



Possible hepatotoxic consequence of nevirapine use in juvenile albino rats

[Posible consecuencia hepatotóxica del uso de nevirapina en ratas albinas juveniles]

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Abstract

Context: Nevirapine (NVP) is used in human immunodeficiency virus exposed neonates. This could present safety concern due to decreased liver metabolizing enzymes activity and renal clearance in neonates.

Aims: To determine the hepatotoxic effect of NVP in juvenile albino rats.

Methods: Juvenile albino rats were weighed, divided into groups and treated orally with 4-32 mg/kg/day of NVP for 14 days including a recovery group. The control groups were treated with water (placebo) and normal saline (solvent). At the end of NVP treatment, rats were weighed and sacrificed, blood was collected and serum extracted. Serum was analyzed for alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP), total bilirubin (TB) and conjugated bilirubin (CB). The liver was harvested via dissection, weighed and evaluated for AST, ALT, ALP, superoxide dismutase (SOD), catalase (CAT), glutathione (GSH), malondialdehyde (MDA) levels and histological damage.

Results: The body, absolute and relative liver weights of rats in NVP treated groups were not significantly different ($p > 0.05$) when compared to placebo. However, serum levels of AST, ALT, ALP, TB and CB were significantly increased ($p < 0.05$) in a dose-dependent manner in NVP-treated groups. Furthermore, liver levels of ALT, ALP, AST and MDA were significantly increased ($p < 0.05$) while SOD, CAT, and GSH were decreased in a dose dependent manner in NVP-treated groups. NVP-treated rats were characterized by varying degrees of hepatic morphological alterations. However, in the recovery group, the effects of NVP were reversed.

Conclusions: This study observed dose-dependent and reversible hepatotoxicity in nevirapine-treated juvenile albino rats.

Keywords: juvenile rats; liver; nevirapine; oxidative stress; toxicity.

Resumen

Contexto: La nevirapina (NVP) se utiliza en los recién nacidos expuestos al virus de inmunodeficiencia humana. Esta podría presentar problemas de seguridad debido a la disminución de la actividad enzimática del metabolismo hepático y la depuración renal en los recién nacidos.

Objetivos: Determinar el efecto hepatotóxico de NVP en ratas albinas juveniles.

Métodos: Se pesaron ratas albinas juveniles, se dividieron en grupos y se trataron oralmente con 4-32 mg/kg/día de NVP durante 14 días, incluyendo un grupo de recuperación. Los grupos controles se trataron con agua (placebo) y solución salina normal (disolvente). Al final del tratamiento con NVP, las ratas fueron pesadas y sacrificadas, se recogió sangre y se extrajo el suero. El suero se analizó para la determinación de alanina aminotransferasa (ALT), aspartato aminotransferasa (AST), fosfatasa alcalina (ALP), bilirrubina total (TB) y bilirrubina conjugada (CB). Los hígados fueron extraídos, pesados y evaluados los niveles de AST, ALT, ALP, superóxido dismutasa (SOD), catalasa (CAT), glutatión (GSH), malondialdehído (MDA) y daño histológico.

Resultados: Los pesos corporal, absolutos y relativos del hígado en los grupos tratados con NVP no fueron significativamente diferentes ($p > 0.05$) en comparación con el placebo. Sin embargo, los niveles séricos de AST, ALT, ALP, TB y CB aumentaron de forma dependiente de la dosis en los grupos tratados con NVP ($p < 0.05$). Además, los niveles hepáticos de ALT, ALP, AST y MDA se incrementaron mientras que SOD, CAT y GSH disminuyeron de forma dependiente de la dosis en grupos tratados con NVP ($p < 0.05$). Las ratas tratadas con NVP mostraron varios grados de alteraciones morfológicas hepáticas. Sin embargo, en el grupo de recuperación, los efectos de la NVP fueron revertidos.

Conclusiones: Este estudio observó una hepatotoxicidad dependiente de la dosis y reversible en ratas albinas juveniles tratadas con nevirapina.

Palabras Clave: estrés oxidativo; hígado; nevirapina; ratas juveniles; toxicidad.

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INTRODUCTION

Human immunodeficiency virus (HIV) pandemic poses a major threat to the lives of infected children. Globally an estimated 3.5 million children are living with HIV, with 10,000 becoming infected daily. Among the estimated 3.5 million children under the age of 15 living with HIV, approximately 91% reside in sub-Saharan Africa (UNAIDS, 2010; WHO, 2011). An estimated 220,000 children became newly infected with HIV in 2014, 190,000 of them in sub-Saharan Africa (UNAIDS, 2010). Mother-to-child transmission is by far the largest source of HIV infection in children below the age of 15 years. In the absence of preventive measures, the risk of a child acquiring the virus from an infected mother ranges from 15 to 25% in industrialized countries, and 25 to 35% in developing countries (Mirochnick et al., 1998). This stimulates the use of highly active antiretroviral therapy in pregnant women, and also, nevirapine (NVP) in HIV-exposed neonates to prevent the risk of transmission of HIV from mother to child during delivery, and breastfeeding (Mirochnick et al., 1998). The use of NVP in neonates and the use of highly active antiretroviral therapy in HIV-positive pregnant women have reduced the rate of HIV transmission by almost 50% in neonates. Nevirapine is a non-nucleoside reverse transcriptase inhibitor. It has high efficacy, favorable lipid profile (Ruiz et al., 2001) and suitability for use during pregnancy and breastfeeding (Horvath et al., 2009). However, the use of NVP as a component of highly active antiretroviral therapy has been associated with hepatotoxicity and skin rash in a large number of studies (Wit et al., 2002). These adverse effects raise concerns about its use, particularly in the perinatal and pediatric settings.

Furthermore, NVP is an inducer of the cytochrome P450 enzyme and requires cytochrome P450 isoenzymes, primarily CYP3A4, for its biotransformation in the liver to hydroxy-metabolites, which are largely excreted in the urine as glucuronide conjugates (Murphy and Montaner, 1996). However, the drug-metabolizing activities of the cytochrome P450-dependent mixed-function oxidases and the conjugating enzymes are substantially lower (50–70% of adult values) in early neonatal life than later (Bartelink et al., 2006). Also, renal clearance which is an

important route of NVP elimination is usually decreased during the neonatal period due to decrease in glomerular filtration rate (Chen et al., 2006). The decreases in drug-metabolizing activities of the cytochrome P450 and renal clearance in neonates may stimulate the accumulation of NVP and its metabolites (Somogyi et al., 1990), which may increase adverse effects on the liver and kidney. The liver is quantitatively and qualitatively the most important site of drug metabolism, although extrahepatic metabolism of drugs is also well recognized, both in the gastrointestinal mucosa and by circulating enzymes such as esterases (Handschin and Meyer, 2005). However, with the advent of parent drugs and metabolite accumulation, its anatomical structure could be impaired, which may further compromise its physiological functions. Therefore, the present study was aimed at investigating the hepatotoxic effect of NVP in juvenile rats, taking into cognizance dose-dependent effects on serum liver function parameters, oxidative stress indices and liver histology.

MATERIAL AND METHODS

Animals

Thirty-five (35) juvenile albino rats of average weight 45 ± 5 g used for this study were obtained from the animal house of the Department of Pharmacology and Toxicology Madonna University, Elele Campus, Rivers State. The juvenile albino rats were kept in seven cages (1-7) of five per cage with free access to food and water *ad libitum*. The cages were ventilated and bedding was replaced every two days, at a room temperature of about 27°C and 12 h light/dark cycle. Animals were handled according to the directive of the 2010 European Parliament and the Council on the Protection of Animals used for scientific purposes.

Drug and chemicals

The pure sample of NVP used for this study was purchased from Shijiazhuang AO Pharmaceutical Import & Export Trading Co., Ltd. Shijiazhuang, China. Reagent kits for assay of transaminases, alkaline phosphatase, total and direct bilirubins

were purchased from ERBA Diagnostic Mannheim GmbH, (Germany). 5,5-Dithiobis (2-nitrobenzoic acid) (DTNB), trichloroacetic acid (TCA), thio-barbituric acid (TBA) were purchased from Sigma-Aldrich Chemical Company (St. Louis, MO, USA). Epinephrine and hydrogen peroxide (H₂O₂) were obtained from Sigma Chemical Company (London, UK). Hematoxylin and eosin stain kit was manufactured by M/S Biolab Diagnostic PVT Ltd India. All other chemicals used in this study were of analytical grade.

Drug administration

Rats in group 1 (placebo control) and group 2 (solvent control) were treated orally with water and normal saline for 14 days respectively. Rats in groups 3 – 6 were treated orally with 4, 8, 16, and 32 mg/kg/day of NVP for 14 days respectively. Rats in group 7, which served as the recovery group were treated with 32 mg/kg/day of NVP for 14 days and were allowed to stay for 14 days after cessation of NVP treatment before sacrifice. Doses of NVP (4, 8, 16 and 32 mg/kg/day) used for this study represented clinical dose, 2, 4, and 8 times the clinical dose (Mugabo et al., 2011). NVP used for this study was suspended in normal saline.

Collection of sample

Rats were sacrificed at the end of drug treatment with the aid of inhalational diethyl ether. Blood samples were collected via cardiac puncture in a sterile non-heparinized container, centrifuged (Analytica, Athens, Greece) at 1200 rpm for 15 min and serum collected and analyzed for liver function parameters. Rats were dissected, the liver was collected, weighed and washed with an ice cold 1.15% KCl solution, and homogenized with 0.1 M phosphate buffer (pH 7.2). It was centrifuged at 1200 rpm for 15 min and supernatant collected and evaluated for liver levels of aminotransferases, alkaline phosphatase, and oxidative stress biomarkers.

Determination of liver function parameters

Determination of alkaline phosphatase (ALP)

This enzyme was assayed using the phenolphthalein method (Babson et al., 1966). The three test tubes containing the sample, a standard, and the control were added 1 mL of distilled. A drop of phenolphthalein monophosphate was added to each of the samples and incubated at 37°C for 5 minutes. The sample (0.1 mL) was added to the first test tube, while a standard solution of ALP (30 U/L) and control serum were added to the second and third tubes, respectively. The tubes were incubated at 37°C for 20 minutes and 1 mL of a color developer was then added to each test tube. Absorbance was measured at 550 nm using a spectrophotometer (Agilent 8453E, USA).

Determination of aspartate aminotransferase (AST) and alanine aminotransferase (ALT)

The method of Reitman and Frankel (1957) was used in the evaluation of aspartate transaminase and alanine transaminase. The sample (0.1 mL) and distilled water were put into separate tubes. Alanine buffer solution (0.5 mL) was added to each test tube for ALT determination while for AST, aspartate buffer solution was added to each test tube. The tubes were incubated at 39°C for 30 min, and 0.3 mL of 2,4-dinitrophenylhydrazine solution was added to each test tube and vortex mixed for 30 minutes. Absorbance was read using a spectrophotometer at 550 nm.

Determination of total bilirubin (TB)

Diazotised sulfanilic acid (0.5 mL) was reacted with bilirubin in diluted serum (0.2 mL serum + 1.8 mL distilled water) and 0.5 mL of methyl alcohol solution in 30 min, which was measured at 540 nm with the aid of a spectrophotometer (Doumas et al., 1973).

Determination of conjugated bilirubin (CB)

Diazotised sulfanilic acid (0.5 mL) was reacted with bilirubin in diluted serum (0.2 mL serum + 1.8 mL distilled water) in 1 minute and was measured at 540 nm with the aid of a spectrophotometer (Watson, 1961).

Antioxidant assay

Determination of superoxide dismutase (SOD)

SOD activity was determined by its ability to inhibit the auto-oxidation of epinephrine determined by the increase in absorbance at 480 nm as described by Sun and Zigma (1978). The reaction mixture (3 mL) containing 2.95 mL of sodium carbonate buffer 0.05 M (pH 10.2), liver homogenate 0.02 mL and epinephrine 0.03 mL in HCL 0.005 N was used to initiate the reaction. The reference cuvette contained buffer 2.95 mL, the substrate (epinephrine) 0.03 mL and water 0.02 mL. Enzyme activity was calculated by measuring the change in absorbance at 480 nm for 5 min. SOD was calculated using the molar extinction coefficient of $\Sigma = 4020 \text{ M}^{-1}\text{cm}^{-1}$.

Determination of catalase (CAT)

CAT activity was determined according to Sinha et al. (1972). It was assayed colorimetrically at 620 nm and expressed as $\mu\text{moles of H}_2\text{O}_2$ consumed/min/mg protein at 25°C. The reaction mixture (1.5 mL) contained 1.0 mL of phosphate buffer 0.01 M (pH 7.0), 0.1 mL of tissue homogenate and 0.4 mL of H_2O_2 2 M. The reaction was stopped by the addition of 2.0 mL of dichromate-acetic acid reagent (5% potassium dichromate and glacial acetic acid were mixed in 1:3 ratio). CAT was calculated using the molar extinction coefficient of $\Sigma = 40 \text{ M}^{-1}\text{cm}^{-1}$.

Determination of reduced glutathione (GSH)

The GSH content of liver tissue as non-protein sulphhydryls was estimated according to the method described by Sedlak and Lindsay (1968). To the homogenate 10% TCA was added, centrifuged. The supernatant (1.0 mL) was treated with 0.5 mL of Ellman's reagent (19.8 mg of DTNB in 100 mL of

sodium nitrate 0.1%) and 3.0 mL of phosphate buffer (0.2 M, pH 8.0). The absorbance was read at 412 nm. GH was calculated using the molar extinction coefficient of $\Sigma = 1.34 \times 10^4 \text{ M}^{-1}\text{cm}^{-1}$.

Determination of malondialdehyde (MDA)

MDA, an index of lipid peroxidation, was determined using the method of Buege and Aust (1978). An aliquot of the supernatant (1.0 mL) was added to 2 mL of TCA-TBA-HCl reagent (1:1:1 ratio, TCA 15%, TBA 0.37%, and HCl 0.24 N) boiled at 100°C for 15 min and allowed to cool. Flocculent materials were removed by centrifuging at 3000 rpm for 10 minutes. The supernatant was removed and the absorbance read at 532 nm against a blank. MDA was calculated using the molar extinction coefficient for MDA-TBA complex of $1.56 \times 10^5 \text{ M}^{-1}\text{cm}^{-1}$.

Histopathological evaluation of the liver

The liver was fixed in 10% formalin, processed routinely and embedded in paraffin wax. Sections of 5 μm thickness was cut, stained with hematoxylin and eosin and examined under the light microscope (Nikon Eclipse E 200) and relevant sections photographed (Nikon D750).

Statistical analysis

Results were expressed as mean \pm SD. Data was subjected to one-way analysis of variance (ANOVA) test, and differences between samples were determined by Dunnett's multiple comparison test. Results were considered to be significant at $p < 0.05$.

RESULTS

This study observed that treatment with 4-32 mg/kg/day of NVP did not produce significant ($p > 0.05$) effects on the body, absolute and relative liver weights when compared to control (Table 1). However, dose-dependent increases in serum AST, ALT, ALP, CB and TB levels were obtained in NVP-treated juvenile rats, which differed significantly at $p < 0.05$ using Dunnett's multiple comparison tests. The observed increases showed 34.7, 87.8, 147.8, and 298.0% in AST levels, 42.6, 82.5, 163.8, and 208.2% in ALP levels and 42.5, 76.5, 143.7, and 296.7% in ALT levels, respectively.

TB levels were increased by 33.3, 70.7, 111.0, and 167.6%, while CB levels were increased by 42.9, 74.2, 126.7, and 210.0%, respectively (Table 2). Furthermore, liver levels of AST, ALT, and ALP were significantly increased ($p < 0.05$) in a dose-dependent manner in NVP-treated juvenile rats. Dose-dependent increases at 4-32 mg/kg/day of NVP represented 48.0, 81.0, 139.7, and 290.9% in ALT levels, 35.4, 75.3, 120.9, and 206.0% in AST levels and 50.3, 91.4, 149.7 and 212.4% in ALP levels (Table 3). Also, in Table 4, the present study observed significant decreases ($p < 0.05$) in liver levels of SOD, CAT and GSH with significant increases ($p < 0.05$) in MDA in a dose-dependent manner in NVP-treated groups. Furthermore,

increases in MDA levels represented 45.2, 90.5, 142.9, and 221.4% at 4-32 mg/kg/day of NVP, respectively (Table 4). However, the effects of NVP observed in the treated groups were restored in the recovery group (Tables 1-4). Histological examination of the liver of 4 mg/kg/day of NVP treated group showed fatty liver while the 8 mg/kg/day treated group showed hepatocytes necrosis (Fig. 1B-C). The group treated with 16 mg/kg/day of NVP showed congested liver, dilation of central vein and sinusoid, while 32 mg/kg of NVP treated group showed liver congestion, hepatomegaly and hepatocytes necrosis (Fig. 1D-E). The liver of the rats in the recovery group showed fatty changes (Fig. 1F).

Table 1. Effects of nevirapine on body and liver weights of juvenile albino rats.

Dose (mg/kg)	Body weight (g)	Absolute liver weight (g)	Relative liver weight (%)
Control	60.3 ± 4.33	3.49 ± 0.04	5.79 ± 0.16
4	65.6 ± 5.22	3.63 ± 0.17	5.54 ± 0.23
8	61.2 ± 6.34	3.54 ± 0.24	5.79 ± 0.14
16	65.4 ± 4.56	3.74 ± 0.18	5.72 ± 0.12
32	62.5 ± 5.33	3.40 ± 0.23	5.44 ± 0.35
32 ^R	64.2 ± 6.83	3.55 ± 0.16	5.55 ± 0.43

Data are expressed as mean ± SD. n=5. No statistically significance was observed at ($p > 0.05$) using ANOVA and Dunnett's multiple comparison tests. 32^R: Recovery group in which the rats were treated with 32 mg/kg/day of NVP for 14 day and were allowed to stay for 14 days after cessation of NVP treatment. Rats in Control group received normal saline solution.

Table 2. Effects of nevirapine on serum liver function parameters of juvenile albino rats.

Dose (mg/kg)	ALT (U/L)	AST (U/L)	ALP (U/L)	CB (µmol/L)	TB (µmol/L)
Control	24.7 ± 2.04 ^a	25.3 ± 2.68 ^a	33.1 ± 3.15 ^a	3.10 ± 0.18 ^a	5.83 ± 0.28 ^a
4	35.2 ± 3.81 ^b	34.1 ± 3.88 ^b	47.2 ± 5.40 ^b	4.43 ± 0.36 ^b	7.77 ± 0.27 ^b
8	43.6 ± 3.61 ^c	47.5 ± 4.66 ^c	60.4 ± 5.70 ^c	5.40 ± 0.27 ^c	9.95 ± 0.34 ^c
16	60.2 ± 5.84 ^d	62.7 ± 4.70 ^d	87.3 ± 4.50 ^d	7.03 ± 0.13 ^d	12.3 ± 1.76 ^d
32	98.0 ± 6.27 ^e	100.7 ± 7.82 ^e	102.0 ± 7.70 ^e	9.61 ± 0.12 ^e	15.6 ± 1.70 ^e
32 ^R	42.3 ± 3.99 ^c	60.0 ± 6.62 ^d	50.6 ± 4.66 ^b	5.60 ± 0.15 ^c	9.71 ± 0.40 ^c

Data are expressed as mean ± SD. n=5. Values with different superscripts on the same column are statistically significant at $p < 0.05$ using ANOVA and Dunnett's multiple comparison tests. ALT: alanine aminotransferase; AST: aspartate aminotransferase; ALP: alkaline phosphatase; CB: conjugated bilirubin; TB: total bilirubin; 32^R: Recovery group in which the rats were treated with 32 mg/kg/day of NVP for 14 day and were allowed to stay for 14 days after cessation of NVP treatment. Rats in Control group received normal saline solution.

Table 3. Effects of nevirapine on liver levels of alkaline phosphatase, and aminotransferases of juvenile albino rats.

Dose (mg/kg)	ALT (U/L)	AST (U/L)	ALP (U/L)
Control	25.2 ± 2.35 ^a	26.3 ± 2.16 ^a	32.4 ± 2.92 ^a
4	37.3 ± 3.12 ^b	35.6 ± 2.14 ^b	48.7 ± 3.50 ^b
8	45.6 ± 4.60 ^c	46.1 ± 3.00 ^c	62.0 ± 5.40 ^c
16	60.4 ± 3.36 ^d	58.1 ± 4.70 ^d	80.9 ± 7.23 ^d
32	98.5 ± 6.31 ^e	80.5 ± 7.80 ^e	101.2 ± 8.47 ^e
32 ^R	44.0 ± 3.12 ^c	45.8 ± 4.03 ^c	58.2 ± 4.10 ^c

Data are expressed as mean ± SD. n=5. Values with different superscripts on the same column are statistically significant at p<0.05 using ANOVA and Dunnett's multiple comparison tests. ALT: alanine aminotransferase; AST: aspartate aminotransferase; ALP: alkaline phosphatase; 32^R: Recovery group in which the rats were treated with 32 mg/kg/day of NVP for 14 day and were allowed to stay for 14 days after cessation of NVP treatment. Rats in Control group received normal saline solution.

Table 4. Effect of nevirapine on liver oxidative stress indices of juvenile albino rats.

Dose (mg/kg)	MDA (nmol/mg protein)	SOD (U/mg protein)	CAT (U/mg protein)	GSH (μmole/mg protein)
Control	0.42 ± 0.07 ^a	7.27 ± 0.09 ^a	9.57 ± 0.01 ^a	6.29 ± 0.02 ^a
4	0.61 ± 0.03 ^b	5.23 ± 0.05 ^b	5.94 ± 0.09 ^b	3.68 ± 0.02 ^b
8	0.80 ± 0.07 ^c	3.53 ± 0.06 ^c	4.04 ± 0.16 ^c	2.34 ± 0.75 ^c
16	1.02 ± 0.02 ^d	2.03 ± 0.10 ^d	2.31 ± 0.05 ^d	1.50 ± 0.05 ^d
32	1.35 ± 0.01 ^e	1.00 ± 0.06 ^e	1.01 ± 0.03 ^e	0.91 ± 0.04 ^e
32 ^R	0.68 ± 0.05 ^b	3.44 ± 0.01 ^c	2.23 ± 0.01 ^d	2.20 ± 0.07 ^c

Data are expressed as mean ± SD. n=5. Values with different superscripts on the same column are statistically significant at p<0.05 using ANOVA and Dunnett's multiple comparison tests. MDA: malondialdehyde; SOD: superoxide dismutase; CAT: catalase; GSH: reduced glutathione; 32^R: Recovery group in which the rats were treated with 32 mg/kg/day of NVP for 14 day and were allowed to stay for 14 days after cessation of NVP treatment. Rats in Control group received normal saline solution.

DISCUSSION

The present study assessed the hepatotoxic profile of NVP in juvenile albino rats. Analysis of organ weight in toxicological studies is an important endpoint for the identification of potentially harmful effects of chemicals. Organ weight can be the most sensitive indicator of the effect of an experimental compound, as significant differences in organ weight between treated and untreated (control) animals may occur in the absence of morphological changes (Bailey et al., 2004). In the present study, juvenile albino rats administered with NVP did not show changes in the body, absolute and relative liver weights.

ALT is a marker enzyme for plasma membrane and endoplasmic reticulum of the liver. It is often employed to assess the integrity of plasma

membrane since it is localized predominantly in the microvilli of the bile canaliculi located in the plasma membrane (Yakubu et al., 2005). ALP and AST are normally localized within the cells of the liver, heart, gill, kidney, muscles and other organs. These enzymes are of major importance in assessing and monitoring liver damage (Yakubu et al., 2005). This study observed dose-dependent increases in serum and liver levels of ALT, AST, and ALP in NVP-treated juvenile albino rats. The observations are signs of liver damage and are in agreement with similar findings in a non-dose-dependent study in adult rats (Martinez et al., 2001; Sule et al., 2012). The observed increases in serum levels of AST, ALT, and ALP could be due to the release of these enzymes into the blood as a result of cellular leakage and loss of functional integrity of liver cell membrane (Drotman and Lawhan, 1979).

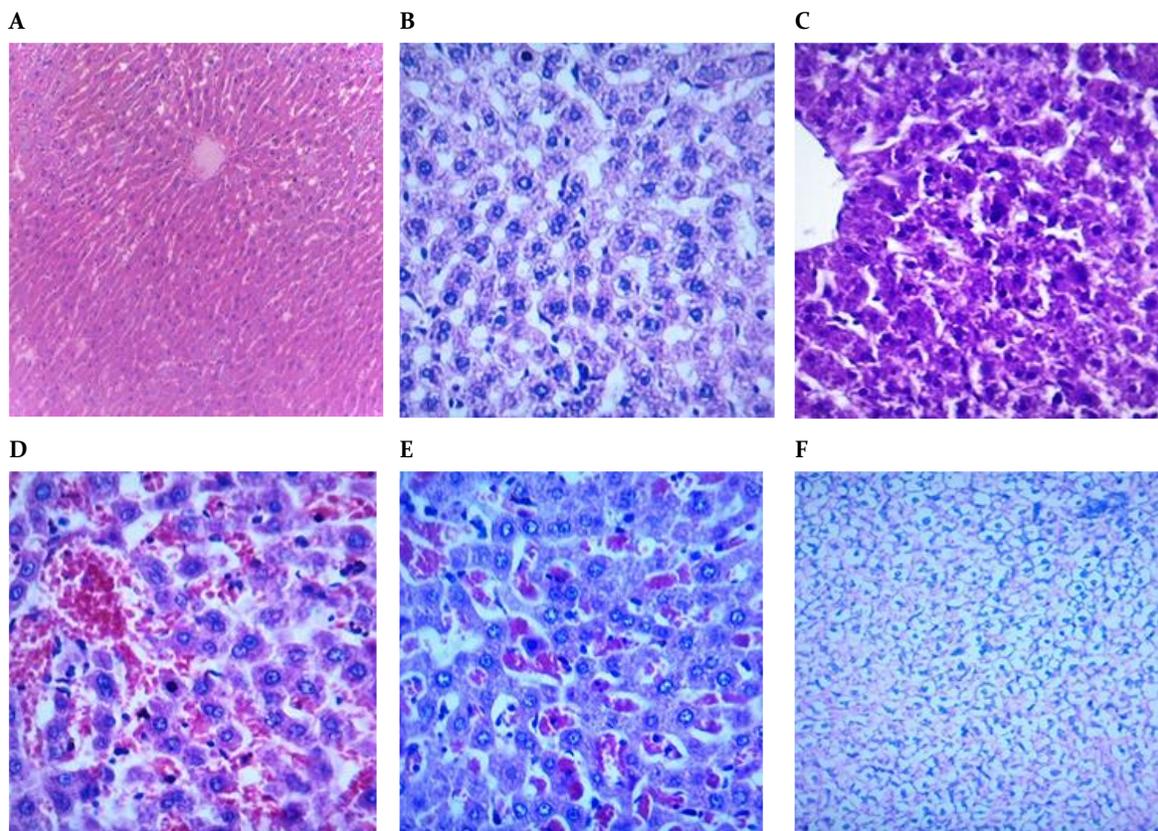


Figure 1. Histopathological evaluation of the rat livers treated with nevirapine (NVP).

(A) The liver of control juvenile albino rat showing normal liver architecture. (B) The liver of juvenile albino rat treated with 4 mg/kg of NVP showing fatty liver. (C) The liver of juvenile albino rat treated with 8 mg/kg of NVP showing hepatocytes necrosis. (D) The liver of juvenile albino rat treated with 16 mg/kg of NVP showing congested liver, dilation of central vein and sinusoid. (E) The liver of juvenile albino rat treated with 32 mg/kg of NVP showing liver congestion, hepatomegaly, and hepatocytes necrosis. (F) The liver of juvenile rat in the recovery group showing fatty liver. (H and E x 200).

Laboratory evaluation of serum bilirubin level is a very sensitive test to substantiate the functional integrity of the liver and severity of necrosis (Meshkibaf et al., 2006). The present study observed dose-dependent increases in serum levels of CB and TB in NVP-treated juvenile albino rats. The elevated serum levels of CB and TB observed in the present study could be attributed to NVP-induced over-production, impaired uptake, conjugation or excretion of unconjugated or conjugated bilirubin from hepatocytes to the bile ducts (Thapa and Walia, 2007).

Furthermore, oxygen is a highly reactive atom that is capable of becoming part of potentially damaging molecules commonly called “free radicals.” Free radical production occurs continuously in all cells as part of normal cellular function. However, excess free radical production

originating from endogenous or exogenous sources might play a significant role in many diseases and drug-induced toxicities (Young and Woodside, 2001). Free radicals are capable of causing oxidative damage to cells thereby impairing their structure and functions (Percival, 1998). Antioxidants, which include SOD, CAT and GSH constitute mutually a supportive team that is capable of stabilizing, or deactivating, free radicals thereby preventing oxidative damage to cells. Antioxidants are absolutely critical for maintaining optimal cellular structure and functions and their levels are yardsticks for oxidative stress (Percival, 1998; Renugadevi and Prabu, 2009). The current study observed dose-dependent decreases in liver levels of SOD, CAT, and GSH in NVP-treated juvenile rats. The observed decreases in the liver levels of SOD, CAT, and GSH could be attributed to NVP-induced

hepatic oxidative stress, which might have led to their depletion (Adaramoye et al., 2013). Also, decreased levels of SOD, CAT, and GSH could be attributed to the direct binding of NVP to the active site of the antioxidants or due to their increased usage in scavenging free radicals induced by NVP (Waisberg et al., 2003).

MDA, which is a primary marker of lipid peroxidation, is one of the final products of the oxidative modification of lipids. The abnormal elevation of MDA level could be associated with cell membrane damage including changes to the intrinsic properties of the membrane, such as fluidity, ion transport, loss of enzyme activity, and protein cross-linking, which may eventually result in cell death (Sharma et al., 2012). The present study observed dose-dependent increases in MDA levels in the liver of NVP-treated juvenile albino rats. This observation indicates hepatic oxidative stress and lipid peroxidation. Exposures to drugs can be highly disruptive to development, producing outcomes ranging from embryonic lethality and congenital malformations, to subtle physiological or morphological alterations that may predispose individuals to diseases that may emerge later in life (Makaji et al., 2011; Schug et al., 2013; Barella et al., 2014). Hepatic morphological changes serve as an important tool for identifying and characterizing liver injury associated with exposure to drugs and toxicants (Sing et al., 2013). The current study observed hepatic morphological alterations in NVP-treated juvenile albino rats which were however, not evident in the recovery group. In the present study, hepatic morphological changes observed in NVP-treated juvenile rats correlate with altered levels of evaluated biochemical parameters and oxidative stress indices. According to Stern et al. (2006) the mechanism of NVP-induced hepatotoxicity remains unknown; however, it was postulated to be immune mediated. Such immune mediation has already been proven in animal models for NVP-induced skin reactions (Popovic et al., 2006). Also, NVP-induced hepatotoxicity has been attributed to direct cell injury or mitochondrial toxicity (Rusmann et al., 2009). However, based on the observations in the present study, oxidative stress could be a possible mechanism of NVP-induced hepatotoxicity.

CONCLUSIONS

This study showed that nevirapine produced dose-dependent and reversible hepatotoxicity in juvenile albino rats. However, the use of nevirapine in human immunodeficiency virus-exposed neonates is safe but may require routine clinical assessment of liver function.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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Author contribution:

Contribution	Adikwu E	Bokolo B
Concepts or ideas	X	
Design	X	X
Definition of intellectual content	X	X
Literature search	X	X
Experimental studies	X	X
Data acquisition	X	X
Data analysis	X	X
Statistical analysis	X	X
Manuscript preparation	X	X
Manuscript editing	X	X
Manuscript review	X	X

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