HISTOPATHOLOGICAL CHANGES DUE TO COMBINED ADMINISTRATION OF DOUVIR-N™ AND FOLIC ACID ON THE KIDNEY OF WISTAR RATS

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ABSTRACT

Acquired immune deficiency syndrome (AIDS) is a disease of the human immune system caused by the Human Immunodeficiency Virus (HIV). Drugs like lamivudine, zidovudine, nevirapine are used in combination as highly active antiretroviral therapy to reduce viral load to undetectable level and improve immunity and prolong survival of patients. However, these antiretroviral drugs are associated with numerous side effects that may be severe and affect patients. Folic acid belongs to the family of medications called vitamins, it is required for healthy skin, hair, liver, nervous system function. The objective of this study was to investigate the effect of Douvir–N™ on the histology of the kidney of adult wistar rats. and the effect of of its combined administration with folic acid. forty albino Wistar rats weighing between 120g – 260g body weights were randomly divided into four groups (10 rats/group). Group A served as control group and received 1ml of distilled water, while rats in group B received 9.29mg/kg bodyweight of Douvir–N™ , Rats in group C received a combination of 9.29mg/kg of Douvir–N™. and 0.07mg/kg of folic acid, rats in group D received 0.07mg/kg of folic acid. At the end of the experimental period, that lasted for 30 days, animals from each group were sacrificed and the kidneys were carefully removed and weighed. Administration of 9.29mg/kg body weight of Dourvir–N™ to the rats in group B showed distorted glomerulus, narrowed Bowman's space and dilated renal tubules. Combined administration of 9.29mg/kg body weight of Douvir–N™ and 0.07mg/kg body weight of folic acid to animals in group C showed mild changes in Bowman's space, and renal tubule whereas the administration of 0.07mg/kg body weight of folic acid alone to rats in group D had no effects on the morphology of the kidney when compared with the control. The findings suggest that Douvir–N™ can significantly distort the cytoarchitecture of the kidney which could be ameliorated by combined administration with folic acid.

Keywords: Douvir–N™, folic acid, Human Immunodeficiency Virus (HIV), Acquired Immunodeficiency Syndrome (AIDS), kidney.

INTRODUCTION

HIV/AIDS was first identified in the United States in 1981 by the U.S centre for disease control and prevention [1], and for the next decade, a diagnosis with the disease was equivalent to a death sentence. In 2012, it was estimated that 35.3 million people were living with HIV and AIDS, out of which; 32.1 million are adults, 17.7 million are women and 3.3 million are children. There were also 1.6 million deaths due to AIDS within this period, out of which 1.2 million deaths were in Sub Saharan Africa [2]. Over three-quarters of these deaths occurred in sub-Saharan Africa[2,3].

Genetic research indicates that HIV originated in West Central Africa during the late nineteenth or early twentieth century.[4] Today, HIV/AIDS is more of a chronic illness. This development was made possible by the introduction of antiretroviral drugs (ARVs) in the 1990s. Highly active antiretroviral therapy (HAART) currently composes of the most effective treatment regimen for HIV/AIDS. HAART has been found to be clinically

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effective, reducing both HIV/AIDS morbidity and mortality[5], and has been cost effective in achieving its clinical outcomes[6,7]. In contrast to these advances, recent evidence suggests that HIV infections rates may have actually risen since the introduction of HAART[8,9]. However, increasing reports of adverse clinical events and toxicities have diminished the enthusiasm generated by HAART. Some of the clinical events include AIDS related insulin resistance, hyperglycemia, which is observed in 30-80% of patients who are on HAART[10]. Reverse transcriptase inhibitors (Lamivudine, Nevirapine and Zidovudine) are drugs that inhibit both types (1 and 2) of HIV reverse transcriptase and also the reverse transcriptase of hepatitis B. The first group of antiretroviral drugs is the Nucleoside Reverse Transcriptase Inhibitors (NRTIs). They were the first type of drug available to treat HIV infection since 1987 and are better known as nucleoside analogues or nukes. HIV needs an enzyme called reverse transcriptase in order to be able to infect healthy cells and reproduce itself in a person's body. Drugs like deoxythymidine, zidovudine, stavudine, lamivudine, abacavir, emtricitabine, tenofovir and zalcitabine slow down the production of the reverse transcriptase enzyme and make HIV unable to infect cells and duplicate itself.[11] The second group of antiretroviral drugs is the Non-Nucleoside Reverse Transcriptase Inhibitors (NNRTIs). These group of drug was approved since 1997 for the treatment of HIV and are generally referred to as non-nucleosides. This group of drugs act by intervening with the transcriptase of the viral RNA into DNA.[12] Nevirapine is an example of a non-nucleoside drug that block the duplication and the spread of HIV.

The most commonly reported side effects when taking HAART include; headache, gastrointestinal upset (nausea, diarrhea, constipation and stomach pains), dizziness, fatigue, difficulty with sleeping, rash, hair loss, muscle and joints aches and pains. A more serious but less common side effect is damage to the liver, severe rash and a condition called lactic acidosis which may cause pancreatitis, liver failure, or renal failure. Also redistribution accumulation of body fat can happen.[13] Antiretroviral drug-related liver injury (ARLI) is a common cause of morbidity, mortality and treatment discontinuation in HIV-infected patients[13]. Prevention and management of ARLI has emerged as major issues among HIV-infected patients in the era of HAART[14]. Virtually every licensed antiretroviral medication has been associated with liver enzyme elevations, although certain drugs may cause liver injury more frequently than others[15].

Antiretroviral drugs reduce viral replication and can reduce mother- to child-transmission of HIV either by lowering plasma viral load in pregnant women or through post exposure prophylaxis in their newborns[16]. While access to services in preventing mother-to-child transmission of HIV has increased, the total number of children being born with HIV has also decreased but HAART in pregnancy has been associated with higher rate of prematurity, pre-eclampsia and gestational diabetes[5]. Most commonly observed birth defects include extra fingers and toes, club feet and cleft palate. This were all associated with first trimester treatment with zidovudine.[17]

Folate is a water-soluble B vitamin that is found naturally in foods such as fruits, dark green vegetables, potatoes, beans and yeast extracts. Folic acid is the synthetic form of folate found in dietary supplements and added to enriched flour and grain products[18]. Growing evidence suggests a potential role of folic acid in invivo and invitro antioxidants actions. When taken before conception, adequate use of folic acid reduces the incidence of Neural tube defects (NTDs) by 50-70% [19]. Neural tube defects are the results of abnormalities in neurulation (closure of neural folds and neuropores to form neural tube). [20, 21] Folate

modulates a number of disorders as a result of its anti- apoptotic and anti-oxidative properties [22]. This includes cardiovascular diseases [23], neural tube and congenital defects[19], subfertility[24] and several malignancies like cancer of the colorectum, lungs, pancreas, esophagus, stomach, cervix, breast[25], neuroblastoma and leukemia [22]. A deficiency of folate may increase blood levels of homocysteine. It also impairs DNA synthesis and cell division. Folate supplementation has been shown to decrease homocysteine levels and to improve endothelial functions[26, 27]. Folate supplementation is associated with improving memory deficits among cognitively impaired subjects. Higher folate intake is correlated with lower risks of Alzheimer's disease[28]. No adverse effects have been associated with consumption of food folates or folic acid in fortified foods[29]. Three primary concerns have been identified as possible adverse effects from excessive levels of supplemental folic acid intake: the masking of pernicious anaemia, which allows the neurological disease of vitamin B12 deficiency to progress unchecked, the disruption of zinc function and the antagonism of medications especially antifolate agents. Each of these consequences presents serious concerns and warrants careful considerations of the evidence. The evidence is weak to non-existent that folic acid has adverse effects by any mechanism than these three [30].

The kidneys are paired retroperitoneal structures normally located between transverse process of T12-L3 vertebrae, with the left kidney more superior in position than the right. The kidneys serve important functions including filtration and excretion of metabolic waste products (urea and ammonia); regulation of electrolytes, fluid, acid-base balance and stimulation of red blood cell production [31]. This work was therefore designed to investigate the effect of combine administration of Douvir-N™ and folic acid on the histopathology of the kidney of Wistar rats.

METHODOLOGY

The drugs used in this study Douvir-N™ is a fixed dose combination of lamivudine, Zidovudine and Nevirapine. It was obtained from the University of Uyo Teaching Hospital (UUTH) Uyo, Nigeria. The drug was manufactured by Cipla pharmaceuticals of India. Folic acid was obtained from Top care pharmacy in Uyo. The drug was manufactured by Vitabiotics Nigeria limited. The drugs were prepared by grinding them using a mortar and pestle to powder form. This was then diluted with 100ml distilled water. The drugs were prepared daily.

Forty adult albino Wistar rats weighing 260g ± 10g body weights were obtained from the animal house of faculty of Basic Medical Sciences, University of Uyo, Nigeria. They were housed in cages and maintained under standard environmental conditions. The rats were fed with standard pellet diet and water. There were randomly divided into 4 groups (10 rats per group) and housed in cages. Douvir-N™ was administered orally twice daily, while, folic acid was administered orally once daily for 30 days. Group A was administered distilled water, they served as control. Group B Douvir-N™ was administered with 9.29mg/kg body weight, Group C was administered a combination of Douvir-N™ (9.29mg/kg) and folic acid (0.07mg/kg), while group D was administered with folic acid alone (0.07mg/kg).

The animals were sacrificed on the 31st day after overnight fast using chloroform inhalation method. The abdominal cavity was dissected through a midline abdominal incision. The kidneys were extracted and rinsed in normal saline and fixed in 10% buffered formalin. They were then processed and stained with the Haematoxylin and Eosin staining method and viewed under the light microscope.

RESULTS

PLATE A: Light microscopic study of the sections obtained from the rats in the control group showed normal histological

appearance of the glomerulus (G), normal Bowman's Space (BS), and normal tubules (T), figure 1.

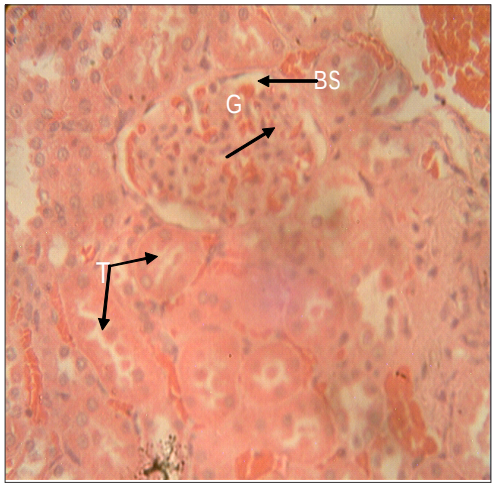

Figure 1: Micrograph of the kidney of control rat showing normal Glomerulus G, Bowman's Space BS and the Tubules T are normal. X400

PLATE B Administration of 9.29mg/kg body weight of Douvir–N[Tm] for 30days to the rats showed distorted glomerulus (G), narrowed Bowman's space (BS), dilated and distorted tubules (T) when compared with the rats in the control group, figure 2.

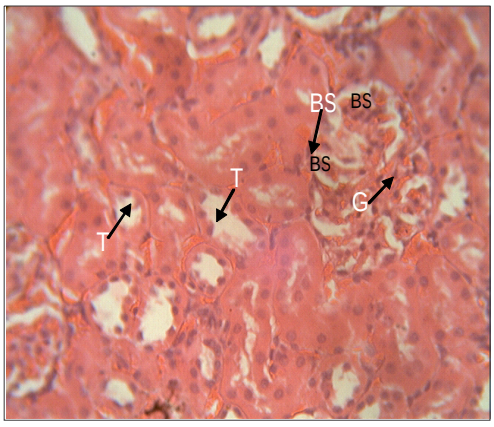

Figure 2: Micrograph of the kidney of a rat administered with 9.29mg/kg body weight of Douvir-N[TM] for 30days showing distorted and shrunken Glomerulus G, narrowed Bowman's Spaces BS, the Tubules T are dilated and distorted , when compared with figure 1. X400

PLATE C The photomicrograph of the kidney of the rats administered with 9.29mg/kg body weight of Douvir–N[Tm] and 0.07mg/kg body weight of folic acid for 30days showed normal glomerulus (G), normal Bowman's space (BS), normal tubules (T), and mild cellular degeneration (CD) when compared with the rats in the control group, figure 3.

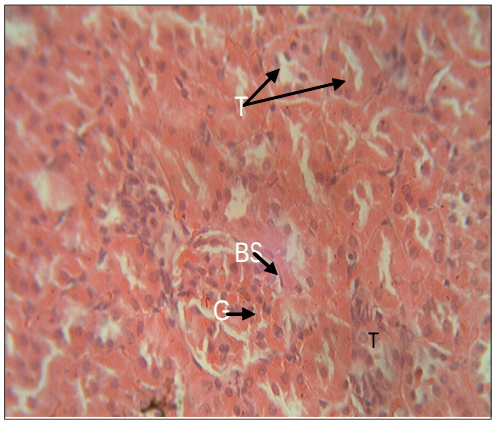

Figure 3: Micrograph of the kidney of a rat administered with 9.29mg/kg body weight of Douvir-N[TM] and 0.07mg/kg of folic acid showing mildly shrunken and distorted glomerulus G, normal Bowman's Space BS, the tubules T appears normal when compared with figure1. X400

PLATE D Administration of 0.07mg/kg body weight of folic acid for 30days to the rats showed normal glomerulus (G), normal Bowman's space (BS), normal tubules (T) and a mild cellular degeneration (CD) when compared with the rats in the control group, figure 4.

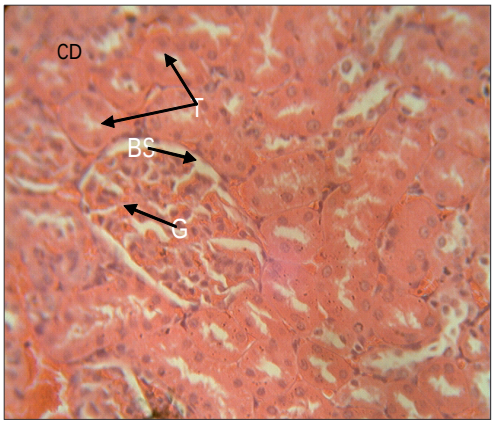

Figure 4: Micrograph of the kidney of a rat administered with 0.07mg /kg body weight of folic acid showing normal Glomerulus, G, normal Bowman's Space BS, the tubules T appears normal when compared with figure 1. X400

DISCUSSION

Highly active antiretroviral therapy (HAART) has been associated with toxicities including those affecting the kidney.[32] Injuries caused by HAART occur via multiple mechanisms, including direct tubular toxicity, allergic reactions, and precipitation of insoluble drug crystals within the renal tubular lumen.[33]

Results obtained from Group A (control) showed no distortions in cellular architecture of the kidney. The glomerulus and tubules were all seen to be intact and normal.

Group B (administered with Douvir-N™) showed distortions and cellular degeneration of the kidney and dilation of the Bowman's capsule. It has been reported that acute renal injury can occur as a result of severe mitochondrial dysfunction and lactic acidosis induced by nucleoside reverse transcriptase inhibitors.[34]A class in which Zidovudine and Lamivudine are members. In addition, it has been reported that non-specific metabolic complications may increase the risk of vascular kidney diseases in patients on HAART. After initiation of Nevirapine containing regimen, acute renal failure is usually observed[34]. The results also agreed with the findings of another study that reported renal derangement and necrosis after treatment with combination therapy, the cellular damage was dose dependent.[36] another study reported enzymatic derangement in Wistar rats administered With Nevirapine.[36]

Group C showed slight changes in the kidney morphology as a result of combination of the Douvir-N™ with folic acid. The folic acid had protective function on the renal epithelial cells and preserved the structural and functional integrity of renal tubules. This must have been as a result of the antioxidant effect of folic acid. It was earlier reported that Neurovite had antioxidant effect on Lamivudine treated Wistar rats.[37] In the last group, there was no significant difference in morphology of kidney between the control group and rats administered with folic acid alone. This shows that folic acid does not damage the renal architecture.

From this research experiment, it can be concluded that Dourvir-N™ led to abnormal morphology of kidney with obvious distortions of the glomerulus and dilation of the tubules. These effects were ameliorated when it was co-administered with folic acid. Therefore, Douvir-N™ should be co-administered with folic acid in the treatment of HIV to prevent renal toxicity.

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