Sub-chronic toxicity evaluation of top-three commercial herbal anti-malarial preparations in the Kumasi metropolis, Ghana

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Conflict of interest

The authors declare that they have no conflict of interest in carrying out any part of this work.
ABBREVIATIONS

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26 DAHD- Daily adult human dose
27 ALP - Alkaline phosphatase
28 ALT- Alanine aminotransferase
29 ANOVA - Analysis of variance
30 AST - Aspartate aminotransferase
31 CHOL- Total cholesterol
32 EDTA - Ethylenediaminetetraacetic acid
33 FBG – Fasting blood glucose
34 GRA - Granulocyte
35 HCT - Hematocrit
36 HDL-C - High-density lipoprotein cholesterol
37 HGB - Haemoglobin
38 HM – Herbal medicinal
39 HMP(s) – Herbal medicinal product(s)
40 HMs - Herbal medicines
41 HP(A)- Herbal product ‘A’
42 HP(B)- Herbal product ‘B’
43 HP(C)- Herbal product ‘C’
44 HP(s) – Herbal product(s)
45 LDL-C - Low density lipoprotein cholesterol
46 LYM - Lymphocyte counts
47 MCH - Mean corpuscular haemoglobin
48 MCHC - Mean corpuscular haemoglobin concentration
49 MCV - Mean corpuscular volume
50 MPV - Mean platelet volume
|   | Abbreviation | Description |
|---|--------------|-------------|
| 3 | MRL          | Maximum residual limit |
| 4 | MXD          | Mixed monocytes, basophil and eosinophils |
| 5 | NaHCO₃       | Sodium carbonate |
| 6 | OECD         | Organisation for Economic Co-operation and Development |
| 7 | PDW          | Platelet distribution width |
| 8 | P-LCR        | Platelet larger cell ratio |
| 9 | PLT          | Platelet count |
| 10 | RBC          | Red blood cell |
| 11 | TG           | Triglyceride |
| 12 | UCCIRB       | University of Cape Coast Institutional Review Board |
| 13 | VLDL-C       | Very low-density lipoprotein cholesterol |
| 14 | WBC          | White blood cell |
| 15 | WHO          | World Health Organization |
Abstract

Purpose: Safety data on commonly used herbal medicinal products (HMPs) and marketed in Ghana is scarce. We assessed the sub-chronic toxicity of three most-patronized commercial antimalarial HMPs in Kumasi, Ghana.

Method: Top-three HMPs (designated HPA, HPB and HPC) were selected after a mini-survey and sub-chronic toxicity evaluation conducted in accordance with OECD 407 guidelines. Control rats received clean water while test groups received daily adult human dose (DAHD), 5xDAHD or 10xDAHD of either HPA, HPB or HPC for 30 days. Rats were sacrificed on day 31 to obtain biochemical, haematology and histology samples for analysis. Data were analysed by one-way ANOVA and post hoc Turkey’s test.

Results: The three HMPs produced alterations in liver morphology predominantly characterized by prominent foci of fatty changes, scattered hepatocytes with intracytoplasmic fat globules and congested central veins and sinusoids. Delicate alveolar with evidence of inflammation and foci of sloughing within rat airway were observed. Alveolar spaces were obscured by debris and inflammatory cells. HPA and HPC produced scattered intensely congested heart vessels while HPB(10) produced haemorrhage and amorphous exudates. All HMPs produced neither treatment-related deaths nor significant change in haematological and biochemical parameters, except for HPA and HPB which decreased (p<0.05) AST and HPB which elevated (p<0.05) FBG.

Conclusion: Data from this study suggest the potential of the herbal products, HPA, HPB and HPC, to cause major organ-system dysfunction or damage. We advise cautious use of these products and recommend further safety evaluation in chronic toxicity models.

Keywords: Surveillance, inflammation, haemolysis, safety, histology, haematology, biochemical, impaired, congestion
1.0 Introduction

Malaria is endemic in Ghana and other Sub-Saharan African countries and it is a leading cause of morbidity and mortality among children under five years and pregnant women in the region. ¹ In 2016, the African WHO region reported 194.4 million malaria cases (90% of the global sum) and 405,000 malaria deaths (91% of the global sum).² ³ Due to the high incidence density of approximately 5 malaria infections per person per year for Sub-Saharan African,⁴ and the high malaria morbidity and mortality rate in the region, antimalarial medications are of great public interest in the region. Because of high cost and poor access to Artemisinin-based Combination Therapy (ACT), the use of plant-based multiherbal preparations (MHPs) for treatment of malaria is common practice in the region.¹ ⁵ Year-round availability, affordability and easy accessibility of plant-based medicines compared to pharmaceutical medicines encourage their use. In fact, over 70% of Sub-Saharan Africans resort to the use of herbal medicines for their primary health needs.⁶ ⁷

In our recent survey, we observed that most of the subjects used herbal medicines solely or in combination with orthodox drug for various health needs including preventive, curative and chronic disease management. The study participants demonstrated high level of trust in herbal medicines and believed that herbal medicines are better curative agents than pharmaceutical medicine.⁸

Most herbal medicinal products (HMPs) have been reported to be effective in treating liver problems,⁹ circulatory and respiratory diseases¹⁰ and malaria.¹¹ In addition, medicinal plants also offer an unlimited and valuable recipe for the discovery of novel drugs. The lead compounds of several well-known pharmaceutical drugs have been discovered from plants. Artemisinin used in treating Plasmodium falciparum malaria was derived from Artemisia annua. The memory enhancer drug, physostigmine, was first isolated from Physostigma venenosum that grows in West Africa. Similarly, the anticancer drug, docetaxel, was originally obtained from Taxus species.

Recent scientific reports show that many medicinal plants employed as alternative medicines have adverse toxic effects comparable to those of pharmaceutical drugs.¹² There have
also been reports on potential mutagenic, carcinogenic, and hepatotoxic effects of some previously studied medicinal plants. Although, the leaves of *Cleistanthus collinus* are believed to possess anticancer properties, they have also been shown to contain a known human poison capable of causing mortality rate of 20-60%. There is also a growing concern regarding contamination and adulteration of over-the-counter HMPs. Although, toxicity profile of some medicinal plants or their extracts have been documented in Ghana, data on pre-market and post-market safety and toxicity are practically unavailable for most antimalarial and other MHPs on the Ghanaian market.

Regular safety surveillance of these commercial products is also lacking thereby creating data gap and raising public health concerns. In our previous studies, we identified commercially available multiherbal products from the Ghanaian market with high levels of heavy metals and banned pesticide. Table I provides a summary of the ethnomedicinal uses and adverse or toxic effects of some medicinal plants employed in Traditional African Medicine. In the present study, we conducted a sub-chronic toxicity evaluation for three of the multiherbal antimalarial products previously analysed for their residual pesticide and heavy metal contents.

### Table I: Ethno-medicinal uses of the medicinal plant constituents

| Medicinal plants       | Ethnomedicinal uses with reference sources                                                                 | Documented safety data                                                                                                                                                                                                 | Herbal product                          |
|------------------------|-------------------------------------------------------------------------------------------------------------|--------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|-----------------------------------------|
| *Cola gigantea*        | Stem barks are used for inflammation and bacterial infections. The plant is used in folklore medicine as a heart anti-depressant. | *C. gigantea* oil extract is believed to possess general cellular toxicity effect due to reactive oxygen species production and oxidative stress. | Time herbal mixture                     |
| *Solanum torvum*       | Leaves and the unripe fruits are used to treat tuberculosis. *S. torvum* plant is used to treat diabetes, and parasitic infections and to reduce oxidative stress on the liver. Extracts from the aerial parts of the plant also have anticancer properties. The plant is also used in Ghana to treat malaria. | Acute administration of *S. torvum* was observed to be safe in broiler chickens. Acute administration of *S. torvum* has the potential in preventing the nephrotoxicity induced by doxorubicin. Aqueous fruit extracts had hypotensive effects and were chronically save in rats. | Acute administration of ethanolic leaf extract was observed to be save in rats. However chronic administration resulted in loss of weight, sluggish movement and significant but reversible hepatoxic effect. |
| *Spathodea campanulata*| Used for the treatment of malaria, cancer and for healing of wounds. | Acute administration of ethanolic leaf extract was observed to be save in rats. However chronic administration resulted in loss of weight, sluggish movement and significant but reversible hepatoxic effect. | Activated spines of *B. buonopozense* is reported to have high biosorption for copper and zinc metals, and may lead to bioconcentration of these metals in *B. buonopozense* plant products. |
| *Bombax buonopozense*  | Used to treat sleeping sickness. Ethanol extract of the stem bark is also believed to have antitypanosomal activities. | Activated spines of *B. buonopozense* is reported to have high biosorption for copper and zinc metals, and may lead to bioconcentration of these metals in *B. buonopozense* plant products. | Activated spines of *B. buonopozense* is reported to have high biosorption for copper and zinc metals, and may lead to bioconcentration of these metals in *B. buonopozense* plant products. |
| *Vernonia amygdaлина*  | Leaf extract of *V. amygdaлина* have been reported to affect multiple stages of Plasmodium life cycle. | Reported to have chromosomal aberrations effect. | Aqueous leaf extract of the plant were observed to have high biosorption for copper and zinc metals, and may lead to bioconcentration of these metals in *B. buonopozense* plant products. |
leukemia and prostate cancer. It is also used for hepatoprotection, nutritional, clinical and veterinary relevance with no serious hepatotoxic effects in rats.

**Ocimum viride**

Ethanol extract of the essential oils from the aerial parts of *O. viride* has anticancer activity against human colorectal adenocarcinoma cells (COLO 205 cell line). *O viride* extract also possess high antimicrobial properties against *Rhizopus stolonifer*, *Aspergillus* sp, and *Fusarium* sp.

**Acadirachta indica**

Different parts of the plant are used to treat malaria, cancer, ulcer, diabetes, dengue fever, chicken pox and dermal complications. Acute and 28-day subacute toxicity tests with *A. indica* fruit oil showed no significant difference in biochemical and haematological parameters, however, at high doses, signs of testicle, liver and kidneys toxicities were observed in histology slides. Seed oil extract was observed by Gandhi and colleagues to cause dose-dependent toxicity on the lungs and central nervous system in both rats and rabbits. An 8-week study with aqueous leaf suspensions showed multi organ toxicities, tremors and loss of weight in goats and guinea pigs.

**Tetrapleura tetraptera**

Used in West Africa for the treatment of malaria, diabetes and hypertension, inflammation, ulcer, and for the management of epilepsy and childhood convulsions. Extracts of the plant also have a well-studied anti-molluscicidal activities for the control of unwanted mollusc vectors.

**Cymbopogon citratus**

Leaf infusion has been used in folklore medicine to treat fever and malaria, inflammatory conditions, antifungal infestations and epilepsy. Oils of *Cymbopogon citratus* showed a dose-dependent significant functional toxicities to stomach and liver of the Wistar rat during 14-day toxicity study at doses higher than 1500 mg/kg body weight, the oil was safe at doses less than 1500 mg/kg body weight.

**Moringa oleifera**

The plant is used for treating malaria, diabetes, cancer, and for the treatment of inflammatory-mediated chronic disorders. *Moringa oleifera* was observed by Asare and colleagues to exhibit genotoxic at supra-supplementation levels of 3000 mg/kg body weight in rats, but in the same study, they observed intake levels ≤1000 mg/kg body weight to be safe in humans. It has also been reported to be safe with no reported toxicity cases in humans.

**Anthoeclista nobilis**

The plant is used to treat diverse health conditions including anti-diabetic, antimalarial, antimicrobial, hypotensive, spasmodic, anti-obesity, antilucerogenic, analgesic, anti-inflammatory, antioxidant, antipyransosmal, antimutagenic and fertility purposes.

**Vitex grandifolia**

The bark of the tree is used as a stomachic and to treat diarrhoea, bronchial complaints, rickets, sores and fever. Also used against malaria, yellow fever, filarial and dengue vector control due to its larvicidal activity. In traditional medicine, the leaves of *V. grandifolia* are used to treat diabetes mellitus and as a diuretic in the treatment of high blood pressure.

**Phyllanthus fraternus**

Used in Ayurveda and Siddha medicine for the treatment of jaundice and possible anti-DNA polymerase activity of the hepatitis virus. The aerial parts of this plant is also believed to have anti-hepatotoxic activity. Hepatoprotective and antioxidant property of the aqueous extract of *P. fraternus* observed by Lata and colleagues on mice previously administered with cyclophosphamide. They observed normalizing of pathological and antioxidant parameters of the cyclophosphamide poisoned mice after *P. fraternus* administration.

**Taabea herbal mixture**

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2.0 Materials and methods

2.1 Mini-survey and selection of herbal antimalarial products

In Ghana, consumers of herbal medicinal products do not need a doctor’s prescription to buy herbal medicine. The herbal medicine consumers (both patients and healthy individuals) buy their herbal products from several sources including herbal centers, pharmacy shops and from those who sell at the roadsides, market places and to travellers in buses or at bus stations. In our previous study, a mini-survey was conducted to determine the most patronized antimalarial, antidiabetic and antihypertensive herbal products on the Kumasi market.\textsuperscript{20,85} From the survey the top-three most patronized multi-herbal antimalarial products, ‘Time Herbal Mixture\textsuperscript{®}’, ‘Taabea Herbal Hixture\textsuperscript{®}’, and Adutwumwaa Malamix\textsuperscript{®} were then selected for further studies (Figure AI).\textsuperscript{20,85}

2.2 Study design

The study design and different stages of the study are presented in Figure I. Stages of the study included herbal product selection, randomization, grouping of the rats and the various studies performed on the rats.
Figure 1: Study design. The macroscopic, haematological, biochemical, relative organ weights and organ histology studies performed on both test group rats and the control rats.
2.3 Animals and treatment schedule

Forty young healthy adult male Sprague-Dawley rats (10-12 weeks old) were used for the study.

The animals were marked to permit easy identification and data handling and randomly assigned to
ten groups of four rats per group (n = 4). Each group was assigned to a cage and all the rats were
kept for 10 days to allow for acclimatization to the laboratory conditions prior to dosing. Animals
were maintained at ambient temperature and humidity with a 12 h light/12 h dark schedule and fed
with standard pelleted rodent feeds and water ad libitum during the acclimatization and
experimental periods. Each of the nine groups of rats was assigned to one herbal antimalarial dose
level and the tenth group that received clean water served as control.

The animals were weighed every week prior to dosing with HPs and before they were sacrificed
under light chloroform anaesthesia on day 30.

The three commercial herbal products (HPs) selected for the study were assigned codes
‘HPA’, ‘HPB’ and ‘HPC’. The equivalent of the recommended daily adult human dose (DAHD) as
stated on the product labels [marked here as HPA(1), HPB(1) and HPC(1)] was used as the
minimum dose and administered to rats. Five and 10 times the DAHD were regarded as middle and
highest doses respectively, and were marked here HPA(5), HPB(5), HPC(5) and HPA(10),
HPB(10) and HPC(10), respectively (Figure 1, Tables II and A1). HPs and sterile water were
administered daily for 30 days via the oral route. Housing, feeding, dosing of animals and daily
observations were carried out as described in the Organisation for Economic Co-operation and
Development’s (OECD 407) guideline. The number of animals assigned per group was in line with
the 3R principle (reduce, refine and replace the use of animals). Care was also taken to avoid
inflicting suffering and pain to the test animals in line with international principles and standards.

The Animal Research: Reporting of In Vivo Experiments (ARRIVE) guidelines as well as the
declaration of Helsinki (revised in 2013) were also obeyed.
### Table II: Dosage forms used in this study compared to daily adult human doses (DAHD)

| Herbal product | Given code | Dosage in test animals (mg/kg body weight) | Dosage in test animals compared to DAHD (daily dosage for 70 kg man) |
|----------------|------------|-------------------------------------------|---------------------------------------------------------------|
| Control group  | Control    | Normal saline                             | 0                                                             |
| Herbal product 1 | HPA(1)     | 469.98                                    | 1x                                                            |
|                | HPA(5)     | 2349.91                                   | 5x                                                            |
|                | HPA(10)    | 4699.80                                   | 10x                                                           |
| Herbal product 2 | HPB(1)     | 399.96                                    | 1x                                                            |
|                | HPB(5)     | 1999.80                                   | 5x                                                            |
|                | HPB(10)    | 3999.60                                   | 10x                                                           |
| Herbal product 3 | HPC(1)     | 774.00                                    | 1x                                                            |
|                | HPC(5)     | 3870.00                                   | 5x                                                            |
|                | HPC(10)    | 7740.00                                   | 10x                                                           |

HPA(1) is the least dose of herbal preparation ‘A’ and equivalent to DAHD indicated, 5 times

HPA(5) and 10 times HPA(10) is the middle and highest doses for herbal preparation ‘A’ respectively. It was repeated for herbal products ‘B’ and ‘C’ with respect to their corresponding DAHD as indicated.

#### 2.4 General observations

Once every day after dosing and during the entire study period, the rats were observed for signs of toxic effects of the test products. The observations include rats feeding, fur colour, self-isolation, signs of pains and death.

#### 2.5 Assessment of haematological parameters
Blood samples for haematology were collected into tubes containing sodium EDTA (1% sodium EDTA in distilled water). Red blood cell (RBC), white blood cell (WBC), granulocyte (GRA), lymphocyte (LYM) counts, haemoglobin (HGB), hematocrit (HCT), mean corpuscular haemoglobin (MCH), mean corpuscular volume (MCV), mean corpuscular haemoglobin concentration (MCHC), platelet (PLT) count, platelet distribution width (PDW), mean platelet volume (MPV) and platelet larger cell ratio (P-LCR) were estimated using haem automated analyzer (Cell Dyne: Model 331430, Abbott Laboratories, IL, USA).

2.6 Biochemical analysis

Rats were weighed 12 hours prior to euthanasia with chloroform and blood samples were collected via cardiac puncture. Five mL of blood was collected into gel separator sample tubes, allowed to clot and centrifuged at 3000 rpm for 15 min. The serum samples were separated, stored at -20°C and used for determination of biochemical parameters using automated biochemistry analyser (ATAC 8000, Elan Diagnostics, CA, USA). Biochemical parameters determined were bilirubin, alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP), total protein (TP), albumin (ALB), globulin (GLO), blood urea nitrogen (BUN), creatinine (CREA), glucose (GLU), low density lipoprotein cholesterol (LDL-C), very low-density lipoprotein cholesterol (VLDL-C), high-density lipoprotein cholesterol (HDL-C), triglyceride (TG) and total cholesterol (CHOL).

2.7 Sperm count and fertility analysis

The analysis was carried out following standard procedures as described by Sreedhar and colleagues. Briefly, small portion of cauda epididymis was cut and crushed in 1 mL of 37°C neutral buffer solution of sodium carbonate (NaHCO₃) to make a homogeneous mixture. Then, 2-3 drops of nigrosine stain were added into the mixture and 10 μL of the homogenate sample was
pipetted on a pre-warmed slide. A minimum of 10 fields were observed to evaluate the sperm count under the high-power light microscope at 40X magnification.

2.8 Histology

Major vital organs, liver, heart, kidney, spleen, lungs and testis, were isolated, weighed and fixed in 10% v/v neutral buffered formalin and processed for histology. Briefly, small portion of each of the organs was carefully excised, dehydrated in graded alcohol and embedded in paraffin. Sections (4-10 µm thick) were prepared, stained with haematoxylin and eosin and mounted on neutral DPX medium. Examination of the slides was done using light microscope.

2.9 Data Analysis

Results were analysed using one-tail ANOVA at 95% confidence interval. Turkey’s post hoc test was carried out on the data using SPSS version 21. GraphPad prism version 8.0 was used for the graphical analysis and data were presented in charts and tables.

Ethical clearance

The study was conducted at the Department of Biochemistry, University of Cape Coast, Ghana. The study was approved by the Research Review Board of the Texila American University and the Institutional Review Board of the University of Cape Coast (UCCIRB). Ethical clearance for study was issued by the UCCIRB (ethical clearance approval number: UCCIRB/EXT/2017/07). The animal study was conducted in line with the Animal Research Reporting of In Vivo Experiments (ARRIVE) guidelines as well as the declaration of Helsinki (revised in 2013).
3.0 Results

3.1 Macroscopic assessment

Regular assessment of general drinking, feeding, appearance and exploratory behaviours (rearing and grooming) during toxicological investigations are important to detect toxicity-related behavioural changes that may be associated with the studied bioactive substance(s).

In this study, general observation from macroscopic assessment of rats during the study did not show any adverse treatment-related changes in their feeding, exploratory behaviours and drinking habits. Incisor heights, fur colour and appearance were also normal.

3.2 Body weight

Administration of certain xenobiotic substances may interfere with the normal feeding and drinking, disruption of gastrointestinal system and hormonal or enzymatic system interference. Feeding deterrents tend to decrease food intake and may lead to growth retardation or loss in body weight. In this study we monitored the weights of the rats to assess the effect of the herbal products on feeding and normal growth.

Generally, we observed weekly increases in weights across the groups for the first three weeks (Figure A1). Weight loss was observed across most of the groups in the fourth week except for the control and the first dose levels of both HPB and HPC. Continual weight loss was also observed in the highest dose group of HPB throughout the study. There were no statistical differences between the control and the compared groups.

3.3 Parameters of haematological function

Therapeutic substances may produce adverse effects that may interfere with immunological process, inhibiting the action of important hormones, enzymes and by means of interfering with normal haematopoiesis. Bioactive substances may inversely influence haematopoiesis resulting in
different forms of anaemia. The body also increase production of different forms of white blood cells in response to xenobiotics as part of normal body’s defence mechanism. Haematological parameters were, therefore, assessed in the present studies to determine the possible toxic effects of the herbal preparations on haematological function.

An increase in white blood cell count (WBC) from 9.67 ± 2.50 HPA(5) to 10.67±1.24 HPA(10) was observed (Table B.1). Substantial increase in mixed cell count (MXD) consisting of monocytes, eosinophils, basophils (%) was also observed from 13.83 ±3.19 HPA(5) to 20.10 ±6.93 HPA(10) and 11.83 ± 3.08 of HPB(5) to 17.13 ± 2.77 of HPB(10) (Table B.2). WBC increased from 8.57 ± 1.85 in HPB(1) to 15.07 ± 2.67 in HPB(5). Substantial MXD (%) increase was also observed from 13.83 ±3.19 HPA(5) to 20.10 ±6.93 in the highest dosed of HPA(4699.80 mg/kg body weight). These increases, however, were statistically not significant when compared to the control group. The HPC did not have significant impact on the haematological parameters when compared to the control group (Table B.3).

Table 1: Summary of weekly weight change and haematology results.

| Parameter     | HPA(1), HPA(5) and HPA(10) | HPB(1), HPB(5) and HPB(10) | HPC(1), HPC(5) and HPC(10) |
|---------------|----------------------------|----------------------------|-----------------------------|
| BWC W1        | NSD                        | NSD                        | NSD                         |
| BWC W2        | NSD                        | NSD                        | NSD                         |
| BWC W3        | NSD                        | NSD                        | NSD                         |
| BWC W4        | NSD                        | NSD                        | NSD                         |
| HCT (%)       | NSD                        | NSD                        | NSD                         |
| MCV (fL)      | NSD                        | NSD                        | NSD                         |
| MCH (pg)      | NSD                        | NSD                        | NSD                         |
| MCHC (g/dL)   | NSD                        | NSD                        | NSD                         |
| Platelet (x10^3/µL) | NSD                  | NSD                        | NSD                         |
| Parameter               | Control | Dosed | Dosed | Dosed |
|-------------------------|---------|-------|-------|-------|
| Lymphocytes (%)         | NSD     | NSD   | NSD   | NSD   |
| MXD (%)                 | NSD     | NSD   | NSD   | NSD   |
| Neutrophils (%)         | NSD     | NSD   | NSD   | NSD   |
| LYM #(x 10^3)           | NSD     | NSD   | NSD   | NSD   |
| MXD #(x 10^3)           | NSD     | NSD   | NSD   | NSD   |
| NEUT #(x 10^3)          | NSD     | NSD   | NSD   | NSD   |
| RDW_SD (fL)             | NSD     | NSD   | NSD   | NSD   |
| RDW.CV (fL)             | NSD     | NSD   | NSD   | NSD   |
| PDW (fL)                | NSD     | NSD   | NSD   | NSD   |

‘NSD’ represent no significant difference between the control group and the dosed group at 95% CI. Key: BWC W represent body weight change for weeks (1, 2, 3 and 4). Red blood cell count (RBC), white blood cell count (WBC), granulocyte count (GRA), lymphocyte count (LYM), haemoglobin (HGB), hematocrit (HCT), mean corpuscular haemoglobin (MCH), mean corpuscular volume (MCV), mean corpuscular hemoglobin concentration (MCHC), platelet count (PLT), platelet distribution width (PDW), mean platelet volume (MPV) and platelet larger cell ratio (P-LCR), Mixed cell count (MXD) consisting of monocytes, eosinophils, basophil.

3.4 Parameters of biochemical function

Aspartate aminotransferase (AST) is primarily found in the cytoplasm and mitochondria of cardiac muscle, liver and skeletal muscle. Alanine aminotransferase (ALT), on the other hand, is found primarily in the cytosol of hepatic cells. During necrosis, liver injury or alteration in hepatocellular permeability, liver enzymes leaks into the blood stream and serum levels of these enzymes, therefore, serve as good markers for assessment of hepatotoxicity. AST and the specific liver enzyme, ALT, are used to assess the integrity of the hepatic cells since they give indication of the degree of hepatocytes degeneration. Indirect bilirubin is formed from the breakdown of RBC’s haemoglobin. It is conjugated to glucuronic acid in the liver (direct bilirubin) and excreted via bile. Bilirubin test is important to access degree of RBC haemolysis and the catabolic function of the liver during bioactive toxicity studies. The kidney plays important role in excreting waste substances including bilirubin and urea from the body. In addition, the liver also plays crucial role in protein and lipid biosynthesis in the body. About 80% of cholesterol used in the mammalian
body is endogenously made. Most cells of the body synthesise cholesterol for their own usage while the liver’s cholesterol is for transportation and other purposes. Most serum endogenous cholesterol therefore has the liver as its origin and their levels may, among other things, tell about the synthetic ability of the liver after drug administration.

Creatinine (the waste product from the normal wear and tear on muscles of the body) and blood urea nitrogen (by-product of protein metabolism) are cleared regularly by the kidney. The levels of serum creatinine and blood urea nitrogen are therefore useful indicators of kidney function. We observed that indirect bilirubin levels increased with dose for HPA but decreased with increasing doses of HPB. Albumin, total and direct bilirubin levels also decreased with increasing doses for HPA. A significantly low AST activity was observed in the middle-dose level of HPA and at all dose levels of HPC when compared with the control group (Figure 2). The effects of HPA and HPB on AST and ALT activities were not dose related. Albumin level was also significantly low in HPB(5) dose group (Figure 1). The least, middle and the highest dose groups of AST were all significantly low when compared to the control group (Figure 1). Low density lipoprotein cholesterol (LDL-C) level non-significantly increased at the dose of HPB(10) while VLDL-C level substantially reduced for HPB(10) group. Reduced HDL-C and total cholesterol levels were also observed for HPB(5). Fasting blood sugar levels for HPB(1) and HPB(10) were significantly higher than in the control group (Figure 2). Most of the lipid parameters for rats in the HPC groups were not significantly different from those of the control group (Figure 2). Similarly, serum creatinine and blood urea nitrogen of all dose groups of HPA, HPB and HPC were not significantly different from the control group (Figure 2).
Figure 1: Liver enzymes, serum proteins and bilirubin. Values are expressed as mean ± SEM. Statistical difference between the test group and the control at 95% CI is marked with * at P-values less than 0.05.
Figure 2: Lipid profile, blood sugar and sperm count. Values are expressed as mean ± SEM. Statistical difference between the test group and the control at 95% CI is marked with * at P-values less than 0.05.
3.5 Relative organ weight

Toxic effects exerted by certain bioactive substances are capable of inducing inflammatory responses in different tissues and organs leading to damage. Inflammation in essential organs may lead to increase in weight and higher organ-to-body weight ratios compared to the normal organs. Organ weights and organ-to-body weight ratio comparison between the treated groups and the control has conventionally been used to evaluate toxic effects of bioactive products. The Society of Toxicologic Pathology considers organ weight evaluation to be an important screening tool in characterizing the toxicity of a bioactive substances in general toxicity studies. In the present study the weights of the heart, liver, kidney, spleen and testis and their relative weights with regards to the body weights were evaluated.

The relative kidney weight of rats in the least dose group of HPC (774.00 mg/kg body weight) was significantly lower than those of the control group. Similarly, the relative lung weight of rats in the highest dose group of HPB (3999.60 mg/kg body weight) was significantly higher when compared with the control group (Figure 3). However, the weights of the heart, the liver, the spleen and the testis of the test groups were not significantly different from the control group.
Figure 3: Relative organ weights per 100g body weight. Values are expressed as mean ± SEM. Statistical difference between the test group and the control at 95% CI is marked with *.

3.6 Histologic assessment

Analysis of the microscopic structure of essential tissues and organs is often recommended in toxicity or safety assessment of bioactive substances. Such histological evaluation is crucial in detecting presymptomatic toxic effects that may not be observed in biochemical analysis. In this
study, we examined the microscopic structures of the heart, lung, liver, kidney, spleen and testis of the experimental animals after exposure to the HMPs.

Infertility among married couples is on the increase in Ghana\textsuperscript{94-95} and finding the cause is a concern to all public health practitioners. Certain bioactive substances impair fertility by adversely affecting spermatogenesis. We therefore, assessed sperm quality and quantity in the present study in order to determine the impact of the herbal preparations on fertility of the male rats.

**Liver:** Liver sections of control rats revealed some level of congestion within the central veins (Figure 4A). Sections from the liver of rats that received HPA(1-10) exhibited congestion within the central veins, sinusoids, large vessels and under the capsule (Figure 4B-D). In addition, foci of necrosis without evidence of inflammation were observed in HPA(1) dose group. Also, scattered individual hepatocytes that showed intracytoplasmic fat globules were seen in rats that received HPA(5) and HPA(10). The liver of rats given HPB(1) exhibited central vein congestion (Figure 4E). Also, liver section from rats administered with HPB(5) and HPB(10) showed normal architecture with congestion of central veins and sinusoids (Figure 4F & 4G). In addition, the hepatocytes of rats in the HPB(5) group revealed prominent foci of fatty changes with mainly microvesicular conformation. Rats in the HPC(1, 5,10) dose groups (Figure 4 H-J) revealed liver sections with congestion within the central veins, sinusoids, large vessels and under the capsule. In addition, a focus of foamy hepatocytes was observed in rats treated with HPC(5).

**Kidney:** Control rats and the group that received HPB(1) showed normal renal architecture with normal glomeruli. Renal tubules and collecting ducts showed mild congestion within their stroma (Figure 4K and 4O). All HPA, HPB and HPC dose groups except HPB(1) showed renal tubules and collecting ducts with mild congestion within their stroma and within the glomeruli (Figure 4). In addition, there were foci of chronic inflammatory change in the highest dose group of HPC (Figure 4T).
**Heart:** Scattered intensely congested heart vessels, without evidence of inflammation, infarction or fibrosis were observed at all dose levels of HPA (Figure 4V-X) and HPC(1, 5, 10) - treated groups (Figure 4Ab-Ad). Intensely eosinophilic muscle fibres, haemorrhage with associated amorphous exudates and isolated muscle fibres as well as scattered intensely congested vessels were seen in the heart section of rats that received HPB(10) (Figure 4Aa).
Figure 4: Photomicrographs of the liver, kidney and heart for the sub-chronic toxicity studies of Sprague-Dawley rats treated with either sterile water, HPA, HPB or HPC for 30 days. Magnification, ×100. Hematoxylin-eosin stain was used.
**Lungs:** Lung sections of rats that received clean water showed foci of sloughing within the airway (Figure 5A). Evidence of chronic inflammation and groups of chronic inflammatory cells filling alveolar spaces and foci of sloughing within the airway were observed in HPA(1) treated rats (Figure 5B). Rats in the HPA(5) group had lungs that showed chronic inflammatory changes dominated by lymphocytes and macrophages with sloughing of epithelial cells. The alveolar spaces were obscured by debris and chronic inflammatory cells (Figure 5C). Lung of rats given HPA(10) also showed moderate chronic inflammatory changes dominated by lymphocytes and macrophages with sloughing of epithelial cells in some areas. The alveolar spaces are obscured by debris and chronic inflammatory cells (Figure 5D). Rats that received HPB(1) showed lung exhibiting thickened alveolar and chronic inflammatory cells with congestion within the lung parenchyma. There was also evidence of non-specific chronic inflammation (Figure 5E). Sections of lung from rats that received HPB(5) showed alveolar with evidence of chronic inflammation in some areas with groups of chronic inflammatory cells. There were foci of sloughing within the airway (Figure 5F). The lung section of rats treated with HPB(10) exhibited alveolar that showed little evidence of inflammation in some areas and foci of sloughing within the airway (Figure 5G). Alveolar with evidence of moderate chronic inflammation in many areas with groups of chronic inflammatory cells filling alveolar spaces were observed in lung section of rats that received HPC (Figure 5H-J). In addition, there were foci of sloughing observed within the airway of HPC(1, 5 &10) treated groups.

**Spleen:** The herbal products (HPA, HPB and HPC) did not affect the histology of the spleens of the rats when compared with the control group (Figure 5K-T).

**Testis:** Sections of testis from control rats and from rats that received different doses of HPA, HPB and HPC showed normal architecture with seminiferous tubules containing cells at all stages of spermatogenesis without any evidence of inflammation or adverse drug effect (Figure 5U-Ad).
Figure 5: Photomicrographs of the lung, spleen, and testis for the sub-chronic toxicity studies of Sprague-Dawley rats treated with either sterile water, HPA, HPB, or HPC for 30 days. Magnification, ×100. Hematoxylin-eosin stain was used.
4.0 Discussion

Herbal medicinal products (HMPs) are commonplace on the Ghanaian market and their use is on the increase due to the growing demand for nature-based products. In spite of the huge patronage and extensive use of HMPs for management of various ailments in Ghana, information regarding their safety is seldom available. The paucity of scientific data on the possible harmful effects of these multi-herbal products prompted the present investigation in which we evaluated the safety of the three most patronized anti-malarial HMPs in the Kumasi metropolis of Ghana in an experimental model of sub-chronic toxicity study. Data from this study did not reveal any significant impairment of haematological function or treatment-related deaths in the various test groups. Red blood cell count and white blood cell count with differential analysis did not reveal any signs of macrocytic or microcytic anaemia (Table B.1-3) in all groups that received the herbal products. While slight elevation was observed in the RBC counts of some groups, the values were not significantly different from those of the control group and as such was considered toxicologically not important. Similarly, HPB (middle and high dose levels) and HPC (low and middle dose levels) increased WBC counts, although values were not statistically different from those of the control. Macroscopic examination of gastrointestinal tract after animals were sacrificed and dissected revealed mild ulcerations in the small intestines of rats that received HPB (high dose).

Our result also showed that sub-chronic administration of HPA (at low and middle doses) and HPC (at all doses) significantly decrease AST in the rats (Figure 1d). These findings suggest either a liver protective effect, chronic toxicity effect or death of the liver cells due to extensive toxic effect of the HPs. It could also be a mixture of some of these effects since the products have been stated to comprise 3-5 different herbal plants with different pharmacological properties. The argument for a possible liver protective effect by HPC is partly supported by previous studies which found one of the active ingredient of HPC ‘Phyllanthus fraternus’ (Table I) to possess anti-
hepatotoxic activity,\textsuperscript{83,96-97} justifying its use in traditional medicine for the treatment of jaundice. This assertion on HPC also correlates well with a clinical trial of the same product which was published at the time this current study was ongoing.\textsuperscript{98} On the other hand, the argument for a possible chronic liver toxicity by the HPs is supported by the previously identified chemical toxicants in these products.\textsuperscript{20,85,99} Histologic findings from this study however supports the induction of liver toxicity by the HMPs. For instance, congestion of sinusoids, within the large vessels and under the capsule were observed in all dose groups of HPA, HPC and HPB(5 and 10).

There were foci of necrosis in HPA(1), hepatocytes with intracytoplasmic fat globules in HPA(5 and 10), hepatocytes with prominent foci of fatty change with mainly microvesicular conformation in HPB(5) group and congestion within the large vessels and under the capsule and a focus of foamy hepatocytes observed in HPC(5). In addition, the fat globules observed in HPA(5 and 10) affected the liver’s synthetic ability leading to a significantly low albumin level in HPA(5). The assertion was strengthened by the fact that the albumin/globulin ratio of HPA (5 and 10) group was much less when compared with control group. A component of the HPC ‘\textit{Vitex grandifolia}’ (Table I) had previously been identified to be toxic to Sprague-Dawley rats\textsuperscript{79} and this conforms well with the toxic observations made in this study.

The fasting blood sugar levels for rats treated with low and high doses of HPB (1 and 10) were significantly higher when compared with the control group. This hyperglycaemia may suggest possible interference with glucose metabolism or injury to the pancreas and a possible predisposition of the rat to diabetes mellitus. The association of hyperglycaemia and development of diabetes mellitus has been well studied.\textsuperscript{100} By extension, since the least dose of HPB(1) (which is the equivalent dose recommended for human use), it is likely that chronic exposure at this dose may predispose the consumer to the risk of diabetes mellitus.

Furthermore, rats administered with HMPs (at all dose levels) exhibited signs of moderate lung toxicity (Fig. 3C). This is evident from lung histology that revealed signs of pulmonary
toxicity characterized by inflammation in all dose groups of HPA and HPB, in addition to congestion and presence of chronic inflammatory cells in rats that received HPC. In addition, the relative weight of the lungs of HPB(10) group was significantly higher than that of the control group. In our recent study, we identified HPs(A, B and C) to have been contaminated with nickel, lead and chromium (residual contents greater than MRL values), and previous studies have also linked Ni overexposure to lung injury, inflammation, fibrosis and cancer of the respiratory tract. Considering the dose-related inflammatory changes in rat lungs and other signs of pulmonary toxicity, it is possible, therefore, that exposure to the Ni content of the HPs may contribute to the observed lung toxicity.

Data from this study further revealed that rats treated with HPB(10) also exhibited gastrointestinal lesions, especially in the small intestine. GIT lesions are typical signs of chromium (VI) toxicity. The induction of GIT lesions by HPB seems to correlate with our recent study where we reported the presence of significantly high levels of Cr above MRL limit in this herbal preparation. Similar morphological changes were observed in the lungs of rats that received HPC with the alveolar showing inflammatory changes dominated by lymphocytes and macrophages and alveolar spaces obscured by debris and chronic inflammatory cells which could be attributed to the heavy metal contaminations previously identified at concentrations above their respective MRL values.

We also observed signs of mild kidney toxicity in rats administered with HPA and HPB (at all dose levels) and moderate kidney toxicity in rats given HPC(1 -10), characterized by congestion within the glomerular capillary and the stroma of the renal tubules and collecting ducts. Foci of chronic inflammation were also seen in rats that received HPC(10). In addition, the relative kidney weight of the rats that received HPC(1) was significantly lower than those of the control group (Figure 4T). The chronic inflammation and possible occurrence of hypoplasia could explain the significantly low relative kidney weight in HPC(1). The observed foci of chronic
inflammation in the present study may be related to the presence of heavy metals (arsenic, chromium and nickel) and pesticides (aldrin and dieldrin) as previously stated and reported in our recent article.\textsuperscript{20} This also correlates with previous findings on chromium (VI),\textsuperscript{102} arsenic,\textsuperscript{106} and aldrin\textsuperscript{101} toxicities.

We observed that the HMPs (HPA, HPB and HPC), at the dose given and duration of administration in this study, did not exert any adverse influence on sperm characteristics. This is evident in the similarity between sperm count and sperm morphology of test rats and those of the control group.

Sub-chronic administration of HPA(1-10) and HPC(1-10) to rats caused moderate myocardial toxicity marked by scattered but intensely congested vessels. The heart sections of rats given HPB(10) show foci of intensely eosinophilic muscle fibres, haemorrhage with associated amorphous exudates, isolated muscle fibres and scattered intensely congested vessels. This effect may in partly be attributable to the high concentration of heavy metals like arsenic in these products.\textsuperscript{20} Association of arsenic and inflammatory damage to the vascular system has been reported in literature. Inflammatory changes and vascular congestion are signs typically associated with arsenic heart toxicity.\textsuperscript{106-107} Although the various medicinal plant components of the antimalarial HMPs investigated in this study may be linked to the toxic effects observed in the rats, we strongly believe that the heavy metal and pesticide contaminants found in these herbal preparations are also playing a major role.

5.0 Conclusion

Data from this study suggest the potential of the herbal products, HPA, HPB and HPC, to cause major organ-system dysfunction or damage. The observed toxic effects of these HMPs may be related in part to their contamination with heavy metals and pesticides. The need for alternative, safe drugs for treatment of malaria is huge. We recommend the use of these herbal products with
caution and suggest further assessment of their safety in a chronic model of toxicity. We also recommend good manufacturing as well as safe farming practices to reduce contamination of herbal medicines with heavy metals, pesticides or other pollutants.

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Competing interests

The authors declare that they have no competing interests.

Authors’ contributions

Frank Adusei-Mensah: involved in conception and design of study, data collection and analysis, drafting of manuscript, approval of the final submission of the manuscript. Carina Tikkanen-Kaukanen: reviewed the manuscript, corrected and improved the scientific quality of the manuscript, gave approval for the final submission of the manuscript. Jussi Kauhanen: reviewed the manuscript, corrected and improved the scientific quality of the manuscript, gave approval for the final submission of the manuscript. Isaac Tabiri Henneh: contributed to data collection and analysis and approved the final submission of the manuscript. Phyllis Elsie Owusu Agyei: contributed to data analysis and approved the final submission of the manuscript. Patrick Kafui Akakpo: contributed to data analysis (histopathology) and approved the final submission of the manuscript.
Martins Ekor: involved in conception and design of study, supervision of data collection and analysis, revision of manuscript for important intellectual content, and submission of final manuscript.

Bibliography

1. Anti-Malaria Drug Policy for Ghana, 2nd Revised Version 2009. http://apps.who.int/medicinedocs/en/d/Js18072en/. Accessed November 6, 2017.

2. WHO | Number of malaria deaths. WHO. http://www.who.int/gho/malaria/epidemic/deaths/en/. Accessed December 29, 2017.

3. WHO | Malaria. WHO. http://www.who.int/mediacentre/factsheets/fs094/en/. Accessed April 18, 2018.

4. Koram K, Barcus MJ, Binka FN, et al. Seasonal malaria attack rates in infants and young children in northern Ghana. The American Journal of Tropical Medicine and Hygiene. 2002;66(3):280-286. doi:10.4269/ajtmh.2002.66.280

5. Fehir LG, Asante KP, Afari-Asiedu S, et al. Seeking treatment for uncomplicated malaria: experiences from the Kintampo districts of Ghana. Malar J. 2016;15. doi:10.1186/s12936-016-1151-7

6. WHO | WHO traditional medicine strategy: 2014-2023. WHO. http://www.who.int/medicines/publications/traditional/trm_strategy14_23/en/. Accessed August 13, 2017.

7. Traditional Medicine Growing Needs and Potential - WHO Policy Perspectives on Medicines, No. 002, May 2002. http://apps.who.int/medicinedocs/en/d/Js2293e/. Accessed November 3, 2017.

8. Adusei-Mensah F, Haaranen A, Kauhanen J, et al. Post-Market Safety and Efficacy Surveillance of Herbal Medicinal Products from Users’ Perspective: A Qualitative Semi-Structured Interview Study in Kumasi, Ghana. Int J Pharm Pharmacol. 2019;3(136). doi:10.31531/25813080.1000136

9. Ekor M. The growing use of herbal medicines: issues relating to adverse reactions and challenges in monitoring safety. Front Pharmacol. 2014;4:177. doi:10.3389/fphar.2013.00177

10. Agbonon A, Eklu-Gadegbeku K, Aklikokou K, Essien K, Akpagana K, Gbeassor M. The effect of Mangifera indica stem bark and Pluchea ovalis roots on tracheal smooth muscle in vitro. Fitoterapia. 2002;73(7-8):619-622. doi:10.1016/S0367-326X(02)00230-7

11. Amoah LE, Kakaney C, Kwansa-Bentum B, Kusi KA. Activity of Herbal Medicines on Plasmodium falciparum Gametocytes: Implications for Malaria Transmission in Ghana. PLoS One. 2015;10(11). doi:10.1371/journal.pone.0142587

12. Wong LL, Lacar L, Roytman M, Orloff SL. Urgent Liver Transplantation for Dietary Supplements: An Under-Recognized Problem. Transplant Proc. 2017;49(2):322-325. doi:10.1016/j.transproceed.2016.11.041

13. Paes-Leme AA, Motta ES, De JM, Dantas FJ, Bezerra RJ, Caldeira-de-Araujo A. Assessment of Aloe vera (L.) genotoxic potential on Escherichia coli and plasmid DNA. J Ethnopharmacol. 2005;102(2):197-201. doi:10.1016/j.jep.2005.06.013

14. Dunnick JK, Nyska A. The Toxicity and Pathology of Selected Dietary Herbal Medicines. Toxicol Pathol. 2013;41(2):374-386. doi:10.1177/0192623312466451
15. Parasuraman S, Raveendran R, Rajesh NG, Nandhakumar S. Sub-chronic toxicological evaluation of cleistanthin A and cleistanthin B from the leaves of Cleistanthus collinus (Roxb.). *Toxicol Rep.* 2014;1:596-611. doi:10.1016/j.toxrep.2014.08.006

16. Eswarappa S, Chakraborty AR, Palatty BU, Vasnaik M. Cleistanthus collinus poisoning: case reports and review of the literature. *J Toxicol Clin Toxicol.* 2003;41(4):369-372.

17. Cohen PA. American roulette--contaminated dietary supplements. *N Engl J Med.* 2009;361(16):1523-1525. doi:10.1056/NEJMep0904768

18. Ntechpa F, Abakar D, Kom B, Nana P, Hamadjida A, Dimo T. Acute and sub-chronic oral toxicity assessment of the aqueous extract leaves of Ficus glumosa Del. (Moraceae) in rodents. *J Intercult Ethnopharmacol.* 2014;3(4):206-213. doi:10.5455/jiec.20140913021547

19. Rosidah null, Yam MF, Sadikun A, Ahmad M, Akowuah GA, Asmawi MZ. Toxicology evaluation of standardized methanol extract of Gynura procumbens. *J Ethnopharmacol.* 2009;123(2):244-249. doi:10.1016/j.jep.2009.03.011

20. Adusei-Mensah F, Essumang DK, Agjie RO, Kauhanen J, Tikkanen-Kaukanen C, Ekor M. Heavy metal content and health risk assessment of commonly patronized herbal medicinal preparations from the Kumasi metropolis of Ghana. *J Environ Health Sci Engineer.* April 2019. doi:10.1016/s0263-2241(09)00373-y

21. Adusei Mensah F, Henneh TI, Ekor M. Pesticide Residue and Health Risk Analysis of Six Commonly Used Herbal Medicinal Products in Kumasi. *TIJPH.* 2018;6(3). doi:10.21522/TIJPH.2013.06.03.Art018

22. Agyare C, Koffuor GA, Boamah VE, Adu F, Mensah KB, Adu-Amoah L. Antimicrobial and Anti-Inflammatory Activities of Pterygota macrorcarpa and Cola gigantea (Sterculiaceae). *Evid Based Complement Alternat Med.* 2012;2012. doi:10.1155/2012/902394

23. Atofani O, Oguntayo H, Areh ET, Adeyemi OS, Kambizi L. Chemical composition, anti-toxoplasma, cytotoxicity, antioxidiant, and anti-inflammatory potentials of Cola gigantea seed oil. *Pharm Biol.* 2019;57(1):154-160. doi:10.1080/13880209.2019.1577468

24. Nguta JM, Appiah-Opong R, Nyarko AK, Yeboah-Manu D, Addo PGA. Medicinal plants used to treat TB in Ghana. *Int J Mycobacteriol.* 2015;4(2):116-123. doi:10.1016/j.ijmyco.2015.02.003

25. Gandhi GR, Ignacimuthu S, Paulraj MG, Sasikumar P. Antihyperglycemic activity and antidiabetic effect of methyl caffeate isolated from Solanum torvum Swartz. fruit in streptozotocin induced diabetic rats. *Eur J Pharmacol.* 2011;670(2-3):623-631. doi:10.1016/j.ejphar.2011.09.159

26. Challal S, Buenafe OEM, Queiroz EF, et al. Zebrafish bioassay-guided microfractionation identifies anticonvulsant steroid glycosides from the Philippine medicinal plant Solanum torvum. *ACS Chem Neurosci.* 2014;5(10):993-1004. doi:10.1021/cn5001342

27. Ramamurthy CH, Subastri A, Suyavaran A, Subbaiah KCV, Valluru L, Thirunavukkarasu C. Solanum torvum Swartz. fruit attenuates cadmium-induced liver and kidney damage through modulation of oxidative stress and glycosylation. *Environ Sci Pollut Res Int.* 2016;23(8):7919-7929. doi:10.1007/s11356-016-6044-3

28. Lu Y, Luo J, Huang X, Kong L. Four new steroidal glycosides from Solanum torvum and their cytotoxic activities. *Steroids.* 2009;74(1):95-101. doi:10.1016/j.steroids.2008.09.011

29. Asase A, Akwetey GA, Achel DG. Ethnopharmacological use of herbal remedies for the treatment of malaria in the Dangme West District of Ghana. *J Ethnopharmacol.* 2010;129(3):367-376. doi:10.1016/j.jep.2010.04.001

30. Abdul Rahuman A, Gopalakrishnan G, Venkatesan P, Geetha K. Isolation and identification of mosquito larvicidal compound from Abutilon indicum (Linn.) Sweet. *Parasitol Res.* 2008;102(5):981-988. doi:10.1007/s00436-007-0864-5

31. Hashemi SR, Zulkifli I, Hair Bejo M, Farida A, Somchit MN. Acute toxicity study and phytochemical screening of selected herbal aqueous extract in broiler chickens. *Int J Pharmaco.* 2008;4(5):352-360.
32. Mohan M, Kamble S, Gadhi P, Kasture S. Protective effect of Solanum torvum on doxorubicin-induced nephrotoxicity in rats. *Food Chem Toxicol.* 2010;48(1):436-440. doi:10.1016/j.fct.2009.10.042

33. Ngulefack TB, Mekhfi H, Dongmo AB, et al. Hypertensive effects of oral administration of the aqueous extract of Solanum torvum fruits in L-NNAME treated rats: Evidence from in vivo and in vitro studies. *Journal of Ethnopharmacology.* 2009;124(3):592-599. doi:10.1016/j.jep.2009.04.057

34. Makinde JN, Amusan OO, Adesogan EK. The antimalarial activity of Spathodea campanulata stem bark extract on Plasmodium berghei berghei in mice. *Planta Med.* 1988;54(2):122-125. doi:10.1055/s-2006-962367

35. Agyare C, Asase A, Lechtenberg M, Niehues M, Deters A, Hensel A. An ethnopharmacological survey and in vitro confirmation of ethnopharmacological use of medicinal plants used for wound healing in Bosomtwi-Atwima-Kwanwoma area, Ghana. *J Ethnopharmacol.* 2009;125(3):393-403. doi:10.1016/j.jep.2009.07.024

36. Akharaiyi FC, Boboeye B, Adetuyi FC. Study of Acute and Sub Chronic Toxicity of Spathodea campanulata P Beav Leaf. *IPCBE.* 2012;41.

37. Iloodgwe EE, Akah PA, Nworu CS. Evaluation of the Acute and Subchronic Toxicities of Ethanol Leaf Extract of Spathodea campanulata P. *Beauv.* International Journal of Applied Research in Natural Products. 2010;3(2):17-21.

38. Mann A, Ifarajimi OR, Adewoye AT, et al. In vivo antitrypanosomal effects of some ethnomedicinal plants from Nupeland of north central Nigeria. *Afr J Tradit Complement Altern Med.* 2011;8(1):15-21.

39. Mustapha S, Dauda BEN, Ikuya YA, Mathew TJ, Aliyu IA, Shaba EY. Removal of Heavy Metals from Aqueous Solutions by Modified Activated Carbon from Bombax buonopozense. *IJIESI.* 2014;3(8):17-24.

40. Abay SM, Lucantoni L, Dahiya N, et al. Plasmodium transmission blocking activities of Vernonia amygdalina extracts and isolated compounds. *Malar J.* 2015;14:288. doi:10.1186/s12936-015-0812-2

41. Omorogie ES, Pal A. Antiplasmodial, antioxidant and immunomodulatory activities of ethanol extract of Vernonia amygdalina del. Leaf in Swiss mice. *Avicenna J Phytomed.* 2016;6(2):236-247.

42. Yedjou CG, Sims JN, Njiki S, Tsabang N, Oungbe IV, Tchounwou PB. VERNONIA AMYGDALINA DELILE EXHIBITS A POTENTIAL FOR THE TREATMENT OF ACUTE PROMYELOCYTIC LEUKEMIA. *Glob J Adv Eng Technol Sci.* 2018;5(8):1-9. doi:10.5281/zenodo.1343591

43. Johnson W, Tchounwou PB, Yedjou CG. Therapeutic Mechanisms of Vernonia amygdalina Delile in the Treatment of Prostate Cancer. *Molecules.* 2017;22(10). doi:10.3390/molecules22101594

44. Adeesanoye OA, Farombi EO. Hepatoprotective effects of Vernonia amygdalina (astereaceae) in rats treated with carbon tetrachloride. *Exp Toxicol Pathol.* 2010;62(2):197-206. doi:10.1016/j.etp.2009.05.008

45. Imafidon CE, Olukiran OS, Ogundipe DJ, Eluwole AO, Adekunle IA, Oke GO. Acetonic extract of Vernonia amygdalina (Del.) attenuates Cd-induced liver injury: Potential application in adjuvant heavy metal therapy. *Toxicol Rep.* 2018;5:324-332. doi:10.1016/j.toxrep.2018.02.009

46. Okwuzu JO, Odeiga P, AdetoroOtubanjo O, Ezechi OC. Cytotoxicity testing of aqueous extract of bitter leaf (Vernonia amygdalina Del) and sniper 1000EC (2,3 dichlorovinyl dimethyl phosphate) using the Allium cepa test. *Afr Health Sci.* 2017;17(1):147-153. doi:10.4314/ahs.v17i1.19

47. Ojiako OA, Nwanjo HU. Is Vernonia amygdalina hepatotoxic or hepatoprotective? Response from biochemical and toxicity studies in rats. *African Journal of Biotechnology.* 2006;5(18). doi:10.4314/ajb.v5i18.55812

48. Sharma M, Agrawal SK, Sharma PR, Chadha BS, Khosla MK, Saxena AK. Cytotoxic and apoptotic activity of essential oil from Ocimum viride towards COLO 205 cells. *Food and Chemical Toxicology.* 2010;48(1):336-344. doi:10.1016/j.fct.2009.10.021
Ihejirika GO. Determination of anti-microbial properties of Ocimum viride concentrations on Rhizopus stolonifer infection and germination of soybean (Glycine max L. Merill). *Archives of Phytopathology and Plant Protection*. 2011;44(19):1894-1900. doi:10.1080/03235408.2010.505798

Bedri S, Khalil EA, Khalid SA, et al. Azadirachta indica ethanolic extract protects neurons from apoptosis and mitigates brain swelling in experimental cerebral malaria. *Malar J*. 2013;12:298. doi:10.1186/1475-2875-12-298

Lucantoni L, Yerbangsa RS, Lupidi G, Pasqualini L, Esposito F, Hlabuuetzel A. Transmission blocking activity of a standardized neem (Azadirachta indica) seed extract on the rodent malaria parasite Plasmodium berghei in its vector Anopheles stephensi. *Malar J*. 2010;9:66. doi:10.1186/1475-2875-9-66

Saleem S, Muhammad G, Hussain MA, Bukhari SNA. A comprehensive review of phytochemical profile, bioactives for pharmaceuticals, and pharmacological attributes of Azadirachta indica. *Phytother Res*. 2018;32(7):1241-1272. doi:10.1002/ptr.6076

Gupta SC, Prasad S, Tyagi AK, Kunnunakkara AB, Aggarwal BB. Neem (Azadirachta indica): An indian traditional panacea with modern molecular basis. *Phytomedicine*. 2017;34:14-20. doi:10.1016/j.phymed.2017.07.001

Deng Y, Cao M, Shi D, et al. Toxicological evaluation of neem (Azadirachta indica) oil: Acute and subacute toxicity. *Environmental Toxicology and Pharmacology*. 2013;35(2):240-246. doi:10.1016/j.etap.2012.12.015

Gandhi M, Lal R, Sankaranarayanan A, Banerjee CK, Sharma PL. Acute toxicity study of the oil from Azadirachta indica seed (neem oil). *Journal of Ethnopharmacology*. 1988;23(1):39-51. doi:10.1016/0378-8741(88)90113-4

Bh A. The toxicity of Azadirachta indica leaves in goats and guinea pigs. *Vet Hum Toxicol*. 1987;29(1):16-19.

Okokon JE, Udokpoh AE, Antia BS. Antimalaria activity of crude extracts of Tetrapleura tetraptera and Copaifera religiosa. *Malar J*. 2011;10:18. doi:10.1186/1475-2875-10-18

Lekana-Douki JB, Oyegue Liabagui SL, Bongui JB, Zatra R, Lebibi J, Toure-Toure S, et al. Antimalarial activity of ethanolic extract of Tetrapleura tetraptera fruit. *Phytother Res*. 2011;4:506. doi:10.1002/ptr.6077

Ojewole JAO, Adewummi CO. Anti-inflammatory and hypoglycaemic effects of Tetrapleura tetraptera (Taub) fruit aqueous extract in rats. *J Ethnopharmacol*. 2004;95(2-3):177-182. doi:10.1016/j.jep.2004.06.026

Ojewole JAO. Analgesic and anticonvulsant properties of Tetrapleura tetraptera (Taub) (Fabaceae) fruit aqueous extract in mice. *Phytother Res*. 2005;19(12):1023-1029. doi:10.1002/ptr.1779

Awe SO, Adewummi CO, Irainloye TA, Ojewole JAO, Olubummi PA, Becker W. Toxicological evaluation of Aridan, tetrapleura tetraptera (Mimosaceae), a molluscicide. *Toxicological & Environmental Chemistry*. 1995;51(1-4):61-68. doi:10.1080/0378878950800750938226

Edet DI, Ikpi GU. Toxicity and Behaviour of *Clarias Gariepinus* (Burchell, 1822) Fingerlings subjected to Piscicidal Plant Extract of Aidon Tetrapleura Tetraptera. *Journal of Applied Sciences and Environmental Management*. 2008;12(3). doi:10.4314/jasem.v12i3.55486

Chukwuocha UM, Fernández-Rivera O, Legorreta-Herrera M. Exploring the antimalarial potential of whole Cymbopogon citratus plant therapy. *J Ethnopharmacol*. 2016;193:517-523. doi:10.1016/j.jep.2016.09.056

Dike IP, Obembe OO, Adebiyi FE. Ethnobotanical survey for potential anti-malarial plants in south-western Nigeria. *J Ethnopharmacol*. 2012;144(3):618-626. doi:10.1016/j.jep.2012.10.002

Francisco V, Costa G, Figueirinha A, et al. Anti-inflammatory activity of Cymbopogon citratus leaves infusion via proteasome and nuclear factor-kB pathway inhibition: contribution of chlorogenic acid. *J Ethnopharmacol*. 2013;148(1):126-134. doi:10.1016/j.jep.2013.03.077
749 66. Boukhatem MN, Ferhat MA, Kameli A, Saidi F, Kebir HT. Lemon grass (Cymbopogon citratus) essential oil as a potent anti-inflammatory and antifungal drugs. Libyan J Med. 2014;9(1):25431. doi:10.3402/ljm.v9.25431

750 67. Silva MR, Ximenes RM, da Costa JGM, Leal LKAM, de Lopes AA, Viana GS de B. Comparative anticonvulsant activities of the essential oils (EOs) from Cymbopogon winterianus Jowitt and Cymbopogon citratus (DC) Stapf. in mice. Naunyn Schmiedebergs Arch Pharmacol. 2010;381(5):415-426. doi:10.1007/s00210-010-0494-9

755 68. Fandohan P, Gnonlonfin B, Laleyie A, Gbenou JD, Darboux R, Moudachirou M. Toxicity and gastric tolerance of essential oils from Cymbopogon citratus, Ocimum gratissimum and Ocimum basilicum in Wistar rats. Food and Chemical Toxicology. 2008;46(7):2493-2497. doi:10.1016/j.fct.2008.04.006

758 69. Prabhu K, Murugan K, Nareshkumar A, Ramasubramanian N, Bragadeeswaran S. Larvicidal and repellent potential of Moringa oleifera against malarial vector, Anopheles stephensi Liston (Insecta: Diptera: Culicidae). Asian Pac J Trop Biomed. 2011;1(2):124-129. doi:10.1016/S2221-1691(11)60009-9

761 70. Adebayo IA, Arsad H, Samian MR. ANTIPROLIFERATIVE EFFECT ON BREAST CANCER (MCF7) OF MORINGA OLEIFERA SEED EXTRACTS. Afr J Tradit Complement Altern Med. 2017;14(2):282-287. doi:10.21010/ajtcam.v14i2.30

766 71. Jaja-Chimedza A, Graf BL, Simmler C, et al. Biochemical characterization and anti-inflammatory properties of an isothiocyanate-enriched moringa (Moringa oleifera) seed extract. PLoS ONE. 2017;12(8):e0182658. doi:10.1371/journal.pone.0182658

768 72. Asare GA, Gyan B, Bugyei K, et al. Toxicity potentials of the nutraceutical Moringa oleifera at suprasupplementation levels. Journal of Ethnopharmacology. 2012;139(1):265-272. doi:10.1016/j.jep.2011.11.009

773 73. Stohs SJ, Hartman MJ. Review of the Safety and Efficacy of Moringa oleifera. Phytother Res. 2015;29(6):796-804. doi:10.1002/ptr.5325

773 74. Anyanwu GO, Nisar-ur-Rehman null, Onyeneke CE, Rauf K. Medicinal plants of the genus Anthocleista—A review of their ethnobotany, phytochemistry and pharmacology. J Ethnopharmacol. 2015;175:648-667. doi:10.1016/j.jep.2015.09.032

774 75. Madubunyi II, Asuzu IU. Pharmacological Screening of Anthocleista nobilis Root Bark. International Journal of Pharmacognosy. 1996;34(1):28-33. doi:10.1076/phbi.34.1.28.13175

778 76. Ngwoke KG, Akwagbulam AG, Erhirhie EO, Ajaghaku DL, Okoye FBC, Esimone CO. Antioxidant, Anti-inflammatory, Analgesic Properties, and Phytochemical Characterization of Stem Bark Extract and Fractions of Anthocleista nobilis. Pharmacognosy Res. 2018;10(1):81-87. doi:10.4103/pr.pr_73_17

780 77. Vitex grandifolia - Useful Tropical Plants. http://tropical.thefarms.info/viewtropical.php?id=Vitex+grandifolia. Accessed April 18, 2018.

783 78. Azokou A, Koné MW, Koudou BG, Tra Bi HF. Larvicidal potential of some plants from West Africa against Culex quinquefasciatus (Say) and Anopheles gambiae Giles (Diptera: Culicidae). J Vector Borne Dis. 2013;50(2):103-110.

784 79. Owolabi MA, Abass MM, Emeka PM, Jaja SI, Nuoli M, Dosa BOS. Biochemical and histologic changes in rats after prolonged administration of the crude aqueous extract of the leaves of Vitex grandifolia. Pharmacognosy Res. 2010;2(5):273-278. doi:10.4103/0974-8490.72322

788 80. Owolabi MA, Abass MM, Emeka PM, Jaja SI, Nuoli M, Dosa BOS. Biochemical and histologic changes in rats after prolonged administration of the crude aqueous extract of the leaves of Vitex grandifolia. Pharmacognosy Res. 2010;2(5):273-278. doi:10.4103/0974-8490.72322

790 81. Rajasubramaniam S, Saradhi PP. Rapid multiplication of Phyllanthus fraternus: a plant with anti-hepatitis viral activity. Industrial Crops and Products. 1997;6(1):35-40. doi:10.1016/S0926-6690(96)00201-4

36
ABSTRACT.

Malamix: A Polyherbal Product for the Treatment of Uncomplicated Malaria in Ghana

ARTICLE INFO

W Tetteh A, Mensah F, O Boadu K, et al. Clinical Evaluation of the Safety and Effectiveness of Adutwumwaa Malamix: A Polyherbal Product for the Treatment of Uncomplicated Malaria in Ghana

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82. Sailaja R, Setty OH. Protective effect of Phyllanthus fraternus against allyl alcohol-induced oxidative stress in liver mitochondria. *J Ethnopharmacol*. 2006;105(1-2):201-209. doi:10.1016/j.jep.2005.10.019

83. Gopi S, Setty OH. Protective effect of Phyllanthus fraternus against bromobenzene induced mitochondrial dysfunction in rat liver mitochondria. *Food Chem Toxicol*. 2010;48(8-9):2170-2175. doi:10.1016/j.fct.2010.05.024

84. Lata S, Singh S, NathTiwari K, Upadhyay R. Evaluation of the Antioxidant and Hepatoprotective Effect of Phyllanthus fraternus Against a Chemotherapeutic Drug Cyclophosphamide. *Appl Biochem Biotechnol*. 2014;173(8):2163-2173. doi:10.1007/s12010-014-1018-8

85. Adusei-Mensah F, Henneh IT, Ekor M. Pesticide Residue and Health Risk Analysis of Six Commonly Used Herbal Medicinal Products in Kumasi, Ghana. *Trop PH*. 2018;6(3):186-195. doi:10.21522/TIJPH.2013.06.03.Art018

86. Sreedhar Naik B, Dangi NB, Sapkota HP, et al. Phytochemical screening and evaluation of anti-fertility activity of Dactyloctenium aegyptium in male albino rats. *Asian Pacific Journal of Reproduction*. 2016;5(1):51-57. doi:10.1016/j.apjr.2015.12.009

87. Lubran MM. Hematologic side effects of drugs. *Ann Clin Lab Sci*. 1989;19(2):114-121.

88. Wang X, Zhang W, Wang Y, et al. Acute and sub-chronic oral toxicological evaluations of quinocetone in Wistar rats. *Regulatory Toxicology and Pharmacology*. 2010;58(3):421-427. doi:10.1016/j.yrtph.2010.08.008

89. Han J-S, Lee B-S, Han S-R, et al. A subchronic toxicity study of Radix Dipsaci water extract by oral administration in F344 rats. *Regulatory Toxicology and Pharmacology*. 2016;81(Supplement C):136-145. doi:10.1016/j.yrtph.2016.07.017

90. Upur H, Amat N, Blazekovic B, Talip A. Protective effect of Cichorium glandulosum root extract on carbon tetrachloride-induced and galactosamine-induced hepatotoxicity in mice. *Food Chem Toxicol*. 2009;47(8):2022-2030. doi:10.1016/j.fct.2009.05.022

91. Kluwe WM. Renal function tests as indicators of kidney injury in subacute toxicity studies. *Toxicology and Applied Pharmacology*. 1981;57(3):414-424. doi:10.1016/0041-008X(81)90239-8

92. Michael B, Yano B, Sellers RS, et al. Evaluation of Organ Weights for Rodent and Non-Rodent Toxicity Studies: A Review of Regulatory Guidelines and a Survey of Current Practices. *Toxicol Pathol*. 2007;35(5):742-750. doi:10.1080/01926230701595292

93. Weingand K, Brown G, Hall R, et al. Harmonization of Animal Clinical Pathology Testing in Toxicity and Safety Studies*. *Fundamental and Applied Toxicology*. 1996;29(2):198-201. doi:10.1006/faat.1996.0022

94. Fiander A. Causes of infertility among 1000 patients in Ghana. *Trop Doct*. 1990;20(3):137-138. doi:10.1177/004947559002000319

95. Donkor ES, Sandall J. Coping strategies of women seeking infertility treatment in southern Ghana. *Afr J Reprod Health*. 2009;13(4):81-93.

96. Ahmed B, al-Howiriny TA, Mathew R. Antihepatotoxic activity of Phyllanthus fraternus. *Pharmazie*. 2002;57(12):855-856.

97. Imafidon CE, Oluikran OS, Oguejiofor DJ, Eluwole AO, Adekunle IA, Oke GO. Acetonic extract of Vernonia amygdalina (Del.) attenuates Cd-induced liver injury: Potential application in adjuvant heavy metal therapy. *Toxicol Rep*. 2018;5:324-332. doi:10.1016/j.toxrep.2018.02.009

98. W Tetteh A, Mensah M, O Boadu K, et al. Clinical Evaluation of the Safety and Effectiveness of Adutwumwaa Malamix: A Polyherbal Product for the Treatment of Uncomplicated Malaria in Ghana
Hallauer J, Geng X, Yang H-C, Shen J, Tsai K-J, Liu Z. The Effect of Chronic Arsenic Exposure in Zebrafish. *Zebrafish*. 2016;13(5):405-412. doi:10.1089/zeb.2016.1252

Development of type 2 diabetes mellitus in people with intermediate hyperglycaemia. https://www.ncbi.nlm.nih.gov/pmc/articles/PMC6516891/. Accessed April 14, 2020.

Mo Y, Jiang M, Zhang Y, et al. Comparative mouse lung injury by nickel nanoparticles with differential surface modification. *J Nanobiotechnology*. 2019;17. doi:10.1186/s12951-018-0436-0

Borneff J, Engelhardt K, Griem W, Kunte H, Reichert J. [Carcinogens in water and soil. XXII. Experiment with 3,4-benzopyrene and potassium chromate in mice drink]. *Arch Hyg Bakteriol*. 1968;152(1):45-53.

Thompson CM, Proctor DM, Suh M, Haws LC, Kirman CR, Harris MA. Assessment of the mode of action underlying development of rodent small intestinal tumors following oral exposure to hexavalent chromium and relevance to humans. *Crit Rev Toxicol*. 2013;43(3):244-274. doi:10.3109/10408444.2013.768596

Bindhu Michael, Barry Yano, Rani S. Sellers, et al. Evaluation of Organ Weights for Rodent and Non-Rodent Toxicity Studies: A Review of Regulatory Guidelines and a Survey of Current Practices. *Toxicol Pathol*. 2007;35(5):742-750. doi:10.1080/01926230701595292

Suzuki H, Tokuriki T, Kamita H, et al. Age-related pathophysiological changes in rat oligomeganephronic hypoplastic kidney. *Pediatr Nephrol*. 2006;21(5):637-642. doi:10.1007/s00467-006-0089-3

Hall EM, Acevedo J, López FG, et al. Hypertension among Adults Exposed to Drinking Water Arsenic in Northern Chile. *Environ Res*. 2017;153:99-105. doi:10.1016/j.envres.2016.11.016

Lantz RC, Hays AM. Role of oxidative stress in arsenic-induced toxicity. *Drug Metab Rev*. 2006;38(4):791-804. doi:10.1080/03602530600980108

Metryka E, Chibowska K, Gutowska I, et al. Lead (Pb) Exposure Enhances Expression of Factors Associated with Inflammation. *Int J Mol Sci*. 2018;19(6). doi:10.3390/ijms19061813

Chibowska K, Baranowska-Bosiacka I, Falkowska A, Gutowska I, Goschorska M, Chlubek D. Effect of Lead (Pb) on Inflammatory Processes in the Brain. *Int J Mol Sci*. 2016;17(12). doi:10.3390/ijms17122140
Figure A.1: Top-three commonly patronized antimalarial herbal medicinal preparations among surveyed participants in the Kumasi metropolis of Ghana. The bars with the star represent the top-three most patronized herbal medicinal products selected for further in vivo sub-chronic toxicity study.
Table A.1: Basic information of the herbal product.

| Drug number | Name of Product       | Conc. (mg/5ml±se m) | Normal DAHD (ml/70kg/day) | Normal DAHD (mg/kg/day) | Indications                      |
|-------------|-----------------------|---------------------|---------------------------|-------------------------|----------------------------------|
| 1           | Taabea Herbal Mixture | 26.11±1.55          | 90                        | 469.98                  | malaria, loss of appetite        |
| 2           | Time Herbal Mixture   | 22.22±2.66          | 90                        | 399.96                  | malaria, loss of appetite, general body pains |
| 3           | Adutwumwaa Malamix    | 43.00±1.16          | 90                        | 774.00                  | Malaria                          |

Determined concentrations in 5mL of each preparation; their daily adult human dose (DAHD) (ml/70kg/day), their determined concentrations for the daily human doses (mg/kg/day), their major constituents and their indications on the label.
Figure A1: Weekly changes in body weight. Values are expressed as mean ±SEM.
### Table B.1: Effect of herbal product HPA on haematological parameters

| Parameter          | Control       | HPA(1)        | HPA(5)        | HPA(10)       |
|--------------------|---------------|---------------|---------------|---------------|
| WBC (x10^3/µL)    | 9.74 ± 0.52   | 8.13 ± 1.31   | 9.67 ± 2.50   | 10.67±1.24    |
| RBC (x10^6/µL)    | 7.81 ±0.23    | 8.07 ±0.40    | 7.42±0.43     | 7.90 ±0.67    |
| HGB (g/dL)        | 14.08 ±0.42   | 14.3 ±0.67    | 12.67 ±0.60   | 13.97 ±0.57   |
| HCT (%)           | 47.40 ±1.34   | 47.63 ±2.06   | 41.83 ±2.66   | 47.87 ±1.94   |
| MCV (fL)          | 60.72 ±1.18   | 59.03 ±0.50   | 56.33 ±0.67   | 61 ±2.60      |
| MCH (pg)          | 18.08 ±0.58   | 17.73 ±0.12   | 17.10 ±0.21   | 17.80 ±0.78   |
| MCHC (g/dL)       | 29.70 ±0.45   | 30.00 ±0.31   | 30.37 ±0.46   | 29.20 ±0.53   |
| Platelet (x10^3/µL)| 1152 ±58.95  | 883.3 ±198.00 | 1060 ±86.53   | 987.3 ±99.55  |
| Lymphocytes (%)   | 75.96 ±3.11   | 77.53 ±3.50   | 57.23 ±15.14  | 63.37 ±9.59   |
| MXD (%)           | 15.02 ±1.87   | 13.83 ±3.19   | 20.10 ±6.93   | 18.47 ±10.10  |
| Neutrophils (%)   | 9.02 ±1.45    | 8.63 ±0.58    | 22.67 ±8.89   | 18.17 ±10.58  |
| LYM #(x 10^3)     | 7.34 ±0.367   | 6.40 ±1.33    | 5.37 ±2.17    | 6.83 ±1.33    |
| MXD #(x 10^3)     | 1.48 ±0.24    | 1.07 ±0.12    | 1.933 ±0.89   | 1.73 ±0.81    |
| NEUT #(x 10^3)    | 0.92 ±0.16    | 0.67 ±0.09    | 2.37 ±1.13    | 2.10 ±1.46    |
| RDW_SD (fL)       | 39.02 ±0.91   | 37.90 ±1.07   | 35.10 ±0.71   | 36.77 ±2.09   |
| RDW.CV (fL)       | 18.30 ±0.44   | 18.23 ±0.35   | 17.23 ±0.87   | 16.50 ±1.18   |
| PDW (fL)          | 11.78 ± 0.17  | 13.43 ± 0.42  | 14.00 ± 2.30  | 11.77 ± 0.18  |
| MPV (fL)          | 9.26±0.11     | 10.07±0.55    | 10.23±0.94    | 9.43±0.23     |
| P_LCR (%)         | 20.18 ±0.89   | 27.93±4.33    | 28.40±8.11    | 21.30±1.42    |

Values are expressed as mean ±SEM. P-values less than 0.05 were considered statistically significant.

**Key:** Red blood cell count (RBC), white blood cell count (WBC), granulocyte count (GRA), lymphocyte count (LYM), haemoglobin (HGB), hematocrit (HCT), mean corpuscular haemoglobin (MCH), mean corpuscular volume (MCV), mean corpuscular hemoglobin concentration (MCHC), platelet count (PLT), platelet distribution width (PDW), mean platelet volume (MPV) and platelet larger cell ratio (P-LCR), Mixed cell count (MXD) consisting of monocytes, eosinophils, basophil.
Table B.2: Effect of herbal product HPB on haematological parameters

| Parameter               | Control       | HPB(1)        | HPB(5)        | HPB(10)       |
|-------------------------|---------------|---------------|---------------|---------------|
| WBC (x10^3/µL)          | 9.74 ± 0.52   | 8.57 ± 1.85   | 15.07 ± 2.67  | 9.13 ± 2.23   |
| RBC (x10^6/µL)          | 7.81 ± 0.23   | 7.33 ± 0.48   | 8.28 ± 0.53   | 7.48 ± 0.50   |
| HGB (g/dL)              | 14.08 ± 0.42  | 13.3 ± 0.63   | 14.43 ± 0.74  | 13.57 ± 0.85  |
| HCT (%)                 | 47.40 ± 1.34  | 44.77 ± 2.43  | 48.5 ± 2.96   | 44.43 ± 3.51  |
| MCV (fL)                | 60.72 ± 1.18  | 61.17 ± 0.72  | 58.57 ± 0.27  | 59.3 ± 2.18   |
| MCH (pg)                | 18.08 ± 0.58  | 18.20 ± 0.32  | 17.47 ± 0.42  | 18.17 ± 0.47  |
| MCHC (g/dL)             | 29.70 ± 0.45  | 29.73 ± 0.23  | 29.80 ± 0.55  | 30.30 ± 0.85  |
| Platelet (x10^3/µL)     | 1152 ± 58.95  | 99.55 ± 44.06 | 1077 ± 117.60 | 1266 ± 260.60 |
| Lymphocytes (%)         | 75.96 ± 3.11  | 81.40 ± 2.23  | 72.03 ± 2.72  | 67.87 ± 4.24  |
| MXD (%)                 | 15.02 ± 1.87  | 13.03 ± 1.93  | 11.83 ± 3.08  | 17.13 ± 2.77  |
| Neutrophils (%)         | 9.02 ± 1.45   | 5.57 ± 1.04   | 16.13 ± 5.72  | 15 ± 3.12     |
| LYM #(x 10^3)           | 7.34 ± 0.367  | 7.07 ± 1.68   | 10.73 ± 1.49  | 6.07 ± 1.35   |
| MXD #(x 10^3)           | 1.48 ± 0.24   | 1.03 ± 0.13   | 1.63 ± 0.18   | 1.57 ± 0.48   |
| NEUT #(x 10^3)          | 0.92 ± 0.16   | 0.47 ± 0.09   | 2.70 ± 1.38   | 1.50 ± 0.57   |
| RDW_SD (fL)             | 39.02 ± 0.91  | 37.50 ± 1.76  | 36.73 ± 1.28  | 37.60 ± 2.17  |
| RDW_CV (fL)             | 18.30 ± 0.44  | 16.33 ± 1.33  | 17.80 ± 1.03  | 17.17 ± 0.09  |
| PDW (fL)                | 11.78 ± 0.17  | 12.40 ± 0.40  | 11.93 ± 0.75  | 13.00 ± 0.67  |
| MPV (fL)                | 9.26 ± 0.11   | 9.80 ± 0.38   | 9.33 ± 0.27   | 10.13 ± 0.37  |
| P_LCR (%)               | 20.18 ± 0.89  | 23.83±2.83    | 20.57±2.64    | 27.13±2.98    |

Values are expressed as mean ±SEM. *P*-values less than 0.05 were considered statistically significant.

**Key:** Red blood cell count (RBC), white blood cell count (WBC), granulocyte count (GRA), lymphocyte count (LYM), haemoglobin (HGB), hematocrit (HCT), mean corpuscular haemoglobin (MCH), mean corpuscular volume (MCV), mean corpuscular hemoglobin concentration (MCHC), platelet count (PLT), platelet distribution width (PDW), mean platelet volume (MPV) and platelet larger cell ratio (P-LCR). Mixed cell count (MXD) consisting of monocytes, eosinophils, basophil.
Table B.3: Effect of herbal product HPC on haematological parameters

| Parameter                  | Control          | HPC(1)           | HPC(5)           | HPC(10)          |
|----------------------------|------------------|------------------|------------------|------------------|
| WBC (x10^3/µL)             | 9.74 ± 0.52      | 11.10 ± 1.04     | 11.77 ± 0.45     | 7.13 ± 2.45      |
| RBC (x10^6/µL)             | 7.81 ± 0.23      | 8.01 ± 0.16      | 7.73 ± 0.53      | 8.06 ± 0.38      |
| HGB (g/dL)                 | 14.08 ± 0.42     | 14.43 ± 0.54     | 13.97 ± 0.56     | 14 ± 0.17        |
| HCT (%)                    | 47.40 ± 1.34     | 47.77 ± 1.67     | 45.03 ± 1.65     | 46.8 ± 0.60      |
| MCV (fL)                   | 60.72 ± 1.18     | 59.6 ± 1.07      | 58.5 ± 2.34      | 58.3 ± 2.91      |
| MCH (pg)                   | 18.08 ± 0.58     | 18.03 ± 0.41     | 18.13 ± 0.64     | 17.43 ± 0.73     |
| MCHC (g/dL)                | 29.70 ± 0.45     | 30.20 ± 0.15     | 31.00 ± 0.17     | 29.93 ± 0.77     |
| Platelet (x10^3/µL)        | 1152 ± 58.95     | 1135 ± 144.60    | 1058 ± 80.71     | 1020 ± 57.30     |
| Lymphocytes (%)            | 75.96 ± 3.11     | 71.93 ± 4.04     | 65.80 ± 6.05     | 72.53 ± 9.12     |
| MXD (%)                    | 15.02 ± 1.87     | 16.80 ± 2.33     | 21.67 ± 3.58     | 11.57 ± 2.39     |
| Neutrophils (%)            | 9.02 ± 1.45      | 11.27 ± 1.73     | 12.53 ± 2.48     | 15.90 ± 6.91     |
| LYM #(x 10^3)              | 7.34 ± 0.367     | 8.00 ± 0.95      | 7.77 ± 0.87      | 4.77 ± 1.09      |
| MXD #(x 10^3)              | 1.48 ± 0.24      | 1.83 ± 0.23      | 2.53 ± 0.41      | 0.90 ± 0.50      |
| NEUT #(x 10^3)             | 0.92 ± 0.16      | 1.27 ± 0.20      | 1.47 ± 0.29      | 1.47 ± 0.97      |
| RDW_SD (fL)                | 39.02 ± 0.91     | 38.50 ± 0.71     | 35.73 ± 1.43     | 35.70 ± 2.85     |
| RDW_CV (fL)                | 18.30 ± 0.44     | 18.23 ± 0.47     | 16.73 ± 0.73     | 17.00 ± 0.76     |
| PDW (fL)                   | 11.78 ± 0.17     | 12.57 ± 0.32     | 12.70 ± 0.66     | 11.67 ± 0.37     |
| MPV (fL)                   | 9.26 ± 0.11      | 9.87 ± 0.30      | 10 ± 0.45        | 9.63 ± 0.19      |
| P_LCR (%)                  | 20.18 ± 0.89     | 25.27 ± 2.04     | 26.40 ± 4.25     | 22.13 ± 2.03     |

Values are expressed as mean ±SEM. P-values less than 0.05 were considered statistically significant.

**Key:** Red blood cell count (RBC), white blood cell count (WBC), granulocyte count (GRA), lymphocyte count (LYM), haemoglobin (HGB), hematocrit (HCT), mean corpuscular haemoglobin (MCH), mean corpuscular volume (MCV), mean corpuscular hemoglobin concentration (MCHC), platelet count (PLT), platelet distribution width (PDW), mean platelet volume (MPV) and platelet larger cell ratio (P-LCR), Mixed cell count (MXD) consisting of monocytes, eosinophils, basophil.
