

Renal histoarchitectural changes in nevirapine therapy: possible role of kolaviron and vitamin C in an experimental animal model.

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Abstract

Background: There is paucity of literature regarding the nephrotoxicity of antiretroviral drugs and its interaction with plant-based adjuvants. This study investigates the attenuating effect of kolaviron in nevirapine- therapy on the histological structure of the kidneys of adult male Sprague-Dawley rats.

Objective: To determine the attenuating influence of anti-oxidant status of kolaviron on the kidneys of experimental animals following nevirapine administration.

Methods: Forty eight pathogen-free adult male Sprague-Dawley rats were used for this study. The animals were divided into 8 groups (A-H) with 6 animals in each group. Group A was given normal saline as the control; group B was given nevirapine; group C was given kolaviron; group D was given vitamin C; group E was given nevirapine and kolaviron; group F was given nevirapine and vitamin C; Group G was given nevirapine and kolaviron (kolaviron withdrawn after day 28) and group H was given corn oil. The experiment lasted 56 days after which the animals were sacrificed, blood samples were collected through cardiac puncture for serum analysis and the kidneys were harvested and prepared for H& E histological examination.

Results: Nevirapine caused histoarchitectural damage in the glomerular apparatus with resultant increase in kidney/body weight ratio ($p < 0.001$). Adjuvant treatment with kolaviron attenuated these nephrotoxic effects. Serum anti-oxidant enzyme (SOD and CAT) activities were significantly reduced in kolaviron and vitamin C treated animals, whereas in the nevirapine group these parameters were significantly elevated ($P < 0.05$). However, co-administration of nevirapine and vitamin C did not improve the histoarchitecture of the kidney.

Conclusion: Adjuvant treatment with kolaviron (an anti-oxidant) for 56 days appears to attenuate the nephrotoxicity of nevirapine in this model.

Keywords: Kidney, histoarchitecture, kolaviron, antiretroviral drugs.

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Introduction

Nevirapine (Nvp) is the first non-nucleoside reverse transcriptase inhibitor (NNRTI) approved by the U.S. Food

and Drug Administration (FDA) for use in combination therapy of Human immunodeficiency virus (HIV-1) infection. Currently, it remains one of the most prescribed antiretroviral drug in developing countries, to prevent vertical transmission (mother-to-child) when used in combination therapy¹. Despite its therapeutic benefits, treatment with Nvp has been associated with a significant incidence of hepatotoxicity and skin rash². Sex related differences in the toxicities of Nvp have been reported with incidence of adverse effects being more common in women than in men and this is related to the higher plasma levels in women^{3,4}.

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Garcinia kola (GK) seed commonly known as “bitter kola” is the fruit of a tropical plant belonging to the family *Guttiferaceae*. It is found in moist forest and grows as a medium sized tree, up to 12m high and 1.2m wide. It is cultivated and distributed throughout West and Central Africa⁵. GK seed is rich in phytochemicals including Garciniabioflavanonones, xanthonones, triterpinones and benzophenones with kolavironones the major components of kolaviron⁶. GK seeds are traditionally used as remedy for liver disease, heartburn, respiratory inflammation and cough⁷. Anti-diabetic properties have been previously reported⁸ and attributed to the bioflavonoid complex (kolaviron) in GK even in fasted normoglycemic and alloxan-diabetic rats. Iwu⁹ showed that GK biflavanones are pharmacologically active with several pharmacokinetic advantages over simple monomeric flavonoids as they survive first-pass hepatic metabolism which inactivates most flavonoids.

The kidneys play a crucial role in excretion of drugs and may thus be vulnerable to drug toxicities. Drug-induced renal injury contributes up to 25% of all cases of acute renal failure¹⁰. Recent interest has focused on complementary and alternative medicines for the treatment of various diseases¹¹ and our previous studies^{8,12} support the increasing utilization of plant-based adjuvants for disease management. Many of the biological functions of flavonoids and phenolic compounds have been attributed to their free radical scavenging and anti-oxidant activities^{12,13}. Studies have also shown the ability of kolaviron (kv) to protect against various noxious compounds like phalloidin, amanita, 2-acetylaminofluorene, carbon tetrachloride, paracetamol, aflatoxin and dimethyl nitrosamine in the liver of rodents¹⁴. With paucity of data reporting potential attenuating influence of kolaviron in any anti-retroviral treatment and in view of the potential side effects of Nvp, the understanding of the mechanism(s) for this side effect should allow for further investigation of the adjuvant use of kolaviron to ameliorate the effects. Based on these considerations, this work investigates the histo-architectural effect of kolaviron on the kidneys of Nvp-treated male Sprague-Dawley rats comparing this with known anti-oxidant vitamin C in an experimental animal model.

Materials and method

Chemicals

Nvp (Aspen) and vitamin C were obtained from Pharmicare Ltd, Port Elizabeth, South Africa and were of analytical grade.

Plant materials

GK seeds were purchased from Eda-Oniyo Ekiti market, Ekiti State, Nigeria. The seeds were taken to the Herbarium unit of the Department of Botany, Obafemi Awolowo University, Ile-Ife, Nigeria for authentication. Samples of it are retained at the herbarium for future reference and allocated voucher specimen number NHI 16745.

Isolation of Kolaviron

Kv was isolated according to the published procedure of⁹ and modified by¹⁵. The outer cover of GK seeds were peeled, sliced and air-dried in the laboratory (25-28°C) and ground into powdered form. The powdered seeds were extracted with n-hexane, in a soxhlet extractor for 24hr. The defatted, dried marc was repacked and then extracted with methanol in a soxhlet extractor. The extract was concentrated and diluted to twice its volume in distilled water and extracted with ethylacetate (6×300ml). The concentrated ethylacetate yielded kolaviron, a golden yellow solid.

Ethical approval

The study protocol was approved by the University of KwaZulu Natal Animal Ethics Committee (Ethical clearance number: Animal/102/14). The animals were bred at the Biomedical Resource Unit (BRU) animal facility of the University of KwaZulu Natal Westville campus, Durban-South Africa. All animals received humane care in accordance with the criteria outlined in the ‘Guide for the Care and Use of Laboratory Animals’ prepared by the National Academy of Science (NAS) and published by the National Institute of Health¹⁶.

Animal model

Forty eight pathogen-free adult male Sprague-Dawley rats aged 9-10 weeks old and weighing approximately 200-250g were used for this study. They were kept in well ventilated plastic cages (36cm long × 24cm wide and 15cm high) under standard animal house conditions at room temperature (28-30°C) and were fed with standard pelleted rat chow from (Meadow feeds a Division of Astral Operations Limited, Durban, South Africa) and given tap water ad libitum.

Experimental design

The animals were randomly distributed to eight treatment groups: A, B, C, D, E, F, G, H with six animals per group (n=6). The animals were allowed 2 weeks of acclimatization before the treatment commenced. Group A received

normal saline as the control, group B were given Nvp at a dose of 1.54mg/kgbw, Group C were given Kv at a dose of 200mg/kgbw, Group D were given vitamin C at a dose of 250mg/kgbw, Group E were given Nvp at a dose of 1.54mg/kgbw and Kv at a dose of 200mg/kgbw, Group F were given Nvp at a dose of 1.54mg/kgbw and vitamin C at a dose of 250mg/kgbw, Group G were given Nvp at a dose of 1.54mg/kgbw and Kv at a dose of 200mg/kgbw (kolaviron was withdrawn after day 28). Group H were given corn oil which served as the vehicle for the administration of Kv. All administration was done daily by oro-gastric gavage. The experiment lasted for 56 days.

Histological examination of tissues

Twenty four hours after the last treatment the animals were humanely sacrificed under excess flouthane® anaesthesia and the kidneys removed and weighed. They were examined for gross pathology and immediately fixed in 10% formal saline. After fixation, the tissues were dehydrated in a graded series of alcohol, cleared in Xylene and embedded in paraffin wax using a cassette. The tissues were sectioned at 4µm thickness using LeicaR RM 2255 microtome and stained with hematoxylin and eosin. The stained slides were cover slipped using DPX mounting glue directly over the tissue section ensuring no air bubbles were trapped. Thereafter, the slides were left overnight to dry and scanned using a Leica SCN 400 (Leica Microsystems GmbH, Wetzlar, Germany) and measured at 250 magnification using image analyser Leica (DMLB) and Leica QWIN software.

Biochemical assays

Blood samples were collected through cardiac puncture and allowed to clot for 30 minutes and then centrifuged

for 15 minutes at 3000 rpm. The serum was decanted into Eppendoff tubes and preserved at -84°C prior to biochemical analysis. SOD levels in the serum were assayed according to the method of Das et al¹⁷. CAT activity was assayed using hydrogen peroxide as substrate according to the method of Aebi¹⁸.

Statistical analysis

The data obtained from all the groups was compiled and statistically analyzed and expressed as mean ± SEM. Since we had a parametric data, differences between groups were compared using One-way ANOVA, with $p < 0.05$ considered significant.

Results

Mortality: No animal died during the experimental period.

Effects of Nevirapine and Kolaviron on kidney/body weight changes:

There was a significant increase in kidney-body weight ratio $p < 0.001$ in nevirapine-treated animals whereas animals in group C treated with kolaviron for same duration did not show any deleterious effect. Further, group D treated animals given vitamin C showed no increase in kidney-body weight ratio significant at $p < 0.05$. But when treated concomitantly with nevirapine in group F, we observed significant increase in kidney-body weight ratio $p < 0.001$. Treatment of nevirapine and kolaviron in group E for 56 days showed significant improvement $p < 0.05$. In contrast, treatment of nevirapine and kolaviron in group G where kolaviron was withdrawn after day 28 showed a significant increase in kidney -body weight ratio $p < 0.001$. Group H administered with corn oil showed no renal injury significant at $p < 0.05$ (See table 1).

Table 1: Kidney and body weight changes across the groups

Groups	Initial BW(g)	Final BW(g)	BW Diff (%)	KW(g)	KWBR ($\times 10^{-3}$)
A	213.5 \pm 0.11	380.0 \pm 5.34	166.5(77.99)	2.11	5.55 \pm 0.08
B	220.5 \pm 5.73	417.0 \pm 5.5	196.5(89.12)	2.40*	5.73 \pm 0.18*
C	202.5 \pm 0.31	356.5 \pm 4.49	154.0(76.05)	1.99	5.59 \pm 0.12
D	243.0 \pm 0.42	392.0 \pm 4.20	149.0(61.32)	1.97*	5.03 \pm 0.13
E	198.5 \pm 0.03	402.5 \pm 5.23	204.0(102.77)	2.13	5.29 \pm 0.06
F	246.5 \pm 0.09	397.5 \pm 5.70	151.0(61.26)	2.34*	5.88 \pm 0.08**
G	232.0 \pm 0.17	390.0 \pm 5.29	158.0(68.10)	2.20	5.62 \pm 0.09
H	212.5 \pm 0.13	390.0 \pm 5.12	177.5(83.53)	2.10	5.37 \pm 0.08

Key:

BW= Body weight, KW=Kidney weight, KWBR=Kidney weight body ratio; *P<0.05;**P<0.01)

Effects of Nevirapine and Kolaviron on antioxidant enzymes

Catalase, which acts as a preventive anti-oxidant enzyme plays an important role in protection against the deleterious effects of lipid peroxidation was significantly decreased following treatment with Nvp alone (p<0.01) and in group with withdrawal of Kv (p<0.05) respectively (Fig 1).

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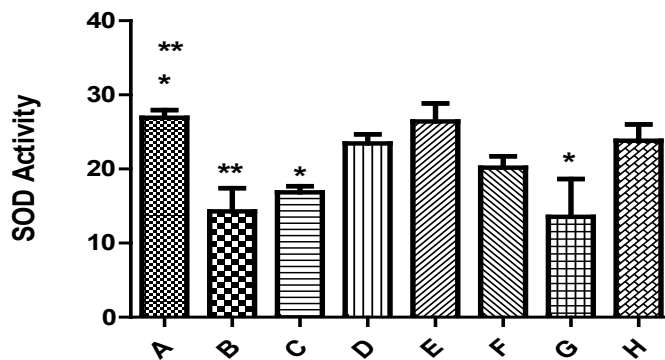


Fig 1: Graphical representation of SOD activity.

Though the activities of SOD in Kv and vitamin C treated groups were not significantly higher than positive control, Nvp treated group was much lower in value than the control (Fig. 2).

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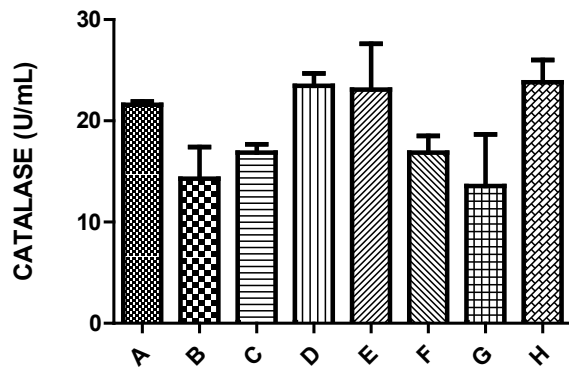


Fig 2: Graphical representation of CAT activity.

Effects of Nevirapine and Kolaviron on the histological changes of the kidney

Histopathological sections of kidneys in control rats revealed adequate preservation of tubular structures with

the presence of glomerular capsule. There were distortions in the kidney histology of nevirapine treated animals with necrosis of glomeruli and atrophy of the proximal and distal convoluted tubules (plate B).

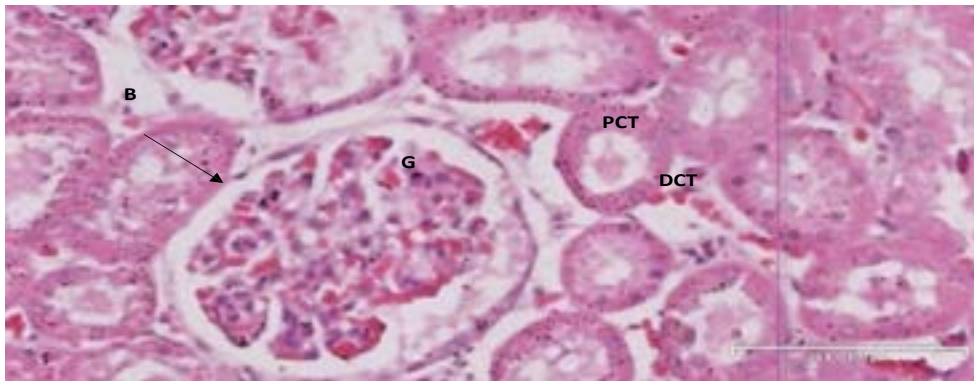


Plate 1: Cross section of kidney of Sprague-Dawley rats in control group. Normal histoarchitecture displayed. (H & Estains).
B=Bowmans capsule, PCT=Proximal convoluted tubules, DCT=Distal convoluted tubules, G= Glomerus.

B

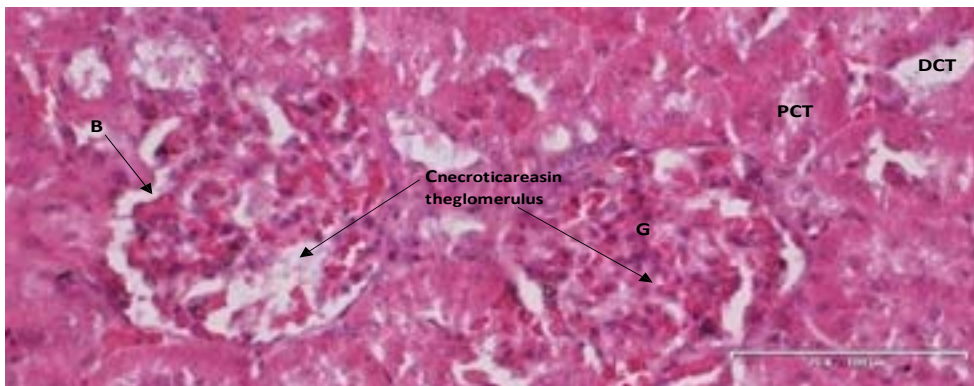
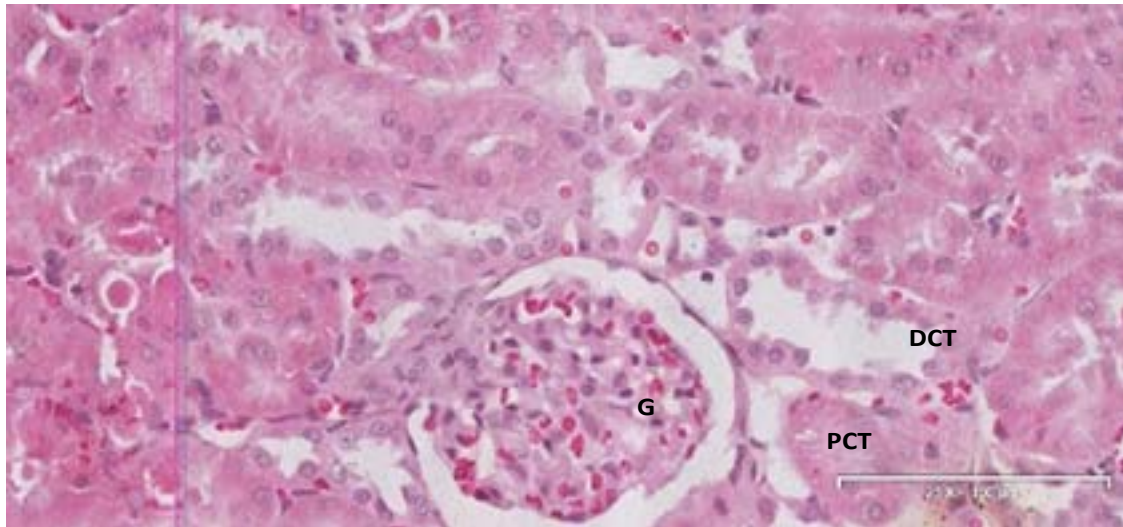


Plate 2: Cross section of kidney of Sprague-Dawley rats treated with nevirapine only. Note the necrosis in glomeruli, atrophied PCT and DCT. (H & Estains).
G=Glomerulus, DCT=Distal convoluted tubule PCT=Proximal convoluted tubules, B=Bowman's space, C=Capillary loops.

C

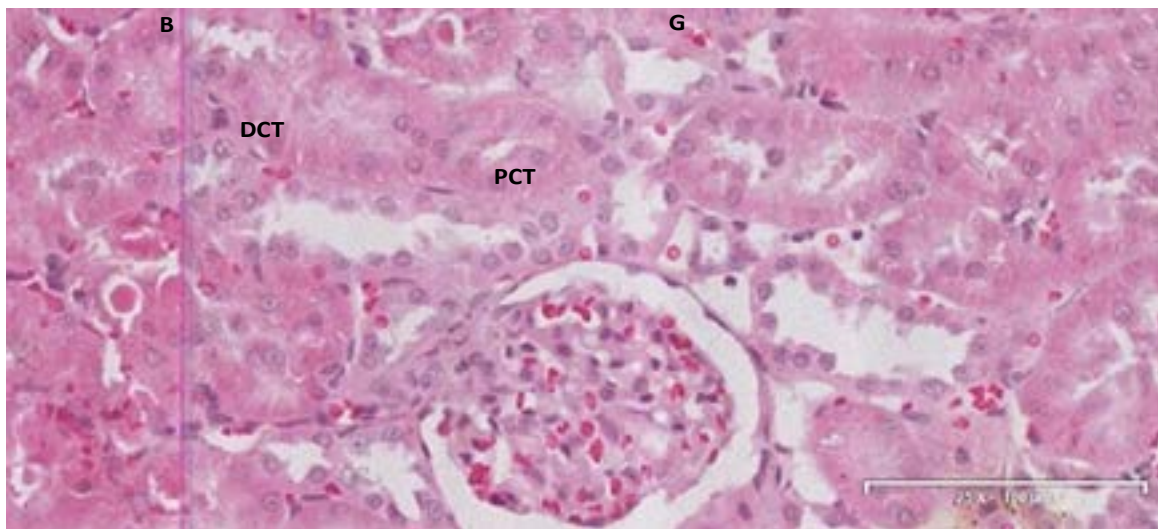
These changes were markedly generalized. There were no observable changes in the histology of the kidney of the groups treated with kolaviron alone, vitamin C alone and Kv with Nvp as these compared favourably with controls (plates 3, 4, 5). Adjuvant treatment with Kv and Nvp in group E showed an improved histological layout of the kidney (plate 5) whereas group G treated with Nvp and

Kv (Kv withdrawn after day 28) showed cellular distortion of the glomeruli with atrophy of Bowman's capsule and space and a generalized hypocellularity (plate 7). Histoarchitectural kidney sections following vitamin C and Nvp co-treatment showed disorganization of the proximal and distal convoluted tubules with generalized hypoplasia (plate 6).



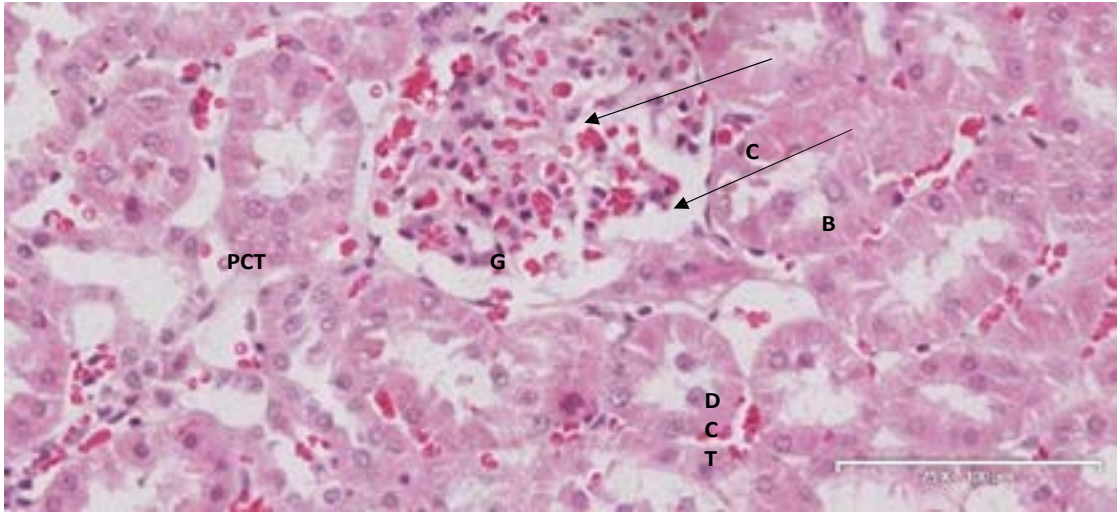
**Plate 3: Cross section of kidney of Sprague-Dawley treated with kolaviron only. Note: Histoarchitecture normal. (H & Estains).
G=Glomerulus, PCT=Proximalconvolutedtubules,DCT=Distalconvolutedtubules,C=Capillary loops.**

D

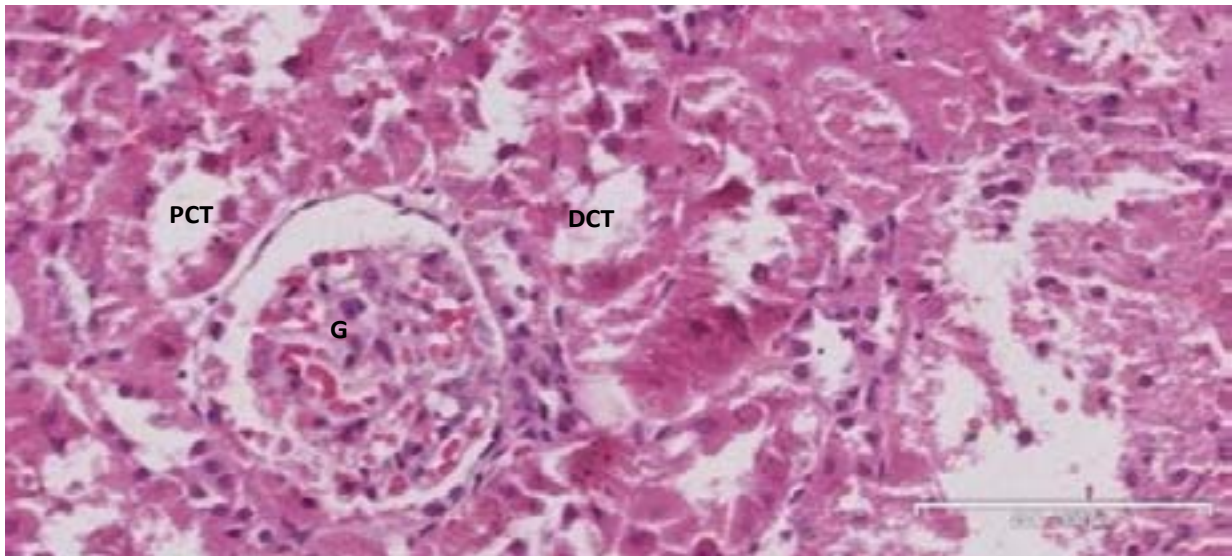


**Plate 4: Cross section of kidney of Sprague-Dawley rats treated with vitamin C only. Histoarchitecture normal. (H & Estains).
B=Bowman'sspace,G=Glomerulus,DCT=Distalconvolutedtubules,PCT=Proximal convoluted tubules.**

E

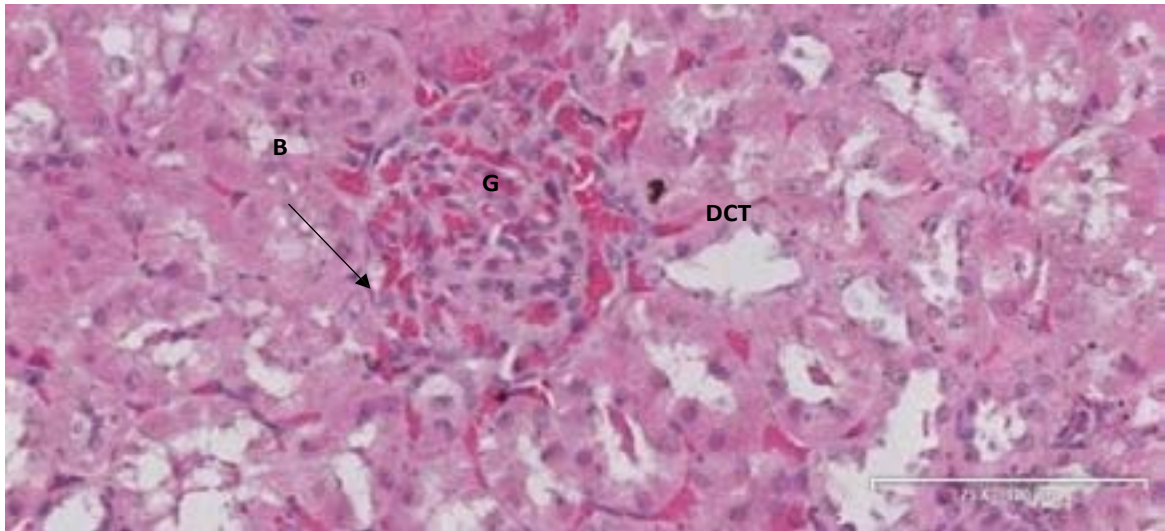


**Plate 5: Cross section of kidney of Sprague-Dawley rats treated with nevirapine and kolaviron. Histoarchitecture normal. (H & Estains).
G=Glomerulus, C=Capillary loops, B=Bowman'sspace, DCT=Distal convoluted tubules, PCT=Proximal convoluted tubules.
F**



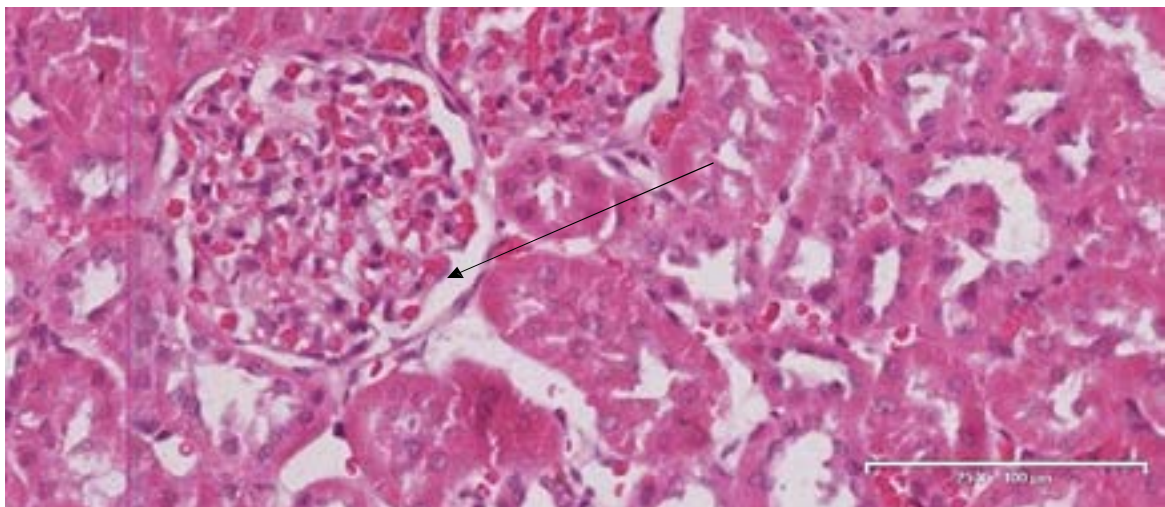
**Plate 6: Cross section of kidney of Sprague-Dawley rats treated with nevirapine and vitamin C. Note: Disorganization in PCT & DCT cellular architecture with generalized hypoplasia. (H & Estains).
PCT=Proximal convoluted tubules, DCT=Distal convoluted tubules.**

G



**Plate7: Cross section of kidney of Sprague-Dawley rats treated with nevirapine and kolaviron (where kolaviron was withdrawn after 28 days).Note: Cellular distortion of glomeruliwithatrophy of Bowman's capsule and space. Generalized hypocellularity. (H & Estains).
G=Glomerulus, B=Bowman's capsule, DCT=Distal convoluted.**

H



**Plate8: Cross section of kidney of Sprague-Dawley rats given cornoil. Histoarchitecture essentially normal.(H & Estains).
G=Glomerulus,B=Bowman'sspace,DCT=Distalconvolutedtubules,PCT=Proximalconvoluted tubules.**

Discussion

The kidney plays a major role in the maintenance of constant volume and composition of the extracellular fluid hence does the basic functions of glomerular filtration, tubular reabsorption and tubular secretion¹⁹. Kidney function can be evaluated by a number of methods. Male rats are particularly a suitable animal model for evaluating tubular lesions because their intra-renal enzyme distribution is similar to that in man²⁰.

Antiretroviral therapy (ART) using Nvp has led to improved wellness of HIV-infected patients by lowering the viral load and hence their positive impact on longevity. However this has come with its potential toxicities on body organs such as the liver with altered oxidative stress status implicated²¹. Histological studies are important in anatomical localization of the toxic or the beneficial actions of various drugs. In this study, the histoarchitecture of the glomeruli, proximal and distal convoluted

ed tubules, present a compromised histological state in the Nvp-treated group with extensive necrosis as well as atrophy of Bowman's capsule and space. Morphological and physiological studies have identified the renal tubule systems as the site of maximum damage by toxicants and hence any direct protective effect would be apparent on the tubule system. This has been observed in the groups treated with Kv as well as vitamin C whereupon this parameter was restored. Our current work on the testes also confirms the deleterious histological changes following ART on testicular tissue²².

Oxidative stress has been recognized as a major pathway of action for most antiretroviral drugs via various mechanisms that may trigger it²³. Anti-oxidant enzymes are inhibited by toxic drugs including Nvp and renal activities of catalase and superoxide dismutase are reduced in this experiment. The reactive oxygen species (ROS) produced directly act on cell components including lipids, proteins and DNA and destroy their structure. The consequence of this is oxidative stress and cell death. Kv is rich in bi-flavonoids which has been ascribed strong anti-oxidant properties²⁴. It could possibly have a reno-protective effect in Nvp-treated rats by exerting its beneficial effects via modulating the anti-oxidant system. Together, the results suggest the beneficial effects of using a free radical scavenger in modulating Nvp-associated nephrotoxicity.

The use of vitamins (C and E) as anti-oxidant supplements in ART has been practiced both in experimental and clinical conditions because they act as 'mop-up' for generated free radicals in the milieu. The effects of Kv and vitamin C in this protocol have tilted towards similarities adjudged from the various parameters assessed. Oxidative stress-induced lipid peroxidation generates numerous electrophilic aldehydes that can attack many cellular targets. They may also inhibit drug-induced apoptosis by inactivating caspase activity which would diminish the efficacy of treatment. The use of anti-oxidants during therapy may enhance therapy by reducing the generation of oxidative-stress induced aldehydes²⁵.

Weight coefficient (organ/BW ratio) is a sensitive, effective and economic indicator of the toxicology and thus important in the identification of target organ by toxicants²⁶. Our investigation reveals that the weight coefficient of kidney tissue in Nvp-treated animals was signifi-

cantly higher than those of controls, further supporting the toxicity of Nvp to this organ. Renotoxicity just like testicular toxicity can manifest in the form of organ swelling, atrophy or hypertrophy²⁷ and the significantly increased KW in Nvp-treated group is consistent with this assertion possibly because of high cytotoxicity-induced tubulo-glomerular atrophy.

While these reports buttress our hypothesis that when massive cellular loss occurs, there is a sharp decline in morphometric parameters which are largely attenuated by anti-oxidant treatment²³, we believe that the precise putative explanations for the structural changes observed may be better explained using stereological techniques in order to generate a 3-dimensional approach. That way a more detailed quantification can be done with the aim of generating relevant approaches to the mechanisms underlying the processes. More so, therapeutic approaches with anti-oxidants in HIV/AIDS treatment presents a challenge to researchers because AIDS itself is a vexing disease with many implications. Therefore, the understanding of the mechanistic information regarding the interactions of the various modalities of biological and exogenous anti-oxidants in the course of therapy would significantly broaden the scope of treatment with consequent reduction of the morbidity and mortality associated with HIV/AIDS.

Conclusion

While studies to elucidate histo-architectural changes in the osmo-regulatory organ (kidney) following antiretroviral therapy is limited, we have highlighted in our experiment that treatment with Nvp disrupts renal histo-architecture leading to compromise in the integrity and biochemical functioning. These perturbations are largely attenuated by adjuvant treatment with Kv which compares favourably with known anti-oxidant (vitamin C) in this experimental animal model. However, further studies (using stereological techniques) are needed to explain the observed effects and provide a mechanistic explanation for Kv action.

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Conflict of interest

All authors declare that there are no conflicts of interest whatsoever.

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