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Toxicological effects of artemether-nevirapine co-administration on serum biochemistry and some organs of Wistar rats

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Abstract

This study investigated toxicological effects of 5 and/or 10 mg/kg artemether (ART_5 or ART_{10}) and nevirapine (NVP) co-administration on serum biochemistry and some organs of Wistar rats. Drugs were administered intraperitoneally to 6 groups (n=6) for 21 days. On day 22, rats were sacrificed, sera obtained to determine electrolyte and antioxidant levels. Liver, kidney, lung and spleen were harvested and weighed for histological studies. Data analysed using one-way ANOVA, Dunnett's post-hoc test and were considered significant at $p \leq 0.05$. There was no difference in superoxide, catalase, Na^+, K^+, Ca^{2+} and HCO_3^- levels in the treated groups. Cl^- decreased ($p \leq 0.05$) in NVP + T_{80} and NVP + ART_{10} administered groups. MDA increased ($p \leq 0.05$) in NVP + ART_{10} group while GPx decreased. No pathological changes were observed in the liver of all treated groups but relative weight increased ($p \leq 0.05$) in NVP-ART_{10} treated group. The kidney of NVP-ART_5 and NVP-ART_{10} treated groups did not differ in relative weight but showed some renal necrosis. The spleen and lung of ART_{10} treated group showed some pathological changes. The changes in relative liver weight, kidney tissue, Cl^-, MDA and GPx levels of ART-NVP administered rats suggest the need for precautionary measures during drug treatment combination.

Keywords: Artemether; Nevirapine; Toxicology; Liver; Kidney; Spleen; Lung, Biochemical parameters

INTRODUCTION

Malaria is known to have devastating impact on people's health and livelihoods with Africa recording the largest burden of morbidity. An estimate of 219 million malaria cases and 435,000 deaths due to malaria occurred worldwide in 2017 making it one of the major problems in developing countries [1]. Artemisinin and its semisynthetic derivatives are a group of drugs used against the parasitic disease due to *Plasmodium falciparum*. It is effective and acts by causing destruction of cell membrane of the parasite, slows down protein synthesis, disorganizes the ribosomes, dilate the nuclear envelope and disintegrate the food vacuoles [2]. Artemether (ART), a relatively lipophilic and unstable drug is the first artemisinin derivative to be developed and found to be more effective than the parent artemisinin; it is used as part of combination therapy in the treatment of malaria.

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Human Immunodeficiency Virus (HIV) remains one of the most serious global health threats of our time. It is a retrovirus that infects cells of the immune system causing impairment in their function. This process can be slowed down with Antiretroviral (ARV) drugs. In 2018, there were 37.9 million people globally living with HIV with 23.2 million accessing ARV therapy and 777,000 deaths from AIDS related illnesses [3]. The use of ARV drugs (in combination) in managing HIV infection has greatly led to a decline in morbidity and mortality associated with the infection. This combination mostly includes the three main classes of ARV drugs for effective management: protease inhibitors, nucleoside reverse transcriptase inhibitors and or non-nucleoside reversed transcriptase inhibitors. Nevirapine (NVP) on the other hand is a non-nucleoside reverse transcriptase inhibitor, also used in combination with other antiretroviral drugs in the management of HIV infections in Nigeria and other parts of the world [4].

There are increasing numbers of HIV-infected patients especially in Africa receiving ART and NVP either alone or in combination with other drugs, creating the potential for drug-drug interaction. Co-administration of these drugs may occur due to possibility of co-infection of malaria with HIV. However, while they are both used to treat these infections, they may exhibit some undesirable adverse effects, which may occur or affect specific organs of the body especially when prescribed for a longer period. Although histopathological studies on the use of these drugs have been reported: on ART [5] and NVP [6] separately but not on co-administration of ART and NVP. The present study therefore investigated the toxicological effect of co-administration of ART and NVP on serum biochemistry and some organs of Wistar rats.

EXPERIMENTAL

Animals. Wistar rats weighing between 180 and 230 g used for this study were obtained from the animal house of the Department of Pharmacology and Therapeutics, Ahmadu Bello University (A.B.U), Zaria. Male and female rats were kept in separate metallic cages. They were fed on normal rodent feed (Vital Feed, Jos-Nigeria) and water *ad libitum*. Animal use was approved by the institutional animal welfare committee of A.B.U., Zaria and animals were handled according to the NIH animal care guidelines [7].

Drugs and chemicals. Artemether (80 mg/ml by Rhone Poulenc International, France) being an oily drug was diluted with 3% v/v tween 80 (T_{80}) to the required volume according to the needed concentrations for administration in animals. The preparation of T_{80} was done by pipetting 3 ml of tween 80 into a bottle, about 20 ml of deionized water was added and shaken vigorously to mix. This was then made up to 100 ml with deionised water. It was again shaken vigorously until mixed thoroughly and the foam was allowed to settle before use. Nevirapine (manufactured by Hetero Drugs Limited, Hyderabad, India) on the other hand which is insoluble in water was also freshly prepared before use by dissolving in small volume of T_{80}. After dissolution, it was made up with the same vehicle according to the required concentrations for administration in animals before use. All other chemicals used were of analytical grades.

Experimental design. Thirty-six (36) rats (males and females) were randomly divided into six groups of six per group. On days 1 to 21 of the experiment, groups 1, 2 and 3 rats received 3% v/v T_{80} while groups 4, 5 and 6 received 30 mg/kg NVP daily before being fed (in a fasting state) for 21 days. In addition to the above treatment from days 15 to 21, groups 1 and 4 received doses of T_{80} (for 7 days), groups 2 and 5 rats received 5 mg/kg ART (ART_5) while groups 3 and 6 rats received 10

mg/kg ART (ART_{10}) [8]. All drugs were administered via intraperitoneal route.

Histopathological studies. On day 22, twenty-four hours after the last drug administration, animals were weighed and then made unconscious in chloroform chamber. They were humanely sacrificed and blood collected into plain bottles through cardiac puncture. The organs (liver, kidney, lung and spleen) were also carefully harvested, weighed and fixed in 10% formal saline. The tissues were further processed, sectioned and stained according to Haematoxylin and Eosin (H and E) technique [9]. The photomicrographs of different organs were taken at various magnifications (100 - 400) using research photographic microscope.

Biochemical Analysis. The blood collected from the rats were labelled and centrifuged at 400 rpm for 5 minutes. The clear serum obtained were stored at -20^{O}C until used to check for the following parameters: malondialdehyde (MDA) as earlier described [10], superoxide (SOD) as earlier described [11], catalase (CAT) as earlier described [12] and glutathione (GPx) as earlier described [13] using commercial kits obtained from Reckon Diagnostics P. Ltd, India. The serum electrolytes (Na^+, K^+, Ca^{2+} Cl^- and HCO_3^-) were analysed using Audicom Electrolyte Analyser (AC9900, USA).

Statistical analysis. Data obtained from organ/body weight ratio were analysed and expressed as means ± standard errors of mean using SPSS (Version 20). One-way analysis of variance (ANOVA) was performed followed by Dunnett's post-hoc test for comparison within group. Value of P less than or equal to 0.05 were considered statistically significant. The analysed results were presented in Tables. The photomicrographs of the organs were also presented in plates.

RESULTS

There was no statistically significant difference in serum electrolytes (Na^+, K^+, Ca^{2+} and HCO_3^-) levels in the experimental groups compared with the control; although Cl^- decreased significantly ($p \leq 0.05$) in NVP + T_{80} and NVP + ART_{10} administered groups when compared with the control (96.2±0.5 versus 102.8±0.3) and (96.0±1.5 versus 102.8±0.3) respectively (Table 1).

There was no statistically significant difference in SOD and CAT levels in all treated groups but the level of MDA increased significantly ($p \leq 0.05$) in NVP + ART_{10} administered group (2.0±0.1 versus 1.3±0.1) while GPx decreased significantly when compared with the control (41.0±1.4 versus 52.3±2.5) – Table 2.

Statistically significant increase ($p \leq 0.05$) was observed in the organ/weight ratio of the liver in ART_{10}-NVP group (Table 3) but no changes were observed in the relative weight of the kidney. There were no pathological changes observed in the liver of all drug treated groups. However, the kidney of the groups treated with NVP-ART_5 and NVP-ART_{10} showed necrosis of renal tubular epithelial cells and obliteration of Bowmans space respectively (Plate I). In addition, congested lung was observed in the groups that received both doses of ART (Plate II). The group treated with ART_{10} showed depletion of lymphocytes at the germinal center of the spleen (Plate III).

DISCUSSION

Treatment of diseases may involve the use of one or more drug combination. Such combination may cause unwanted effects that may lead to death. Occurrence of malaria may not be without immune suppression and this is considered as one of the opportunistic infections in an immunosuppressed individual which may necessitate co-administration of ART and NVP.

The study revealed no significant changes in the blood level of electrolytes measured but a significant decrease in Cl^- in NVP and NVP-ART_{10} administered groups was observed. This may probably be as a result of cell membrane damage or a mild alteration in membrane permeability which can alter electrolyte balance. Serum electrolytes such as Na^+, Cl^-, HCO_3^- and K^+ are some of the parameters that are used in determining kidney function [14]. Increase or decrease in their levels may be an indicator of abnormal kidney function. Therefore, significant decrease in Cl^- observed may be a sign of slight instability in kidney function and this is in line with the histology results presented in this study. Disturbance in acid-base balance may be due to a decrease in chloride and bicarbonate concentrations in the blood which is in line with earlier observation [15] although this excludes bicarbonate.

Table 1: Effects of Artemether-Nevirapine Co-administration on Serum Electrolytes in Wistar Rats

NVP ART	T_{80} T_{80}	T_{80} ART_5	T_{80} ART_{10}	NVP T_{80}	NVP ART_5	NVP ART_{10}
SE (mmol/l)						
Na^+	138.5±1.2	142.6±1.3	137.5±1.2	137.0±1.2	137.8±1.2	136.0±1.7
K^+	4.0±0.2	4.1±0.2	4.3±0.2	4.1±0.2	3.8±0.2	4.2±0.4
HCO_3^-	25.5±1.4	26.4±0.5	23.2±1.2	24.0±0.9	23.5±0.7	22.0±0.1
Ca^{2+}	2.4±0.1	2.5±0.2	2.4±0.1	2.5±0.1	2.4±0.2	2.5±0.1
Cl^-	102.8±0.3	101.4±1.3	99.7±1.6	96.2±0.5*	100.8±1.4	96.0±1.5*

T_{80}= 3% v/v tween 80, ART_5 = 5 mg/kg artemether, ART_{10} = 10 mg/kg artemether, NVP = nevirapine (30 mg/kg), Na^+=Sodium, K^+=Potassium, HCO_3^- =Bicarbonate, Ca^{2+}=Calcium, Cl^-=Chlorine, SE = Serum Electrolyte; N = 6 per group, Data are mean ± SEM using, SPSS. Statistically significant *$p \leq 0.05$, (ANOVA, followed by Dunnett's post hoc test).

Table 2: Effects of Artemether-Nevirapine Co-administration on Antioxidant and Lipid Peroxidation Levels in Wistar Rats

NVP ART	T_{80} T_{80}	T_{80} ART_5	T_{80} ART_{10}	NVP T_{80}	NVP ART_5	NVP ART_{10}
MDA	1.3±0.1	1.8±0.1*	1.7±0.1	1.3±0.1	1.6±0.2	2.0±0.1**
SOD	1.90±0.16	1.97±0.15	2.07±0.22	1.92±0.12	1.90±0.12	1.75±0.06
CAT	50.50±3.15	48.67±3.81	46.67±3.27	46.83±3.08	48.00±2.29	47.00±2.67
GPx	52.3±2.5	45.5±3.1	50.0±3.1	46.5±2.3	50.2±2.4	41.0±0.4*

MDA = Malondialdehyde, SOD = Superoxide dismutase, CAT = Catalase, GPx = Glutathione, T_{80}= 3% v/v tween 80, ART_5 = 5 mg/kg artemether, ART_{10} = 10 mg/kg artemether, NVP = nevirapine (30 mg/kg), N = 6 per group, Values are mean ± SEM **S**tatistically significant *$p \leq 0.05$, **$p \leq 0.001$ (ANOVA, followed by Dunnett's post hoc test).

Table 3: Body and Organ Weights of Rats Co-administered with Artemether and Nevirapine

NVP ART	T_{80} T_{80}	T_{80} ART_5	T_{80} ART_{10}	NVP T_{80}	NVP ART_5	NVP ART_{10}
BW (g)	205.1±6.9	209.8±12.9	201.1±9.9	205.8±10.9	204.5±12.1	186.7±5.3
Brain	0.66±0.03	0.66±0.05	0.61±0.02	0.66±0.03	0.76±0.07	0.74±0.07
Kidney	0.74±0.03	0.75±0.05	0.70±0.02	0.69±0.02	0.78±0.03	0.73±0.04
Liver	3.19±0.17	3.28±0.07	3.37±0.04	3.30±0.22	3.60±0.11	3.83±0.05*
Lung	0.96±0.11	0.79±0.04	0.89±0.13	0.89±0.09	1.13±0.13	0.95±1.58
Spleen	0.53±0.05	0.55±0.05	0.62±0.05	0.49±0.02	0.56±0.02	0.56±0.04

T_{80}= 3% v/v tween 80, ART_5 = 5 mg/kg artemether, ART_{10} = 10 mg/kg artemether, NVP = nevirapine (30 mg/kg), BW=Body weight, N = 6 per group, Values are mean ± SEM. **S**tatistically significant *$p \leq 0.05$, (ANOVA, followed by Dunnett's post hoc test).

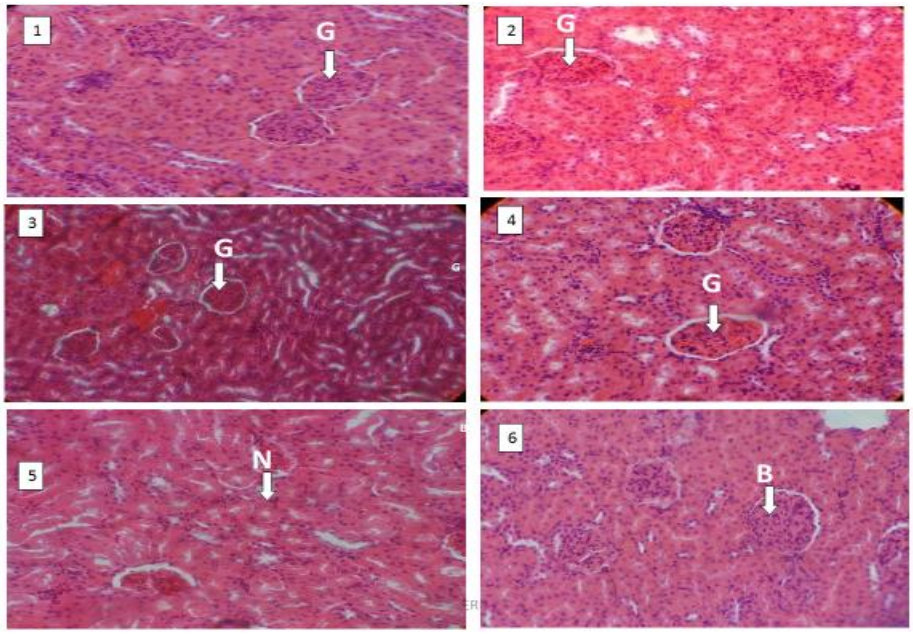

Plate I: Section of kidney from rats treated as follows:

(1) 3% v/v tween 80 - normal glomerulus (G), H & E stain (Mag. X 400). (2) ART 5 mg/kg - normal glomerulus, (G), H & E stain (Mag. X 400) (3) ART 10 mg/kg - normal glomeruli (G), H & E stain (Mag. X 100) (4) NVP - normal glomeruli (G), H & E stain (Mag. X 100) (5) ART 5 mg/kg + NVP - necrosis of renal tubular epithelial cells (N), H & E stain (Mag. X 400) (6) ART 10 mg/kg + NVP - obliteration of Bowmans space (B), H & E stain (Mag. X 400)

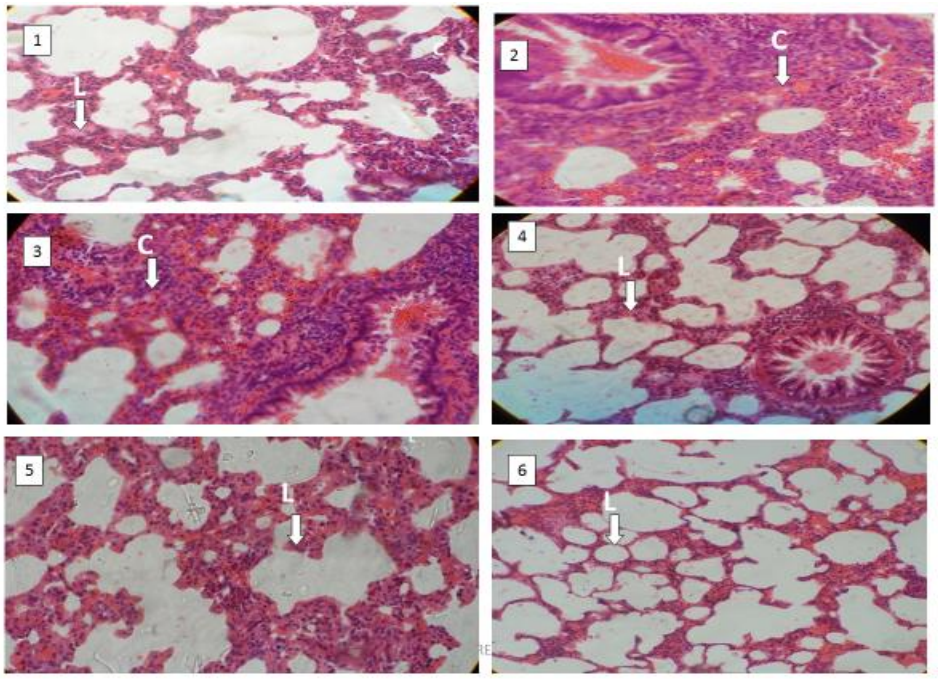

Plate II: Section of lung from rats treated as follows:

(1) 3% v/v tween 80 - normal lung architecture (L), H & E stain (Mag. X 400) (2) ART 5 mg/kg - congested lung (C), H & E stain (Mag. X 400) (3) ART 10 mg/kg - congested lung (C), H & E stain (Mag. X 400) (4) NVP - normal lung architecture (L), H & E stain (Mag. X 400) (5) ART 5 mg/kg + NVP - normal lung architecture (L), H & E stain (Mag. X 400) (6) ART 10 mg/kg + NVP - normal lung (L), H & E stain (Mag. X 400)

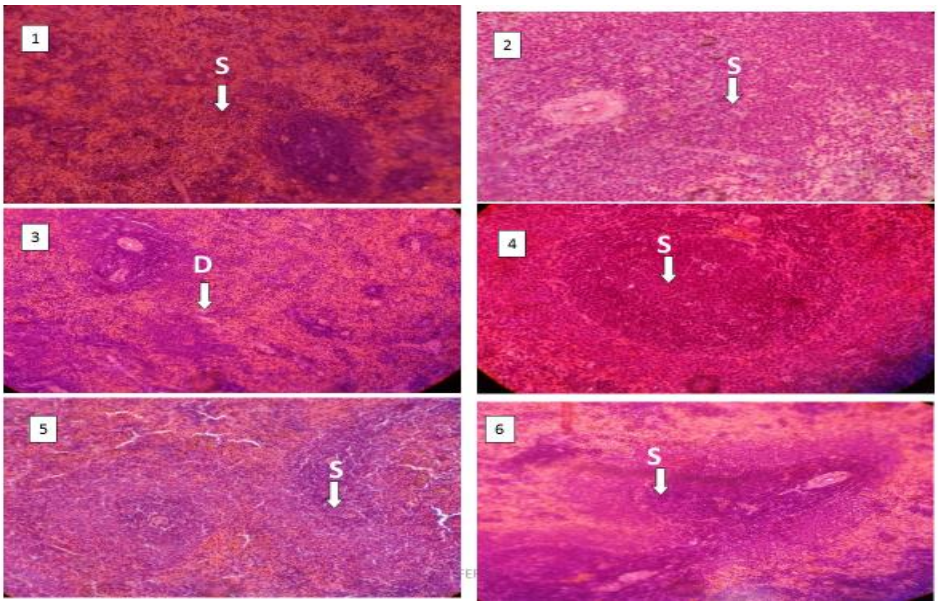

Plate III: Sections of spleen from rats treated as follows:
(1) 3% v/v tween 80 – normal spleen (S), H & E stain (Mag. X 200) (2) ART 5 mg/kg – normal spleen (S), H & E stain (Mag. X 200) (3) ART 10 mg/kg - depletion of lymphocytes at germinal centre (D), H & E stain (Mag. X 400) (4) NVP - normal spleen (S), H & E stain (Mag. X 200) (5) ART 5 mg/kg + NVP - normal spleen (S), H & E stain (Mag. X 200) (6) ART 10 mg/kg + NVP - normal spleen (S), H & E stain (Mag. X 400)

It was earlier reported that the importance of serum electrolytes is correlated with their involvement in many vital activities including muscle contraction, maintenance of acid-base balance and nerve impulse conduction [16].

Antioxidants like MDA, CAT, GPx and SOD are substances that inhibit or delay oxidation of a substrate. They are present in minute quantity and easily oxidized by reactive oxygen species (ROS) in biological systems. They decrease the rate at which the ROS react with cellular components like lipid membrane, DNA and protein. This may eventually protect against DNA damage as well as cellular dysfunction and cell death. Decrease in the level of GPx as observed in this study may be connected with in balance between ROS production and antioxidant defence resulting in oxidative stress. This may cause deregulation of cellular functions and eventually lead to various pathological conditions [17]. It may also be associated with the altered histology observed. Glutathione (GPx) is involved in the maintenance of redox balance in the cellular compartments, which is of great biological importance because it allows fine turning of the cellular redox environment during stress and under normal conditions. A central nucleophilic cysteine residue is responsible for high reductive potential of GPx. It scavenges cytotoxic H_2O_2 and react non-enzymatically with other ROS [17]. Significant increase in MDA observed showed exposure of animal organ to oxidative stress and this may have implications especially in prolonged co-administration.

In the present study, there were no statistically significant changes in the relative weight of the organs harvested (kidney, lung and spleen) except the liver. Although the histological study demonstrated necrosis of renal tubular epithelial cells and obliteration of Bowmans space of the kidney in the ART_5-NVP and ART_{10}-NVP groups respectively. These effects were not observed in the groups administered with the two drugs separately but in the co-administration. Therefore, the need to further investigate the actual mechanism responsible for these observed effects.

Organ weight has been reported to be one of the most sensitive drug toxicity indicators and its changes often precedes morphological changes [18]. The slight increase in relative liver weight in ART_{10}-NVP administered group may be viewed as a toxicity indicator although this was not observed in the histology but our previous study on biochemical analysis showed significant increase in alkaline phosphatase [19] which is an indication of liver toxicity. It should also be noted that the major site of metabolic clearance of foreign compounds is the liver [20] and therefore referred to as an important organ in any toxicological study.

The kidney functions to keep the electrolyte concentration constant in spite of body changes. The metabolized and non-metabolized toxic materials are removed from the body by the kidney. Some of these drugs and metabolites cause injury to the kidney, this probably explain the effect observed in the present study. The function of the kidney is to maintain total body homeostasis by excreting metabolic wastes and regulating intracellular fluid volume, electrolytes and acid base balance; any alteration in the above functions means failure in the integrity of the kidney or kidney tissue which may result in kidney failure.

Histological examination is the golden standard for evaluating treatment related pathological changes in tissue and organs [21]. It is a sensitive and crucial parameter used in determining cellular changes that may possibly occur in any target organs [22]. The histopathological effect on the kidney observed in ART-NVP administered group confirmed those results obtained from our previous study on biochemical parameters [19].

Administration of ART alone in the present study showed congested lung and depletion of lymphocytes at germinal centre of spleen. Reasons for the above results are not clear but may probably be due to the effect of the drug on normal architecture of the lung and spleen. This effect may also be related to the ability of ART to cause destruction of parasite cell membrane and affecting protein synthesis [2] which is the mechanism through which ART brings about its activity. Lungs are reported to be pharmacologically active organ and affect the blood concentration of drugs when given intravenously [23]; this is because the lung can take up, retain, metabolized and delay the release of many drugs which may probably lead to increase in activity or toxicity. ART has been very effective when used either alone or in combination with other antimalarial drugs in the treatment of malaria but systemic toxicity of the drug is also a possibility; therefore, caution needs to be exercised when used therapeutically. It should be noted that previous works had reported toxic effect of ART on the liver when administered alone [5,24,25] but not on the lungs and the spleen. Interestingly, NVP was observed to abolish the histopathological effect caused by ART on the spleen and the lung; the reason for this is yet unknown. Therefore, the need for further study to unravel the mechanism behind the observed effects.

Conclusion. The changes observed in both the kidney tissue and liver relative weight as well as observed altered Cl^-, MDA and GPx levels suggest precautionary measures during ART and NVP treatment combination in HIV and malaria co-morbidity

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