. Numerous generic versions of 3TC are available [5].

Lamivudine is able to reduce HIV viral load and increase CD4 cell counts in the majority of people when taken in combination with at least two other antiretroviral drugs [6]. It is effective against HIV-1 and HIV-2. Some evidence indicates that 3TC can penetrate the central nervous system [5]. 3TC is given in combination, usually with a protease inhibitor or a non-nucleoside reverse transcriptase inhibitor and at least one other nucleoside or nucleotide reverse transcriptase

***Corresponding Author Address:** Peter A. I, Department of Anatomy University of Uyo, Nigeria; E-mail: aniekanpeter@uniuyo.edu.ng*

inhibitor, particularly AZT (zidovudine,) or abacavir. A study has shown that it reduces disease progression and AIDS death by 66% when added to AZT monotherapy in both AZT-experienced and AZT-naïve patients [7].

Most antiretroviral agents have been associated with hepatic toxicity. NRTIs have been found to cause hepatic staetosis, generally after more than six months of therapy [8]. One of the risk factors for the development of severe hepatotoxicity in a nevirapine treated cohort was the baseline CD4 cell count of less than 50cells/mm3 [9, 10]. There is dearth of literature on the toxic effect of lamivudine on the liver. This study was designed to investigate the histochemical effects of this drug on the liver of Wistar rats.

METHODOLOGY

Twenty male Wistar rats were used for this study. The animals were handled according to the guidelines for the treatment of laboratory animals. The rats were divided into 2 groups of 10 rats each. Group A served as the control and were administered with 1 ml of distill water. Group B was treated with 4.28mg/kg of lamivudine daily for 30 days. After which the rats where sacrificed using chloroform inhalation method and the liver was harvested, processed and stained using PAS crocian blue for iron, carcino- embryonic antigen (CEA) and cytokeratin- 7 (CK-7) Immunochemistry methods. Stained slides were viewed using light microscope. 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 were extracted into fresh test tubes and stored in a refrigerator for analysis of aspartate aminotransaminase test (AST), alanine aminotransaminase test (ALT), alkaline phosphotase (ALP). Data was analyzed using student T-test.

RESULTS

Fig. 1 Photomicrograph of the histology of the liver of group A administered with distill water. Showing normal liver architecture; the central vein (V), hepatocytes plates (H), sinusoidal spaces (S) and nuclei (N) are all normal. Fig 2 Photomicrograph of the histology of the liver of group B administered with 4.28mg/kg of lamivudine daily for 30 days. Showing moderate distortion of liver cellular architecture; the central vein (V) are dilated, hepatocytes plates (H) are swollen, sinusoidal spaces (S) dilation and pyknotic nuclei (N). Fig 3 Photomicrograph of the histology of the liver of group A administered with distill water. Showing normal liver expression of CEA by hepatocytes. Fig 4 Photomicrograph of the histology of the liver of group B administered with 4.28mg/kg of lamivudine daily for 30 days. Showing increased expression of CEA by hepatocytes. Fig 5 Photomicrograph of the histology of the liver of group C administered with distill water. Showing normal liver expression of Ck-7 by hepatocytes. Fig 6 Photomicrograph of the histology of the liver of group D administered with 4.28mg/kg of lamivudine daily for 30 days. Showing increased expression of Ck-7 by hepatocytes.

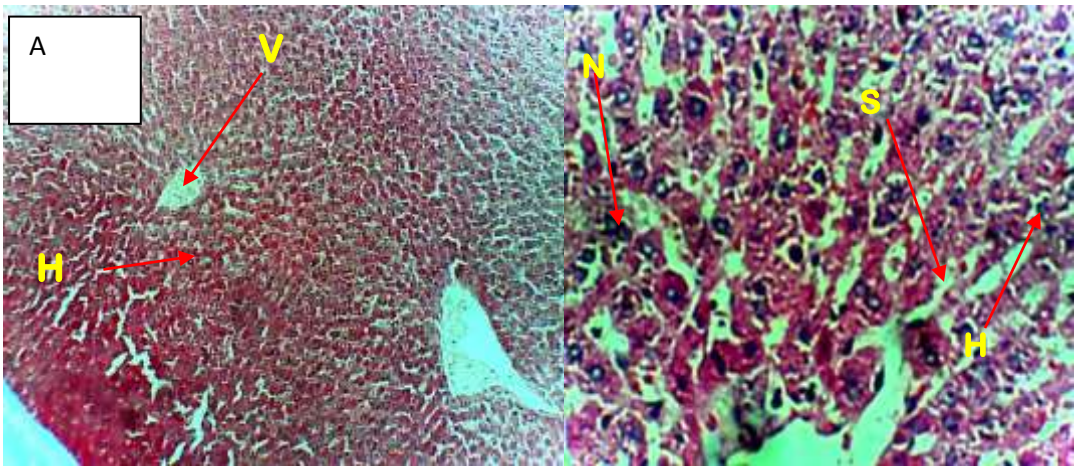

Fig 1 Photomicrograph of the histology of the liver of group A administered with distill water PAS crocian blue for iron control X 100 X 400. Central vein (V), hepatocytes plates (H), sinusoidal spaces (S) and nuclei (N)

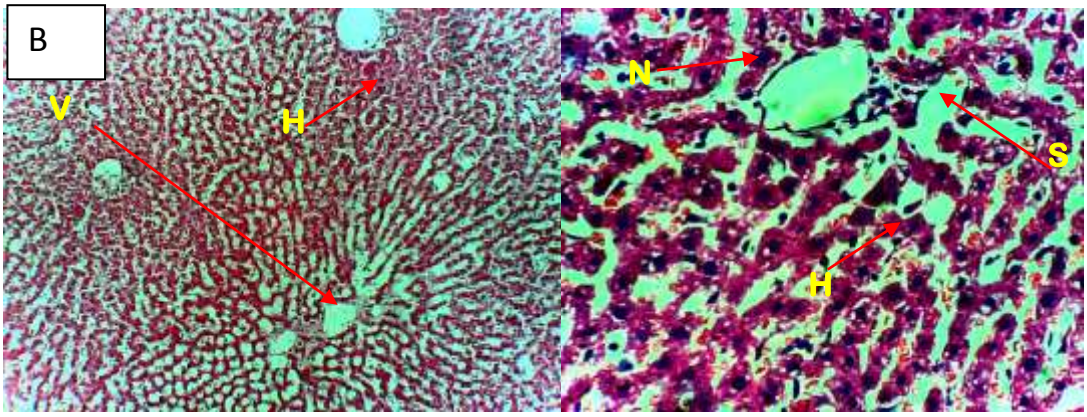

Fig 2 Photomicrograph of the histology of the liver of group B administered with 4.28mg/kg of lamivudine PAS Crocian blue for iron x 100 x 400. Central vein (V), hepatocytes plates (H), sinusoidal spaces (S) and nuclei (N)

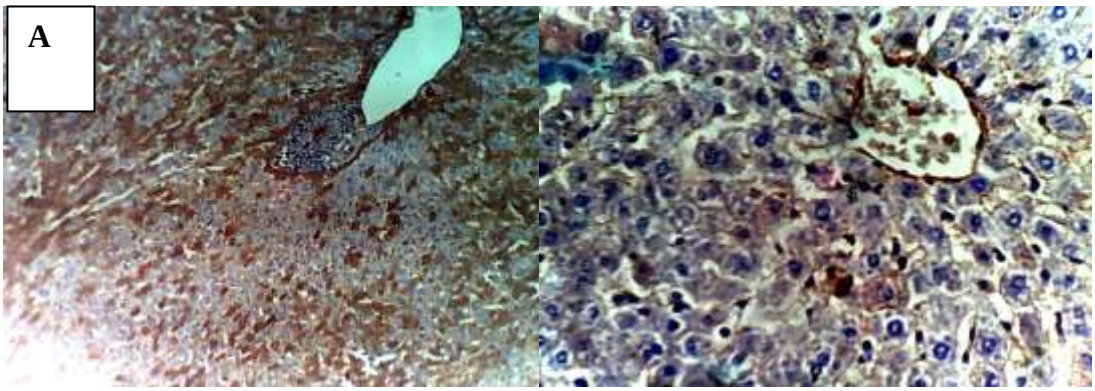

Fig 3 Photomicrograph of the histology of the liver of group A administered with distill water CEA X 100 and X 400

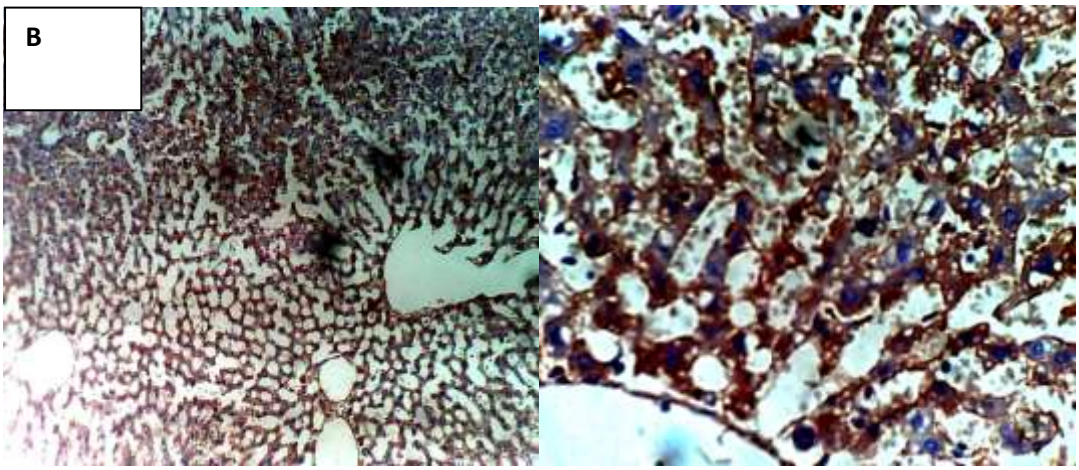

Fig 4 Photomicrograph of the histology of the liver of group B administered with

4.28mg/kg of lamivudine CEA X 100 X 400

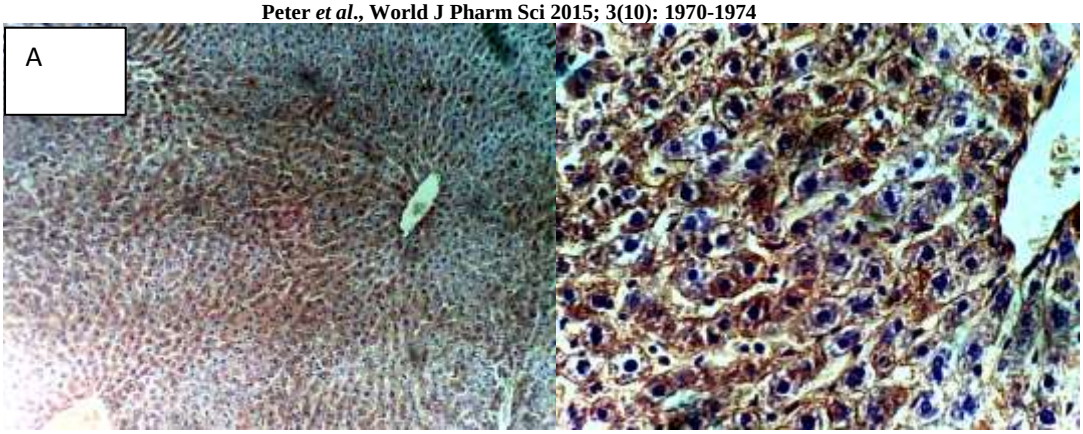

Fig 5 Photomicrograph of the histology of the liver of group A administered with distill water CK-7 X 100 X 400

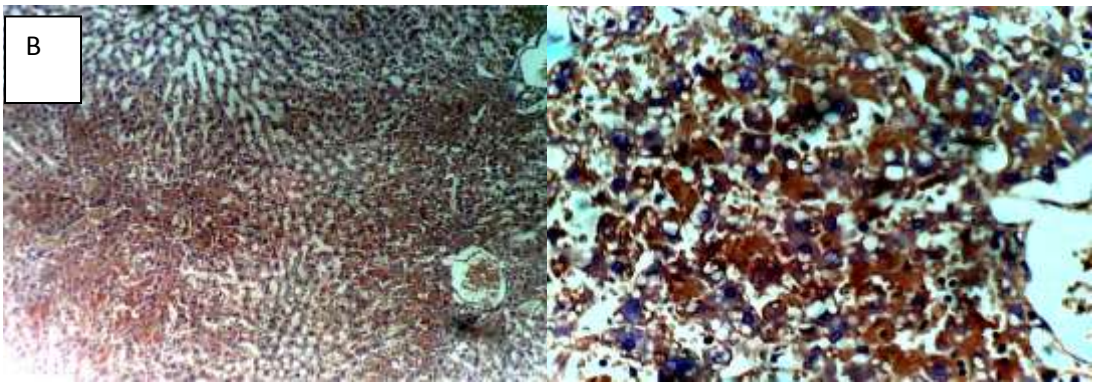

Fig 6 Photomicrograph of the histology of the liver of group B administered with 4.28mg/kg of lamivudine CK-7 X 100 and x400

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
A	59.80 ± 13.47	34.80± 1.83	111.40 ± 9.57
B	82.00 ± 11.00*	38.80 ± 6.94	125.80 ± 6.40*

***** = significant difference from control group at p = 0.05

DISCUSSION

The benefits of antiretroviral drugs are compromised by numerous side-effects, adverse clinical events and toxicities. Drugs are important causes of liver injury, more than 900 drugs, toxins, and herbs have been reported to cause liver injury [11]. This study was designed to investigate the Histochemical effects of lamivudine on the liver of Wistar rats The results obtained from this study revealed that oral administration of lamivudine had toxic effects on the liver, with moderate distortion of liver cellular architecture; dilatation of the central vein, sinusoidal spaces and pyknotic nuclei changes with no depletion of iron stores as shown in the color intensity of PAS crocian blue for iron as compared with control group. Iron stores are usually depleted in chronic liver injury but in our study there was no depletion likely due to the short duration of the study. Liver damage might lower the standard of life of HIV patients on medication and can eventually lead to non-adherence or withdrawal of medication. This finding is supported by histological findings that earlier reported that lamivudine can distort the cytoarchitecture of the

cerebellum [12]. In general, severe hepatic injuries have been documented to occur in HAART patients, regardless of the treatment regime [13].

ALT, AST and ALP are liberated into the blood whenever liver cells are damaged and increased plasma enzymes activity is a sensitive index of hepatic damage [14, 15] Neither of these enzymes is specific to the liver but ALT occurs in much higher concentration in the liver than elsewhere [15]. Therefore, the increased serum AST and ALP activity in this study more specifically reflects hepatic damage [14, 15]. This agreed with the immunohistochemistry findings which revealed increased expression of CEA and CK-7 suggestive of liver damage.

CEA is a nonspecific marker for cancers [16] and liver inflammation caused by hepatitis or chemotherapy [16]. There is a close relationship between gastric CEA values and the degree of gastric inflammation [17]. The study of cytokeratin expression has provided a valuable insight into the biliary microanatomy of the liver in health and disease. A study has shown increased expression of CK-7 in liver disease [18]. The increase CEA value in our study suggests that there was liver injury.

Drug-related toxicity leads to poor medication adherence and ultimate virological failure [19]. This is why studies on adverse drug reactions are very important and relevant, to identify potential risk and prevent of drug toxicity. In conclusion, chronic administration of lamivudine leads to toxic changes in the histology and biochemical indices in the liver of Wistar rats, strict monitoring of liver function should be a routine in patients taking lamivudine.

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