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EFFECT OF CO-ADMINISTRATION OF ARTEMETHER AND NEVIRAPINE ON HEPATO-RENAL FUNCTIONS IN WISTAR RATS

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ABSTRACT

Malarial and Human Immuno-deficiency Virus (HIV) infections occur together in many parts of the world creating the need for co-administration of antimalarial and antiretroviral drugs with potential for drug interactions. This study investigated the effect of co-administration of artemether (ART) and nevirapine (NVP) on liver enzymes and kidney functions in both non-immuno-compromised and immuno-compromised Wistar rats. Animals were divided into six (6) groups of 6 rats each. Groups 4, 5 and 6 received 30 mg/kg NVP daily for 21 days. Groups 1, 2 and 3 received 3%$^{v}/_{v}$ Tween 80 (T_{80}) from days 1-21; and in addition groups 2 and 3 received 5 mg/kg ART (ART_5) and 10 mg/kg ART (ART_{10}) respectively from days 15-21. Groups 5 and 6 also received ART_5 and ART_{10} respectively in addition to NVP from days 15 to 21 and all drugs were administered intraperitoneally. On day 22, animals were sacrificed and sera obtained. Alanine transaminase (ALT), alkaline phosphatase (ALP) and aspartate transaminase (AST) were determined using standard kinetic methods. Total protein, albumin, creatinine and urea levels were determined using enzyme selectra XL machine. In a separate experiment, the above protocol was repeated in rats administered (immuno-compromised) with dexamethasone 20 mg/kg on day 1 followed by booster doses of 10 and 5 mg/kg on days 8 and 15 respectively. Statistically significant increases (p<0.05) in ALP and ALT were observed in NVP alone and NVP-ART_{10} groups in both non-immuno-compromised and immuno-compromised rats respectively. In immuno-compromised rats, significant increase (p<0.05) in ALP was also observed in NVP-ART_{10} group. No changes were observed in total protein, albumin and urea in both groups. However, a significant increase (p<0.05) in creatinine was observed in NVP-ART_{10} administered group in both non-immuno-compromised and immuno-compromised rats. Alterations in ALP, ALT and creatinine observed suggest impairment in normal liver and kidney functions, hence the need for precautionary measures when ART and NVP are co-administered.

Keywords: Artemether, nevirapine, co-administration, kidney function, liver enzymes

INTRODUCTION

Malaria remains a global problem with over 80% cases occurring in Africa and large parts of Asia (WHO, 2015). It is responsible for an estimated 300-500 million acute illnesses and 2 million deaths in both tropical and sub-tropical regions of the world (WHO, 2012). This has necessitated a vigorous treatment with effective antimalarial drugs such as artemisinins. Artemether (ART) belongs to artemisinins; a

well tolerated drug used in combination with other antimalarial drugs in most malaria endemic areas. ART is the first artemisinin derivative incorporated on a wide scale into ACTs; it is effective and still widely used in tropical regions of the world.

HIV is known to be one of the world's most serious health and developmental challenges with affected people mostly residing in low and middle income countries particularly in sub-Saharan Africa (UNAIDS, 2012). Globally, it was reported that there were approximately 36.7 million people living with HIV at the end of 2016 with 1.8 million people becoming newly infected (WHO, 2016); with this figure, there is a need for urgent attention. Treatment of HIV involves the use of combination antiretroviral therapy to attack the virus as well as other drugs to preventing opportunistic infections (like malaria) that may occur due to immune-compromised system. Nevirapine (NVP) is a non-nucleoside reverse transcriptase inhibitor used for prophylaxis and treatment of HIV infections (Bekker *et al*., 2012); in combination with other antiretroviral drugs.

Malaria and HIV infections are highly prevalent most especially in sub-Saharan Africa with HIV-infected patients at high risk of being infected with malaria. This may call for co-administration of antimalarial and antiretroviral therapies. Some antiretroviral and antimalarial drugs are known to be metabolized by the CYP450 enzyme; hence, the chance for drug-drug interaction when co-administered. Toxicological effects of NVP alone on the kidney and liver have been studied and this has been concluded to be the most hepatotoxic compared to lamivudine and stavudine (Sule *et al*., 2012). Malaria and HIV occur together in many parts of the world creating the need for co-administration of antimalarial (ART)) and antiretroviral drugs (NVP) with potential for drug interactions.

It was earlier reported that NVP affects pharmacokinetic profiles of ART (Kredo *et al*., 2011) establishing the need for more clinical investigation. Also there were other reports indicating possible regenerating effect of ART on hepatocellular damage (Oguntibeju *et al*., 2011; Ejiofor *et al*., 2009).

Kidney and liver are very important organs in metabolising and detoxifying any drug, therefore they are important target in any toxicological evaluation. Based on this background, this study seeks to evaluate the toxicological effects of administering ART and NVP separately, and in combination on liver enzymes and kidney functions in both non-immuno-compromised and immuno-compromised Wistar rats.

MATERIALS AND METHODS:

Drugs

Drugs used were ART (80 mg/ml) by RhomePoulene International, France, NVP manufactured by Hetero Drugs Limited, Hyderadab, India and dexamethasone manufactured by JinLing Pharmaceutical, Zhejiang, China. NVP which does not dissolve in water was suspended in 3%$^{v}/_{v}$ T_{80} (3 ml of T_{80} in deionised water) and made up to the required volume with the same vehicle before administration. ART on the other hand being an oily drug was also diluted with 3% $^{v}/_{v}$ T_{80} to the required volume based on the needed concentration and it was freshly prepared just before administration.

Animals

Wistar rats weighing 180-230 g obtained from the Animal House of the Department of Pharmacology and Therapeutics, Ahmadu Bello University, Zaria, Nigeria were used

for this study. They were kept in clean plastic cages floored with wood shavings in a well-ventilated room, placed on standard rodent feed with free access to water. They were grouped according to their weight and sexes. The animals were used according to the guidelines of NIH publication on animal care (NIH, 1985) and approved by Animal Ethical Committee of Ahmadu Bello University, Zaria-Nigeria.

Methods

Animal grouping and Treatment

Rats were divided into 6 of 6 in each group. Group 1 animals received the vehicle, 3% $^{v}/_{v}$ T_{80}, up to day 21. Groups 4, 5 and 6 received 30 mg/kg NVP daily for 21 days. Groups 2 and 3 received T_{80} from days 1-21; and in addition received 5 mg/kg ART (ART_5) and 10 mg/kg ART (ART_{10}) respectively from days 15-21. Groups 5 and 6 also received ART_5 and ART_{10} respectively in addition to NVP from days 15 to 21. All drugs were administered intraperitoneally. In a separate experiment, the above protocol was repeated in rats administered with dexamethasone 20 mg/kg on day 1 followed by booster doses of 10 and 5 mg/kg on days 8 and 15 of the experiment respectively (Anafi *et al*., 2014).

At the end of the treatment period on day 22, animals were sacrificed and sera obtained for biochemical analysis. Alanine transaminase (ALT), alkaline phosphatase (ALP) and aspartate transaminase (AST) were determined using standard kinetic methods. Total protein, albumin, creatinine and urea levels were determined using enzyme selectra XL machine.

Biochemical Analysis

On day 22 of the experiment, animals were sacrificed and blood collected into plain bottles. The blood samples were transferred into test tubes and allowed to stand for a few minutes to clot and then centrifuged at 4000 revolutions per minutes for 5 minutes. The serum was obtained by centrifugation and stored at 4^0C for subsequent analysis of ALT, ALP and AST enzyme levels using commercial kits according to the manufacturer's instructions and as described by Henry (1964). Creatinine, albumin and serum urea nitrogen were determined by the method described by Henry (1974).

Statistical Analysis

The results were expressed as mean ± SEM and subjected to one-way Analysis of variance (ANOVA) followed by Dunnett's post-hoc test to detect if there was any significant difference among and within groups. Differences were considered statistically significant at $p<0.05$ and results presented in figures.

RESULTS

In non-immuno-compromised rats, statistically significant increases ($p<0.05$) in ALP were observed in NVP alone and NVP-ART_{10} groups (Figure 1). In immuno-compromised rats, significant increases ($p<0.05$) in ALP were also observed in NVP-ART_{10} group while ALT was significantly increased in both NVP and NVP-ART_{10} groups (Figure 2). No statistically significant changes were observed in total protein, albumin and urea in both non-immuno-compromised and immune-compromised rats (Figures 3 and 4). A slight elevation of AST was observed in NVP administered rats of non-immuno-compromised group when compared with the control, although not statistically significant. However, a significant increase ($p<0.05$) in creatinine was observed in NVP-ART_{10} administered group in both non-immuno-compromised and immune-compromised rats (Figures 3 and 4).

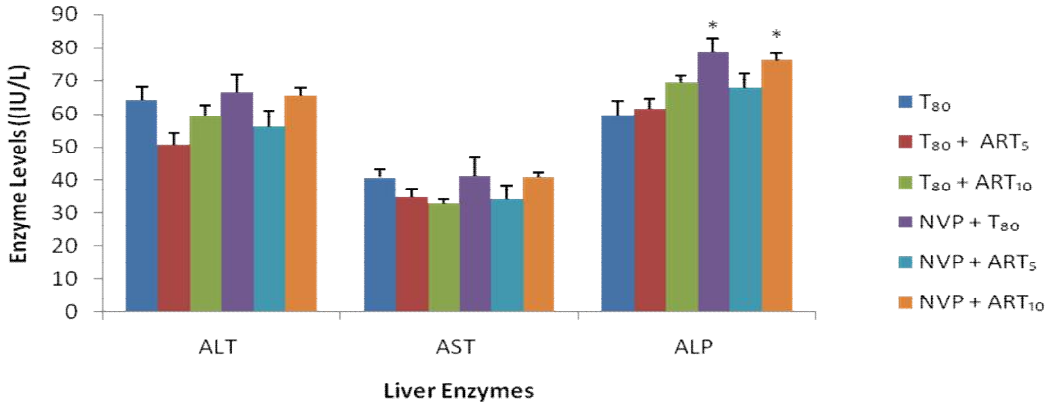

Figure 1: Effects of Artemether-Nevirapine Co-administration on Liver Enzymes in Non-immune-compromised Wistar Rats

ALT = Alanine aminotransferase, AST = Aspartate aminotransferase, ALP = Alkaline phosphatase. T_{80}= 3% v/v Tween 80, ART_5 = 5 mg/kg artemether, ART_{10} = 10 mg/kg artemether, NVP = nevirapine, N = 6 per group, Data are mean ± SEM using, SPSS**.** Statistically significant *p< 0.05, (ANOVA, followed by Dunnett's post hoc test).

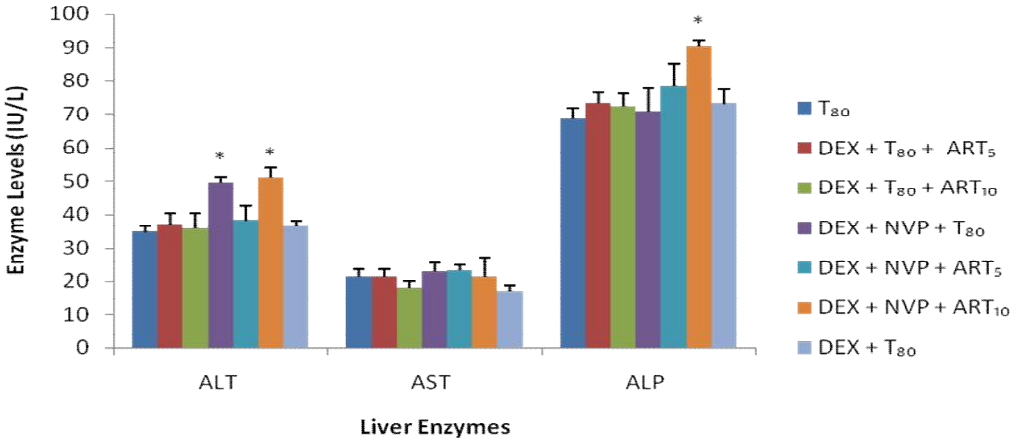

Figure 2: Effects of Artemether-Nevirapine Co-administration on Liver Enzymes in Immuno-compromised Wistar Rats

ALT = Alanine aminotransferase, AST = Aspartate aminotransferase, ALP = Alkaline phosphatase. T_{80}= 3% v/v Tween 80, Dex=dexamethasone, ART_5 = 5 mg/kg artemether, ART_{10} = 10 mg/kg artemether, NVP = nevirapine, N = 6 per group, Data are mean ± SEM using, SPSS**.** Statistically significant *p< 0.05, (ANOVA, followed by Dunnett's post hoc test).

DISCUSSION

This study assessed the effects of ART-NVP co-administration on liver enzyme in non-immune-compromised and immuno-compromised Wistar rats. The significant increase in the levels of ALP observed in NVP and ART_{10}-NVP groups in both non-immune-compromised and immuno-compromised Wistar rats may be an indication of hepatic toxicity. Studies however have reported significant increase in the level of liver enzymes of animals treated with NVP (Umar *et al*., 2008; Adaramoye *et al*., 2012) and other antiretroviral drugs (Kayode *et al*., 2011). This study is in line with that report although different dosage and route of administration were used in this study. The facts that increase in the level of liver enzymes in nevirapine administration have earlier been reported is an indication that administration of ART did not ameliorate the toxic effect of NVP on the liver in the present study.

The liver enzymes monitored are biomarkers for assessing hepatic damage and their activities in the blood; these played a significant role in the investigation and diagnosis of effects of drugs on the liver cells (Dada and Omokhodion, 2007). Marker enzymes and other biochemical indices are important and effective tools in the diagnosis of diseases as well as toxicity that may arise as a result of drug administration.

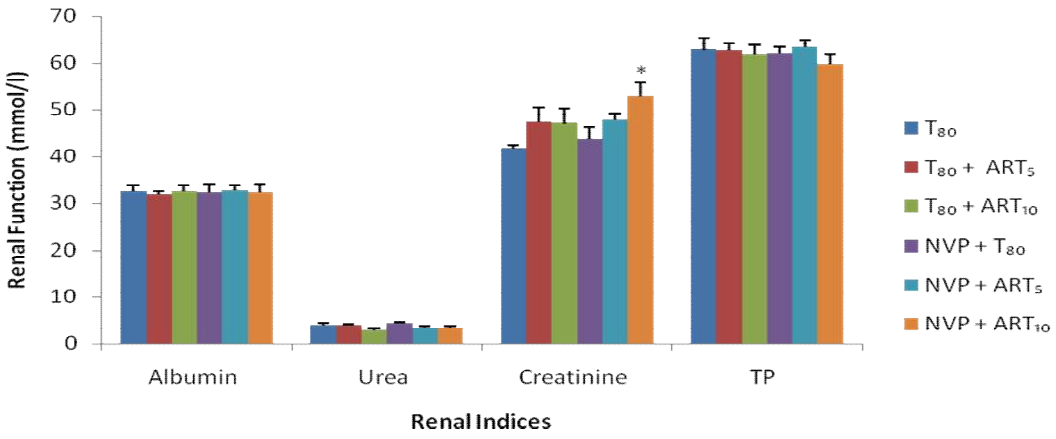

Figure 3: Effects of Artemether-Nevirapine Co-administration on Renal Indices in Non-immune-compromised Wistar Rats

TP = Total protein, T_{80}= 3% v/v Tween 80, ART_5 = 5 mg/kg artemether, ART_{10} = 10 mg/kg artemether, NVP = nevirapine, N = 6 per group, Data are mean ± SEM using, SPSS**.** Statistically significant *$p < 0.05$, (ANOVA, followed by Dunnett's post hoc test).

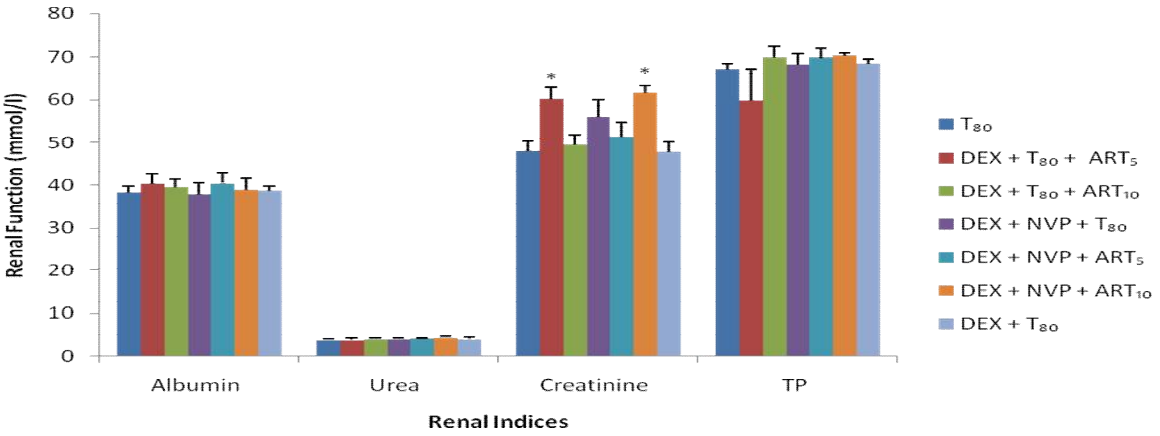

Figure 4: Effects of Artemether-Nevirapine Co-administration on Renal Indices in Immuno-compromised Wistar Rats

TP = Total protein, T_{80}= 3% v/v Tween 80, DEX=dexamethasone, ART_5 = 5 mg/kg artemether, ART_{10} = 10 mg/kg artemether, NVP = nevirapine, N = 6 per group, Data are mean ± SEM using, SPSS. Statistically significant *p< 0.05, (ANOVA, followed by Dunnett's post hoc test).

A group of rats co-administered with ART-NVP showed a slight elevation of ALT and AST compared to the control. The elevation of these enzymes in the plasma might be as a result of release of the enzymes from some tissues indicating tissue damage due to drug administration. ALT and AST elevation has been reported in several studies as a condition showing necrosis of hepatocytes (Macfarlane *et al*., 2000; Sulkowski, 2004; Onyesom, 2012). Sule *et al*., (2012) had also reported a significant increase in the liver enzymes in NVP treated rats; the study also showed that NVP was the most hepatotoxic of the antiretroviral drugs studied. This was also supported by an earlier report that severe hepatic reactions to HAART was attributed to NVP component of the drug combination (Johnson and Baraboutis, 2000; Martinez *et al*., 2001). Kayode *et al*. (2011) also reported that all ARV medications cause elevation in liver enzymes. Evidence from this investigations showed that NVP is somehow toxic to the liver. However, the results of the effect of co-administration of ART and NVP investigated in the present study showed mild hepatic damage which when accumulate over a long period of time may cause more harm to the liver. This observed effect may be due to the presence of NVP which have been reported earlier to be toxic to the liver. In addition, the results also showed that ART did not reverse the observed effect on the liver by NVP. Effects of co-administration of ART and NVP on albumin, urea and total protein in normal and immuno-compromised rats studied showed no significant difference between the control and the drug treated groups. However, significant elevation of creatinine level observed calls for caution since creatinine is one of the biochemical markers implicated in renal tissue damage. Akomolafe *et al*.,2012 had also reported significant increase in creatinine in rats administered with doses of ART in *Plasmodium berghei* infected rats. Although, the present study did not observe this effect in ART alone administered group in non-immunne-compromised rats but in

combination with NVP as well as in immune-compromised ART_5 group. The changes observed in the present study may be due to the presence of ART as reported in other study (Akomolafe *et al*., 2012).

Blood Urea Nitrogen (BUN) and creatinine are sensitive biochemical indices for the evaluation of renal function (Ogawa, 1992), therefore increase in their levels are implicated in kidney diseases. The fact that the result of the present study showed a significant elevation of creatinine with no effect on BUN may be a threat to renal function. Although a slight variation in albumin and total protein levels were also observed between the control and ART-NVP treated groups which may also be an indication of mild impairment of liver function.

Plasma urea, creatinine and electrolytes are the most sensitive biochemical markers used in the assessment of renal tissue damage; this is because urea and creatinine are excreted through the kidneys, while the electrolytes are reabsorbed in the tubules (Akomolafe *et al*., 2012). Increased levels of urea and creatinine have been implicated in kidney diseases, such as acute glomerulonephritis, nephrosclerosis and tubular necrosis (Pari and Murugan, 2006). In this study, there was no clear evidence of kidney damage; however, there is a need for appropriate protective measures when ART and NVP are co-administered.

Co-administration of ART and NVP may not be absolutely safe; alterations in ALP, ALT and creatinine observed suggest impairment in normal liver and kidney functions, hence the need for precautionary measures when ART and NVP are to be used concurrently.

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