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Original Research

Renal implication of nevirapine use in juvenile albino rats

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ABSTRACT

Background: Nevirapine (NVP) is excreted by the kidney, therefore, use in human immunodeficiency virus exposed neonates could be of safety concern due to decreased renal function associated with the neonatal period. This study, therefore, investigated the nephrotoxic profile of NVP in juvenile albino rats. **Materials and Methods:** Juvenile albino rats used for this study were divided into seven groups A-G of five rats each. Rats in groups A and B were treated with water and normal saline as placebo and solvent control, respectively. Rats in groups C-G were treated orally with 4-32 mg/kg/day of NVP for 14 days, respectively including a recovery group. At the end of drug administration, rats were weighed and sacrificed. Blood was collected; serum extracted and evaluated for creatinine (Cr), urea (U), uric acid (UA), albumin (Ab), total protein (TP), and electrolytes (K^+, Na^+, Cl^- and HCO_3^-). Kidneys were harvested, weighed and evaluated for superoxide dismutase (SOD), catalase (CAT), glutathione (GSH), malondialdehyde (MDA) levels, and histological changes.

Results: The body, absolute, relative kidney weights, and serum electrolytes were not significantly ($P > 0.05$) altered in the NVP treated rats in comparison to placebo control. However, serum levels of Ab and TP were decreased, whereas Cr, U, UA were increased significantly ($P < 0.05$) and in a dose-dependent manner in NVP-treated rats. Kidney levels of MDA were increased, whereas SOD, CAT, and GSH levels were decreased significantly ($P < 0.05$) and in a dose-dependent manner in NVP-treated rats. Kidneys of NVP-treated rats showed dose-dependent tubular necrosis. However, NVP-induced changes in all evaluated parameters were restored in the recovery group. **Conclusion:** This study observed dose-dependent and reversible renal toxicity in NVP treated juvenile albino rats. The use of NVP in neonates may be safe; however, neonatal renal function assessment is advice before and with NVP use.

KEY WORDS: Juvenile rats, nevirapine, oxidative stress, renal toxicity

INTRODUCTION

Each year, over 1.5 million new-born children are infected with human immunodeficiency virus (HIV) in the world with the highest in sub-Sahara Africa. Most HIV-infected children acquire the infection through mother-to-child transmission (MTCT) of HIV, which can occur during pregnancy, labor and delivery, or during breastfeeding. In the absence of any intervention, the risk of MTCT of HIV is 15-30% in non-breastfeeding populations; breastfeeding by an infected mother increases the risk by 5-20% to a total of 20-45% [1]. MTCT of HIV accounts for the vast majority of the 2.5 million children under the age of 15 years who are estimated to be living with HIV, almost 90% of them in sub-Saharan Africa [2-4]. An estimated 56,700 HIV-positive babies were born each year in the absence of the prevention of mother to child intervention [5]. Most infants who are HIV-infected die under the age of 2 years and about 33% die under the age of 1 year in the absence of antiretroviral therapy [6,7]. This necessitates the use of highly active antiretroviral therapy (HAART) in HIV-positive pregnant women and also, the use of nevirapine (NVP) in HIV-exposed neonates [8]. NVP is a first generation non-nucleoside reverse transcriptase inhibitor that is used as a component of HAART [9]. The use of NVP in children has contributed to decrease in mortality and morbidity associated with HIV. However, the use of NVP in neonates could be of concern because at birth, kidneys are anatomically and functionally immature, and as a result, the renal function in the new-born is limited [10-12].

Furthermore, NVP metabolites are largely excreted in the urine as glucuronide conjugates with <3% of NVP excreted unchanged [13]. Renal excretion of medications is dependent on glomerular filtration rate, renal tubular secretion, and reabsorption. Neonatal period is characterized by minimal glomerular filtration, which is about 30-40% that of adults and active tubular secretion of drugs is about 20-30% that of adults. Therefore, drugs like NVP that depend on renal function for elimination could be cleared slowly by neonates and may accumulate leading to nephrotoxicity [14,15]. The kidney is the primary organ responsible for elimination of waste products, toxins, and drugs from the body. It has many other functions

which include; water and electrolyte homeostasis, maintenance of plasma osmolarity, acid-base balance, and the production and secretion of hormones [16,17]. Due to its involvement in drug excretion, the renal arterioles and glomerular capillaries are vulnerable to the toxicological effects of drugs [18]. The inability of the kidney to excrete drugs due to decreased renal clearance could lead to drug accumulation which may impair its anatomical structure, thereby compromising its physiological functions [19]. Therefore, this study was designed to evaluate the dose-dependent toxicological effects of NVP on serum renal function indices, oxidative stress indices, and kidney histology of juvenile rats.

MATERIALS AND METHODS

Animals and Drugs

This study used thirty-five juvenile albino rats of the average weight of 45 ± 5 g obtained from the animal house of the Department of Pharmacology and Toxicology, Madonna University, Elele, Rivers State. The rats were kept in seven cages (A-G) of five rats per cage at room temperature in natural lighting conditions and had free access to water and standard rodent feed *ad-libitum*. AO Pharmaceutical Shijiazhuang, China supplied the pure sample of NVP used for this study. This study used 4, 8, 16, and 32 mg/kg of NVP which represent 2, 4, 8, and 16 times the clinical dose [20]. NVP was suspended in normal saline [21].

Drug Administration

Rats in group A (placebo control) and B (solvent control) were treated orally with water and normal saline for 14 days, respectively. Rats in groups C-F were treated orally with 4, 8, 16, and 32 mg/kg/day of NVP orally for 14 days, respectively. Rats in group G which served as the recovery group were treated with 32 mg/kg/day of NVP for 14 days and were sacrificed 14 days after cessation of NVP treatment.

Collection of Sample

After the last treatments, rats were fasted overnight and sacrificed with the aid of inhalational diethyl ether. Blood samples were taken by cardiac puncture into the sterile non-heparinized container. Serum was obtained after centrifugation at 1200 rpm for 15 min and was used for the evaluation of renal function parameters. The kidneys were collected through dissection and washed in an ice cold 1.15% KCl solution and centrifuged at 1200 rpm for 15 min after kidneys were homogenized with 0.1 M phosphate buffer (pH 7.2). The supernatant was collected and evaluated for kidney levels of oxidative stress biomarkers.

Determination of Relative Kidney Weight

At sacrifice, the weights of the juvenile rats and kidneys were determined and the relative kidney weight (%) was calculated as shown below:

$$\text{Relative kidney weight} = \frac{\text{Absolute kidney weight (g)}}{\text{Body weight at sacrifice (g)}} \times 100$$

Evaluation of Renal Function Parameters and Kidney Oxidative Stress Indices

Serum urea (U) was analyzed using diacetylmonoxime method [22], creatinine (Cr) was estimated using Jaffe's deproteinization method 1886 [23], whereas uric acid (UA) was measured according to Sanders *et al*., 1980 [24]. Albumin (Ab) was measured as described by Tietz *et al*., (1994) [25] and total protein (TP) content was assayed as described by Plummer, (1971) [26]. Superoxide dismutase (SOD) level was assayed according to Sun and Zigma (1978) [27], catalase (CAT) level was evaluated as reported by Sinha *et al*., 1972 [28], reduced glutathione (GSH) was measured using the method of Sedlak and Lindsay (1968) [29] and malondialdehyde (MDA) was determined as reported by Buege and Aust (1978) [30]. Potassium and sodium were determined using flame photometric methods, whereas chloride and bicarbonate levels were determined using titrimetric methods.

Histopathological Examination of the Kidney

Kidneys were excised, cleaned by blotting with filter paper and were fixed in 10% formalin. Kidney tissues were processed and embedded in paraffin wax. Sections of 5 μ thickness were cut, stained with hematoxylin and eosin and examined under the light microscope and relevant sections photographed.

Statistical Analysis

The results are presented as mean ± standard deviation and were analyzed for statistical significance using analysis of variance and Dunnett's multiple comparison test. The values with $p < 0.05$ were considered statistically significant.

RESULTS

This study observed that treatment with NVP did not produce significant ($P > 0.05$) effects on the body, absolute, relative kidney weights, and serum electrolytes when compared to control [Tables 1 and 2]. In Table 3, serum levels of Cr, U, and UA were significantly ($P < 0.05$) increased in a dose-dependent manner in NVP-treated rats. The percentage increases represent 43.8%, 87.6%, 147.6%, and 223.8% (Cr), 49.1%, 87.3%, 155.8%,

Table 1: Effects of NVP on body and kidney weights of juvenile albino rats

Dose (mg/kg)	Body weight (g)	Absolute kidney weight (g)	Relative kidney weight (%)
Control	65.3±5.33	0.46±0.04	0.71±0.09
4	66.9±4.22	0.48±0.03	0.72±0.05
8	68.2±7.34	0.44±0.04	0.65±0.06
16	63.5±8.56	0.45±0.07	0.71±0.02
32	62.7±7.33	0.47±0.01	0.75±0.03
32®	65.2±6.83	0.53±0.03	0.81±0.07

NVP: Nevirapine. Data are expressed as mean±SD. n=5. 32®: Recovery group, SD: Standard deviation

and 277.3% (U) and 53.2%, 83.0%, 131.2%, and 184.4.0% (UA) at 4-32 mg/kg/day of NVP, respectively. On the other hand, serum Ab and TP levels were significantly ($P < 0.05$) decreased in a dose-dependent manner in NVP-treated juvenile rats [Table 3]. Serum Ab levels were decreased by 31.1%, 50.5%, 68.6%, and 83.1%, whereas TP levels were decreased by 31.5%, 46.6%, 66.4%, and 84.5% at 4-32 mg/kg/day of NVP, respectively. However, observed effects of NVP on Cr, U, UA, Ab, and TP levels were reversed in the recovery group (32®) [Table 3]. Furthermore, significant ($P < 0.05$) and dose-dependent decreases in kidney levels of SOD, CAT and GSH with increases in MDA levels were observed in NVP-treated rats. The observed increases in the kidney MDA levels represent 35.1%, 67.6%, 91.9%, and 197.3% at 4-32 mg/kg of NVP, respectively [Table 4]. Furthermore, the percentage decreases in kidney antioxidant levels represent 35.2%, 49.2%, 68.0%, and 85.2% (SOD), 31.7%, 49.0%, 67.2%, and 87.0% (CAT), and 31.0%, 50.1%, 66.6%, and 83.2% (GSH) at 4-32 mg/kg/day of NVP, respectively. Interestingly, observed effects of NVP on kidney levels of SOD, CAT, GSH, and MDA were reversed in the recovery group (32®) [Table 4].

Table 2: Effects of NVP on serum electrolytes of juvenile albino rats

Dose (mg/kg)	Na^+ (mmol/l)	K^+ (mmol/l)	Cl^- (mmol/l)	HCO_3- (mmol/l)
Control	105.0±6.21	2.20±0.16	100.6±7.20	12.2±1.00
4	104.2±8.32	2.27±0.43	105.0±6.66	10.0±1.21
8	109.0±7.40	2.30±0.52	98.2±4.32	13.7±1.31
16	118.1±8.51	2.40±0.11	114.6±8.15	11.1±1.72
32	124.1±6.06	2.45±0.15	120.1±8.02	14.1±3.21
$32^{(R)}$	103.1±7.32	2.22±1.32	110.1±7.03	12.1±1.41

NVP: Nevirapine. Data are expressed as mean±SD, *n*=5, 32®: Recovery group, SD: Standard deviation

Table 3: Effects of NVP on serum renal function parameters of juvenile albino rats

Dose (mg/kg)	Cr (mg/dL)	U (mg/dL)	UA (mg/dL)	Ab (g/dL)	TP (g/dL)
Control	1.05±0.05[a]	26.0±2.09[a]	1.09±0.04[a]	3.31±0.08[a]	6.57±0.09[a]
4	1.51±0.08[b]	36.9±2.35[b]	1.67±0.07[b]	2.28±0.09[b]	4.50±0.06[b]
8	1.97±0.05[c]	48.7±2.86[c]	2.00±0.06[c]	1.64±0.09[c]	3.51±0.09[c]
16	2.60±0.06[d]	66.5±1.73[d]	2.52±0.08[d]	1.04±0.02[d]	2.21±0.09[d]
32	3.40±0.04[e]	98.1±5.28[e]	3.10±0.09[e]	0.56±0.07[e]	1.02±0.07[e]
32®	1.88±0.06[c]	38.2±2.69[b]	2.26±0.05[c]	1.54±0.06[c]	4.63±0.10[b]

NVP: Nevirapine, Cr: Creatinine, U: Urea, UA: Uric acid, Ab: Albumin, TP: Total protein. 32®: Recovery group. Data are expressed as mean±SD. n=5. Values with different superscripts on the same column differ significantly at *P*<0.05 ANOVA and Dunnett's multiple comparison test

Furthermore, the kidney of juvenile rat treated with 4 mg/kg of NVP showed tubular necrosis while rat treated with 8 mg/kg of NVP showed necrosis and dilated tubules. Kidney of juvenile rat treated with 16 mg/kg of NVP showed tubular necrosis with inflammatory materials in the tubular lumen whereas 32 mg/kg of NVP treated rat showed extensive tubular necrosis with inflammatory materials in the tubular lumen. However, necrotic changes were not evident in the kidney of rat in the recovery group [Figure 1a-f].

DISCUSSION

The kidneys which play vital roles in the elimination of drugs and metabolites could be prone to drug and metabolite-induced damage [31]. This makes the toxicological assessment of the biological interactions between drugs, metabolites and the kidney of clinical importance and an essential yardstick for the safety of drugs [32]. Body and organ weight evaluations are an integral part of the toxicological assessment of chemical substances [33]. In the present study, juvenile albino rats administered with NVP did not show significant changes in body, absolute, relative kidney weights and serum electrolytes. This observation is consistent with findings in adult rats treated with NVP [34]. U is the major nitrogen-containing metabolic product of protein metabolism. Cr is synthesized primarily in the liver from the methylation of glycocyamine and is removed from the blood, chiefly by the kidneys, through glomerular filtration. UA is the major product of purine nucleotides; adenosine and guanosine. Serum levels of Cr, U and UA are essential clinical endpoints for the assessment of renal function [35]. Elevations in their serum levels could be correlated with impairment in renal function [36,37]. This study observed impaired kidney function in NVP-treated juvenile rats as evidenced by dose-dependent increases in serum levels of Cr, U, and UA. Ab and TP provide an extremely sensitive test for both glomerular and tubular function. Lowered serum Ab and TP are associated with an excess loss in the glomerulus and could be associated with impaired kidney function [38,39]. Serum levels of Ab and TP were decreased in dose-dependent manner in NVP-treated rats. The observed decreases in serum Ab and TP concentrations in this study indicate kidney damage [40].

Antioxidants play an important role in reactive oxygen species cascade reaction. SOD transforms superoxide radical ($O2^-$) into hydrogen peroxide (H_2O_2), and CAT removes H_2O_2 and

Table 4: Effect of NVP on kidney oxidative stress indices of juvenile albino rats

Dose (mg/kg)	SOD (U/mg protein)	CAT (U/mg protein)	GSH (μmole/mg protein)	MDA (nmole/mg protein)
Control	6.74±0.07[a]	10.5±0.22[a]	6.01±0.09[a]	0.37±0.02[a]
4	4.37±0.18[b]	7.17±0.05[b]	3.90±0.17[b]	0.50±0.02[b]
8	3.43±0.27[c]	5.36±0.13[c]	3.00±0.14[c]	0.62±0.02[c]
16	2.16±0.08[d]	3.45±0.07[d]	2.01±0.03[d]	0.71±0.03[d]
32	1.00±0.07[e]	1.37±0.15[e]	1.01±0.14[e]	1.10±0.03[e]
32®	4.45±0.12[b]	5.24±0.12[c]	3.74±0.19[b]	0.75±0.03[d]

SOD: Superoxide dismutase, CAT: Catalase, GSH: glutathione, MDA: malondialdehyde, NVP: Nevirapine. 32®: Recovery group. Data are expressed as mean±SD. *n*=5. Values with different superscript on the same column differ significantly at *P*<0.05 ANOVA and Dunnett's multiple comparison test

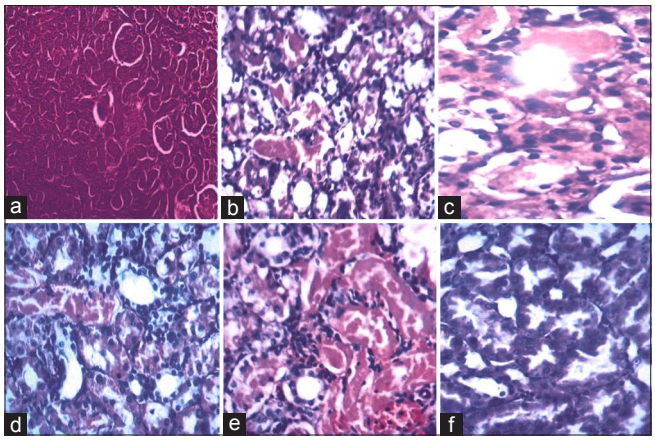

Figure 1: (a) Photomicrograph of control kidney of juvenile rat treated with water for 14 days showing normal kidney architecture, (b) photomicrograph of the kidney of juvenile rat treated with 4 mg/kg of NVP for 14 days showing tubular necrosis, (c) photomicrograph of the kidney of juvenile rat treated with 8 mg/kg of NVP for 14 days showing tubular necrosis and dilated tubules, (d) photo micrograph of the kidney of juvenile rat treated with 16 mg/kg of NVP for 14 days showing tubular necrosis with inflammatory materials in the tubular lumen, (e) photo micrograph of the kidney of juvenile rat treated with 32 mg/kg of NVP for 14 days showing extensive tubular necrosis with inflammatory materials in the tubular lumen, (f) photomicrograph of the kidney of juvenile rat in the recovery group without evident necrosis (H and E, ×200)

limits hydroxyl radical formation. GSH acts directly as an antioxidant and also participates in catalytic cycles of several antioxidant enzymes such as GSH peroxidase and GSH reductase [41-43]. Studies have shown that decreases in the levels of these antioxidants indicate oxidative stress. In this study, kidney levels of SOD, CAT and GSH were decreased in a dose-dependent manner in NVP-treated juvenile rats which were, however, restored in the recovery group. The observed decreases in the levels of SOD, CAT, and GSH are consistent with similar findings in a non-dose dependent study in adult albino rats [44]. This observation could be attributed to NVP-induced renal oxidative stress (via the generation of oxidative radicals) leading to the depletion of the antioxidants [44,45]. In addition, observed decreases in levels of SOD, CAT, and GSH may also be due to their increased usage in scavenging free radicals induced by the NVP [46].

MDA, a primary marker of oxidative stress and lipid peroxidation is one of the final products of the oxidative modification of lipids. It is responsible for cell membrane damage, including changes to intrinsic properties of membranes, such as fluidity, ion transport, and loss of enzyme activity and protein cross-linking which may eventually result in cell death [47]. The observations in this study showed dose-dependent increases in MDA levels in the kidneys of NVP-treated juvenile rats. This confirmed the involvement of lipid peroxidation in NVP-induced renal toxicity [48,49]. Furthermore, examination of the kidneys of NVP-treated rats showed dose-dependent tubular necrosis; however, kidneys of juvenile rats in the recovery group showed absence of tubular necrosis. The mechanisms of NVP-induced renal toxicity is yet unknown. However, studies suggest that antiretroviral drug-induced renal toxicity could be associated with the stimulation of cell death through the activation of mitogen-activated protein kinase, transport defect through the inhibition of transport pumps, direct kidney damage, and oxidative stress through mitochondrial toxicity [50]. Interestingly, observation in this study add credence to the involvement of oxidative stress in NVP-induced renal toxicity as evidenced by decreased SOD, CAT, and GSH and increased MDA levels. In addition, observations in this study showed that NVP-induced renal toxicity could be reversible.

CONCLUSION

This study showed that NVP produced dose-dependent and reversible renal toxicity in juvenile albino rats. The use of NVP in neonates may be safe; however, this study recommends routine clinical assessment of renal function in HIV exposed neonates before and with NVP use.

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