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EVALUATING OMEGA-3 AND SELENIUM IN MITIGATING HAART-INDUCED BLOOD TOXICITY IN WISTAR RATS

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Abstract: Highly active antiretroviral therapy (HAART) or combination antiretroviral therapy is a treatment regimen typically comprising of combination of three or more drugs from two or more classes of antiretroviral drugs. HAART-induced haemotoxicity is well reported with oxidative stress partly blamed for the insult on tissues. There is paucity of information on the possible ameliorating effects of common antioxidants (Omega 3 and Selenium) on HAART-induced haemotoxicity in wistar rats and hence this study. Sixteen male Wistar rats weighing 120 – 250g used for the study were divided into four groups of 4 rats each viz group 1 (control), group 2 (HAART-only), group 3 (HAART + Omega 3) and group 4 (HAART + Selenium). Duration of daily administration and treatment was six weeks. Results showed a significantly decreased ($p<0.05$) red blood cell count in the HAART-only group compared with control but which was significantly increased in the HAART + Omega 3 and HAART+ Selenium groups compared with HAART-only group ($p<0.05$ in each case). Hemoglobin concentration was significantly reduced ($p<0.05$) in the HAART-only group compared with control but significantly increased in HAART + Omega 3 and HAART + Selenium groups compared with HAART-only group ($p<0.05$). Packed cell volume was significantly reduced in the HAART-only group compared with control but which was significantly higher in HAART + Omega 3 and HAART + Selenium groups compared with HAART-only group ($p<0.05$). No significant difference was observed in mean corpuscular volume among the groups. The mean corpuscular hemoglobin was significantly reduced in the HAART-only group compared with the control ($p<0.05$). Mean corpuscular hemoglobin concentration was significantly decreased ($P<0.05$) in the HAART-only group compared with control but increased in the HAART + Omega 3 and HAART + Selenium compared with HAART only groups ($p<0.05$ in each case). We therefore conclude that co-administration of Omega 3 or Selenium ameliorates HAART-induced haemotoxicity in Wistar rats.

Keywords: HAART, Selenium, Omega 3, ameliorate, haemotoxicity.

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INTRODUCTION

Highly active antiretroviral therapy (HAART) is a treatment regimen typically comprising of a combination of three or more drugs from two or more classes of anti-retroviral drugs.[1] The National Institute on Drug Abuse, NIDA describes HAART as a customized combination of different classes of medications prescribed by a physician based on such factors as the patient's viral load, strain of the virus, CD4 cell count, and other considerations like disease symptoms and which must be taken every day for life.

The introduction and wide spread use of HAART have led to marked reduction in AIDS-related morbidities and mortalities due to diversity of regimen and its interference at different sites in the life cycle of the virus. This effect is also attributed to the extended durability of viral suppression.[3]Advent of HAART has also brought dramatic improvement in overall prognosis with appreciable impact on the management of HIV infection, reduction in viral replication as well as survival and quality of life. [4] Moreso, introduction of HAART has demonstrated remarkable success in reducing health care cost for HIV-positive individuals and incidences of opportunistic infections,

Like most drug combination regimens, HAART has been associated with several drugs-related problems. This is easily understood because the various classes of antiretroviral drugs, Nucleoside/tide reverse transcriptase inhibitors. (NRTI/NERTI), no nucleoside/tide reverse transcriptase inhibitors, (NNRTI/NNtRTI), protease inhibitors (PI), integrase strand inhibitors (INSTIs), Fusion inhibitors (FIs) and chemokine receptor antagonists (CCR5- Antagonists) all have their side effects.[6,7]So, despite the numerous benefits of HAART regimen, studies have linked a number of side effects and toxicities to its administration including hepatotoxicity, [8] nephropathy, myopathy etc.[9] HAART-associated toxicities including hematological toxicities have evolved as main reasons to discontinue or modify antiretroviral therapy since rational alternatives are not readily available. [5,10,8]

The haemotoxicity of HAART is well documented.

Macrocytosis effect of lamivudine has been reported on the neutropenic and anaemia effects of HAART[11 5,] HAART administration has also been associated with low concentrations of haemoglobin, packed cell volume and lymphocytes[14] as well as red cell aplasia. The administration of HAART has been linked to a systemic expression of oxidative stress biomarkers like GPx SOD, CAT, and MDA to explain the possible mechanisms of tissue injury.

It was based on the above that known and readily available antioxidants (Selenium and Omega 3) were chosen to test their ability to prevent or reduce haemotoxicity in male rats treated with HAART.

MATERIALS AND METHODS

Experimental design

Sixteen male Wistar rats were separated into four (4) groups of four rats each. Group 1 was control, group 2 HAART-only, group 3, HAART + Omega 3 and group 4, HAART + Selenium. Group 1 received 0.003ml/g body weight of Tween 80/water solution. Group 2 (HAART-only) was given 25mg/kg 3TC, 25mg/kg – TDF and 3.8mg/kg – DTG (0.00167ml of HAART solution per gram rat). Group 3 (HAART + Omega 3) was administered with 600mg/kg of 0mega 3 plus 25mg/kg 3TC, 25mg/kg – TDF and 3.8mg/kg – DTG). Group 4 (HAART + Selenium) was given 25mg/kg 3TC, 25mg/kg – TDF and 3.8mg/kg – DTG plus 0.3mg/kg of Selenium. All rats had unrestricted access to water and rat chow for the six (6) weeks duration of experiment. All animals were

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given their respective solutions/drugs by oral gavaging once daily for the duration of experimentation. Rats were weighed regularly and the doses of their drugs adjustedaccordingly.

Experimental animals: Sixteen male adult Wistar rats weighing 120 – 250g used for the study were kept in wooden cages and housed in the Animal House of the Department of Human Physiology, University of Calabar, Calabar. They were raised under a 12 hours light – 12 hours darkness cycle at room temperature and used for the study after one week of acclimatization.

Drugs: A combined ART drug/HAART (Mylan Laboratories, Ltd, India) composed of 300mg Lamivudine (3TC), 300mg TenovirDisoproxil Fumarate (TDF) and 50mg Dolutegravir (DTG) per tablet was obtained from Antiretroviral Unit of Infections Disease Hospital (IDH) Calabar, Nigeria.

Emzor Omega 3® fish oil (1000mg/capsule) containing Eicosapentaenoic acid NLT (18%) and Docoxahexaenoic acid NLT (12%) made by Gujarat LiquiPharmacaps Pvt, Ltd, GLPL, India and Selenium as Sodium Selenite (200µg/capsule) made by Bactolac Pharmaceutical Inc. USA were obtained from Bez Pharmacy, Etta Agbor Road, Calabar, Nigeria.

Drugs preparation and dosage

Extrapolation of HAART dose: The HAART doses for rats were extrapolated from human doses for HAART using formula developed by [21]

$$= \text{Animal NOAEL (mg/kg)} \times \frac{\text{weight}_{\text{animal}}\ [\text{kg}]}{\text{Weight}_{\text{human}}\ [\text{kg}]}^{1-0.67}$$

$$= \text{Animal dose (mg/kg)} \times \frac{\text{Animal } K_m}{\text{Human } K_m}$$

Animal equivalent dose mg/kg = Human dose (mg/kg) x K_m ratio

Where (K_m) = correction factor estimated by dividing the average body weight (kg) of species to its body surface area (m^2)].

Tween-80/water solution: Tween 80® (Laborfine Chem. PVT Ltd, India) was used as solvent in the study. Ten (10) drops of Tween-80 were placed in a graduated sample bottle and then made up to 40mls using portable water and administered at a dose of 0.003ml/g of rat.

Stock solution of HAART: Each tablet of HAART (50mg Dolutegravir, 300mg Lamivudine and 300mg Tenovirdisoproxil Fumarate) was grind to powder, dissolved in four (4) drops of Tween 80 and then made up to 20mls with water to give a concentration of 2.5mg, 15mg and 15mg of Dilutegravir, Lamivudine and Tenovir respectively per ml of solution of HAART.

Stock solution of Omega 3: Content of six capsules of Omega 3 (6000mg) was dissolved in 4 drops of Tween 80® and marked up to 30ml with water giving a concentration of 200mg/ml solution.

Stock solution of Selenium: This was prepared by dissolving 2000µg (content of ten capsules) in 4 drops of Tween 80® and marked up to 40mls with water giving a concentration of 50µg/ml of solution

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Collection of samples: At the end of six (6) weeks of experimentation, animals were anaesthesized with chloroform and blood samples taken from them via intracardiac puncture. Samples were then immediately transferred into pre-labelled EDTA bottles for determinate of haematological parameters.

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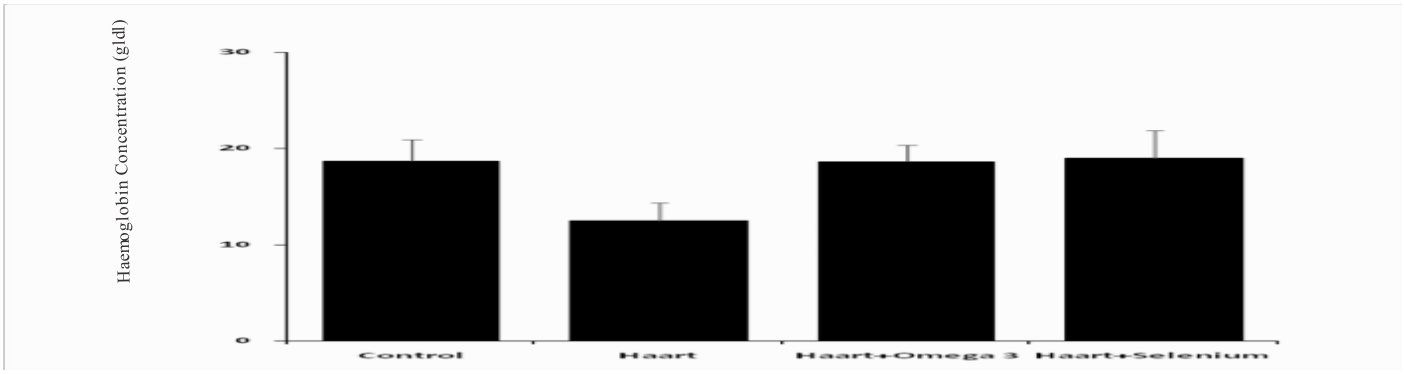

Evaluation of haematological parameters: Haematological parameters were evaluated using Auto-Haem Analyzer (Counlter Electronics, Luton, Bedfordshire, UK) having standard calibrations according to manufacturer's instruction.[22]

Statistical analysis: Data were presented as mean ± standard error of mean (SEM). Data were analysed using analysis of variance (ANOVA), followed with a post hoc test of least significant differences. A value of $p<0.05$ was considered statistically significant. All analyses were conducted using Statistical Package for the Social Sciences (SPSS) version 25.

Ethical approval: Approval for this study was obtained from the Animal Research and Ethics Committee of the Faculty of Basic Medical Sciences, University of Calabar, Calabar – Nigeria (Approval No. 108 PHY 3821).

RESULTS

Red blood cell count ($x10^6$/L): Red blood cell count (RBC) in the HAART-only group (2.94±0.95) was significantly lower compared with control (6.05±1.71) with $p<0.01$. It was however significantly increased ($p<0.05$) in the HAART + Omega 3 (7.08±1.07) and HAART + Selenium (7.86) groups compared with HAART-only group
(2.94±0.95) as shown in Fig. 1.

Hemoglobin concentration (g / d l):
Hemoglobin concentration was significantly reduced ($p<0.05$) in HAART-only (12.1±1.90) compared with control (18.1±1.16) groups. It was however significantly higher ($p<0.05$) in the HAART + Omega 3 (18.73±1.97) and HAART + Selenium (18.85±2.21) compared with HAART-only (12.1±1.90) groups as presented in Fig. 2.

Packed cell volume (%): Comparison of packed cell volume (PCV) in different groups shows a significant decrease ($p<0.05$) in PCV in HAART-only group (31.032 ± 4.26) compared with control (49.91±3.08). It was however significantly higher ($p<0.05$) in HAART + Omega 3 (48.85±5.00) and HAART + Selenium (55.89±4.85) compared with HAART-only (31.32±4.26) groups. This is shown in Fig. 3.

Mean corpuscular volume (L): Mean corpuscular volumes (MCV) in control (71.25±13.74), HAARTonly (75.25±10.56), HAART + Omega 3
(68.75±6.95) and HAART + Selenium (72.25±12.01) groups were statistically insignificant when compared with themselves as shown in Fig. 4.

Mean Corpuscular hemoglobin (pg): Mean corpuscular hemoglobin (MCH) in HAART-only (19.83±2.77) was significantly lower ($p<0.05$) compared with control (24.92±4.09) groups as in Fig. 5.

Meancorpuscular hemoglobin concentration (g/dl): Meancorpuscularhaemoglobinconcentration (MCHC) in the HAART-only group (22.98±4.02) was significantly reduced ($p<0.05$) compared with control

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(39.2±4.53). This was however significantly increased ($p<0.05$) in HAART + Omega 3 (35.75±3.30) and HAART + Selenium (34.05±3.70) groups compared with the control group as in Fig. 6.

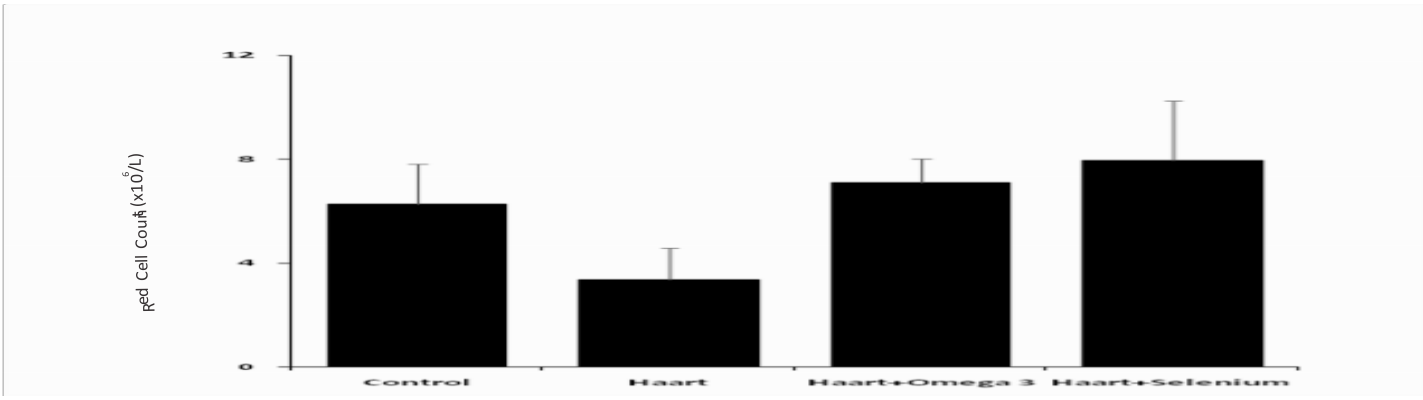


Fig. 1: RBC count in the different experimental groups. Values are expressed as mean ± SD, n = 4.
a = $p < 0.05$ vs Control b = $p < 0.05$ vs Hart.

Experimental groups Fig. 2: Hemoglobin (Hb) concentration in the different experimental groups. Values are expressed as mean ± SD, n = 4.
a = $p < 0.05$ vs Control b = $p < 0.05$ vs Hart.

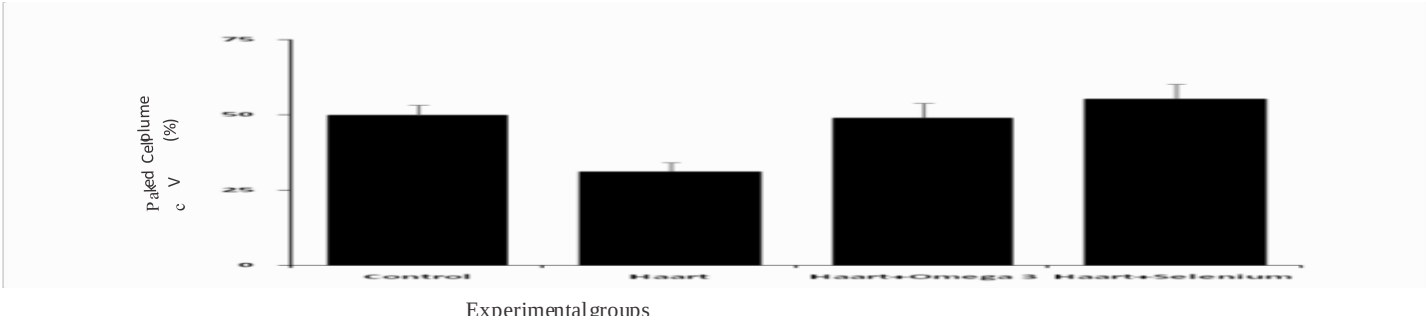
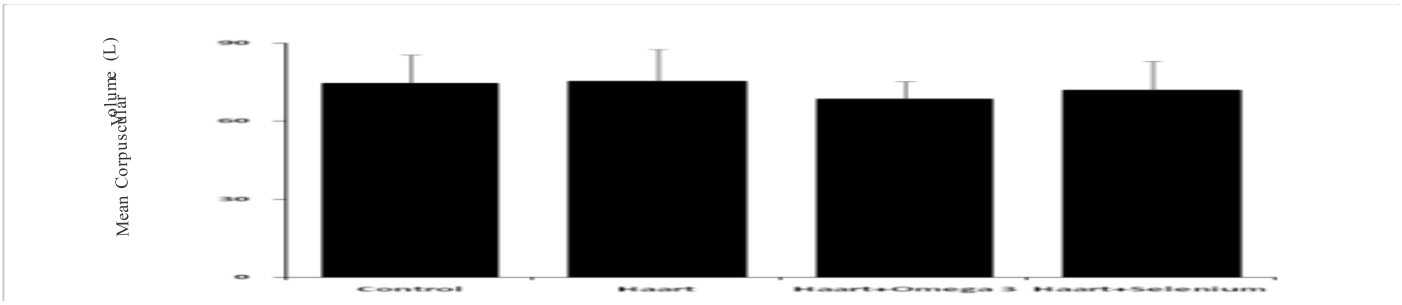


Fig. 3: Packed cell volume in the different experimental groups.
Values are expressed as mean ± SD, n = 4.
a = $p < 0.05$ vs Control b = $p < 0.05$ vs Haart.



Experimental groups Fig. 4: Mean corpuscular volume in the different experimental groups. Values are expressed as mean ± SD, n = 4 No significant difference among groups.

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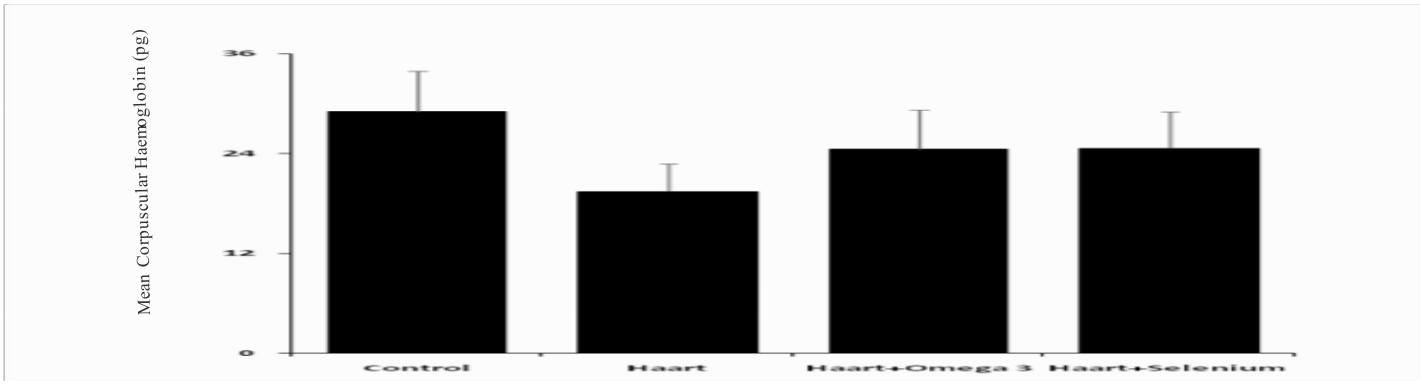

Fig. 5: Mean corpuscular hemoglobin in the different experimental groups. Values are expressed as mean ± SD, n = 4. a = p < 0.05 vs Control.

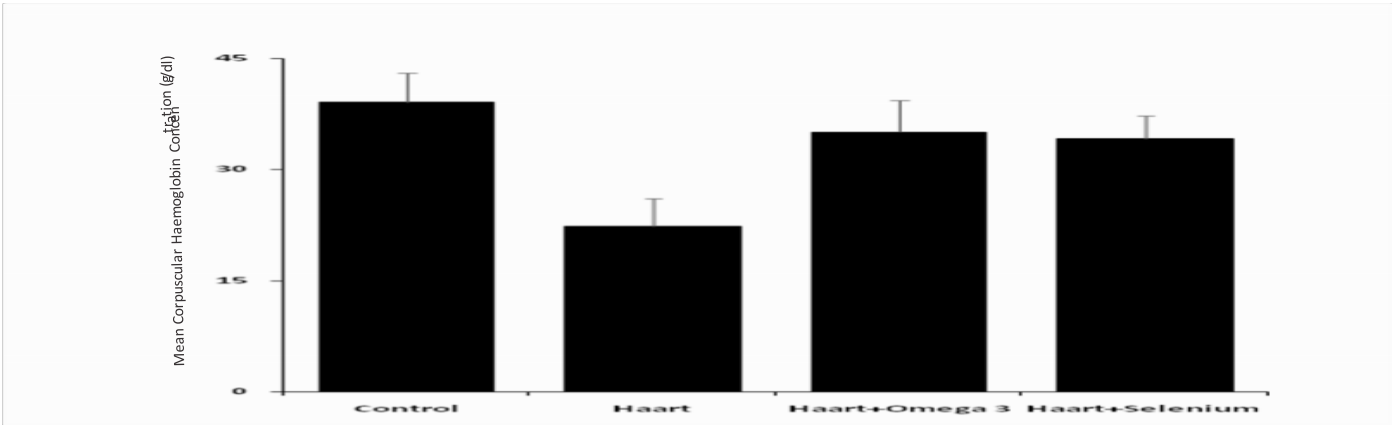

Fig. 6: Mean corpuscular hemoglobin concentration in the different experimental groups. Values are expressed as mean ± SD, n = 4.

a = p < 0.05 vs Control b = p < 0.05 vs Haart.

DISCUSSION

This study shows that administration of HAART significantly reduced red blood cell count, packed cell volume, hemoglobin, mean corpuscular hemoglobin and mean cell hemoglobin concentration. However, co-administration of Omega 3 or Selenium ameliorated the changes in Hb, PCV and RBC account.

Start your discussion with the key findings from this work before any other comments

Introduction of HAART regimen into the treatment of

HIV/AIDS was seen as a major breakthrough in management of HIV with improved clinical conditions and better prognosis. However, this achievement has been known to have its draw-backs including haemotoxicity from the cock-tail, a situation which may compound HIV-related hematological alterations in infected people. Oxidative stress is one of the mechanisms of tissue injury by HAART.[20 ,17, 18]

The significantly reduced RBC count in the HAART-only group compared with control could have been due to HAART-induced haemotoxicity also observed by Kayodeet al .[14] The haemotoxicity might have been primary as was observed by who noted that HAART/Lamivudine causes red cell aplasia. The decrease in RBC count was prevented following co-administration of Omega 3 or Selenium with HAART likely due to their Antioxidant effects.23 ,24,25, 26 The observed decrease in PCV in the HAART-only group compared with control is similar

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to the findings from a previous study in[14] as packed cell volume is directly related to red blood cell count. In pure red cell aplasia, there is interference with erythropoietin mechanisms.[16,27] This might have been due to the RBC aplasia induced by HAART/Lamivudine.[15,16] The reduction in PCV was however prevented/reversed by administration of Omega 3 or Selenium, a situation which may also be attributed to the antioxidant effects of Omega 3 and Selenium.

The significant decrease in Hb in HAART-only group compared with control noted in our study is similar to the observation by.[14-16] This might have been due to interference with the mechanisms for hemoglobin synthesis by HAART. This was prevented following co-administration of HAART with Omega 3 or Selenium resulting in a rather increase in Hb concentration in HAART + Omega 3 and HAART + Selenium groups compared with HAART-only group.

Our results did not show any significant changes in MCV among the groups. Mean corpuscular volume (MCV) refers to the average volume of single RBC.[28]The findings of low RBC count, PCV and Hb concentration in the presence of a normal MCV, points to the fact that anemia induced by HAART is normocytic in nature.[27]

Mean corpuscular hemoglobin (MCH) is the quantity of Hb present in one RBC [27] and is low in hypochromic anemias. The findings of decreased MCH in the HAART-only group compared with control shows that HAART causes a hypochromic type of anemia.

Our study revealed a significant decrease in mean corpuscular hemoglobin concentration (MCHC) in HAART-only compared with control groups. Mean corpuscular hemoglobin concentration is the concentration of Hb in one RBC and is the most important absolute value in diagnosis of anaemia. The reduced value of this index compared with control confirmed that anaemia of HAART origin is hypochromic in nature .[29] From our results, this index was significantly increased in HAART + Omega 3 and HAART + Selenium compared with HAART-only groups showing that the two antioxidants could prevent HAART-induced hypochromic in red blood cells.

CONCLUSION

From the foregoing, we conclude that administration of Omega 3 or Selenium with HAART prevents HAART-induced derangement in RBC count, PCV, Hb, MCH and MCHC in rats.

RECOMMENDATION:

From findings in this study, we recommend the administration of Omega 3 or Selenium with HAART as prophylaxis against hem toxicity induced by HAART.

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