ASN- PH-020919
ISSN: 2315-537X

International Journal of Herbs and Pharmacological Research
IJHPR, 2014, 3(4):60-67
www.antrescentpub.com

E- ISSN: 2384-6836

RESEARCH PAPER

ZIDOVUDINE THERAPY: EFFECT(S) ON HISTOLOGY OF THE KIDNEY OF WISTER RATS

Naiho A.O., Nwaokolo K.M. and Odigie M.O.
Department of Human Physiology, Faculty of Basic Medical Sciences, College of Health Sciences, Delta State University, Abraka, Delta State, Nigeria.
Corresponding author: Email: obidikealex@yahoo.com

Received: 24th July, 2014 **Accepted: 15th September, 2014** **Published: 30th September, 2014**

ABSTRACT

This study investigated the histo-morphological effect(s) of Zidovudine therapy on the kidney of Wister rats. Fifteen Wistar rats obtained from the Animal house of the College of Medicine, Ambrose Alli University, Ekpoma, Edo State, Nigeria, were used for this study. A three week period of acclimatisation was allowed. They were subsequently weighed and randomly stratified into a control (A; n=5) and two treatment groups - B (low dose; n=5) and C (high dose; n=5). Groups B and C received varied doses of Zidovudine (6mg/kg and 12mg/kg respectively). After the completion of a 21 day treatment exercise, renal tissues were harvested from each of the groups and taken for histo-morphologic analysis The results showed significant body weight reduction in the treatment groups as compared to the control ($p<0.05$) with mild to moderate derangement in the kidney sections of group B (high dose). It can be concluded therefore, that while low doses of zidovudine can reduce body weight of the animals with no obvious deleterious effects, higher dose however, has capacity to induce histomorphologic alterations in renal tissues of rats.

Key words: ARV drug, Renal parameters, Body weight, Kidney, Zidovudine

INTRODUCTION

Pneumocystis carinii is an opportunistic infection (Prekates, et al., 1997) associated with malignant neoplasies like Kaposi's sarcoma (CDC, 1981a; 1981b). The "hydra-headed" monstrous virus involved is now referred to as the Human Immunodeficiency Virus (HIV), which is currently known to be notorious and responsible for the Acquired Immunodeficiency Syndrome (AIDS) (UNAIDS, 1998; Ajakaiye, 2002). It has been considered as the worlds greatest pandemic ever known to devastate mankind (UNAIDS, 1998) and most prevalent among persons of reproductive age; about one third of whom are expectant parents.

In Nigeria for example, literature has it that an estimated number of 2.95 million of the populace are living with HIV/AIDS (Ogundipe, 2009). This huge statistical report has continued to challenge the research community, the academia, governments, media organizations, religious groups and several non-governmental organisations the world over. A pointer to the veracity of believing this report however, is the "relative porosity" of the routes for the spread of this infection. One of the routes by which the infection can spread is in utero, usually referred to as mother–to–child transmission (MTCT) (Marinda 2011; Arya 2010) and could involve oral means as breastfeeding (Liang 2009), during parturition (child birth) (Arya 2010) and even post-natally (UNAIDS/WHO, 1998; Morgan *et al*., 1990; Adjorlolo-Johnson *et al*., 1994; Andreasson *et al*., 1993).

To help curtail the excesses and devastating menace of this monster called HIV, the WHO in 2000, recommended inhibitors of the analogues of the reverse transcriptase enzymes, found in Nucleotides that lack phosphate group polymers (Nucleosides), as regimens for people living with HIV/AIDS (Menéndez-Arias, 2002; Clavel and Hance, 2004). Such Nucleoside analogues include (but not limited to) Zidovudine (Azidothymine--AZT), Didanosine, Nevirapine and Lamivudine. Generally, anti-Retrovirals (ARVs) work by inhibiting the enzyme (reverse transcriptase) which HIV uses to synthesize a prototype DNA for itself, using the host's DNA as a template (Gulick et al., 1997; Imamichi, 2004).

Although these drugs have been scientifically proven to be efficacious in improving the immunity status of sufferers and reducing the viral load to undetectable level as well as elongating the survival of victims (Yazdanpanah 2009), researchers are however sceptical as to the adverse effect(s) that may be associated with an absolute dependency on them by people living with HIV/AIDS. For instance, AZT has been reported to manifest with several side effects, with the commonest being Hematological toxicity (severe anaemia and neutropenia), lactic acidosis, nausea, vomiting, headache, dizziness, fatigue, insomnia, kidney disorders, weakness and muscle pain at the early weeks of its administration (Ministry of Health, Zambia National Formulary Committee, 2008; Sharma 2011). Even though polypharmacy (multiple drug administration) plus adjustments in diet will usually tame some of the effects (McCord 2008; Payer 2010), the toxicity may worsen with time due to tachyphylaxis from the virus (Fisher 2001; Somarriba 2010; Ahoua 2011REF).

Physiologically, the oral administration of zidovudine usually results in first pass metabolism of the drug in the liver, a situation that tends to limit its bioavailability to receptors for therapeutic effect (Thomas et al., 2006; Hurst et al., 2007; Varma et al., 2010a; Varma et al., 2010b).). Hence, other possible route to bypass the liver has been considered as well. Therefore, this study was intends to investigate the effect of zidovudine on the histomorphologic architecture of the renal organ.

MATERIALS AND METHODS

Animals: Fifteen (15) male albino rats, not more than three months of age (each) where obtained from the College of Medicine, at Ambrose Alli University (AAU), Ekpoma, Edo State. The reason for restricting their age to 3 months was because laboratory study of lifespan is currently only feasible for short-lived species, and rats happen to show short life expectancy (about 3 years). This signifies that as they age, there's bound to be a decline in the renal clearance of the study drug (zidovudine) due to decline in age and by implication, renal functionality. This circumstance usually increases bioavailability and therefore, may thwart the regimen and the therapeutic effect that was desired per concentration per body weight of subject (Wister rat).

Nevertheless, the rats were transported to the animal house of the College of Health Sciences at the Delta State University (DELSU), Abraka, Delta State, Nigeria, where they were housed in a wooden cage. They were weighed and confirmed to weigh between 180-250g, and thereafter stratified into experimental groups A and B (A = low dose; and B = high dose). Group C served as the control. The rats were fed with rat chow from Bendel Foods Ltd and allowed to acclimatize for a period of one week after which they were re-weighed. The reason for re-weighing the rats was to be sure that the new environment (DELSU environment) had no negative effect on their body initial weight, as this will be indicative of a bad acclimatisation process.

Ethical Approval: Ethical consent and approval was granted by the Research and Ethics committee of the College of Health Sciences, Delta State University, Abraka, Delta State, Nigeria.

Drugs: Zidovudine (the targeted ARV) was obtained from the anti-retroviral unit of the Central Hospital, Agbor, in Delta State. The drugs were analytically graded and administered based on the concentration (in gram/dm^3) per unit weight (kg equivalence of between 180-250g) of each rat. The reason for the "drug stoichiometry" was to avoid over dosage as all drugs are lethal above certain dose. Issues of drug-drug interactions were also put into cognisance. For instance, stavudine and doxorubian are known inhibitors of zidovudine. So as a precautionary measure, the rats were never given such (both after and before purchases were made).

Drug Administration: The rats (15 in total) were as stated earlier, grouped into three (A, B and C). Experimental groups were administered with varying doses of Zidovudine (in water) for a period of 21 days, while group C rats received only normal feed and water. Specifically, group A received 6mg/kg (low dose) while group B received

12mg/kg (high dose) of Zidovudine. The experimental rats were constantly monitored and their body weight changes recorded on weekly basis using an electronic balance. The doses administered to the experimental animals were however adjusted to be commensurate with any observed weight gain or loss.

Sacrifice and Tissue Extraction: 12 hours following the last treatment, the animals (per group) were re-weighed and immediately given a cervical dislocation (after anaesthetizing with chloroform). The reason for doing this 12-hour post drug prandial was to give adequate time for proper drug circulation and metabolism in vivo. Thereafter, the pancreas, stomach and kidneys were carefully removed and fixed in a 10% formal-saline for 48 hours. The reason for using formal-saline was to keep the tissues at constancy with their ex-vivo environment.

Tissue Processing: With the assistance of a certified anatomist from the Department of Human Anatomy and Cell Biology in DELSU, the harvested organs (pancreas, stomach and kidneys) were subjected to standard tissue processing techniques that include dehydration, impregnation, embedding, sectioning and staining with Haematoxylin and Eosin (H and E) for appropriate microscopy. Photomicrographs were then captured with the aid of a 5.0 mega pixel microscope of about 500 resolution capacity, having a serial port for connection with a computer's Universal Serial Board (USB) (see below).

Statistical Analysis: All the data obtained in this study were expressed as Mean ± SEM (standard error of mean). Significant differences were tested using ANOVA with margin of error valued at 0.05. Where $p<0.05$, results were considered statistically significant as appropriate (see below).

RESULTS

On body weight, the results showed a tremendous weight difference in the control group (206.6 ± 92.4)g as compared to rats in the experimental groups A (administered low dose) and B (administered high dose), who's weights were 1.86±83.18 and 190±84.97 grams respectively.

Table 1: Comparisons of body weight of control versus low and high dose rats, treated with zidovudine

Group	Weight Difference	t-cal	t-tab	P-value
Control	3.4±1.52			
Low Dose (Group A)	1.2±0.08	3.23	2.31	P < 0.05
High Dose (Group B)	0.0 ± 0.1	5.0	2.31	P < 0.05

Results are presented in gram, mean ± SD (n = 5).

The results showed a statistically significant reduction in the body weight of the experimental animals as compared to the control. In fact, there was a continuous weight loss in the low and high dose groups as shown in the table.

On renal tissue microscopy, it was observed that the control tissue sections presented intact tissue cytoarchitecture with intact cortical glomeruli, tubules and interstitium (Figure 2). Tissue sections of group A and B treated with low and high concentration of Zudovudine, showed moderate interstitial congestion (Figures 3 and 4).

DISCUSSION

The observed significant reduction in the body weight of the treated rats coincides with the findings from previous studies on Zidovudine. Specifically, Lamperth *et al.* (1991) observed that AZT-treated animals lost 10% of their original weight by the end of the 3rd month of the experiment. Similarly, the observed interstitial congestions in the treated kidney sections serve as a pointer to the ensuing events at the cellular and molecular levels. This is supported by the assertions of Izzedine *et al*. (2005) and Daugas *et al*. (2005) that antiretroviral drug can cause renal failure through a variety of mechanisms that includes direct renal tubular toxicity (ATN, Fanconi-like syndrome, distal

tubular acidosis, obstructions (crystal deposition in the kidney), and glomerular lesions. Indeed, this seeming Zidovudine's capacity to induce renal tissue damage would definitely aggravate HIV's capacity to induce glomerular and vascular or tubule-interstitial nephropathies (Weiner et al., 2003), which are leading cause of end-stage renal disease (ESRD) especially among black sufferer patients (D'Agati et al., 1998),

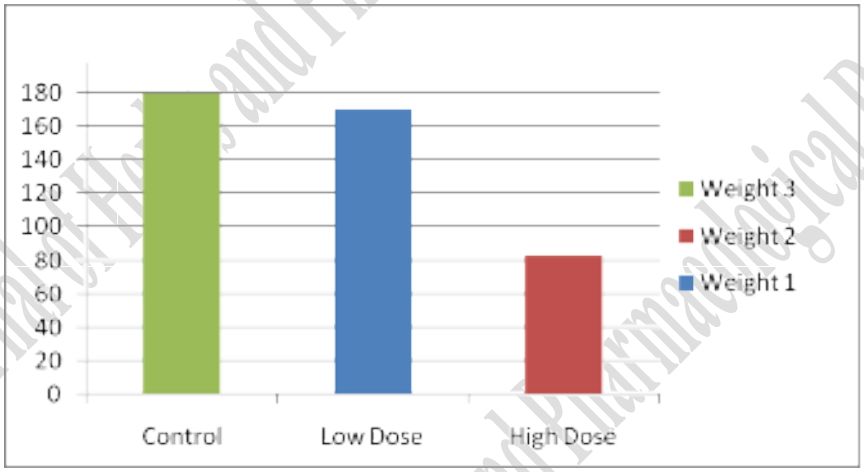

Figure 1: A Bar chart showing mean body weights of rats in gram against zidovudin concentration in milligrams

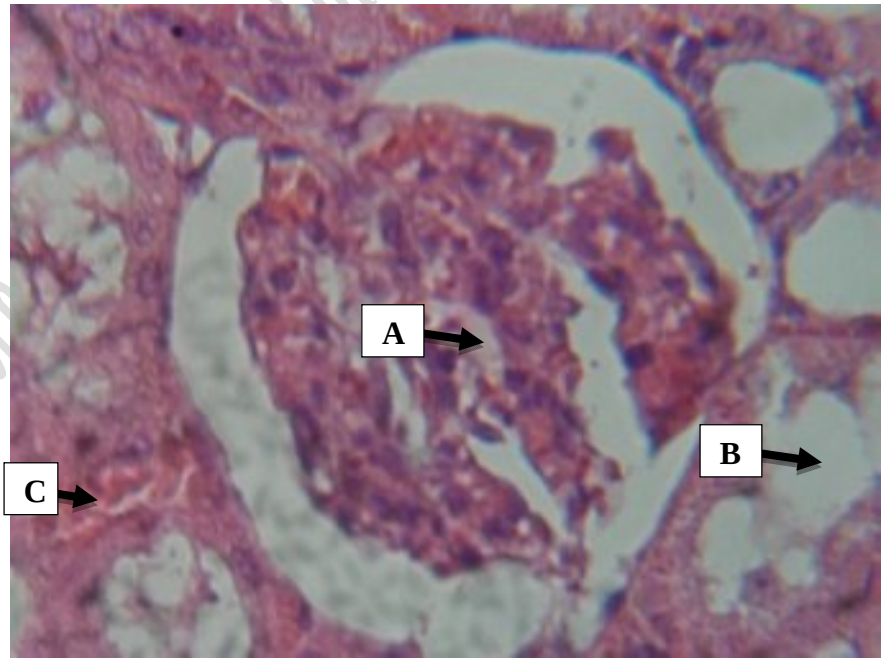

Figure 2: Photomicrograph of rat kidney (H&E x400) showing cortical glomeruli (A), tubules (B) and interstitium (C)

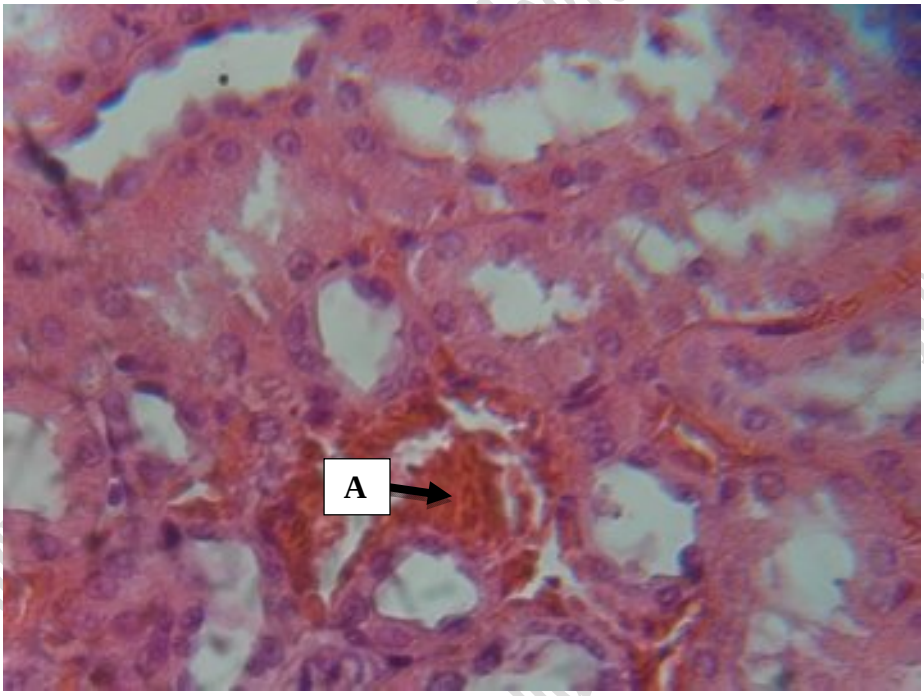

Figure 3: Photomicrograph of rat kidney (H&E x400) treated with low concentration of Zudovudine showing moderate interstitial congestion (A).

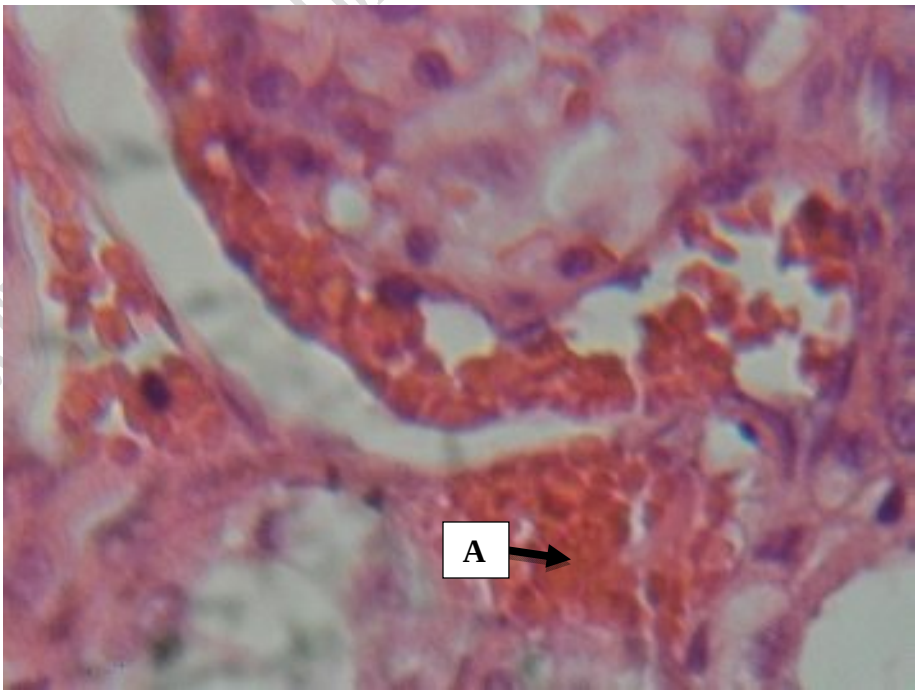

Figure 4: Photomicrograph of rat kidney (H&E x400) treated with high concentration of Zudovudine for 3 weeks showing moderate interstitial congestion A.

Undeniably, the tremendous advancements achieved in the management of HIV remains one the great scientific feats of the 21st century. Even available reports have shown that mortality rate for HIV-positive individuals have declined considerably and continue to do so (Killian 2011; Bhaskaran 2008; Giusti 2011). However, the concerns that the indispensability of antiretroviral drugs comes with it a lot of challenges especially a number of troubling side effects (Barsoum, 2006) can no longer be ignored. The fact that these drug-induced side effects can diminish patients' quality of life and contribute to an increase the rate of cardiovascular events and *diabetes* (Escote 2011; Tien 2008; Tebas 2008; Palios 2012) is indeed a course for concern.

It is obvious therefore, that Zidovudine has the capacity to induce body weight reduction and histopathological alterations in organ systems, with their attendant negative consequences on HIV patients that would surely compound the vulnerability status of HIV patients to infections. It is our opinion that there is a need to develop a much more efficient chemotherapeutic strategy that would ensure a holistic clinical management of HIV patients, especially those that are highly susceptibility to drug induced adverse effects.

ACKNOWLEDGEMENT

Our thank goes to the Head of Department Dr Ese Adegor for her assistance towards this research.

REFERENCES

Adjorlolo-Johnson, G., De Cock, K. M., Ekpini, E., Vetter, K.M., Sibailly, T., Brattegaard, K., Yavo, D., Doorly, R., Whitaker, J.P., Kestens, L., Ou, C.Y., George, J.R. and Gayle, H.D. (1994). Prospective comparison of mother-to-child transmission of HIV-1 and HIV-2 in Abidjan, Ivory Coast. *JAMA*; 272:462–466.

Ahoua, L., Umutoni, C., Huerga, H., Minetti, A., Szumilin, E., Balkan, S., Olson, D.M., Nicholas, S. And Pujades-Rodriguez, M. (2011). "Nutrition Outcomes of HIV-Infected Malnourished Adults Treated With Ready-to-Use Therapeutic Foodin Sub-Saharan Africa: a Longitudinal Study." *J Int AIDS Soc*; 14:2.

Ajakaiye, D.O. (2002). Elements of Socio-Economic Burden of HIV/AIDS in Nigeria, pp.1-11. In: D. Olu Ajakaiye and O.F. Odumosu (Eds.): Socio-economic Burden of HIV/AIDS Epidemic in Nigeria. NISER, Ibadan.

Andreasson, P.A., Dias, F., Naucler, A., Andersson, S. and Biberfeld, G. (1993). A prospective study of vertical transmission of HIV-2 in Bissau, Guinea-Bissau. *AIDS*; 7: 989-993.

Arya, M., Levison, J. and Giordano, T.P. (2010). "Ongoing Barriers to HIV Testing During Pregnancy: A Need for Media Campaigns Addressing Low Knowledge about Perinatal HIV Transmission among Women in the United States." *AIDS Patient Care STDs*; 24.2: 71-72.

Barsoum, R.S. (2006). Chronic kidney disease in the developing world. *N. Engl. J. Med*; 354:997-999

Bhaskaran, K., Hamouda, O., Sannes, M., *et al*. (2008). "Changes in the Risk of Death After HIV Seroconversion Compared with Mortality in the General Population." *JAMA*; 300(1): 51-59.

Centers for Disease Control (1981): Kaposi's sarcoma and Pneumocytosis pneumonia among homosexual men – New York City and California. *MMWR*; 30:305-308.

Centers for Disease Control (1981): Pneumocytosis pneumonia –Los Angeles. *MMWR*; 30:250-252.

Clavel, F. and Hance, A.J. (2004). HIV Drug Resistance. *N Engl J Med*; 350: 1023-1035.

D' Agati, V., Suh, J.I. and Carbone, L. (1998). Pathology of HIV-associated nephropathy: A detailed morphologic and comparative study. *Kidney Int*; 35: 1358-1370

Daugas, E., Rougier, J.P. and Hill, G. (2005). HAART related nephropathies in HIV-infected patients. *Kidney Int*; 67: 393-403.

Escoté, X, Miranda, M., Veloso, S., Domingo, P., Alonso, C., Olona, M., López-Dupla, M., Aguilar, C., Peraire, J., Viladés, C., García-Pardo, G., Sirvent, J.J., Vendrell, J., Richart, C. and Vidal, F. (2011). Lipodystrophy and insulin resistance in combination antiretroviral treated HIV-1-infected patients: implication of resistin. *J. Acquir Immune Defic Syndr*; 57: 16-23.

Fisher, K. (2001). "Wasting and Lipodystrophy in Patients Infected with HIV: A Practical Approach in Clinical Practice." *AIDS Read*; 11.3: 132-133, 137-140, 147.

Giusti, A., Penco, G. and Pioli, G. (2011). "Vitamin D Deficiency in HIV-Infected Patients: a Systematic Review."*Nutr Diet Supp;* 3: 101–111.

Gulick, R.M., Mellors, J.W., Havlir, D., Eron, J.J., Gonzalez, C., McMahon, D., Richman, D.D., Valentine, F.T., Jonas, L., Meibohm, A., Emini, E.A. and Chodakewitz, J.A. (1997). Treatment with indinavir, zidovudine, and lamivudine in adults with human immunodeficiency virus infection and prior antiretroviral therapy. *N Engl J Med*; 337: 734-739.

Imamichi, T. (2004). Action of anti-HIV drugs and resistance: Reverse transcriptase inhibitors and protease inhibitors. *Curr Pharm Design*; 10: 4039-4053.

Izzedine, H., Launay-Vacher, V. and Deray, G. (2005). Antiviral drug-induced nephrotoxicity. *American Journal of Kidney Disease*; 45: 804-817.

Killian, M. S. and Levy, J. A (2011). "HIV/AIDS: 30 Years of Progress and Future Challenges." *Eur J Immunol* 41.12 (2011): 3401-11.

Lamperth, L., Dalakas M.C., Dagani, F., Anderson, J. and Ferrari, R. (1991). Abnormal skeletal and cardiac muscle mitochondria induced by zidovudine (AZT) in human muscle in vitro and in an animal model. *Lab Invest*; 65(6):742-51.

Liang, K., Gui, X., Zhang, Y.Z., et al. (2009). "A Case Series of 104 Women Infected With HIV-1 via Blood Transfusion Postnatally: High Rate of HIV-1 Transmission to Infants through Breast-Feeding." *J Infect Dis*. 200.5: 682-6.

McCord, A.(2008). Milk thistle may help improve liver health in people with HIV and hepatitis C. *Proj Inf Perspect*; Sep. (46):18.

Marinda, E. T., Moulton, L. H., Humphrey, J. H., *et al.* (2011). "In Utero and Intra-Partum HIV-1 Transmission and Acute HIV-1 Infection during Pregnancy: Using the BED Capture Enzyme-Immunoassay as a Surrogate Marker for Acute Infection." *Int J Epidemiol*; 40.4: 945-954.

Menéndez-Arias, L. (2002). Targeting HIV: antiretroviral therapy and development of drug resistance. *Trends Pharmacol Sci*; 23: 381-388.

Ministry of Health, Zambia National Formulary Committee (2008). *Standard Treatment Guidelines, Essential Medicines List, Essential Laboratory Supplies for Zambia*. 2nd ed. Lusaka, Zambia: Zambia Ministry of Health.

Morgan, G., Wilkins, H.A., Pepin, J., Ousman, J., Brewster, D., Whittle H, *et al*. (1990). AIDS following mother-to-child transmission of HIV-2. *AIDS*; 4:879-882.

Ogundipe, A. (2009). Federal Government of Nigeria: National Policy on HIV/AIDS. National Agency for the Control of AIDS (NACA). pp. 1-54. Available at http://nigeria.unfpa.org/pdf/ntpol.pdf

Payer BA, Reiberger T, Rutter K, *et al*. (2010). Successful HCV eradication and inhibition of HIV replication by intravenous silibinin in an HIV-HCV co-infected patient. *J Clin Virol*; 49.2:131-3.

Palios, J., Kadoglou, N.P.E. and Lampropoulous, S. (2012). The Pathophysiology of HIV-/HAART-Related Metabolic Syndrome Leading to Cardiovascular Disorders: The Emerging Role of Adipokines. *Exp Diabetes Res*; 2012: 103063. Epub Dec. 8. Available at http://www.ncbi.nlm.nih.gov/pmc/articles/PMC3235775/

Prekates, A., Kyprianou, T., Paniara, O. and Roussos, C. (1997). Pneumocystis carinii pneumonia in a HIV-seronegative patient with untreated rheumatoid arthritis and CD4+ T-lymphocytopenia. *Eur Respir*; *J*; 10: 1184–1186.

Sharma, B. (2011). "Anti-HIV-1 Drug Toxicity and Management Strategies." *Neurobehav HIV Med*; 327-340.

Somarriba, G., Neri, D., Schaefer, N. and Miller, T.L. (2010). "The Effect of Aging, Nutrition, and Exercise during HIV Infection." *HIV AIDS* (Aukl) 2: 191-201.

Tebas P. (2008). Insulin resistance and diabetes mellitus associated with antiretroviral use in HIV-infected patients: pathogenesis, prevention, and treatment options. *J Acquir Immune Defic Syndr*. 49 (Suppl 2): S86-S92.

Thomas, V. H., Bhattachar, S., Hitchingham, L., Zocharski, P., Naath, M., Surendran, N., Stoner, C. L. and El-Kattan, A. (2006). The road map to oral bioavailability: an industrial perspective. *Expert Opin Drug Metab Toxicol*; 2(4): 591-608.

Tien PC et al. (2008). Antiretroviral therapy exposure and insulin resistance in the Women's Interagency HIV study. *J Acquir Immune Defic Syndr*; 49(4):369-376.

UNAIDS (1998). Report on the Global HIV/AIDS epidemic. Geneva, UNAIDS. Available at http://data.unaids.org/Publications/IRC-pub06/epiupdate98_en.pdf

Varma, M. V., Ambler, C. M., Ullah, M., Rotter, C. J., Sun, H., Litchfield, J., Fenner, K. S. and El-Kattan, A. F. (2010a). Targeting intestinal transporters for optimizing oral drug absorption. *Curr Drug Metab*; 11(9): 730-742.

Varma, M. V., Obach, R. S., Rotter, C., Miller, H. R., Chang, G., Steyn, S. J., El-Kattan, A. and Troutman, M. D. (2010b). Physicochemical space for optimum oral bioavailability: contribution of human intestinal absorption and first-pass elimination. *J Med Chem*; 53 (3): 1098-1108.

Yazdanpanah, Y., Fagard, C., Descamps, D., Taburet, A.M., Colin, C., Roguebert, B., Katlama, C., Pianox, G., Jacomet, C., Piketty, C., Bollens, D., Molina, J.M. and Chene, G (2009). "High Rate of Virologic Suppression with Raltegravir Plus Etravirine and Darunavir/Ritonavir among Treatment-Experienced Patients Infected with Multidrug-Resistant HIV: Results of the ANRS 139 TRIO Trial." *Clin Infect Dis*; 49.9: 1441-1449.

AUTHORS CONTRIBUTION

This research work was carried out by Nwokolo K.M and Odigie M.O under the supervision of Dr Naiho A.O who convieved the idea of this research, determine its aim and objective, and designed the experimental protocol.