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# ACUTE AND SUB-ACUTE TOXICITY EVALUATION OF DISULPHIDE DERIVATIVE OF DIHYDROARTEMISININ

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## ABSTRACT

Dihydroartemisinin (DHA), the active metabolite of artemisinins, possesses anticancer activity but its usefulness may be limited by its short half-life. Recently, a persulphide (or disulphide) derivative of DHA (sDHA) has been synthesized with the aim of extending its half-life. This study evaluated the acute and sub-acute toxicity profile of sDHA in comparison with the parent drug, DHA in Sprague-Dawley rats. $LD_{50}$ of DHA and sDHA were determined using Lorke's method. Thereafter, 0, 10, 20 and 30% of their $LD_{50}$ were administered i.p., once daily for 28 days to different groups of rats (n=6 per group). Body weights, as well as hepatic, renal and hematological indices were evaluated. $LD_{50}$ values obtained for sDHA and DHA were 374.17 and 574.46 mg/kg i.p., respectively. Body weights were unaffected, serum levels of AST, ALT and ALP were elevated ($p<0.001$), but urea and creatinine were unaltered by sDHA and DHA. Additionally, both drug treatments resulted in alterations in liver-to-body weight ratio and liver microstructure. Kidney-to-body weight ratio was not affected by both drugs, but they caused mild glomerular inflammation and vascular degeneration. Furthermore, they reduced RBC, Hb, PCV, and platelet levels ($p<0.001$). The drugs also caused elevations in total and differential WBC counts, except basophil. The effects on all the parameters by both drugs were comparable, except on RBC, wherein sDHA induced a higher level of reduction ($p<0.05$). Putting the results together, both drugs have

comparable toxicity profiles, although, sDHA reduces erythrocytes more and has a lower $LD_{50}$.

**KEYWORDS:** Dihydroartemisinin, endodisulphide, endoperoxide, Lorke's method

**INTRODUCTION**

Cancers are among the leading causes of morbidity and mortality worldwide, with approximately 14 million new cases and over 8.2 million cancer related deaths annually.[1] Whereas, some tumors are benign and may be harmless, solid malignancies require some form of treatment, which includes surgery, radiotherapy, chemotherapy or use of targeted agents, but chemotherapy remains the first-line treatment.[2,3] In some situations, combination of treatment options are considered to improve antitumor efficacy as the efficacies of monotherapies may not be satisfactory.[2,4-6] Chemotherapy employs cytotoxic drugs which are mostly designed to affect dividing cells, making proliferating normal tissues like the bone marrow to be at risk of adverse effects.[7] Improvement of quality of life and survival of cancer patients would therefore depend greatly on development of highly effective drugs that selectively kill malignant cells. Many chemotherapeutic agents show good responses and survival, however, their adverse effects, high cost and high frequency of administration often lead to discontinuation, dose reduction, and likely emergence of drug resistance. Thus, the availability of highly effective and affordable anticancer agents with tolerable adverse effects is key in cancer chemotherapy.

Dihydroartemisinin (DHA), a potent antimalarial drug, has been shown to possess significant anticancer activity against a variety of human cancer models including, pancreatic, leukemic, osteosarcoma, colorectal ovarian, and lung cancer cells.[8-10] DHA is a derivative of artemisinin (the active component of the leaves of *Artemisia annua*), and also the active metabolite of all artemisinin compounds. The pharmacophore ring system of the sesquiterpene lactol of DHA contains an endoperoxide moiety which is common to all artemisinins. The drug is chemically unstable, displays a marked propensity to undergo ring opening of the lactol and rearrangement under neutral conditions. This results in the formation of a new biologically active peroxide, which in turn rapidly decays to the inert end product, deoxyartemisinin.[11] As a result of this instability, DHA and other artemisinins have very short elimination half-lives, which is attributed to poor cure rates and high rates of recrudescence of the drugs. Consequently, more stable artemisinin compounds with longer half-lives are more desirable in chemotherapy of cancer and microbial infections. This

concern has resulted in the structural modification and synthesis of the sesquiterpene lactol endodisulphide derivative of DHA (Fig. 1) through substitution of the peroxide oxygen atoms in the pharmacophore ring system of its sesquiterpene lactol with persulphide (or disulphide) group.[12] This new artemisinin drug is believed to have a longer half-life, however, it is not yet known whether it would maintain or possess a higher anticancer activity in comparison with its parent drug. It is also not known whether this new drug would have tolerable toxicological profile as the parent drug.

The objective of this study was to determine the acute and sub-acute toxicity profiles of DHA and the disulphide derivative in rats.

## MATERIALS AND METHODS

### Drugs, chemicals and reagents

Pure powder of dihydroartemisinin and its disulphide derivative were obtained from the Department of Medicinal and Pharmaceutical Chemistry, Faculty of Pharmacy, University of Uyo, Nigeria. Tamoxifen citrate (Cytotam®) tablets (Cipla Pharmaceuticals Ltd. Verna, Goa 403710, India), and cyclophosphamide (Endoxan®) tablets (Zyus Biogen- Baxter, Mumbai, India), were purchased from the pharmacy unit of the University of Uyo Teaching Hospital, Uyo, Nigeria. Liver function assay kit (Sigma-Aldrich Inc., St. Louis, MO 63178, USA), kidney function assay kit (Sigma-Aldrich Inc., St. Louis, MO 63178, USA), hematological profile assay reagents (Norma Diagnostika GmbH, Untertullnerbach, Austria), and Polysorbate, Tween® 80 (Sigma-Aldrich and Merck, KGaA, Darmstadt, Germany) were also purchased.

### Animals

Adult male Sprague-Dawley rats (weighing 180-200 g) were purchased fromthe Animal House, College of Medicine, University of Lagos, Lagos, Nigeria. The animals were housed three per cage, and maintained under standard conditions. They were fed with rodent chow, given free access to clean water, and kept under 12 h light-dark cycle at room temperature (25±2°C). Animals were handled with care and all experiments were done in accordance with the approval guidelines of the Ethics Committee On Animal Experiments of the University of Port Harcourt, Nigeria.

**Methods**

**Acute toxicity study ($LD_{50}$ determination)**

The median lethal doses ($LD_{50}$) of dihydroartemisinin (DHA) and its disulphide derivative (sDHA) were determined with rats, using the Lorke's method.[13] Briefly, twenty-six Sprague-Dawley rats were fasted overnight prior to the experiment and divided into two groups, containing eighteen and eight, respectively, for phases 1 and 2 of the study.

Phase 1: The rats were divided into 6 groups (each containing three rats) and administered single doses of sDHA (250, 500 or 1000 mg/kg, i.p) and DHA (250, 500 or 1000 mg/kg, i.p.). The mice were observed closely for mortality, behavioral changes and other symptoms of toxicity over a period of 24 h.

Phase 2: The rats were divided into four groups (each containing one rat) and administered single doses of sDHA (300, 350, 400 or 450 mg/kg, i.p.) and DHA (550, 600, 650 mg or 750 mg/kg, i.p.), and observed over 24 h for mortality and other signs of toxicity. The doses used in phase 2 were based on the results that were obtained in phase 1 (doses between the highest nonlethal and least lethal doses were selected).

**Sub-acute toxicity study**

Forty-two Sprague-Dawley rats were divided into seven groups (n=6 per group), and administered sDHA (37.42, 74.83 and 112.25 mg/kg, i.p.) and DHA (57.45, 114.89 and 172.34 mg/kg, i.p.), and vehicle, 30 % Tween 80 (0.2 mL) once daily for 28 days. The doses of sDHA and DHA used correspond, respectively, to 10, 20 and 30 % of their $LD_{50}$. On the next day after drug treatment, the animals were anaesthetized deeply with diethylether and sacrificed by cervical dislocation. The thoracic region was opened to expose the heart, and blood was collected by cardiac puncture into anticoagulant containing sample bottles and hematological analysis was performed. Another aliquot of blood was transferred into plain centrifuge tubes, allowed to coagulate for 30 min at room temperature and then centrifuged at 3000 rpm. The supernatant serum was collected and used for estimation of biochemical parameters- aspartate transaminase (AST), alanine transaminase (ALT), alkaline phosphatase (ALP), urea, and creatinine. Body weights of the animals were also monitored before and after the experiment. Liver and kidney of animals were removed, cleared of adhering tissues, weighed and the relative organ weight (organ-to-body weight ratio) for each animal was obtained. The liver and kidney tissues were then preserved immediately in buffered (10 %) formaldehyde and processed for histopathological studies.

**Hematological parameters analysis**

White blood cell (WBC), red blood cell (RBC), platelet, and WBC differential (neutrophil, lymphocyte, monocyte and eosinophil, and basophil) counts were determined using a Hematology Auto-Analyzer (Sysmex 1-5-1 Wakinohama-Kaigandori Chuo-ku, Kobe 651-0073, Japan). The levels of hemoglobin, packed cell volume, and mean corpuscular hemoglobin, and mean corpuscular hemoglobin concentration were equally measured.

**Biochemical parameters analysis**

Serum levels of aspartate transaminase (AST), alanine transaminase (ALT), alkaline phosphatase (ALP), urea, and creatinine were measured using an automated Clinical Chemistry Analyzer (Sylectra Pro S, Model: 13-9693, Netherland).

**Histopathological examination**

Tissues were processed for histological studies using the haematoxylin and eosin (H&E) staining method, as described by Drury and Wallington.[14] Briefly, fixed liver and kidney tissues were dehydrated using graded concentrations of ethanol and defatted (or cleared) in xylene. Once cleared, tissues were infiltrated (impregnated) with soft paraffin wax at 58°C in three successions at an hour interval. Thereafter, the tissues were embedded in molten paraffin wax and then blocked out. Tissue blocks were sectioned (5-7 µm thickness) with a rotary microtome, fixed on clean albuminized slides, and stained with haematoxylin and eosin (H&E). The slides were dehydrated with alcohol and cleared in xylene again before they were mounted and observed under light microscope (Leica CMX, Germany). All alterations from the control slides were noted and photographed using 400x objective.

**Statistical analysis**

The results are presented as mean±SEM for each group. Differences among groups were analyzed using one-way analysis of variance (ANOVA) followed by Dunnette's test for pair wise comparisons. Data were analyzed using GraphPad Prism 5 software and values were considered significant at $p < 0.05$.

## RESULTS

**Acute toxicity study ($LD_{50}$ determination)**

Intraperitoneal administration of disulphide derivative of dihydroartemisinin (sDHA) did not cause mortality at 250 mg/kg, but all animals that were injected 500 and 1000 mg/kg were killed in phase 1 (Table 1). In the phase 2, mortality occurred only in rats that were injected ≥

400 mg/kg sDHA (Table 1). Further, intraperitoneal administration of dihydroartemisinin (DHA) did not cause mortality or serious clinical signs of toxicity in rats at 250 and 500 mg/kg but mortality occurred in group that was administered 1000 mg/kg in phase 1 (Table 2). In addition, there was mortality in all DHA treated groups in phase 2, except the 550 mg/kg treated group (Table 2). There was lethargy in the treated rats compared to control, and animals exhibited twitching before death.

The median lethal dose ($LD_{50}$) of both drugs was calculated using Eq. 1 below:

$LD_{50} = \sqrt{(D_0 \times D_{100})}$ Eq. 1 Lorke[13]

[$D_0$ representing, highest dose that did not cause mortality; and $D_{100}$, lowest dose that caused mortality],

$LD_{50}$ of DHA $= \sqrt{(550 \times 600)\ mg/kg}$

$= 574.46$ mg/kg i.p. in rats

$LD_{50}$ of sDHA $= \sqrt{(350 \times 400)\ mg/kg}$

$= 374.17$ mg/kg i.p. in rats

**Sub-acute toxicity study**

**Body weights monitoring**

Body weights were increased over time in control, sDHA and DHA treated rats compared to their initial (Day 1) body weights (Table 3).

**Biochemical parameters**

Serum levels of alanine transaminase (ALT), aspartate transaminase (AST), and alkaline phosphatase (ALP) enzymes in sDHA and DHA treated rats were significantly ($p < 0.001$) increased in comparison with control rats (Table 4). When compared, no significant difference existed between the values of sDHA and DHA in all the enzymes. In addition, there were no changes in the serum levels of urea and creatinine of sDHA and DHA treated rats when compared with values obtained in control rats (Table 4).

**Hematological parameters**

White blood cell (WBC) counts in all sDHA and DHA treated rats were significantly increased ($p < 0.001$) compared to the control (Table 5). On the contrary, red blood cell

(RBC) and platelet counts in sDHA and DHA treated rats were significantly decreased ($p < 0.001$) compared to control (Table 5). When sDHA values were compared with those of DHA, only the RBC values were significantly different ($p < 0.05$), the values of the former being lower (Table 5).

Hemoglobin (Hb) and packed cell volume (PCV) were significantly reduced in sDHA and DHA -treated rats compared to control, but the sDHA and DHA values were not significantly ($p > 0.05$) different from each other (Table 6). Mean corpuscular hemoglobin (MCH) and mean corpuscular hemoglobin concentration (MCHC) values in all treated rats were also significantly decreased when compared with the control values (Table 6). In addition, the values of sDHA and DHA were not significantly different from each other at all dose levels for MCH and MCHC (Table 6).

Neutrophil count was significantly increased in all the treatment groups compared to control group. Lymphocyte, monocyte and eosinophil counts were also significantly increased in all the treatment groups. Basophils were not found in control and DHA treated rats, but were present in sDHA-treated groups (Table 7). The values of sDHA and DHA were not significantly different from each other at all dose levels for all the WBC differential counts (Table 7).

**Weight and histology of liver and kidney**

There was significant increase ($p < 0.05$) in liver-to-body weight ratios that were obtained in all DHA treated, and also in 103.92 mg/kg sDHA treated rats when compared with control (Table 8). There was no significant change ($p > 0.05$) in the kidney-to-body weight ratio in all the treated groups of rats when compared with the kidney-to-body weight ratio that was obtained in control rats (Table 8).

Histology of liver of animals that were treated with vehicle (control) showed normal histology with normal cellular profile of hepatic tissues, hepatic artery, hepatic vein, and nucleus within a normal cellular architecture (Figs. 2A and 3A). The livers of sDHA-treated rats showed mild to moderate degrees of inflammation, vacuolation, hepatocytic hyperplasia, cellular degeneration, and pyknotic nuclei, which were present in a dose-dependent fashion (Figs. 2A, 2B, 2C and 2D). Livers of rats that were treated with DHA (54.77 mg/kg) showed inflammation and formation of pyknotic nucleus (Figure 3B), but for rats that received higher

doses of DHA, there was also vacuolation and hepatocytic hyperplasia, when compared with control group (Figs. 3A, 3C and 3D).

Histological analyses of kidneys of control rats revealed normal cell architecture and cellular profiles of proximal and distal convoluted tubules, glomerulus and other renal tissues (Figs. 4A and 5A). Kidneys of rats treated with sDHA (34.64 mg/kg) showed mild inflammation and formation of numerous pyknotic nuclei, but no cellular abnormality occurred, when compared with control group (Figs. 4A and 4B). Aside from the above, vascular degeneration was also observed in kidneys of the rats that received 69.28 and 103.92 mg/kg of sDHA (Figs. 4A, 4C and 4D). Furthermore, kidneys of DHA administered rats did not show cellular abnormality, except the groups that received 109.54 and 164.32 mg/kg which showed presence of mild inflammation, vascular degeneration and numerous pyknotic nuclei (Figs. 5A, 5B, 5C and 5D).

**Table: 1 Mortality rate following single intraperitoneal injection of different concentrations of disulphide-substituted dihydroartemisinin (sDHA) over 24 h in Sprague-Dawley rats (Acute toxicity study)**

| **Phase 1** | | | | |
|---|---|---|---|---|
| Dose (No. of rats per group) | 250 mg/kg (3) | 500 mg/kg (3) | 1000 mg/kg (3) | |
| No. dead | 0 | 3 | 3 | |
| **Phase 2** | | | | |
| Dose (No. of rats per group) | 300 mg/kg (1) | 350 mg/kg (1) | 400 mg/kg (1) | 450 mg/kg (1) |
| No. dead | 0 | 0 | 1 | 1 |

**Table: 2. Mortality rate following single intraperitoneal injection of different concentrations of dihydroartemisinin (DHA) over 24 h in Sprague-Dawley rats (Acute toxicity study).**

| **Phase 1** | | | | |
|---|---|---|---|---|
| Dose (No. of rats per group) | 250 mg/kg (3) | 500 mg/kg (3) | 1000 mg/kg (3) | |
| No. dead | 0 | 0 | 3 | |
| **Phase 2** | | | | |
| Dose (No. of rats per group) | 550 mg/kg (1) | 600 mg/kg (1) | 650 mg/kg (1) | 750 mg/kg (1) |
| No. dead | 0 | 1 | 1 | 1 |

**Table: 3. Body weights following 28 days daily treatment of disulphide-substituted derivative of dihydroartemisinin (sDHA) and its parent drug, dihydroartemisinin (DHA) in Sprague-Dawley rats**

| Groups | Body weight (g) | |
|---|---|---|
| | Day 1 | Day 28 |
| **Control** | 124.00±7.50 | 142.20±9.12* |
| sDHA, 34.64 mg/kg | 112.00±3.41 | 124.80±2.27* |
| sDHA, 69.28 mg/kg | 104.00±3.23 | 112.50±3.13* |
| sDHA, 103.92 mg/kg | 112.00±4.60 | 121.00±2.42* |
| DHA, 54.77 mg/kg | 124.00±5.65 | 136.30±7.42* |
| DHA, 109.54 mg/kg | 123.00±6.75 | 130.30±4.92* |
| DHA, 164.32 mg/kg | 120.00±5.13 | 128.50±2.74* |

Values are expressed as mean±SEM, n = 6 rats per group.

* Indicates significant values compared to Day 1 body weights at $p < 0.05$.

**Table: 4. Effects of sub-acute administration of disulphide-substituted derivative of dihydroartemisinin (sDHA) and its parent drug, dihydroartemisinin (DHA) on serum levels of some biochemical indices of liver and kidney functions in Sprague-Dawley rats,**

| Groups | ALT (IU/L) | AST (IU/L) | ALP (IU/L) | Urea (mg/dL) | Creatinine (mg/dL) |
|---|---|---|---|---|---|
| Control | 38.50±1.78 | 117.7±7.76 | 61.83±1.30 | 4.35±0.93 | 0.68±0.02 |
| sDHA, 34.64 mg/kg | 76.67±1.52* | 226.50±6.20* | 157.00±3.40* | 4.48±0.63 | 0.68±0.06 |
| sDHA, 69.28 mg/kg | 87.83±4.19* | 242.30±9.59* | 160.70±3.73* | 4.51±0.33 | 0.65±0.07 |
| sDHA, 103.92 mg/kg | 99.67±1.99* | 263.00±6.29* | 170.20±5.58* | 4.44±0.55 | 0.71±0.05 |
| DHA, 54.77 mg/kg | 85.67±3.87* | 206.80±2.56* | 136.30±4.42* | 4.42±0.67 | 1.14±0.19 |
| DHA, 109.54 mg/kg | 100.30±1.61* | 249.30±6.62* | 152.70±6.06* | 4.39±0.85 | 1.08±0.21 |
| DHA, 164.32 mg/kg | 102.50±3.43* | 273.30±3.74* | 162.80±3.15* | 4.37±0.09 | 0.85±0.01 |

Values are expressed as mean±SEM, n = 6 rats per group.

* Indicates significant values compared to control group at $p < 0.001$.

ALT: Alanine transaminase, AST: Aspartate transaminase, ALP: Alkaline phosphatase.

**Table: 5. Effects of sub-acute administration of disulphide-substituted derivative of dihydroartemisinin (sDHA) and its parent drug, dihydroartemisinin (DHA) on white blood cell (WBC), red blood cell (RBC) and platelet counts in Sprague-Dawley rats**

| Groups | WBC ($x\ 10^3\ mm^{-3}$) | RBC ($x\ 10^6\ mm^{-3}$) | Platelet ($x\ 10^3\ mL^{-1}$) |
|---|---|---|---|
| Control | 6.07±0.23 | 7.86±0.11 | 756.70±34.19 |
| sDHA, 34.64 mg/kg | 7.75±0.18* | 4.69±0.10*† | 546.50±15.14* |
| sDHA, 69.28 mg/kg | 7.73±0.18* | 3.55±0.26*† | 502.50±32.72* |
| sDHA, 103.92 mg/kg | 7.63±0.20* | 3.63±0.29*† | 472.30±32.28* |
| DHA, 54.77 mg/kg | 7.26±0.15* | 6.59±0.23* | 619.20±10.10* |

| | | | |
|---|---|---|---|
| DHA, 109.54 mg/kg | 7.52±0.19* | 5.51±0.17* | 528.20±16.85* |
| DHA, 164.32 mg/kg | 7.78±0.13* | 5.92±0.40* | 452.20±46.29* |

Values are expressed as mean±SEM, n = 6 rats per group.

*Indicates significant values compared to control group, $p < 0.001$.

†Indicates significant values compared to corresponding values of DHA, $p < 0.05$.

**Table: 6. Effects of sub-acute administration of disulphide-substituted derivative of dihydroartemisinin (sDHA) and its parent drug, dihydroartemisinin (DHA) on red blood cell indices in Sprague-Dawley rats.**

| Groups | Hb ($g\ dL^{-1}$) | PCV (%) | MCH (pg) | MCHC ($g\ dL^{-1}$) |
|---|---|---|---|---|
| Control | 14.72±0.37 | 40.17±1.05 | 19.72±0.27 | 35.08±0.69 |
| sDHA, 34.64 mg/kg | 11.14±0.20* | 32.00±0.96* | 15.45±0.23* | 30.05±2.072* |
| sDHA, 69.28 mg/kg | 10.40±0.20* | 30.33±1.23* | 13.98±0.33* | 20.49±1.48* |
| sDHA, 103.92 mg/kg | 10.21±0.31* | 26.67±2.11* | 13.21±0.20* | 16.35±0.74* |
| DHA, 54.77 mg/kg | 12.48±0.27* | 37.50±1.43* | 16.66±0.29* | 30.08±1.30* |
| DHA, 109.54 mg/kg | 12.35±0.14* | 30.17±0.98* | 14.11±0.23* | 26.10±1.61* |
| DHA, 164.32 mg/kg | 11.57±0.23* | 29.17±1.35* | 12.04±0.28* | 21.37±0.65* |

Values are expressed as mean±SEM, n = 6 rats per group.

* Indicates significant values compared to control group at $p < 0.001$.

Hb: Hemoglobin, PVC: Packed cell volume, MCH: Mean corpuscular hemoglobin, MCHC: Mean corpuscular hemoglobin concentration.

**Table: 7. Effects of sub-acute administration of disulphide-substituted derivative of dihydroartemisinin (sDHA) and its parent drug, dihydroartemisinin (DHA) on white blood cell differential counts in Sprague-Dawley rats**

| Groups | Neutrophils ($x10^3\ mm^{-3}$) | Lymphocytes ($x10^3\ mm^{-3}$) | Monocytes ($x10^3\ mm^{-3}$) | Eosinophils ($x10^3\ mm^{-3}$) | Basophils ($x10^3\ mm^{-3}$) |
|---|---|---|---|---|---|
| Control | 1.53±0.08 | 5.37±0.64 | 0.08±0.01 | 0.03±0.01 | 0.00±0.00 |
| sDHA, 34.64 mg/kg | 2.27±0.07* | 7.44±0.16* | 0.12±0.01* | 0.17±0.01* | 0.10±0.00* |
| sDHA, 69.28 mg/kg | 2.23±0.10* | 7.59±0.15* | 0.15±0.01* | 0.20±0.01* | 0.10±0.00* |
| sDHA, 103.92 mg/kg | 2.15±0.10* | 7.83±0.06* | 0.14±0.01* | 0.22±0.02* | 0.10±0.00* |
| DHA, 54.77 mg/kg | 1.82±0.06* | 7.48±0.06* | 0.11±0.00* | 0.17±0.02* | 0.00±0.00 |
| DHA, 109.54 mg/kg | 1.85±0.06* | 7.52±0.06* | 0.12±0.01* | 0.17±0.02* | 0.00±0.00 |
| DHA, 164.32 mg/kg | 1.98±0.05* | 7.85±0.08* | 0.14±0.01* | 0.18±0.02* | 0.03±0.02* |

Values are expressed as mean±SEM, n = 6 rats per group.

* Indicates significant values compared to control group at $p < 0.001$.

**Table: 8. Effects of sub-acute administration of disulphide-substituted derivative of dihydroartemisinin (sDHA) and its parent drug, dihydroartemisinin (DHA) on liver and kidney weights of Sprague-Dawley rats.**

| Groups | Organ-to-body weight ratio (x $10^{-3}$) | |
|---|---|---|
| | Liver | Kidney |
| Control | 35.16±0.64 | 4.92±0.07 |
| sDHA, 34.64 mg/kg | 35.74±0.05 | 4.01±0.14 |
| sDHA, 69.28 mg/kg | 36.69±0.12 | 4.44±0.14 |
| sDHA, 103.92 mg/kg | 43.56±0.14* | 4.30±0.10 |
| DHA, 54.77 mg/kg | 43.27±0.05* | 4.40±0.14 |
| DHA, 109.54 mg/kg | 45.49±0.07* | 4.45±0.06 |
| DHA, 164.32 mg/kg | 48.35±0.64* | 5.45±0.21 |

Values are expressed as mean±SEM, n = 6 rats per group.

* Indicates significant values compared to control group at $p < 0.05$.

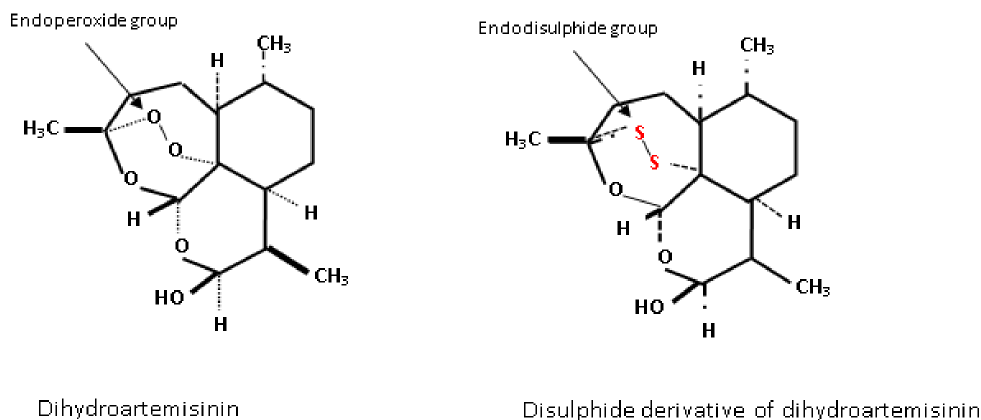
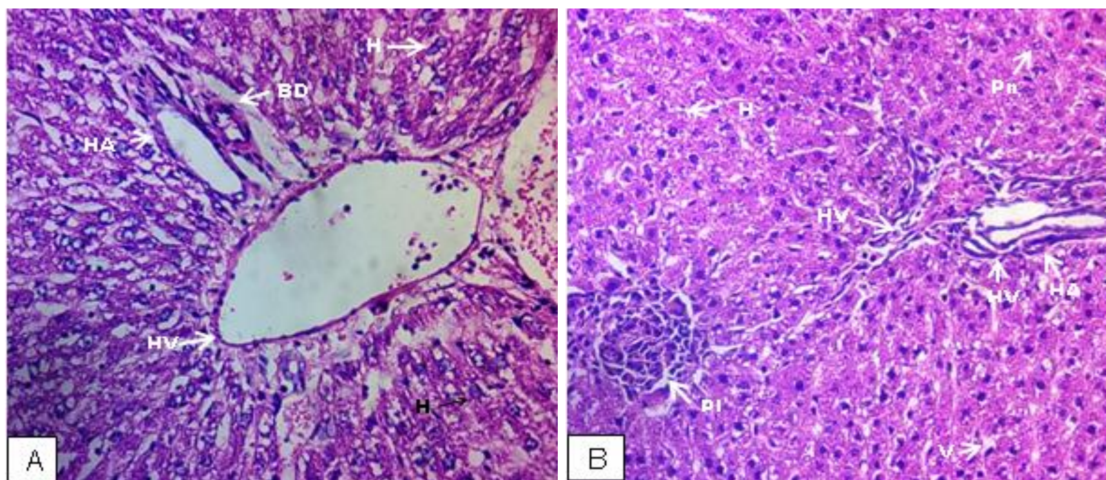



**Figure 1**

**Figure: 1. Chemical structures of dihydroartemisinin and its disulphide derivative**



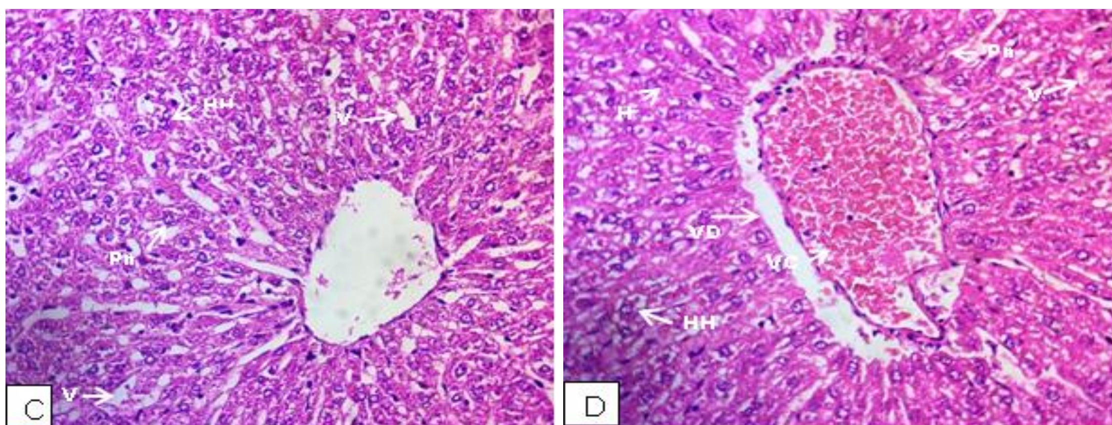



**Figure: 2. Photomicrographs of liver of Sprague-Dawley rats following subacute dihydroartemisinin disulphide-derivative (sDHA) treatment, H&E staining (400x)**

A: Vehicle (Control) treated group; liver histology showing normal cellular profile of hepatic artery (HA), hepatic vein (HV), bile duct (BD) and hepatocytes (H) within normal cellular architecture.

B: sDHA (34.64 mg/kg) treated group; liver histology showing presence of vacuolation (V), periportal inflammation (PI) and pyknotic nucleus (Pn).

C: sDHA (69.28 mg/kg) treated group; liver histology showing presence of inflammation, vacuolation (V), hepatocytic hyperplasia (HH) and pyknotic nucleus (Pn)

D: sDHA (103.92 mg/kg) treated group; liver histology showing presence of vacuolation (V), hepatocytic hyperplasia (HH), vascular degeneration (VD) and pyknotic nucleus (Pn).

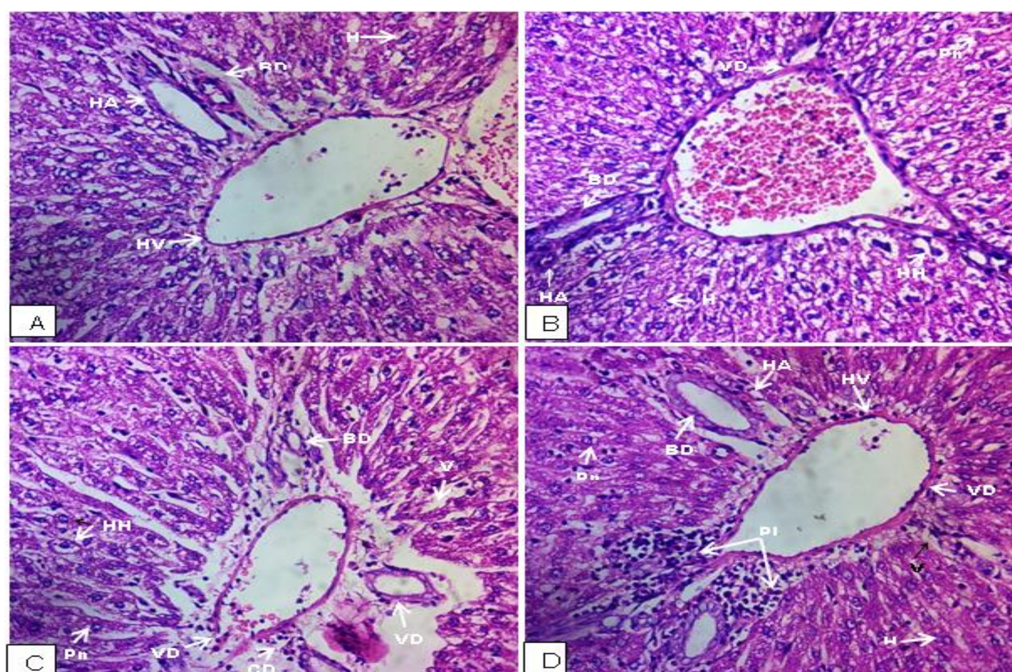



**Figure: 3. Photomicrographs of liver of Sprague-Dawley rats following subacute dihydroartemisinin (DHA) treatment, H&E staining (400x)**.

A: Vehicle (Control) treated group; liver histology showing normal cellular profile of hepatic artery (HA), hepatic vein (HV), bile duct (BD) and hepatocytes (H) within normal cellular architecture.

B: DHA (54.77 mg/kg) treated group; liver histology showing inflammation and presence of hepatocytic hyperplasia (HH), vascular degeneration (VD) and pyknotic nucleus (Pn).

C: DHA (109.54 mg/kg) treated group; liver histology showing vacuolation (V), hepatocytic hyperplasia (HH), vascular degeneration (VD), cellular degeneration (CD) and presence of pyknotic nucleus (Pn).

D: DHA (164.32 mg/kg) treated group; liver histology showing presence of periportal inflammation (PI), vascular degeneration (VD) and pyknotic nucleus (Pn).

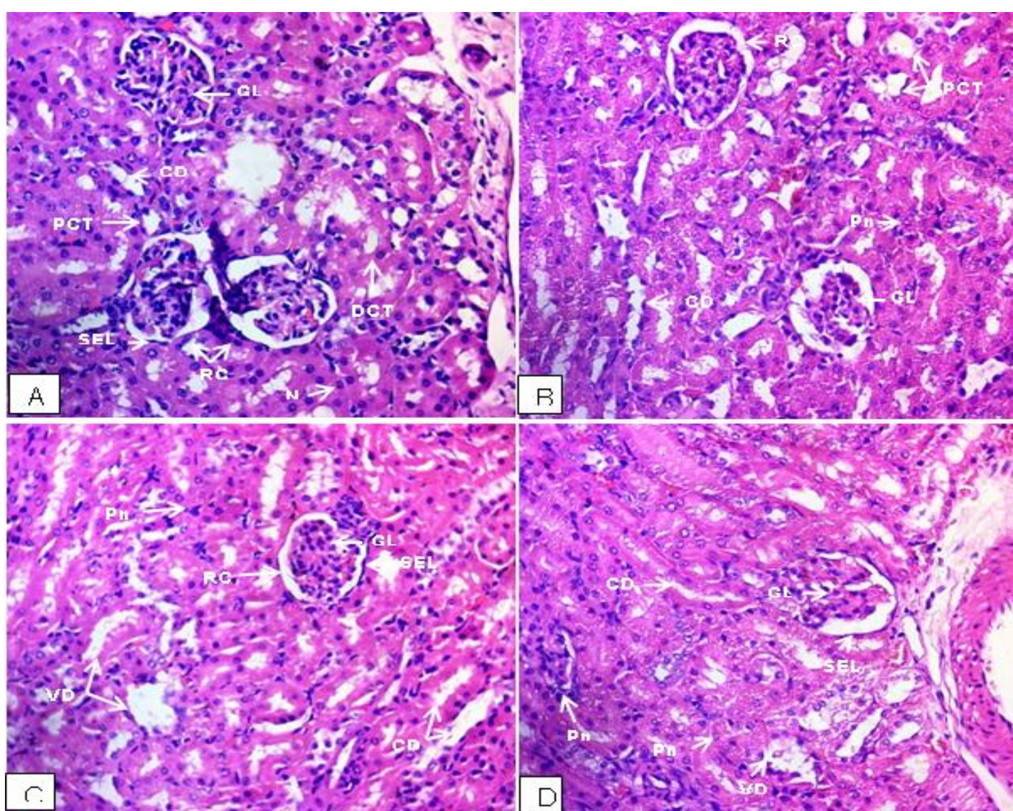



**Figure: 4. Photomicrographs of kidney of Sprague-Dawley rats following subacute dihydroartemisinin disulphide-derivative (sDHA) treatment, H&E staining (400x)**

A: Vehicle (Control) treated group; kidney histology showing normal cellular profile of proximal (PCT) and distal convoluted tubules (DCT), collecting ducts (CD), renal corpuscle (RC) containing glomerulus (GL) and lined with squamous epithelial (SEL) within a normal cellular architecture.

B: sDHA (34.64 mg/kg) treated group; kidney histology showing presence of numerous pyknotic nuclei (Pn), but without cellular abnormality.

C: sDHA (69.28 mg/kg) treated group; kidney histology showing vascular degeneration (VD) and numerous pyknotic nuclei (Pn).

D: sDHA (103.92 mg/kg) treated group; kidney histology showing vascular degeneration (VD) and numerous pyknotic nuclei (Pn).

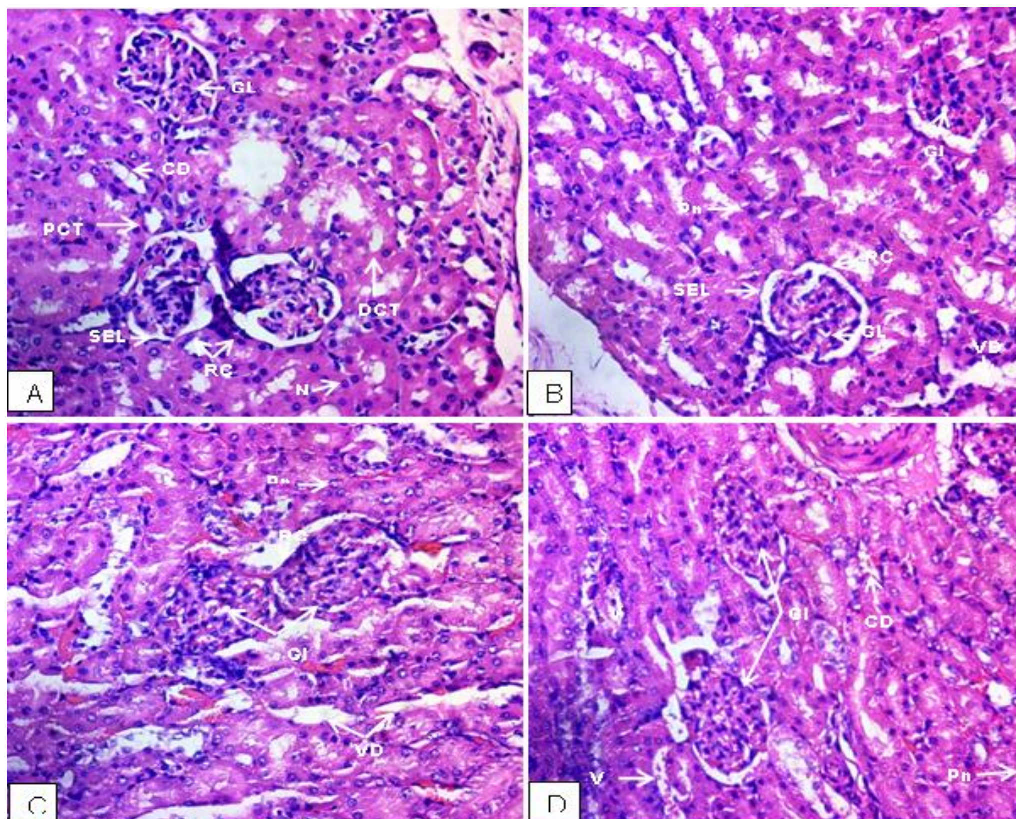



**Figure: 5. Photomicrographs of kidney of Sprague-Dawley rats following subacute dihydroartemisinin (DHA) treatment, H&E staining (400x)**

A: Vehicle (Control) treated group; kidney histology showing normal cellular profile of proximal (PCT) and distal convoluted tubules (DCT), collecting ducts (CD), renal corpuscle (RC) containing glomerulus (GL) and lined with squamous epithelial (SEL) within a normal cellular architecture.

B: DHA (54.77 mg/kg) treated group; kidney histology showing normal cellular profile without any obvious abnormality.

.C: DHA (109.54 mg/kg) treated group; kidney histology showing presence of glomerular inflammation (GI), vascular degeneration (VD) and pyknotic nuclei (Pn).

D: DHA (164.32 mg/kg) treated group; kidney histology showing presence of vacuolation (V), glomerular inflammation (GI), vascular degeneration (VD) and pyknotic nuclei (Pn).

## DISCUSSION

In the recent past, many cancer research projects have been focused on developing new chemotherapies either by exploring the anticancer ability of novel compounds or assessing anticancer potentials of drugs conventionally used in other clinical diseases. Artemisinin derivatives, including dihydroartemisinin (DHA) have been shown to possess anti-cancer activities.[9] However, a major drawback in their clinical efficacies is their short half-lives, which range from few minutes to two hours.[15-18] The pharmacological activity of artemisinins is directly linked to their structures[19,20] and even minor structural changes of the drugs could profoundly modify their pharmacodynamics and pharmacokinetics, as well as their toxicological indices. Recently, Bassey[12] has synthesized disulphide substituted derivative of DHA which is believed to have a longer half-life than the parent drug. The present study reports the acute and sub-acute toxicological profiles of DHA and the disulphide substituted derivative (sDHA).

The results of the acute toxicity study revealed that sDHA has a lower median lethal dose ($LD_{50}$) than DHA, 346.41 and 547.72 mg/kg i.p. in the rat, respectively. $LD_{50}$ is a fundamental toxicological index that measures the potential of a compound to cause lethality in animals, specifically fifty percent in a given population. It is also an important determinant of the safety margin or therapeutic index of a drug.[21-23] In this study, the Lorke's method[13] was used to determine the $LD_{50}$, which uses few number of animals. From the result, a lower concentration of sDHA produced lethality, and is therefore likely to be more toxic and have lesser safety margin than the parent drug, DHA.

Three doses (low, mid and high) corresponding to 10, 20 and 30 % of the determined $LD_{50}$ of DHA (54.77, 109.54 and 164.32 mg/kg) and sDHA (34.64, 69.23 and 103.92 mg/kg) were selected for the sub-acute toxicity study. From the results, there was normal weight gain in all treated rats during sub-acute administration, indicating that DHA and the disulphide derivative do not adversely affect growth of rats. Change in body weight by drugs and chemicals is indicative of toxicity and this becomes clinically significant when there is more than a 10 % loss in body weight.[24,25] Further, organ weight is an important indicator of physiological and pathological status of animals, and relative organ weight (organ-to-body-weight ratio) is fundamental to establish exposure of an organ to injury or otherwise.[24,26] The liver and kidney are among primary organs that can be exposed to such injuries (metabolic reactions) caused by toxicants.[24] In this study, the relative organ weight of the

kidney was unaltered by both drugs, but there was change in that of the liver- an increase occurring during treatment with both drugs. Alteration in the relative weight of the liver suggests that the two drugs may induce toxicity to the liver during the treatment period, whereas their lack-of-effect on the relative kidney weight indicates that the drugs may not alter renal function. Assessment of the serum levels of hepatic enzymes- aspartate transaminase (AST), aspartate transaminase (ALT), and alkaline phosphatase (ALP) further indicated abnormality in liver function. Hepatocellular damage is characterized by elevation in serum levels of AST, ALT and ALP, which often results from leakage of the enzymes into the serum.[27,28] ALT is localized primarily in the cytosol of hepatocytes and is considered as the most sensitive marker of hepatocellular damage than the other enzymes.[27,29] The observed elevations in the serum levels of all three enzymes by DHA and sDHA in this study is therefore highly indicative of damage to liver cells. Additionally, DHA and sDHA caused dose-dependent histopathological changes, including inflammation, vacuolation, and hepatocyte hyperplasia. This provides evidence that prolong use of the drugs will alter liver function in rats.

In addition, DHA and sDHA did not alter urea and creatinine levels. They also did not cause obvious abnormality in kidney cellular profile, although, both drugs (but mostly sDHA) caused mild inflammatory and vascular changes in the histology of the organ. Nevertheless, the lack of marked changes in serum levels of creatinine and urea implies that the mild histological changes seen in DHA and sDHA treated rats may not be significant enough to affect kidney function and may be considered as clinically insignificant. More so, the relative organ weight of the kidney was unaltered by both drugs, indicating strongly that the drugs are not likely to adversely affect the kidney.

Furthermore, DHA and sDHA caused decreases in red blood cells (RBC) and platelets. Expectedly, hemoglobin level and packed cell volume (PCV) were also decreased. Mean corpuscular hemoglobin (MCH) and mean corpuscular hemoglobin concentration (MCHC) values were equally decreased by DHA and the derivative. This indicates that both agents are capable of causing bleeding and also induce anemia if used over long duration. The effects on these hematological parameters by both drugs were comparable, except on RBC wherein sDHA induced a higher level of reduction. The implication of this is that the disulphide derivative of DHA may induce a higher degree of anemia than the parent drug. In addition, the drugs caused comparable levels of elevation in total white blood cell (WBC) count and

also in the relative counts of the WBC subtypes, except basophil. This effect on WBC by the agents may be useful in cancer chemotherapy as leucopenia and immunosuppression are features that occur commonly during cancer chemotherapy, either due to the disease or anticancer drugs.

## CONCLUSIONS

Concluding from the results obtained, the disulphide derivative of dihydroartemisinin has a lower $LD_{50}$ and would therefore exhibit a higher acute toxicity than dihydroartemisinin. Both drugs may have no adverse effect on the kidney, but induce hepatic toxicity, anemia, increased WBC production, and reduction in platelets and erythrocytes. Overall, dihydroartemisinin and its disulphide derivative have a comparable sub-acute toxicity profile, but the disulphide derivative induces a greater level of erythrocyte reduction than dihydroartemisinin.

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