Assessing the Risk of Birth Defects Associated With Exposure to Highly Active Anti-Retroviral Therapy during Organogenesis in Rats

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The use of anti-retroviral drugs during pregnancy has increased since the demonstration of reduction of mother-to-child transmission of HIV with highly active antiretroviral therapy (HAART). The risk of HAART cannot be ruled out; data are generally limited or varied. This study intends to thoroughly assess the teratogenic effect of HAART on the organogenesis stage of fetal development using animal model. Pregnant rats were divided into 13 groups with 12 animals per group. The therapeutic doses of drug administration were done to simulate the treatment pattern in APIN HIV/AIDS Clinic of the University of Lagos Teaching Hospital, Nigeria (find detailed treatment groups in methodology). Six rats in each group were randomly selected and sacrificed on day 20 by cervical dislocation prior to day 21 of gestation and the foetuses were harvested through abdominal incision for physical examination. Blood samples were collected from the 1st filial rats of the remaining six animals for biochemical and haematological examination. The liver, kidney, heart and brain of all the sacrificed animals were used for histopathological examination. There were significant ($P \leq 0.05$) low birth weights of the foetuses of the animals that were treated with HAART. Results also revealed a reduction ($P \leq 0.05$) in the platelets counts, WBC and RBC of most treatment groups at the first filial generation. Significant ($P \leq 0.05$) elevations in the levels of AST and UA in the foetuses of the animals treated with HAART were also observed. It can be concluded that administration of single and combined antiretrovirals have potential teratogenic effect.

Key words: HAART, Organogenesis, HIV, Birth defects

INTRODUCTION

Human immunodeficiency virus (HIV) infection is a worldwide problem [1] and increasing numbers of women are entering pregnancy on multiple anti-retroviral agents [2, 3]. Thus, the use of anti-retroviral drugs during pregnancy has increased since the demonstration of reduction of mother-to-child transmission (MTCT) of HIV-1 first with zidovudine monotherapy and more recently with highly active antiretroviral therapy (HAART) regimens [3, 4]. The report of WHO showed that the percentage (%) of pregnant women having HIV, receiving drugs for Prevention of Mother to Child Transmission (PMTCT) increased from 15% in 2005 to 45% in 2008 and 54% in 2009 in sub Saharan Africa [5].

The teratogenic risk of most of the anti-retroviral agents cannot be ruled out; human studies are lacking, animal studies are either positive for fetal risk or lacking as well, data are generally limited or varied [6, 7]. Current guidelines recommend 3- or 4-drug regimens when initiating antiretroviral therapy in adults and many of these antiretroviral drugs have not been tested extensively for teratogenic potentials [8]. Zidovudine and efavirenz have had indications of potential teratogenicity in humans, although data are not consistent for either of these drugs [7]. Data from the European Collaborative Study (ECS) and the UK National Study of HIV in Pregnancy and Childhood did not detect an increased risk of defects with ARV exposure [9, 10]. However, the analysis of data from the Pediatric AIDS Clinical Trials Group (PACTG) that studied 185 subjects suggested an increased risk of ventricular septal heart defects after zidovudine exposure in the first trimester [11] and animal data suggest a potential increased risk for central nervous system and facial defects among cynomolgus monkeys after first trimester exposure to efavirenz, but human data are inconclusive [11, 12]. More so, Saitoh et al, 2005 and the report of Perinatal HIV Guidelines, 2009, considered efavirenz, a non-nucleoside analogue, as a potential teratogen on the basis of both animal data and case reports [8, 13].

The present guideline in the management of HIV infected pregnant women that required combination of antiretroviral regimens and concomitant use of drugs such as cotrimoxazole may definitely increase risk [7]. The potential of antiretroviral agents to cross the placental has been well documented [14] and cautious approach to the use of anti-retrovirals during pregnancy (despite their beneficial effects) becomes even highly important because, after the thalidomide disaster of the 1960s, it has become apparent and more accepted that the developing embryo could be highly vulnerable to certain environmental agents (including drugs) that have negligible or non-toxic or beneficial effects to adult individuals.

A number of theoretical mechanisms have sug-

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gested potential modification of fetal toxicity (as seen in combination therapies) to include the induction of biochemical or metabolic changes (with negative consequences on the fetus) in the maternal system that would not have arisen in the use of a single drug or the combination increasing the dose available to interact with fetal mechanism leading to negative effects or changes that are absent in the use of the drug singly. An important mechanism of induction of fetal toxicity by teratogenic agents is the induction of oxidative stress in the fetal system [15]. Oxidative stress can cause cell death and even moderate oxidation can trigger apoptosis, while more intense stresses may cause necrosis [16].

Generally, there is paucity of data on the teratogenic potentials of antiretroviral agents and additional data on their risks during pregnancy are needed [7]. Therefore, this study intends to assess the teratogenic effect of single and combination antiretroviral agents on the organogenesis stage of fetal development and also investigate the possible roles of vitamin C (an antioxidant) in modulating the teratogenic effects of these agents on the fetus using animal model. The findings obtained from this study may give a directional surveillance in humans and possible generation of more reliable and consistent data.

METHODOLOGY

Drugs

The anti-retroviral drugs were obtained from the Aids Prevention Initiative in Nigeria (APIN) HIV/AIDS Clinic of the University of Lagos Teaching Hospital (LUTH), Lagos-Nigeria

- Lamivudine, 3TC (150 mg / tablet)
- Zidovudine, AZT (300 mg / tablet)
- Nevirapine, NVP (200 mg / tablet)
- Lopinavir/ritonavir, LPV (Aluvir®) (200/50 mg /tablet)

Animals

Sexually matured adult Albino rats (male and female) with average weight of 160 g were obtained from Laboratory Animal Centre of College of Medicine, University of Lagos, Nigeria. The animals were authenticated in Zoology department, Faculty of Science, University of Lagos, Nigeria. They were made to acclimatize for two weeks before the commencement of the experiment. The animals were fed on Pfizer Animal Feed cubes and water ad libitum. The investigation conforms to The Guide for the Care and Use of Laboratory Animals published by the U. S. National Institutes of Health (NIH Publication No. 85-23, revised 1996) for studies involving experimental animals. Ethical clearance for use of animals for this research was obtained from the College of Medicine University of Lagos, Research Ethics Committee.

Cycle Determination

After two weeks of acclimatization, vaginal smears of female rats were done for viewing under the microscope to determine the females that will be receptive to the males during mating. Vaginal secretion was collected (in the morning between 8:00 and 9:00 a.m.) with a plastic pipette filled with 10µ L of normal saline by inserting the tip into the rat vagina, but not deeply. Vaginal fluid was then placed on glass slides to observe under a light microscope (x 10 magnification) according to the method of Marcondes *et al.* [17]. The pro-oestrus stage is the receptive state that was microscopically checked out for [18]. The vaginal smears of mated female rats were assessed for the presence of sperm plug. The first day to see sperm plug was taken as day 1 of pregnancy

Treatment Groups

Rats have an average gestational period of 21 days. Exposure period (period in which drugs were administered) was stage of organogenesis (days 6–17).

Pregnant rats were divided into 13 groups with 12 animals per group. The therapeutic doses of drug administration were done to simulate the treatment pattern in APIN HIV/AIDS Clinic of the University of Lagos Teaching Hospital (LUTH), Lagos-Nigeria.

Group 1 (control group) received distilled water (10 ml/kg); Group 2 received a combination of AZT (9 mg/kg), 3TC (5 mg/kg) and NVP (6 mg/kg); Group 3 received a combination of AZT (9 mg/kg), 3TC (5 mg/kg), NVP (6 mg/kg) and Vitamin C (10 mg/kg); Group 4 received a combination of 3TC (5 mg/kg), AZT (9 mg/kg) and LPV (12/3 mg/kg); Group 5 received a combination of 3TC (5 mg/kg), AZT (9 mg/kg), LPV (12/3 mg/kg) and Vitamin C (10 mg/kg); Group 6 received NVP (6 mg/kg). Group 7 received NVP (6 mg/kg) and Vitamin C (10 mg/kg); Group 8 received 3TC (5 mg/kg); Group 9 received 3TC (5 mg/kg) and Vitamin C (10 mg/kg); Group 10 received AZT (9 mg/kg); Group 11 received AZT (9 mg/kg) and Vitamin C (10 mg/kg); Group 12 received LPV (12/3 mg/kg); Group 13 received LPV (12/3 mg/kg) and Vitamin C (10 mg/kg).

Morphological and Histopathological Examination

Six rats in each group were randomly selected, subjected to light ether anesthesia and sacrificed on day 20 by cervical dislocation prior to day 21 of gestation and the fetuses were harvested through abdominal incision for physical examination. The tail length, crown-rump length, umbilical cord length, total weight (includes weight of fetus and placenta) and weights of fetus were recorded. After the physical examination, the harvested fetuses were taken for gross histopathological analysis at the Morbid Anatomy Department of the College of Medicine, University of Lagos. The histopathological features observed were formation of digital rays, neural tube defects, cleft palate and general growth abnormalities.

Biochemical and Haematological Examination

The six other rats in each group were allowed to litter and the litters were allowed to grow for a period of at least one month after which six litters were randomly selected from each of the groups. Blood samples were collected from the animals for biochemical and haematological examination. The fully automated clinical chemistry analyzer (Hitachi 912, Boehringer Mannheim, Germany) was used to determine the levels of Aspartate aminotransferase (AST), Alanine aminotransferase (ALT), Alkaline phosphatase (ACP),

Urea, Creatinine, Uric acid, Albumin, Total protein, Bilirubin, Cholesterol, Triglyceride, High density lipoproteins (HDL), Low density lipoproteins (LDL) and the fully automated clinical haematological analyzer (Pentra-XL 80, Horiba ABX, USA) was also used to determine the levels of white blood cells, red blood cells, hemoglobin, hematocrit (packed cell volume), platelet, lymphocyte count and percentage, neutrophil percentage, mean red cell volume (MCV), mean cell hemoglobin concentration (MCHC) and mean cell hemoglobin (MCH)

Histopathological Examination

The liver, kidney, heart and brain of all the sacrificed animals were fixed in 10% formalin in labeled bottles. Tissues were processed routinely and embedded in paraffin wax. Sections of 5 μ thickness were cut, stained with haematoxylin and eosin and examined under the light microscope by a pathologist.

Statistical analysis

Results were expressed as mean ± SEM. The data were subjected to one way analysis of variance (ANOVA) test and differences between samples were determined by Dunnett's Multiple Comparison Test, using the Graph Pad Prism (statistical) software. MANOVA analysis was also used to speculate the level of variations in between test groups. Results were considered to be significant at $P \leq 0.05$.

RESULTS

The anti-retroviral agents were administered to the rats at doses used clinically according to the standard guidelines. The drugs were administered orally from day 6 through day 17 of pregnancy which is the period of organogenesis.

The result on Table 1 showed the percentage (%) of live foetuses in all the groups to be 100 %. The mean number of live foetuses ranged from 6.5–8.5 and there was no record of fetal resorptions in all the groups.

Table 2 results showed that the mean tail length (TL) ranged from 0.9765 ± 0.0025 cm to 1.176 ± 0.0265 cm. There were significant reductions ($p \leq 0.05$) in the mean tail lengths in groups 2, 5, 6, 7, 8, 10, 12 and 13 compared to the control group. The mean umbilical cord length (UCL) ranged from 1.300 ± 0.0750 cm to 2.306 ± 0.0565cm. The mean umbilical cord lengths were significantly reduced ($p \leq 0.05$) in all the treatment groups compared to the control group. The mean crown rump length (CRL) also ranged from 4.673 ± 0.1263 cm to 5.412 ± 0.0742 cm. There were significant reductions ($p \leq 0.05$) in the mean crown rump lengths in all the treatment groups (except groups 3 and 4) compared to the control group. Furthermore, the mean total weight (TW) ranged from 2.399 ± 0.0441 g to 4.428 ± 0.0501 g and there were significant reduction ($p \leq 0.05$) in mean total weight in all the treatment groups compared to the control group. The results also revealed the mean fetal weight (FW) ranged from 1.934 ± 0.0227 g to 3.683 ± 0.330 g and significant reduction ($p \leq 0.05$) were observed in all the treatment groups compared to the control group.

Table 3 results show no gross malformations (neural tube defects, cleft palate abnormalities or abnormal digital rays' formation) in the fetuses of all the treated groups.

Figure (a–c) show results from the histopathological analysis of harvested organs of the litters. Analysis showed that the hearts were pathologically normal in all the groups (treatment and control groups). The Histology of the brains revealed a mild to moderate edema in all the groups (including control group). The liver tissues revealed intra-cytoplasmic vacuolization/inclusions in groups 2, 3, 4, 5, 6, 7, 9, 10, 12 and central vein (and/or sinusoidal) congestions in groups 3, 6, 10 and 12. There was also mild necro- and periportal inflammation in group 13. The results of kidneys revealed fatty infiltration with or without mild vascular congestion and vacuolization of tubular cells in groups 3, 4, 6, 10, 11, 12, and 13.

Tables 4a and 4b results show the effect of in utero ARV exposure on biochemical parameters at first filial. There were statistically significant increases ($p \leq 0.05$) in Aspartate Transaminase (AST) levels in all groups when compared with the control group. Uric acid (UA) levels were also significantly ($p \leq 0.05$) increased in all the groups except group 8 (Lamivudine–treated group) when compared with the control group. Results showed statistically significant reduction ($p \leq 0.05$) in Creatinine levels in all the groups in comparison with control. Alanine Transaminase (ALT) levels were significantly increased ($p \leq 0.05$) in groups 4, 8 and 12 when compared with control, while there were statistically significant decrease ($p \leq 0.05$) in ALT levels in the other groups when compared with control. Urea levels were significantly increased ($p \leq 0.05$) in groups 2, 3, 4, 5, and 10 when compared with control, while there were statistically significant decrease ($p \leq 0.05$) in Urea levels in groups 6, 7, 11 and 13 in comparison with control. Groups 8, 9 and 12 showed no statistically significant difference in Urea levels. Albumin (ALB) levels were statistically significantly decreased ($p \leq 0.05$) in groups 2, 3, 6, 7, 8, 10 and 11 when compared with control. There were statistically significant reductions ($p \leq 0.05$) in total protein (TP) levels in all the groups when compared with control. Furthermore, cholesterol levels were significantly increased ($p \leq 0.05$) in groups 12 and 13 when compared with control, while groups 4 and 6 show statistically significant decreases ($p \leq 0.05$) in Cholesterol levels compared with control. The triglyceride (TG) results showed statistically significant increased ($p \leq 0.05$) in groups 11 and 13, while other groups except group 10 shows statistically significant decreases ($p \leq 0.05$) in TG levels compared with control. Alkaline Phosphatase (ALP) levels revealed significant increases ($p \leq 0.05$) in groups 3, 6, 10, 11 and 13 when compared with control, while groups 7, 9, and 12 showed significant decreased ($p \leq 0.05$) in ALP levels compared with control. The bilirubin (BIL) levels were significantly increased ($p \leq 0.05$) in groups 2, 3, 4, 5 and 12 when compared with control. The low density lipoprotein (LDL) levels were increased ($p \leq 0.05$) in groups 12 and 13 when compared with control, while the high density lipoprotein (HDL) levels were reduced ($p \leq 0.05$) in groups 3, 4, 5, 6, 7, 9, 10, 11, and 13 when compared with control.

Tables 5a and 5b results shows the effect of *in utero*

Table 1 Incidence of resorptions and live fetuses in treated animals.

Groups	Stage of drug exposure	Percentage (%) of live fetuses	Average number of live fetuses	Number of resorptions
1	Organogenesis	100	8.5	0
2	Organogenesis	100	8.5	0
3	Organogenesis	100	8.5	0
4	Organogenesis	100	7.5	0
5	Organogenesis	100	7.5	0
6	Organogenesis	100	8.0	0
7	Organogenesis	100	8.0	0
8	Organogenesis	100	7.5	0
9	Organogenesis	100	6.5	0
10	Organogenesis	100	8.0	0
11	Organogenesis	100	8.0	0
12	Organogenesis	100	7.0	0
13	Organogenesis	100	8.0	0

Table 1 show the incidence of fetal resorptions and average number of live fetuses recorded in control and treated animals.
Group 1: Control group. Group 2: AZT (9 mg/kg), 3TC (5 mg/kg) and NVP (6 mg/kg). Group 3: AZT (9 mg/kg), 3TC (5 mg/kg), NVP (6 mg/kg) and Vitamin C (10 mg/kg). Group 4: 3TC (5 mg/kg), AZT (9 mg/kg) and LPV (12/3 mg/kg). Group 5: 3TC (5 mg/kg), AZT (9 mg/kg), LPV (12/3 mg/kg) and Vitamin C (10 mg/kg). Group 6: NVP (6 mg/kg). Group 7: NVP (6 mg/kg) and Vitamin C (10 mg/kg). Group 8: 3TC (5 mg/kg). Group 9: 3TC (5 mg/kg) and Vitamin C (10 mg/kg). Group 10: AZT (9 mg/kg). Group 11: AZT (9 mg/kg) and Vitamin C (10 mg/kg). Group 12: LPV (12/3 mg/kg). Group 13: LPV (12/3 mg/kg) and Vitamin C (10 mg/kg).

Table 2 Incidence of growth retardation and size abnormality in fetuses of treated rats.

Groups	TL (cm) [mean ± SEM]	UCL (cm) [mean ± SEM]	CRL (cm) [mean ± SEM]	TW(g) [mean ± SEM]	FW (g) [mean ± SEM]
1	1.15 ± 0.12^{ab}	2.30 ± 0.23^{a}	5.41 ± 0.31^{a}	4.42 ± 0.21^{a}	$3.64 \pm 0.14^{*a}$
2	$0.98 \pm 0.11^{*de}$	$1.59 \pm 0.38^{*cd}$	$4.74 \pm 0.23^{*cd}$	$2.40 \pm 0.18^{*g}$	$1.93 \pm 0.09^{*f}$
3	1.17 ± 0.11^{a}	$1.812 \pm 0.28^{*b}$	5.45 ± 0.27^{a}	$3.63 \pm 0.14^{*b}$	$3.17 \pm 0.12^{*b}$
4	$1.06 \pm 0.27^{*bcde}$	$1.30 \pm 0.29^{*e}$	5.18 ± 0.43^{b}	$3.46 \pm 0.15^{*c}$	$2.91 \pm 0.27^{*c}$
5	$0.96 \pm 0.06^{*e}$	$1.47 \pm 0.23^{*d}$	$4.84 \pm 0.30^{*cd}$	$2.74 \pm 0.20^{*de}$	$2.24 \pm 0.16^{*d}$
6	$1.05 \pm 0.05^{*bcde}$	$1.88 \pm 0.13^{*b}$	$4.83 \pm 0.24^{*cd}$	$2.53 \pm 0.09^{*f}$	$2.06 \pm 0.06^{*e}$
7	$1.05 \pm 0.06^{*bcde}$	$1.95 \pm -0.10^{*b}$	$4.92 \pm 0.19^{*cd}$	$2.57 \pm 0.11^{*f}$	$2.06 \pm 0.09^{*e}$
8	$1.07 \pm 0.62^{*bcd}$	$1.77 \pm 0.19^{*bc}$	$4.67 \pm 0.49^{*d}$	$2.64 \pm 0.10^{*ef}$	$2.13 \pm 0.08^{*e}$
9	1.10 ± 0.10^{ab}	$1.60 \pm 0.20^{*cd}$	$4.74 \pm 0.37^{*cd}$	$2.59 \pm 0.10^{*f}$	$2.05 \pm 0.08^{*e}$
10	$1.07 \pm 0.07^{*bcd}$	$1.91 \pm 0.23^{*b}$	$4.72 \pm 0.24^{*cd}$	$2.75 \pm 0.15^{*de}$	$2.26 \pm 0.14^{*d}$
11	1.15 ± 0.18^{ab}	$1.93 \pm 0.15^{*b}$	$4.94 \pm 0.33^{*c}$	$2.77 \pm 0.23^{*d}$	$2.23 \pm 0.18^{*d}$
12	$1.04 \pm 0.71^{*cde}$	$1.79 \pm 0.19^{*b}$	$4.74 \pm 0.39^{*cd}$	$2.65 \pm 0.07^{*ef}$	$2.16 \pm 0.06^{*de}$
13	$0.99 \pm 0.67^{*de}$	$1.77 \pm 0.30^{*bc}$	$4.69 \pm 0.26^{*cd}$	$2.61 \pm 0.11^{*f}$	$2.12 \pm 0.11^{*e}$

Table 2 shows the incidence of growth retardation (measured as tail length, crown-rump length and umbilical cord length) and size abnormality (measured as total and fetal weight) in control and treated rats.
Results are presented as Mean ± SEM (n = 6).
^{abcdefg} represent MANOVA analysis, results with the same alphabet in a column showed same levels of variations (disparity) in comparison with the control group.
* represents result of ANOVA analysis where $p \leq 0.05$ as compared to the control (Group 1)
Groups 1–13: as in Table 1.
TL is tail length; CRL is crown-rump length; UCL is umbilical cord length; TW is total weight of foetus and placenta; FW is foetal weight; SEM is standard error of mean
Exposure period was stage of organogenesis (days 6–17).

ARV exposure on hematological parameters at first filial. Results showed statistically significant reductions ($p \leq 0.05$) in white blood cells counts, packed cell volume (PCV), red blood cells (excepts 2, 3 and 10), Platelet counts (excepts 2, 3 and 12) and hemoglobin (except groups 8 and 9) in all the groups when compared with control. Analysis of the mean corpuscular hemoglobin (MCH) showed a statistically significant reduction ($p \leq 0.05$) in groups 3, 7, 8, 9, 10 and 12 when compared with control. The neutrophil percentages (%) were significantly increased ($p \leq 0.05$) in groups 5, 12 and 13 and reduced ($p \leq 0.05$) in all other groups when compared with control. The results further showed significant increase ($p \leq 0.05$) in lymphocyte percentages (%) in groups 2, 10 and 11 and decreased ($p \leq 0.05$) in groups 4, 5, 12 and 13 when compared with control.

Table 3 Incidence of gross malformations in foetuses following anti-retroviral exposure.

Groups	Total number of fetuses studied	Formation of digital ray	Neural tube defects	Cleft palate abnormalities	Comments
1	17	0/17	0/17	0/17	Normal
2	17	0/17	0/17	0/17	Normal
3	17	0/17	0/17	0/17	Normal
4	15	0/15	0/15	0/15	Normal
5	15	0/15	0/15	0/15	Normal
6	16	0/16	0/16	0/16	Normal
7	16	0/16	0/16	0/16	Normal
8	15	0/15	0/15	0/15	Normal
9	13	0/13	0/13	0/13	Normal
10	16	0/16	0/16	0/16	Normal
11	16	0/16	0/16	0/16	Normal
12	14	0/14	0/14	0/14	Normal
13	16	0/16	0/16	0/16	Normal

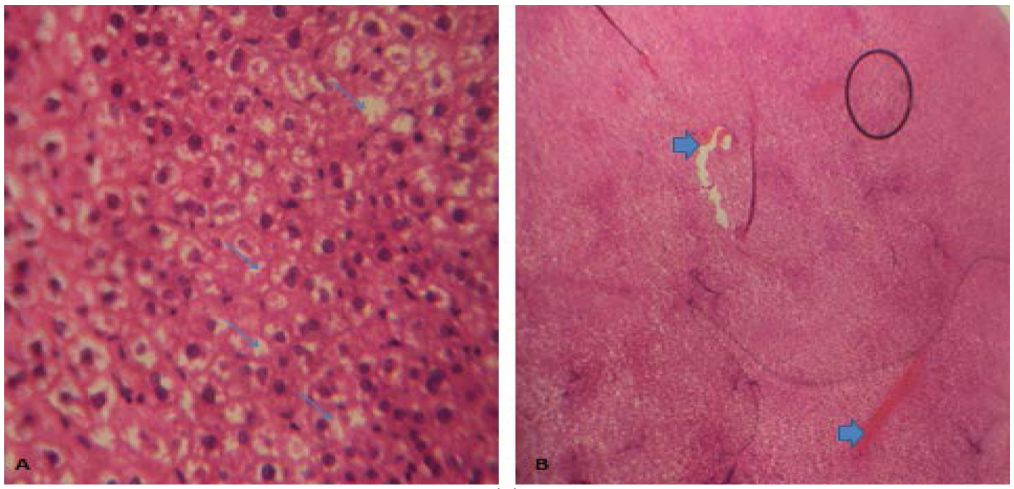
Table 3 shows the incidence of gross malformations in foetuses following antiretroviral (single and combination) exposure.
Foetuses were grossly examined for formation of digital rays, neural-tube defects, and cleft palate abnormalities.
Groups 1–13: as in Table 1.
Exposure period was stage of organogenesis (days 6–17).

DISCUSSION

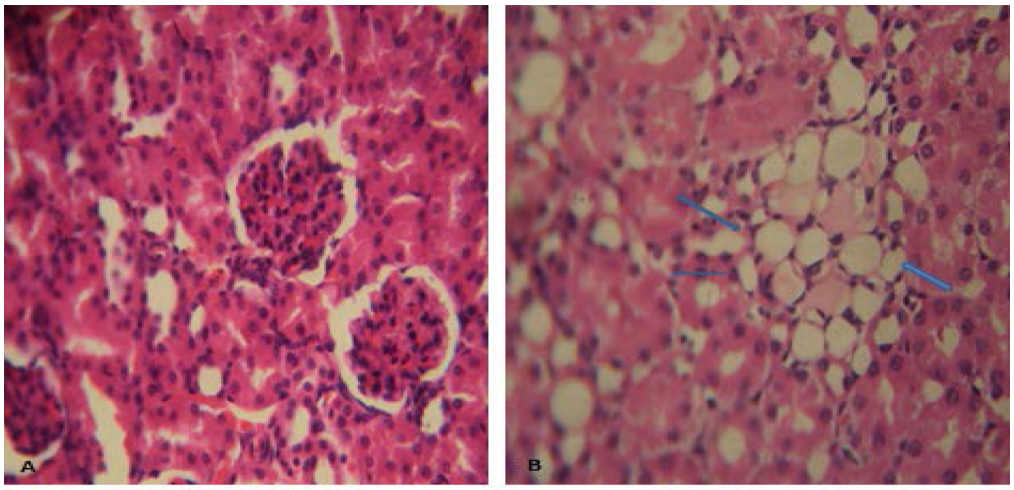
It is a general principle that the administration of any drug to a pregnant patient is to be avoided, because of possible fetal damage. However, increasing numbers of women are entering pregnancy on multiple anti-retroviral agents [2, 3] and the use of antiretroviral drugs during pregnancy has increased since the demonstration of reduction of mother-to-child transmission (MTCT) of HIV-1 first with zidovudine monotherapy and more recently with highly active antiretroviral therapy (HAART) regimens [3, 4].

The data obtained from this investigation employed both ANOVA and MANOVA statistical analysis procedures. The ANOVA analysis revealed the significant differences {$p \leq 0.05$} across test groups compared with the control group. While the MANOVA analysis showed the levels/degree of variations in the test groups as highlighted in the results. It was observed from the present study that the foetal weight of litters decreased ($p \leq 0.05$) across the groups when compared with the control group with the Zidovudine /Lamivudine/Nevirapine combinations giving the maximum foetal weight reduction (1.934 ± 0.0227g). There were also reductions in the foetal weight plus placental weight in all the groups as compared to the control group with maximum reduction in Zidovudine/Lamivudine/Nevirapine combinations (2.399 ± 0.0441g). Earlier studies have shown that children with low birth weight (LBW) have an increased risk of developing diabetes [19, 20], obesity [21] and reduced intelligence [22] later in life.

There were also reductions in the crown-rump lengths of the foetuses that received both single and combined ARV as compared to the control. Crown-rump length (CRL) is the measurement of the length of human embryos and foetuses from the top of the head (crown) to the bottom of the buttocks (rump). It is typically determined from ultrasound imagery and can be used to estimate gestational age. The measurement of CRL is useful in determining the gestational age (menstrual age starting from the first day of the last menstrual period) and thus the expected date of delivery (EDD). Some unconfirmed studies have shown that the crown-rump length is proportional to the umbilical cord length (UCL); the UCL as found in this study, reduced statistically ($p \leq 0.05$) compared to the control group. Reductions in CRL and foetal size/weight are an indication of growth retardation (stunted growth) and have clinical implications for threatened abortion [23] and the risk of spontaneous abortion in assisted conceptions [24]. These observations in animals have positive correlations with human's studies (HIV pregnant women on combination antiretroviral therapy) [25, 26, 27]. These findings may also postulate however, that low birth weight outcomes and risk for preterm birth are not due to exposure to the combined antiretrovirals alone, but are expected occurrence even on single agent exposure. It has been postulated that exposure to HAART or other antiretroviral combinations, which often contains at least one PI, leads to insulin resistance and endothelial inflammation, with a concomitantly increased risk of pre-eclampsia (a condition in which hypertension arises in pregnancy in association with significant amounts of protein in the urine) [28]. Pre-eclampsia, in turn, is associated with LBW and preterm birth [29]. Researchers concluded at the 18th Conference on Retroviruses and opportunistic Infections in Boston that premature births and LBW in HIV-positive women continue to be a concern, and need to be anticipated and managed actively [30]. Studies have suggested that individuals who were born with low birth weight may be at increased risk for certain chronic conditions in adulthood. These conditions include high blood pressure, type-2 (adult-onset) diabetes and heart disease. When these conditions occur together, they are called metabolic syndrome. One study found that men who weighed less than 3,000g at birth were 10 times more likely to have metabolic syndrome than



(a)



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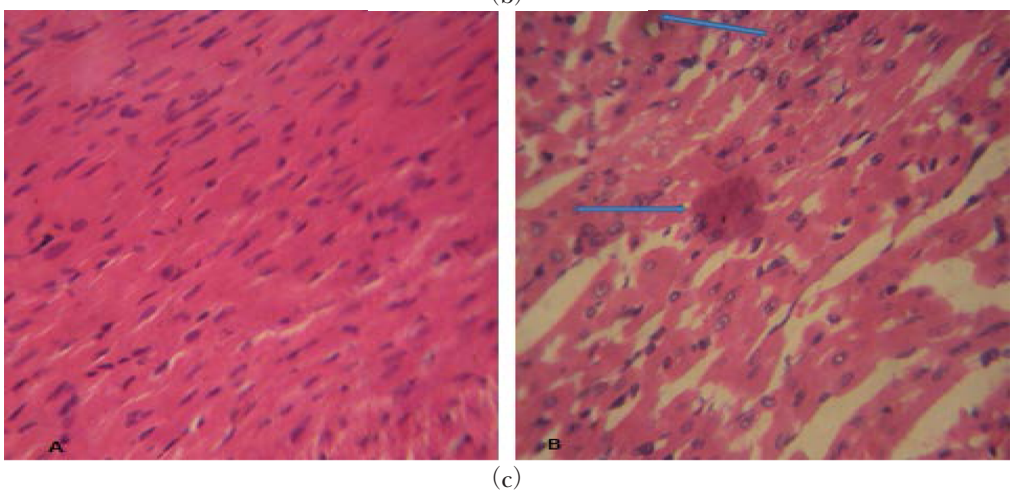

(c)

Figure (a) Microscopic (photomicrograph) illustration of liver from litter (first filial). A shows hepatocytes (thin arrows) with intracytoplasmic inclusions as seen in group 2 (Zidovudine/Lamivudine/Nevirapine), group 4 (Lamivudine/Zidovudine/Lopinavir/ritonavir), group 7 (Nevirapine/Vitamin C) and group 9 (Lamivudine/Vitamin C), magnification × 400. B shows congested hepatic central veins (thick arrow heads) as seen in groups 3 (Zidovudine/Lamivudine/Nevirapine/Vitamin C), 5 (Lamivudine/Zidovudine/Lopinavir/ritonavir/Vitamin C), 6 (Nevirapine), 10 (Zidovudine), and 12 (Lopinavir/ritonavir), magnification × 400. (b) Microscopic (photomicrograph) illustration of kidney from litter (first filial). A shows the normal kidney (control group) magnification × 400. B shows focal areas of fat infiltration (thick arrows) surrounded by normal renal tubules as seen in the group 3 (Zidovudine/Lamivudine/Nevirapine/VitaminC), group 4 (Lamivudine/Zidovudine/Lopinavir/ritonavir) and group 9 (Lamivudine/Vitamin C) magnification × 400. (c) Microscopic (photomicrograph) illustration of heart from litter (first filial). A shows the normal heart (control group), magnification × 400 B shows areas of mild hypertrophy of myocytes as seen in the group 13 (Lopinavir/ritonavir/Vitamin C), magnification × 400

Table 4a Effect of *in utero* ARV exposure on biochemical parameters at first filial

Groups	BIOCHEMICAL PARAMETER						
	AST (µ/L)	UA (µmol/L)	CRE (µmol/L)	ALT (µ/L)	UREA (mmol/L)	ALB (g/L)	TP (g/L)
1	34.52 ± 1.15^{f}	0.81 ± 0.11^{g}	56.11 ± 1.72*^{a}	79.38 ± 3.43*^{bc}	6.52 ± 0.42^{b}	39.73 ± 2.28^{ab}	73.29 ± 2.30^{a}
2	160.27 ± 14.85*^{c}	2.39 ± 0.22*^{b}	20.29 ± 2.72*^{fg}	59.88 ± 3.85*^{d}	7.23 ± 0.36*^{a}	21.25 ± 3.27*^{d}	42.08 ± 2.20*^{cd}
3	109.27 ± 17.78*^{de}	1.54 ± 0.14*^{d}	18.89 ± 9.89*^{g}	63.50 ± 10.96*^{cd}	8.48 ± 2.11*^{a}	23.73 ± 6.41*^{d}	49.23 ± 3.49*^{c}
4	398.58 ± 33.84*^{a}	1.14 ± 0.17*^{ef}	38.84 ± 5.18*^{c}	204.10 ± 27.24*^{a}	8.367 ± 0.30*^{a}	40.58 ± 2.04^{ab}	63.65 ± 2.61*^{ab}
5	118.10 ± 14.20*^{d}	1.38 ± 0.05*^{de}	38.71 ± 1.73*^{c}	68.28 ± 2.40*^{cd}	9.57 ± 1.17*^{a}	40.08 ± 1.14^{ab}	65.88 ± 2.21*^{ab}
6	140.28 ± 12.57*^{c}	2.28 ± 0.26*^{bc}	23.25 ± 6.18*^{fg}	24.70 ± 1.50*^{e}	4.95 ± 0.71*^{b}	23.33 ± 5.87*^{d}	45.00 ± 0.90*^{cd}
7	98.72 ± 12.94*^{de}	3.40 ± 0.22*^{a}	25.73 ± 4.31*^{ef}	28.14 ± 2.88*^{e}	4.80 ± 0.77*^{b}	24.14 ± 8.18*^{d}	43.50 ± 2.89*^{cd}
8	50.71 ± 3.19*^{f}	0.87 ± 0.06*^{fg}	39.50 ± 1.65*^{c}	91.30 ± 3.41*^{b}	6.97 ± 0.56^{b}	43.42 ± 2.87*^{a}	67.73 ± 2.81*^{ab}
9	95.70 ± 13.64*^{de}	1.45 ± 0.17*^{de}	48.44 ± 2.09*^{b}	67.77 ± 3.11*^{cd}	6.63 ± 0.51^{b}	41.41 ± 3.27^{ab}	66.73 ± 3.67*^{ab}
10	151.2 ± 7.84*^{c}	1.412 ± 0.08*^{de}	34.26 ± 1.84*^{cd}	41.35 ± 0.22*^{de}	11.38 ± 0.27*^{a}	35.85 ± 1.43*^{c}	62.87 ± 0.42*^{b}
11	97.35 ± 11.44*^{de}	1.38 ± 0.11*^{de}	30.72 ± 2.60*^{de}	62.18 ± 5.49*^{cd}	4.700 ± 0.22*^{b}	34.11 ± 3.80*^{c}	42.46 ± 0.50*^{cd}
12	263.67 ± 18.64*^{b}	2.20 ± 0.66*^{c}	34.15 ± 2.09*^{cd}	86.85 ± 1.96*^{b}	6.68 ± 2.77^{b}	37.97 ± 3.12^{abc}	62.45 ± 5.98*^{b}
13	94.60 ± 5.89*^{e}	1.31 ± 0.06*^{de}	25.31 ± 0.99*^{ef}	25.46 ± 0.63*^{e}	5.16 ± 0.31*^{b}	38.44 ± 1.44^{abc}	42.22 ± 0.27*^{cd}

Table 4a shows the effect of *in utero* ARV exposure on biochemical parameters at first filial.
Results are presented as Mean ± SEM (n = 6).
^{abcdefg} represent MANOVA analysis, results with the same alphabet in a column showed same levels of variations (disparity) in comparison with the control group.
* represents result of ANOVA analysis where $p \leq 0.05$ as compared to the control (Group 1)
Groups 1–13: as in Table 1.
AST is Aspartate Transaminase; UA is Uric Acid; CRE is Creatinine; ALT is Alanine Transaminase; ALB is Albumin; TP is Total Protein; SEM is standard error of mean. Exposure period was stage of organogenesis (days 6–17).

Table 4b Effect of *in utero* ARV exposure on biochemical parameters at first filial (cont'd).

Groups	BIOCHEMICAL PARAMETER					
	CHOL (mmol/L)	T.G (mmol/L)	ALP (µ/L)	BIL (µmol/L)	LDL (mmol/L)	HDL (mmol/L)
1	2.00 ± 0.45^{cd}	1.58 ± 0.21*^{cd}	339.40 ± 24.39^{ef}	3.25 ± 0.28^{c}	0.15 ± 0.05^{c}	1.05 ± 0.05^{ab}
2	2.02 ± 0.16^{cd}	1.10 ± 0.11*^{ds}	353.15 ± 17.50^{de}	4.30 ± 0.38*^{ab}	0.12 ± 0.04^{c}	0.87 ± 0.28^{bc}
3	2.15 ± 0.17^{c}	0.18 ± 0.16*^{f}	493.57 ± 34.71*^{a}	4.08 ± 0.291*^{ab}	0.12 ± 0.04^{c}	0.73 ± 0.25*^{cde}
4	1.33 ± 0.16*^{e}	1.23 ± 0.16*^{e}	348.63 ± 23.75^{ef}	4.05 ± 0.16*^{abc}	0.15 ± 0.05^{c}	0.78 ± 0.10*^{cde}
5	1.52 ± 0.33^{de}	1.28 ± 0.17*^{de}	327.13 ± 17.31^{f}	3.65 ± 0.26*^{bc}	0.12 ± 0.04^{c}	0.83 ± 0.15*^{bcd}
6	1.35 ± 0.38*^{e}	1.07 ± 0.08*^{de}	406.92 ± 30.04*^{c}	3.63 ± 0.35^{be}	0.17 ± 0.08^{c}	0.63 ± 0.81*^{def}
7	1.54 ± 0.43^{de}	1.10 ± 0.17*^{de}	384.14 ± 20.42*^{cd}	3.50 ± 0.26^{bc}	0.12 ± 0.25^{c}	0.64 ± 0.13*^{def}
8	1.97 ± 0.24^{cd}	1.01 ± 0.04*^{de}	362.42 ± 26.98^{de}	3.35 ± 0.42^{c}	0.13 ± 0.05^{c}	1.18 ± 0.18^{a}
9	2.02 ± 0.23^{cd}	1.05 ± 0.05*^{e}	253.37 ± 31.01*^{g}	3.47 ± 0.53^{bc}	0.12 ± 0.04^{c}	0.58 ± 0.10*^{ef}
10	2.41 ± 0.97^{bc}	1.400 ± 0.07^{cd}	449.51 ± 33.49*^{b}	3.67 ± 0.39^{bc}	0.23 ± 0.22^{c}	0.86 ± 0.17*^{bcd}
11	2.20 ± 0.17^{c}	2.08 ± 0.08*^{b}	408.50 ± 17.23*^{c}	3.68 ± 0.43^{bc}	0.10 ± 0.00^{c}	1.20 ± 0.09*^{a}
12	2.81 ± 0.52*^{b}	1.07 ± 0.23*^{de}	235.38 ± 24.53*^{g}	4.67 ± 0.54*^{a}	0.62 ± 0.10*^{a}	0.90 ± 0.26*^{bc}
13	4.03 ± 0.61*^{a}	4.17 ± 0.41*^{a}	465.38 ± 39.31*^{ab}	3.57 ± 0.41^{bc}	0.48 ± 0.21*^{b}	0.47 ± 0.13*^{f}

Table 4b shows a continuation of the effect of *in utero* ARV exposure on biochemical parameters at first filial.
Results are presented as Mean ± SEM (n = 6).
^{abcdefg} represent MANOVA analysis, results with the same alphabet in a column showed same levels of variations (disparity) in comparison with the control group.
* represents result of ANOVA analysis where $p \leq 0.05$ as compared to the control (Group 1)
Groups 1–13: as in Table 1.
CHOL is Cholesterol; TG is Triglycerides; ALP is Alkaline Phosphatase; BIL is Bilirubin; LDL is Low Density Lipoprotein; HDL is High Density Lipoprotein; SEM is standard error of mean. Exposure period was stage of organogenesis (days 6–17).

the men who weighed more than 4,390g at birth [31]. The pathological results as seen in this study showed that the heart was normal in all the groups except in the Zidovudine group and Lopinavir/ritonavir/ Vitamin C group where focal areas of calcification in the heart and mild hypertrophy of myocytes were seen respectively. Hypertrophic cardiomyopathy (HCM) (seen in group 13) is a condition in which the heart muscle becomes thick. The thickening makes it harder for blood to leave the heart, forcing the heart to work harder to pump blood. Hypertrophic cardiomyopathy is often asymmetrical; meaning one part of the heart is thicker than the other parts. The condition is usually passed down through families (inherited) (the

Table 5a Effect of *in utero* ARV exposure on hematological parameters at first filial.

GROUPS	HEMATOLOGICAL PARAMETER				
	WBC (10^3 /µL)	HB (g/dL)	PCV	MCV (fl)	MCH (pg)
1	16.80 ± 0.198^{a}	15.80 ± 0.258^{a}	48.43 ± 0.296^{a}	57.28 ± 0.851^{cd}	18.83 ± 0.260^{b}
2	8.300 ± 0.294*^{d}	12.83 ± 0.370*^{d}	37.95 ± 0.722*^{e}	60.17 ± 0.443*^{b}	18.45 ± 0.354^{b}
3	12.82 ± 0.311*^{b}	13.95 ± 0.564*^{b}	41.07 ± 0.627*^{cd}	58.16 ± 1.380^{c}	16.82 ± 0.517*^{d}
4	9.100 ± 0.177*^{c}	13.57 ± 0.364*^{c}	43.22 ± 0.838*^{c}	58.68 ± 1.366^{c}	19.08 ± 0.316^{ab}
5	9.333 ± 0.159*^{c}	13.55 ± 0.306*^{c}	42.48 ± 0.282*^{c}	60.03 ± 0.568*^{b}	19.37 ± 0.279^{a}
6	8.433 ± 0.236*^{d}	12.35 ± 0.136*^{d}	37.58 ± 0.232*^{e}	56.37 ± 0.594^{d}	17.88 ± 0.341^{c}
7	9.167 ± 0.484*^{c}	12.82 ± 0.324*^{d}	38.70 ± 0.489*^{d}	54.33 ± 0.738*^{de}	15.93 ± 0.422*^{d}
8	9.150 ± 0.253*^{c}	15.03 ± 0.288^{a}	43.73 ± 0.321*^{c}	53.28 ± 1.103*^{de}	14.94 ± 0.323*^{e}
9	9.367 ± 0.385*^{c}	14.97 ± 0.307^{a}	42.63 ± 0.924*^{c}	54.08 ± 0.806*^{de}	15.27 ± 0.508*^{d}
10	15.32 ± 0.351*^{a}	14.95 ± 0.245^{a}	45.00 ± 0.562*^{b}	53.13 ± 0.345*^{de}	17.28 ± 0.174*^{c}
11	15.30 ± 0.382*^{a}	14.15 ± 0.382*^{b}	43.50 ± 0.202*^{c}	51.32 ± 1.805*^{e}	19.17 ± 0.303^{ab}
12	11.20 ± 0.365*^{b}	12.77 ± 0.254*^{d}	39.45 ± 0.793*^{d}	55.17 ± 0.222*^{d}	17.87 ± 0.270*^{c}
13	9.950 ± 0.291*^{c}	8.367 ± 0.253*^{e}	26.82 ± 0.247*^{f}	64.88 ± 0.663*^{a}	19.18 ± 0.196^{ab}

Table 5a shows the effect of *in utero* ARV exposure on hematological parameters at first filial.
Results are presented as Mean ± SEM (n = 6).
^{abcdefg} represent MANOVA analysis, results with the same alphabet in a column showed same levels of variations (disparity) in comparison with the control group.
*represents result of ANOVA analysis where $p \leq 0.05$ as compared to the control (Group 1) Groups 1–13: as in Table 1
WBC is White Blood Cells; HB is Hemoglobin; PCV is Packed Cell Volume; MCV is Mean Corpuscular Volume; MCH is Mean Corpuscular Hemoglobin; SEM is standard error of mean. Exposure period was stage of organogenesis (days 6–17).

Table 5b Effect of *in utero* ARV exposure on hematological parameters at first filial (cont'd).

GROUPS	HEMATOLOGICAL PARAMETER					
	MCHC (g/dL)	PLAT (10^4 /µL)	NEUT. (%)	LYM (%)	MONO (%)	RBC (10^3 /µL)
1	32.57 ± 0.438^{d}	730.0 ± 6.593^{a}	28.80 ± 0.286^{c}	62.98 ± 0.717^{c}	7.883 ± 0.361^{d}	8.378 ± 0.134^{b}
2	34.45 ± 0.369*^{b}	718.0 ± 6.792^{b}	27.02 ± 0.158*^{d}	66.05 ± 0.268*^{ab}	6.933 ± 0.370^{e}	8.217 ± 0.111^{b}
3	35.87 ± 0.331*^{a}	706.7 ± 10.230^{b}	26.17 ± 0.484*^{d}	64.77 ± 0.903^{b}	9.067 ± 0.735^{b}	8.000 ± 0.242^{b}
4	31.05 ± 0.970^{e}	649.7 ± 13.290*^{d}	29.35 ± 0.315*^{c}	60.12 ± 0.425*^{d}	10.15 ± 0.231*^{a}	7.235 ± 0.075*^{cd}
5	32.67 ± 0.186^{d}	639.8 ± 12.080*^{de}	32.00 ± 0.481*^{c}	58.70 ± 0.589*^{e}	9.150 ± 0.249*^{b}	6.975 ± 0.042*^{e}
6	32.97 ± 0.209^{d}	542.3 ± 10.150*^{f}	27.40 ± 0.324*^{c}	63.62 ± 0.726^{c}	9.667 ± 0.481*^{a}	6.438 ± 0.148*^{f}
7	34.85 ± 0.671*^{b}	673.5 ± 12.380*^{c}	26.55 ± 0.457*^{c}	64.27 ± 0.544^{b}	9.183 ± 0.593*^{b}	7.583 ± 0.292*^{c}
8	31.92 ± 0.547^{e}	622.5 ± 15.130*^{e}	26.18 ± 0.624*^{c}	64.72 ± 0.659^{b}	9.100 ± 0.747^{bc}	7.193 ± 0.167*^{d}
9	31.92 ± 0.547^{e}	622.5 ± 15.130*^{e}	27.07 ± 0.307*^{c}	63.32 ± 0.890^{c}	9.617 ± 0.888^{a}	7.167 ± 0.146*^{d}
10	33.07 ± 0.0989^{c}	661.0 ± 14.760*^{c}	24.98 ± 0.454*^{e}	66.10 ± 0.493*^{a}	8.233 ± 0.260^{c}	8.543 ± 0.094^{a}
11	32.38 ± 0.314^{d}	622.5 ± 12.360*^{e}	25.85 ± 0.255*^{d}	65.75 ± 0.341*^{a}	8.567 ± 0.194^{c}	6.387 ± 0.122*^{f}
12	31.70 ± 0.803^{e}	707.7 ± 56.500^{b}	47.50 ± 1.087*^{ab}	48.12 ± 1.476*^{f}	4.867 ± 0.062*^{f}	7.032 ± 0.069*^{de}
13	28.57 ± 0.481*^{f}	338.5 ± 4.731*^{g}	49.57 ± 1.140*^{a}	41.52 ± 0.544*^{g}	9.200 ± 0.457*^{b}	4.083 ± 0.031*^{g}

Table 5b shows a continuation of the effect of *in utero* ARV exposure on hematological parameters at first filial.
Results are presented as Mean ± SEM (n = 6).
^{abcdefg} represent MANOVA analysis, results with the same alphabet in a column showed same levels of variations (disparity) in comparison with the control group.
* represents result of ANOVA analysis where $p \leq 0.05$ as compared to the control (Group 1)
Groups 1–13: as in Table 1.
MCHC is Mean Corpuscular Hemoglobin Concentration; NEUT is Neutrophils; LYM is Lymphocytes; MONO is Monocytes; RBC is Red Blood Cells; SEM is standard error of mean. Exposure period was stage of organogenesis (days 6–17).

observed mild HCM in this study could thus be due to inheritance). Once HCM has been identified in a family, immediate testing of all family members will help to identify those at risk. Children often do not show signs of HCM; the first sign many children display is sudden cardiac arrest. HCM is believed to be a result of several problems (defects) with the genes that control heart muscle growth. The myopathy may also be due to infection, disordered metabolism, nutritional excess or deficiency, toxic agents, autoimmune processes, or degeneration. The cause of a hypertrophic cardiomyopathy may however remain unknown [32]. Zidovudine related cardiomyopathy has been reported though the frequency remains quite rare. One of the side effects of the NRTIs (also rare but potentially life-threatening) is cardiomyopathy probably related to mitochondrial toxicity caused by NRTIs [33]. It should be noted however that children who have moderately symptomatic conditions according to the HIV Pediatric Classification System (Clinical Categories) may present with cardiomyopathy as a clinical presentation of HIV infection and not a consequence of ART. Also

in HIV infected patients, the HIV-associated dilated cardiomyopathy is of major interest. It corresponds to a dilated and less contractile left ventricle.

The results from this study showed hepatic pathological presentations ranging from central vein and/or sinusoidal congestion and necrosis, intracytoplasmic vacuolization/inclusions, and mild necro- and periportal inflammation. These presentations are significant as they are absent (not observed) in the control group. These observations are therefore probably due to the in utero exposure of the infants to the antiretrovirals. However, (Lamivudine group) and (Zidovudine/Vitamin C group) showed normal (similar to control) presentations. It thus may mean that Lamivudine exposure does not adversely affect the liver (hepatic system) of the fetus in utero and Vitamin C was effective (probably due to its anti-oxidant activity) in modulating the adverse effect of in utero exposure to Zidovudine. These modulatory effect was absent for other groups. Liver toxicity is common with HAART and occurs in up to 6% of patients [34]. Their occurrence depends on the drug classes or agents used as well as on pre-existing liver dysfunction. The level of liver toxicity ranges from mild and fully reversible liver enzyme elevation to rare but rapidly occurring, occasionally fatal, liver failure. Nevirapine and ritonavir have been associated with severe hepatotoxicity and hepatic failure with several fatalities linked to nevirapine [35] and in year 2000, the U.S. Food and Drug Administration issued a black box label on nevirapine, warning that it could cause severe liver damage, including liver failure. Chronic, high-dose therapy with Zidovudine is associated with significant side effects, including hepatotoxicity and deducing from the results reported in this study vitamin C was effective (probably due to its anti-oxidant activity) in modulating the possible hepatotoxicity that may occur due to in utero exposure to Zidovudine. Pathological presentations of the kidney showed effects ranging from normal presentations to fatty vacuolation of tubular cells and mild vascular congestion. Fatty infiltration is a deposit of fat in tissues, especially between cells. It is also the presence of fat vacuoles in the cell cytoplasm. It should be noted that infiltration is the pathological diffusion or accumulation in a tissue or cells of substances not normal to it or in amounts in excess of the normal. A fatty change represents the intracytoplasmic accumulation of triglyceride (neutral fats).

Aspartate Transaminase (AST) values were significantly higher ($p \leq 0.05$) in all the treatment groups compared to the control group. The results for ALT shows a significant increase ($p \leq 0.05$) in groups 4, 8 and 12 when compared with control, while there was a statistically significant decrease ($p \leq 0.05$) in ALT levels in the other groups when compared with control. Alkaline Phosphatase (ALP) levels showed statistically significantly increases ($p \leq 0.05$) in groups 3, 6, 10, 11 and 13 when compared with control, while groups 7, 9, and 12 show statistically significant decreases ($p \leq 0.05$) in ALP levels compared with control. Groups 2, 4, 5, and 8 show non-statistically significant difference in ALP levels when compared to control. Bilirubin (BIL) levels were statistically significantly increased ($p \leq 0.05$) in groups 2, 3, 4, 5 and 12 when compared with control while other groups show non-statistically significant differences in BIL levels when compared to control. These imbalances in liver function tests showed that the ARVs administered to pregnant mother-rats in one way or the other caused an effect observable in the fetal results at the first filial and relatively corroborate the histopathological results which showed changes in liver cells constitution ranging from central vein congestion to intracytoplasmic inclusion/vacuolation in all the treatment groups (except the Lamivudine and the Zidovudine plus Vitamin C group). Case reports also exist about liver failure occurring on indinavir, atazanavir, efavirenz and nelfinavir [36, 37]. Hepatic toxicity with hyper-bilirubinemia was described under Zidovudine (AZT) + Lamivudine (3TC) + Efavirenz therapy.

Though these liver toxicities observable with the use of ARVs in patients have not been correlated in literature with possible toxicities (or effects) in fetuses/offsprings, the results obtained from this study showed that these toxicities are possibilities that can be seen (to occur) in the fetuses at first filial. The use of an anti-oxidant in this study, (vitamin C) did not significantly affect these changes in liver biochemical parameters.

Uric acid levels were elevated significantly ($p \leq 0.05$) in all the groups except group 8 (Lamivudine–treated group). Results also showed a statistically significant reduction ($p \leq 0.05$) in Creatinine levels in all the groups when compared with control. Urea levels was significantly raised ($p \leq 0.05$) in groups 2, 3, 4, 5, and 10 when compared with control, while there was a statistically significant decrease ($p \leq 0.05$) in Urea levels in groups 6, 7, 11 and 13 when compared with control. Groups 8, 9 and 12 showed no statistically significant difference in Urea levels. Serum uric acid can be elevated due to reduced excretion by the kidneys. Renal toxicity has been experienced with only a few ARVs particularly Indinavir and Tenofovir (which were not included in this study). With Indinavir, more than 20 % of patients have persistent asymptomatic leukocyturia associated with a gradual loss of renal function without urological symptoms [38]. However, renal failure is rare [39]. Animal studies showed a dose-related nephrotoxicity with tenofovir, although several case reports have suggested its occurrence, severe renal toxicity occurs rarely and was not observed in the major clinical trials with tenofovir [40]. Acute renal failure and proximal tubulopathy, nephrogenic diabetes insipidus and rarely hypo-phosphatemicosteomalacia have been reported [41]. Studies also show that the use of tenofovir is also associated with a modest decline in creatinine clearance in comparison to patients never treated with tenofovir [42].

Hematological results from this study showed a statistically significant ($p \leq 0.05$) reduction in White Blood Cell counts (i.e. leukopenia) in all the groups when compared with control. The results showed a general leukopenia suggestive of a decreased immune function and increased susceptibility to infection subsequent to the in utero exposure to these drugs at the stage of organogenesis in all groups (implicating all the ARVs used in this study). Neutrophilia (increase in Neutrophils) was observed to be significant in groups 12 and 13. Neutrophilia is mainly as a result of acute

bacterial infections, inflammation, tissue necrosis e.g. myocardial infarction or burns. Platelet counts were reduced (thrombocytopenia) in most of the groups suggesting that a potential reduction in ability to form clots or an increase in bleeding episodes may be observed in infants exposed in utero to these ARVs. This however has not been documented in human studies. Hemoglobin is the iron-containing oxygen-transport metalloprotein in the red blood cells of all vertebrates as well as the tissues of some invertebrates. Hemoglobin in the blood carries oxygen from the respiratory organs (lungs or gills) to the rest of the body (i.e. the tissues) where it releases the oxygen to burn nutrients to provide energy to power the functions of the organism, and collects the resultant carbon dioxide to bring it back to the respiratory organs to be dispensed from the organism. Some of the antiretroviral drugs (especially zidovudine) are myelo-suppressive, especially with respect to the red cells, and therefore lead to anemia [43]. Most commonly affected are patients with advanced HIV infection and pre-existing myelosuppression, on chemotherapy or co-medication with other myelotoxic drugs such as co-trimoxazole, pyrimethamine, amphotericin B, ribavirin, and interferon, or with other antiretroviral drugs. 5 to 10% of patients taking zidovudine develop anemia – usually during the first 3 months of therapy, but sometimes even after years on treatment [36]. Red cell counts were reduced (anemia) in most of the groups as well as reduced hemoglobin levels in all the groups suggesting that a potential reduction in ability of the blood (through the RBCs) to carry oxygen to tissues leading to tissue hypoxia and or necrosis in infants exposed in utero to these ARVs.

Pathological presentations of the brain showed (mild) brain edema. The observed cerebral edema occurred across all the groups (treatment and control). This generality suggests that the pathology is possibly through causes other than the antiretrovirals (both single agents and combinations), as the control group was not given drugs yet the cerebral edema presented in the histology of the brain.

CONCLUSION

It can be concluded that administration of single and combined antiretrovirals have potential teratogenic effect shown by significant growth retardation as evident by reduced birth weight and crown-rump length. Gross pathological analysis showed no teratogenic effects indicated by absence of neural tube defects, digital rays and cleft palate abnormalities. Histopathology studies reveal possible antiretroviral-induced teratogenicity on the liver and kidneys; effect on the heart was unclear and absent in the brain. Biochemical results showed marked increases in liver function tests particularly Aspartate Transaminase (AST), significant increase in uric acid and a marked decrease in creatinine levels at the first filial subsequent to in utero ARVs exposure. Hematological results reveal significant reductions in WBC and RBC counts, Hemoglobin levels, Packed Cell Volume and Platelet counts due to the in utero exposure to ARVs at the first filial. Ascorbic acid did not produce any significant modulatory effect on the teratogenic effects of ARVs in single and combination therapy on fetal development. Further researches such as use of other animal species or controlled human studies (which will determine particular effects in humans) are necessary.

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REFERENCES

1) Pasupathi P, Ramachandran T, Sindhu PJ, Saravanan G, and Bakthavathsalam G. Enhanced Oxidative Stress Markers and Antioxidant Imbalance in HIV Infection and AIDS Patients. J. Sci. Res. 2009; 1(2): 370−380.
2) Minkoff H, Ahdieh L, Massad LS, Anastos K, Watts DH, Melnick S, Muderspach L, Burk R, Palefsky. J. The effect of highly active antiretroviral therapy on cervical cytologic changes associated with oncogenic HPV among HIV-infected women. AIDS 2001; 15(16): 2157−2164
3) Cooper ER, Charurat M, Mofenson L. Combination antiretroviral strategies for the treatment of pregnant HIV-1-infected women and prevention of perinatal HIV-1 transmission. J Acquir Immune Defic Syndr 2002; 29: 484−494.
4) Connor EM, Sperling RS, Gelber R. Reduction of maternal-infant transmission of human immunodeficiency virus type 1 with zidovudine treatment: Pediatric AIDS Clinical Trials Group Protocol 076 Study Group. N Engl J Med 1994; 331: 1173−1180
5) World Health Organization. Children and AIDS, 2010. www.unicef.org/esaro/Stocktaking_Key_Facts.pdf
6) Watts DH, Li D, Handelsman E. Assessment of birth defects according to maternal therapy among infants in WITS. J Acquir Immune Defic Syndr 2007; 44: 299−305.
7) Watts DH. 2007. Teratogenicity Risk of Antiretroviral Therapy in Pregnancy. Current HIV/AIDS Reports 2007; 4: 135−140
8) Perinatal HIV Guidelines Working Group. Public Health Services Task Force. Recommendations for use of antiretroviral drugs in pregnant HIV-1-infected women for maternal health and interventions to reduce perinatal HIV-1 transmission in the United States, 2009.
9) Patel D, Thorne C, Fiore S, Newell ML and the European Collaborative Study. Does highly active antiretroviral therapy increase the risk of congenital abnormalities in HIV-infected women? J Acquir Immune Defic Syndr. 2005; 40: 116−8
10) Townsend CL, Willey BA, Cortina-Borja M, Peckham CS, Tookey PA. Antiretroviral therapy and congenital abnormalities in infants born to HIV-1-infected women in the United Kingdom and Ireland, 1990–2007. AIDS 2009; 23: 519−24
11) The Antiretroviral Pregnancy Registry: Interim report. 1/1/89-7/31/2009.
12) Nightingale SL. From the food and drug administration. J Am Med Assoc. 1998; 280: 1472
13) Saitoh A, Hull AD, Franklin P, and Spector SA. Myelomeningocele in an infant with intrauterine exposure to efavirenz. Journal of Perinatology 2005; 25(8): 555−556.
14) Poirier MC, Olivero OA, Walker DM, Walker VE. Perinatal genotoxicity and carcinogenicity of anti-retroviral nucleoside analog drugs. Toxicol Appl Pharmacol 2004; 199(2): 151−16.
15) Shepard TH. Teratogenicity of therapeutic agents. Curr Probl Pediatr 1979; 10: 1−42.
16) Lennon SV, Martin SJ, Cotter TG. 1991. Dose-dependent induction of apoptosis in human tumour cell lines by widely diverging stimuli. Cell Prolif. 24(2), 203−14.
17) Marcondes FK, Bianchi FJ and Tannon AP. Determination of estrous cycle phase of rats: some helpful consideration. Brazilian journal of Biology 2002; 62(4a): 609−614
18) Long JA and Evans HM. The oestrous cycle in the rat and its associated phenomena. Mem. Univ. Calif 1922; 6: 1−148
19) Rich-Edwards JW, Colditz GA and Stampfer MJ. Birthweight and the risk for type 2 diabetes in adult women. Ann Intern Med 1999; 130: 278−84.
20) Gillman MW, Rifas-Shiman S, Berkey CS, Field AE and Colditz

GA. Maternal gestational diabetes, birth weight,and adolescent obesity, Pediatrics 2003; 111(3): e221–e226
21) Singhal BS, Gorospe JR and Naidu S. Megalencephalic leukoencephalopathy with subcortical cysts. J Child Neurol 2003; 18: 646–52
22) Matte TM. Bresnahan M and Begg E. Influence of Varition in Birth weight within Normal Range and Within Sibships on IQ at Age 7 Years: Cohort Study. British Medical Journal 2001; 323: 310–314
23) Reljič M. The significance of crown–rump length measurement for predicting adverse pregnancy outcome of threatened abortion. Ultrasound Obstet Gynecol 2001; 17: 510–512
24) Choong S, Rombauts L, Ugoni A, Meagher S. 2003. Ultrasound prediction of risk of spontaneous miscarriage in live embryos from assisted conception. Ultrasound Obstet Gynecol 2003; 22: 571–577.
25) Schulte J, Ken D, Thomas S, Beverly B, Glenn F. Declines in Low Birth Weight and Preterm Birth among Infants Who Were Born to HIV-Infected Women during an Era of Increased Use of Maternal Antiretroviral Drugs: Pediatric Spectrum of HIV Disease, 1989–2004. Pediatrics 2007; 119: e900
26) Beckerman K, Albano J, Martinez-Tristani M, Seekins D, Storfer S, David N, Vannappagari V, Watts DH, Scheurle A, Tilson H. For The APR Steering Committee. Preterm Birth, low birth weight and fetal antiretroviral exposure: Estimated gestational age and birth weight data from singleton live births, 1989 through 31 January 2009. XVIII International AIDS Conference (AIDS 2010), Vienna, Austria.
27) Paige LW, Miguel M, Kathleen M, Susan B, Michael DH, Lynne MM, PACTG 219C Team. Neurodevelopment and In Utero Antiretroviral Exposure of HIV-Exposed Uninfected Infants. Pediatrics 2010; 125: e250
28) Wimalasundera RC, Larbalestier N, Smith JH, deRuiter A, Thom SA, Hughes AD. Pre-eclampsia, antiretroviral therapy and immune reconstitution. Lancet 2002; 360: 1152–1154.
29) Vatten LJ, Skjaerven R. Is pre-eclampsia more than one disease? Br J Obstet Gynaecol 2004; 111: 298–302
30) Leach-Lemens C. AIDSmap News, March 2011
31) Valsmakis G. Causes of Intrauterine Growth Restriction and the Postnatal Development of the Metabolic Syndrome. Annals of the New York Academy of Sciences 2006; 1092: 138–147.
32) Maron BJ, McKenna WJ, Danielson GK. American College of Cardiology/European Society of Cardiology clinical expert consensus document on hypertrophic cardiomyopathy. A report of the American College of Cardiology Foundation Task Force on Clinical Expert Consensus Documents and the European Society of Cardiology Committee for Practice Guidelines. J Am Coll Cardiol 2003; 42(9): 1687–713.
33) Hoffmann C, Rockstroh JK, Kamps BS. HIV Medicine. Flying Publisher – Paris, Cagliari, and Wuppertal, 2006.
34) Becker S. Liver toxicity in epidemiological cohorts. Clin Infect Dis 2004; 38: S49–55.
35) Bjornsson E and Olsson R. Suspected drug-induced liver fatalities reported to the WHO database. Dig Liver Dis 2006; 38: 33–8.
36) Carr A and Cooper DA. 2001. Adverse effects of antiretroviral therapy. Lancet 2001; 356: 1423–30.
37. Clark SJ, Creighton S, Portmann B, Taylor C, Wendon JA, Cramp ME. Acute liver failure associated with antiretroviral treatment for HIV: a report of six cases. J Hepatol 2002; 36: 295–301.
38) Dieleman JP, van Rossum AM, Stricker BC, Sturkenboom MC, de Groot R, Telgt D, Blok WL, Burger DM, Blijenberg BG, Zietse R, Gyssens IC. Persistent leukocyturia and loss of renal function in a prospectively monitored cohort of HIV-infected patients treated with indinavir. J Acquir Immune Defic Syndr 2003; 32: 135–142.
39) Kopp JB. Renal Dysfunction in HIV-1-infected Patients. Curr Infect Dis Rep 2002; 4: 449–460.
40) Scott JD, Wolfe PR, Bolan RK, Guyer B. Serious renal impairment occurs rarely with use of tenofovir DF. HIV Clin Trials 2006; 7: 55–8.
41) Parsonage MJ, Wilkins EG, Snowden N, Issa BG, Savage MW. The development of hypophosphataemic osteomalacia with myopathy in two patients with HIV infection receiving tenofovir therapy. HIV Med 2005; 6: 341–6.
42) Mauss S, Berger F, Schmutz G. Antiretroviral therapy with tenofovir is associated with mild renal dysfunction. AIDS 2005; 19: 93–5.
43) De Jesus E, Herrera G, Teofilo E, Gerstoft J, Buendia CB, Brand JD, Brothers CH, Hernandez J, Castillo SA, Bonny T, Lanier ER, Scott TR; CNA30024 Study Team. Abacavir versus zidovudine combined with lamivudine and efavirenz, for the treatment of antiretroviral-naive HIV-infected adults. Clin Infect Dis 2004; 39: 1038–46.