



Hematological changes and nitric oxide levels accompanying high-dose artemether-lumefantrine administration in male guinea pigs: Effect of unsweetened natural cocoa powder

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ABSTRACT

Background: Unsweetened natural cocoa powder (UNCP), prepared after removal of the cocoa butter, is a common beverage in Ghana. It possesses antimalarial prophylactic property and has a beneficial effect on blood components. **Aim:** The aim of this study was to determine whether regular dietary supplement of UNCP mitigates high-dose (HD) artemether-lumefantrine (A-L)-induced hematological disorders and to determine the effect on nitric oxide (NO) levels. **Materials and Methods:** Adult male guinea pigs (300 g - 350 g) were randomly divided into 5 groups of 6 guinea pigs each. Among the 5 groups, 3 groups were treated with UNCP (300, 900, and 1500 mg/kg body weight) for 14 days. A-L (75 mg/kg) was administered from the 12th to 14th day. One of the remaining 2 groups received distilled water only, i.e., vehicle control group (VCG) while the other received 75 mg/kg A-L only, i.e., negative control group (NCG). Blood samples from all groups were obtained by cardiac puncture (day 15) followed by hematological and NO analysis. **Results:** A-L reduced white blood cells (WBC) by 31.87%, lymphocyte count by 45.99%, hemoglobin by 11.72%, hematocrit by 18.56%, and platelet count by 33.08% in the NCG. Administration of various doses of UNCP increased WBC and lymphocyte count ($P > 0.05$) compared to the NCG. UNCP and A-L combination caused an increase in NO levels when compared to the VCG. **Conclusion:** Regular consumption of UNCP by guinea pigs increases plasma NO and restores some hematological disorders induced by a 3-day HD A-L administration.

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INTRODUCTION

Plants have been consumed or exploited for their medicinal purposes from time immemorial for as long as history has been recorded. Three categories of herbs are distinguished in Cherokee medicine, namely, food herbs, medicinal herbs, and poison herbs [1]. The food herbs are mild in action and are

expected to have very low toxicity. One of such food herbs is the plant *Theobroma cacao*. The species *T. cacao* belongs to the class *Equisetopsida* and family *Malvaceae*. Cocoa is a major nutraceutical in Ghana and most parts of Africa. As a food herb, it is used by most indigenous people either in a raw state using the bean or the pod, as dark chocolate, and as a beverage in a powdered form obtained from the cocoa bean. Research

works are currently directed at gathering plant materials from the rain forests and other places for their potential medicinal values [2].

Cocoa contains water-soluble polyphenols (also called flavanols), which include catechins, epicatechin, procyanidins, anthocyanins, and leucoanthocyanins [3]. The quantitative analysis of unsweetened natural cocoa powder (UNCP) has also been well researched and proven that even flavanol contents are not likely to change during different manufacturing processes [4-6]. Several polyphenols such as 14 N-phenylpropenoyl-L-amino acids, N-[4'-hydroxy-(E)-cinnamoyl]-L-tryptophan, and N-[4'-hydroxy-3'-methoxy-(E)-cinnamoyl]-L-tyrosine have also been identified [7-9]. Pharmacologically, cocoa is known to exert antioxidant [10], anti-inflammatory [11,12], antimalarial, and anti-asthmatic properties [13-15]. Studies have also shown that UNCP could protect the liver, kidney, and heart against chemical injury in animals [2,3,10]. The antioxidant properties of these flavanols are ascribed partly to their structural characteristics, and it represents the molecular basis for their radical-scavenging property [3,10]. In spite of cocoa's antimalarial activity, its ability to increase nitric oxide (NO) production [16-18], white blood cell (WBC), and platelet counts (PLT) without changing hematocrit (HCT) and hemoglobin (HGB) levels could be of immense importance in malaria-infected individuals [19].

Artemether-lumefantrine (A-L) is a preferred artemisinin-based antimalarial in Ghana. Studies have shown that artemether can induce changes in hematological profiles in rats, which may aggravate anemia in malaria patients [20]. Besides, A-L has been found to reduce red blood cell count (RBC), HGB, and packed cell volume (PCV) in patients under treatment [21]. Though the WHO-recommended artemisinin-based combination therapy (ACT) [22,23] has impressive parasitocidal properties both *in vivo* and *in vitro*, there have been issues of treatment failures, resistance, and increasing cases of toxic effects [24,25]. Some countries have, therefore, considered increasing the dose of A-L used in the management of malaria to arrest the issue of resistance [26]. However, increase in dose implies that there will be increased side effects, adverse reactions, and other toxic effects [27]. A-L administration has been found to reduce NO levels. On the contrary, other studies have also shown that A-L increases NO levels as a compensatory mechanism in cases of reduced NO levels [16,26].

UNCP, prepared after removal of the cocoa butter from powdered cocoa beans, is taken several times on a daily basis as a beverage by patients while on treatment with A-L, a common practice in Ghana. Hematological parameters are one of the vital indices monitored during malaria treatment.

It is against this background that this study was conducted to investigate the effect of UNCP on the hematological parameters and NO levels during high-dose (HD) A-L administration.

The result of this study would serve as one of the scientific premises for discovering the beneficial effect of UNCP during its simultaneous consumption with A-L during malaria treatment.

MATERIALS AND METHODS

Preparation of UNCP Solution

The UNCP is a non-alkalized cocoa from Hords Company Ltd. Calculated amount (9.6 g) of Brown Gold Natural Cocoa Powder (Batch number BT620IT; FDA/DK 06-070) manufactured in Accra by Hords Company Ltd., Ghana, was dissolved in warm distilled water (40 ml) with stirring, making a concentration of 240 mg/ml (of the UNCP). This was administered to the guinea pigs in groups 3, 4, and 5 at their respective doses (i.e. 300, 900, and 1500 mg/kg) via oral gavage.

Phytochemical Analysis

Phytochemical analysis was conducted to determine the various constituents in the unsweetened natural cocoa [28].

Saponin Test

About 0.5 g of UNCP was added to water in a test tube. The test tube was shaken to observe foam formation.

Tannins Test

About 0.5 g of UNCP was dissolved in 80% of aqueous methanol (10 cm³). Freshly prepared iron (III) chloride solution was added and the color change was observed.

Flavanoids Test

About 0.1 g of UNCP was added to 80% ethanol (15 cm³). To the filtrate was added magnesium turnings followed by concentrated HCl (0.5 cm³), and observed for color changes within 10 minutes.

Cardiac Glycoside Test

About 0.5 g of UNCP was dissolved in chloroform (2 cm³) in a test tube, after which concentrated sulfuric acid was carefully added down the side of the test tube to form a lower layer.

Animal Experimentation

30 adult male guinea pigs weighing between 300 g and 350 g were purchased from the Animal Experimentation Department of the Noguchi Memorial Institute for Medical Research, University of Ghana, Legon. These animals were chosen according to their weight. The guinea pigs were acclimatized to laboratory environment (20-23°C) with a 12 h light-darkness cycle for 7 days prior to experimentation. The guinea pigs had access to standard laboratory diet and water *ad-libitum*. The study protocol was approved by the

departmental Ethical and Protocol Review Committee and the Noguchi Memorial Institute for Medical Research Institutional Animal Care and Use Committee with protocol approval number: 2013-01-3E.

Experimental Design

The adult male guinea pigs were randomly assigned to 5 groups with each group containing 6 guinea pigs. Three groups received the UNCP at doses of 300, 900, and 1500 mg/kg per body weight, respectively, for 14 days, with two other groups serving as controls [15]. Animals in one of the two controls were given only A-L at a dose of 75 mg/kg body weight (negative control group [NCG]) whereas those in the other group were given distilled water (vehicle control group [VCG]) [20].

Nearly, 9.6 g of the UNCP was weighed and dissolved in 40 ml of hot distilled water and stirred continuously until homogeneous mixture was formed (everything went into solution). This was given to the animals daily for 14 days.

Preparation of A-L Solution

A concentration of 20 mg/ml of the artemether was prepared and administered to the guinea pigs in the various groups at a dosage of 75 mg/kg body weight via oral route using the oral gavage for 3 days (i.e. from 12th to 14th day). Dosage was calculated with reference to the dose of artemether in the drug combination. To achieve this, 70 tablets of A-L (Novartis Coartem[®]) dispersible tablets (20/120 mg), which is equivalent to 1400 mg of artemether, was dissolved in 70 ml of distilled water and stirred until obtaining a completely homogeneous mixture.

In all cases, fresh solutions of UNCP and A-L were prepared before each dosing. The guinea pigs were, thus, observed daily for a total period of 14 days.

- Group 1: Control (distilled water only)/vehicle control (CTRL)
- Group 2: 75 mg/kg A-L (last 3 days)/negative control (COART)
- Group 3: Cocoa 300 mg/kg (14 days) + 75 mg/kg A-L (last 3 days) (300)
- Group 4: Cocoa 900 mg/kg (14 days) + 75 mg/kg A-L (last 3 days) (900)
- Group 5: Cocoa 1500 mg/kg (14 days) + 75 mg/kg A-L (last 3 days) (1500).

Calculation of Human Equivalent Doses (HED)

Conversion of animal doses to HED was based on basal surface area. HEDs were calculated according to a study by Reagan-Shaw *et al.*, 2008, [29] using the formula:

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

Taking note of the value of km factors (i.e., body weight, kg/surface area, m²) for adult and guinea pigs to be 37 and 8, respectively, and an average weight of Ghanaian to be 70.0 kg, the HED and number of teaspoonful of UNCP at the various dose levels are calculated as follows:

$$300 \text{ mg/kg} = 65 \text{ mg/kg, which is equivalent to } 4540 \text{ mg for a } 70 \text{ kg man} = 4.54 \text{ g daily.}$$

Assuming 1 teaspoonful of UNCP is 2.5 g, 300 mg/kg UNCP corresponds to approximately 2 teaspoonful of UNCP daily.

$$900 \text{ mg/kg} = 194.6 \text{ mg/kg HED, which is equivalent to } 13622 \text{ mg/70 kg man} = 13.42 \text{ g daily (equivalent to } 5.4 \text{ teaspoonful daily).}$$

$$1500 \text{ mg/kg} = 324.3 \text{ mg/kg HED, which is equivalent to } 22701 \text{ mg/70 kg man} = 22.7 \text{ g daily (equivalent to } 9 \text{ teaspoonful daily).}$$

$$75 \text{ mg/kg} = 16.22 \text{ mg/kg HED, which is equivalent to } 1135.4 \text{ mg/70 kg man} = 1140 \text{ mg of A-L daily.}$$

Food and Water Intake and Body Weight

Food from Ghana Agro Food Company Ltd. and water consumption by the animals in the various groups was monitored both morning and evening throughout the study. Body weights were measured using an electronic balance daily before dosing of the animals, and any change was noted. Besides, the animals were monitored for any clinical observations.

Hematological Studies

Guinea pigs were euthanized with 50 mg/kg chloroform by exsanguination and 2 ml of blood was sampled by cardiac puncture and transferred into EDTA-2k test tubes for immediate analysis. An automated hematology analyzer (KX-2IN, Sysmex Corporation, Japan) was used to estimate the counts of the various parameters considered in this study.

NO Level Determination

The nitrite concentration in the plasma was measured as an index of NO levels by Griess reagent system (South Africa) according to the manufacturer's instruction.

In this measurement, the total NO kit by R&D Systems was used in this study. In this system, nitrate is converted to nitrite using nitrate reductase, after which the total nitrite is measured.

The principle of this assay determines NO concentration based on the enzymatic conversion of nitrate to nitrite by nitrate reductase. The reaction is followed by colorimetric detection of nitrite as an azo dye product of the Griess Reaction. The Griess Reaction is then based on the two-step diazotization reaction, in which acidified NO₂⁻ produces a nitrosating agent, which reacts with sulfanilic acid to produce the diazonium ion. This ion is then coupled to N-(1-naphthyl) ethylenediamine to form the chromophoric azo-derivative, which absorbs light at 540-570 nm.

Statistical Analysis and Data Evaluation

Statistical analysis of the data was done using GraphPad Prism Software version 5.0. Results were expressed as mean \pm standard error of mean, $n = 6$. Statistical analysis was performed using one-way analysis of variance (ANOVA) followed by Student–Newman–Keuls *post-hoc* test. Statistical significance was set at $P < 0.05$.

With the NO experiment, data were expressed as \pm standard deviation (SD), Dunnett's multiple comparison test was used in this *post-hoc* analysis. All statistical analyses were performed using GraphPad prism 5 software.

RESULTS

Phytochemical Analysis

Phytochemical analysis of UNCP showed the presence of saponins, flavonoids, tannins, and cardiac glycosides.

Food and Water Intake and Body Weight

There was no increase in food consumption. Water consumption and urine output, however, increased except in the VCG and NCG. Changes in body weights were not significant. Animals that received 75 mg/kg A-L only showed signs of piloerection on day 13.

Effects of A-L and UNCP on WBC Count

Figure 1a shows that A-L administration was accompanied by a reduction in WBC count in the NCG (Coartem®) by 31.87% as compared to the VCG ($P > 0.05$). Administration of UNCP at doses of 300, 900, and 1500 mg/kg body weight restored the WBC levels during concomitant administration with A-L ($P = 0.1158$).

Effects of A-L and UNCP on Neutrophil Count

Figure 1b shows that A-L administration increased the neutrophil count of the NCG (Coartem®) by 30.20% as compared to the VCG ($P = 0.06$). Administration of UNCP at a dose of 300 mg/kg body weight decreased the neutrophil count by 90.38% as compared to the NCG (Coartem®). In addition, UNCP administration at doses of 900 and 1500 mg/kg restored these neutrophil levels during A-L administration ($P = 0.06$).

Effects of A-L and UNCP on Monocyte Count

Figure 1c shows that A-L administration insignificantly caused a slight increase in monocyte count in the NCG (Coartem®) by 2.55% as compared to the control group ($P > 0.05$). Administration of UNCP at a dose of 300 mg/kg caused an insignificant decrease in monocyte count compared to the NCG. At a dose of 1500 mg/kg UNCP, however, levels of monocyte count during A-L administration increased by 24.03% ($P > 0.05$).

Effects of A-L and UNCP on Lymphocyte Count

Figure 1d shows that A-L administration decreased the lymphocyte count of the NCG (Coartem®) by 45.99% as compared to the VCG ($P > 0.05$). Administration of UNCP at doses of 300, 900, and 1500 mg/kg body weight restored these lymphocyte count levels by 36.49%, 43.95%, and 56.19%, respectively ($P = 0.0014$), during A-L administration.

Effects of A-L and UNCP on HGB Level

Figure 1e shows that A-L administration decreased HGB level of the NCG (Coartem®) by 11.72% as compared to the VCG ($P > 0.05$). Administration of UNCP at doses of 300, 900, and 1500 mg/kg body weight restored the HGB levels during A-L administration ($P > 0.05$).

Effects of A-L and UNCP on Eosinophil Count

Figure 1f shows that administration of A-L and UNCP did not significantly influence eosinophil count although it increased by 6.03% in the NCG compared to the VCG ($P = 0.1370$). Administration of UNCP at a dose of 300 mg/kg body weight increased eosinophil count by 25.64% compared to the NCG. In addition, comparing with eosinophil count of the NCG, UNCP at 900 mg/kg body weight caused a decrease of 110.81% while that of the 1500 mg/kg restored the eosinophil levels during the simultaneous administration of A-L by 16.58% ($P > 0.05$).

Effects of A-L and UNCP on RBC

Figure 1g shows that A-L administration decreased RBC count of the NCG (Coartem®) by 6.43% as compared to the VCG ($P > 0.05$). Prophylactic administration of UNCP with A-L at doses of 300, 900, and 1500 mg/kg body weight restored the decreased levels of the RBC count by 4.17%, 5.55%, and 12.55%, respectively ($P > 0.05$).

Effects of A-L and UNCP on Basophil Count

Figure 1h shows that the administration of A-L and UNCP did not significantly influence basophil count although it increased by 7.273% in the NCG compared to the NCG ($P > 0.05$). Administration of UNCP and A-L at a dose of 300 mg/kg increased basophil by 8.06% compared to the NCG. UNCP at 900 mg/kg caused a decrease at 34.14% while that of the 1500 mg/kg body weight restored the decreased levels of basophils by 39.56% ($P > 0.05$).

Effects of A-L and UNCP on HCT Level

Figure 1i shows that A-L administration decreased HCT count in the NCG by 18.56% as compared to the VCG ($P < 0.05$). Administration of UNCP at doses of 300, 900, and 1500 mg/kg body weight restored HCT levels during A-L administration by 6.72%, 6.44%, and 11.37%, respectively ($P > 0.05$).

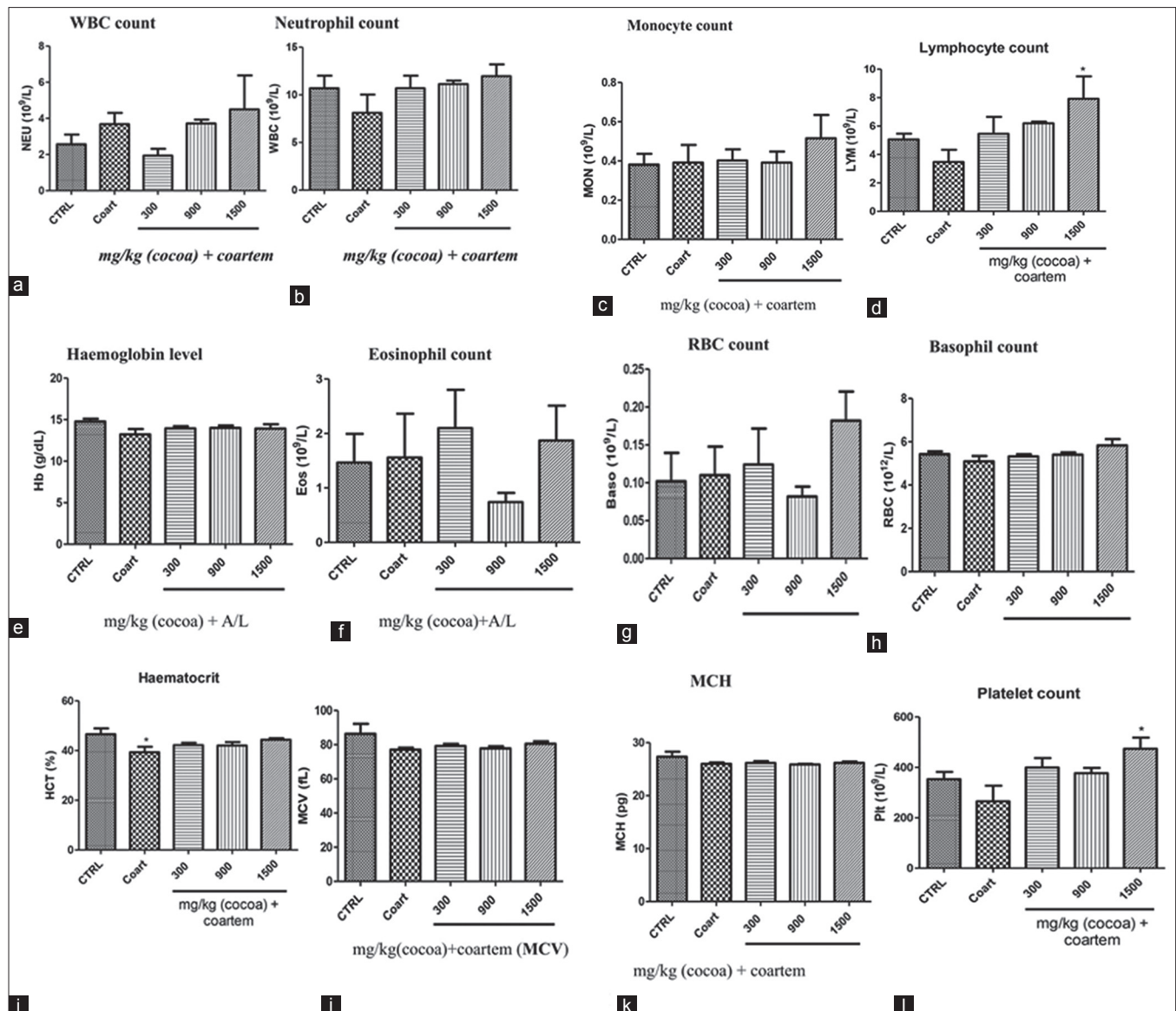


Figure 1: Effect of unsweetened natural cocoa powder (low dose [LD] = 300, medium dose [MD] = 900, and high dose [HD] = 1500 mg/kg) on some hematological parameters of guinea-pigs, (a) White blood cells. (b) Neutrophil count. (c) Monocyte count. (d) Lymphocyte count. (e) Hemoglobin level. (f) Eosinophil count. (g) Red blood cell count. (h) Basophil count. (i) Hematocrit. (j) Mean corpuscular volume. (k) Mean corpuscular hemoglobin. (l) Platelet count during 75 mg/kg artemether-lumefantrine administration. Values are mean \pm standard error of mean ($N=6$). *means $P < 0.05$, ** $P < 0.01$, and *** $P < 0.001$ compared to the vehicle control group (one-way analysis of variance, followed by a Dunnett's multiple comparison test)

Effects of A-L and UNCP on Mean Corpuscular Volume (MCV)

Figure 1j shows that A-L administration insignificantly decreased MCV count of the NCG by 12.21% compared to the vehicle controls ($P > 0.05$). Administration of UNCP at doses of 300, 900, and 1500 mg/kg body weight restored the MCV levels ($P > 0.05$).

Effects of A-L and UNCP on Mean Corpuscular Hemoglobin (MCH)

Figure 1k shows that the administration of A-L and UNCP did not significantly influence MCH although MCH decreased

by 5.1% in the NCG compared to the VCG ($P > 0.05$). Administration of UNCP at a dose of 300 mg/kg body weight increased MCH by 0.61% compared to the NCG. In addition, comparing with MCH of the NCG, UNCP at 900 mg/kg body weight caused a reduction of 0.39% while that of the 1500 mg/kg body weight reversed these levels when administered with A-L ($P > 0.05$).

Effects of A-L and UNCP on PLT

Figure 1l shows that A-L administration decreased PLT of the NCG by 33.08% as compared to the VCG ($P > 0.05$). Administration of UNCP with A-L at doses of 300, 900, and

1500 mg/kg body weight restored the decreased levels of the PLT by 33.62%, 29.70%, and 44.02%, respectively ($P < 0.05$ for UNCP administration at 1500 mg/kg body weight).

Effects of A-L and UNCP on NO

Figure 2 shows the effect of UNCP (300, 900, and 1500 mg/kg) on nitrite (NO metabolite) concentrations in the plasma of guinea pigs during A-L administration. Values are mean \pm SD ($n = 5$) and $*P < 0.05$, $**P < 0.01$, and $***P < 0.001$ when compared to the A-L control (one-way ANOVA followed by a Dunnett's multiple comparison test). The low-dose UNCP (300 mg/kg) + A-L produced the greatest increase in NO followed by MD UNCP + A-L, which were both statistically significant, and then HD UNCP + A-L.

NO Levels

The group administered with UNCP at a dose of 300 mg/kg recorded the highest increase of 149.71% in NO ($P < 0.05$) followed by the administration of UNCP at a dose of 900 mg/kg with 34.25% ($P < 0.05$), then group with UNCP at a dose of 1500 mg/kg group at 4.88% ($P < 0.05$) when compared to the VCG [Figure 2].

DISCUSSION

Erythrocytic indices such as erythrocyte or RBC counts, HCT or PCV, and HGB are important indicators of the functional state of the erythron. Erythrocyte counts reflect the total number of RBC per unit volume of circulating blood whereas HGB determinations indicate the oxygen-carrying capacity of blood, and HCT determinations show the proportion of blood that is made up of cellular elements and the proportion that

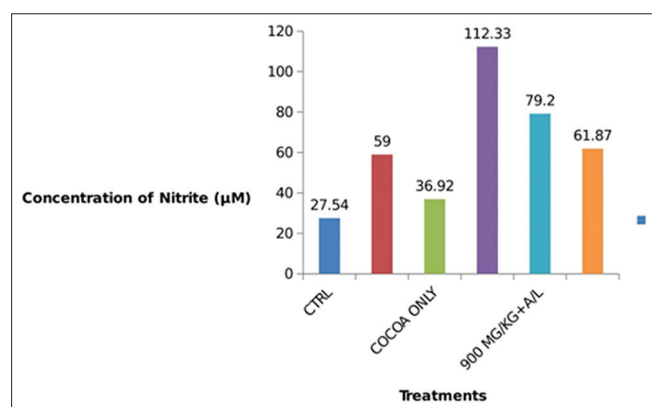


Figure 2: Effect of unsweetened natural cocoa powder (low dose [LD] = 300, medium dose [MD] = 900, and high dose [HD] = 1500 mg/kg) on nitrite concentration in plasma of guinea pigs during artemether-lumefantrine (A-L) administration. Values are mean \pm standard deviation ($n = 5$) and $*P < 0.05$, $**P < 0.01$, and $***P < 0.001$ compared to the control (one-way analysis of variance followed by a Dunnett's multiple comparison test). The LD unsweetened natural cocoa powder (300 mg/kg) + A-L produced the greatest increase (147.33 ± 117.78 , $P < 0.05$, i.e., 149.71%) in nitric oxide followed by MD unsweetened natural cocoa powder + A-L (79.21 ± 36.24 , $P < 0.05$, i.e., 34.25%) and then HD unsweetened natural cocoa powder + A-L (61.88 ± 3.83 , $P < 0.05$, i.e., 4.88%) when compared to the A-L only group

is plasma [30]. This study imitated the normal duration of Coartem used in Ghana (i.e. 3 days). UNCP at a dose of 75 mg/kg has also been used in previous studies as HD UNCP [19].

From the results, A-L administration decreased the levels of WBC count, lymphocyte count, HGB, RBC count, and PLT, as observed in Figure 1a, c, e, h, and l, respectively, in the NCG (Coartem®). Normally, these reduced indices imply bone marrow depression, autoimmune hemolytic anemia, systemic lupus, or severe hemorrhage. The results concurred with similar results documented by the previous researchers that ACTs are known to cause embryonic erythrocyte depletion and delay erythroid differentiation [31].

The characteristic peroxide lactone structure of the artemether is indispensable for its antimalarial property, thus the splitting of this endoperoxide bridge by heme iron species in the guinea pigs may have resulted in the release of reactive oxygen that induced oxidative stress and caused the decreases that were observed with A-L administration. UNCP restored the decreased levels of HCT, HGB, and RBC, as observed in the A-L control group. Though not studied in this research, this might have been due to the antioxidant activity of UNCP, which is able to mop up the free radicals produced by the artemether moiety. The increased levels with UNCP (particularly with HCT levels) may be observed in dehydrated states as cocoa has been reported to possess diuretic effects. This was also confirmed in the general observations made, especially in the UNCP-treated groups as a general increase in diuresis and water consumption. The changes in body weight remained as an insignificant reduction. The decreased levels of WBC and lymphocyte count with A-L administration observed from the results concur with the previous work by Wang *et al.* [32] who also reported similar results, and that derivatives of artemisinin exhibited potent immunosuppressive activity by decreasing WBC and lymphocytes. However, UNCP restored the decreased levels of WBC ($P > 0.05$) and lymphocyte ($P < 0.05$) count as observed with A-L administration alone. The increase in WBC of the experimental guinea pigs meant that the administration of cocoa could boost the immune system, since these cells are important in protecting and fighting infections. This supports and explains the earlier observations that cocoa promotes superlative health by strengthening the immune system and prevents many viral diseases [19].

UNCP restored the decreased PLT as observed in A-L [Figure 1l]. The decreased PLT levels may be indicative of the presence of liver disease caused by the hepatotoxic effect of the HD A-L. Normally, PLT diminishes due to PLT trapping leading to enlarged spleen (splenomegaly). Splenomegaly due to artemether has been known since decades [33].

The mean corpuscular values (MCV and MCH), obtained from the RBC count, HCT, and HGB, are usually useful in elucidating and classifying anemia morphologically; they represent an estimation of the alterations in size and HGB content of individual RBC [30,34]. Results of this study showed that there were no significant differences in MCV and MCH during UNCP intake following A-L administration, and this may

be due to the absorption of iron from cocoa (non-heme iron source), which is not high, since the absorption of non-heme iron is less efficient as compared to heme iron sources [35]. Besides, the polyphenols contained in UNCP are known to decrease the absorption of non-heme iron [36,37]. The effect on the RBC notwithstanding HGB levels was not significantly affected. Perhaps, a more prolonged intake of cocoa could have had an effect that may be worth further investigating.

The differential leukocyte counts reflect the systemic status of an animal in relation to its response and adjustment to injurious agents, stress, and/or deprivation; the indices are of value in confirming or eliminating a tentative diagnosis, in making a prognosis and guiding therapy [30]. It could further provide information on the severity of an injurious agent, the virulence of an infecting organism, the susceptibility of a host, and the nature, severity, and duration of a disease process [38]. From the results, the administration of A-L and UNCP did not have any influence on neutrophil count, monocyte count, eosinophil count, and basophil count, as seen with Figure 1b, d, f, and g. This observation may be due to the resiliency of the bone marrow which is able to resist the effects of some chemical agents and also the short-term administration of both A-L and UNCP. The inconsistency in basophil numbers as observed in the results following the administration of A-L followed by UNCP, and even sometimes, their total absence in blood of rodents (guinea pigs) is a normal occurrence. The increase in lymphocyte observed with UNCP administration may also be due to the fact that lymphocytes can also originate from sites other than the bone marrow such as the thymus [14,30].

The UNCP and A-L combination increased the levels of NO as compared to the other groups [Figure 2]. These no increase in the animals that received both UNCP and A-L could be attributed to the flavonoid content of the unsweetened natural cocoa [14]. Flavonoid-rich chocolate and cocoa drinks have been found to increase NO levels. Other studies have shown that A-L increases the level of oxidants such as superoxides (O_2^-) and peroxides (H_2O_2), leading to oxidative stress. This further leads to a reduction in NO levels, since superoxides and peroxides are considered as NO scavengers [14,16-18]. The rise in NO observed in the A-L administered group could be as a result of a compensatory mechanism trying to restore the NO level, which is in line with other findings [16]. The high increase in NO levels observed in the cocoa and A-L combinations, especially in the animals receiving 300 mg/kg and 900 mg/kg of UNCP, may be indicative of enhanced protective effects of UNCP at these dose levels. This is extremely important in view of the effect of A-L on hematology as observed by other researchers [20]. Other studies have also revealed that the highest antihypertensive effect (due to NO vasodilator effects) of an orally administered flavonol-rich cocoa powder to spontaneously hypertensive rats was at a dose of 300 mg/kg and 900 mg/kg [39,40]. From our calculations, optimum effect of UNCP in preventing potential A-L induced hematotoxicity lies between 300 and 900 mg/kg. This corresponds to between 2 teaspoonful and 6 teaspoonful of UNCP daily [29]. It is evident from the above that a daily dose of 300, 900, and 1500 mg/kg corresponds to 4.54 g, 13.42 g, and 22.70 g UNCP daily, respectively.

The normal recommended use of UNCP in Ghana as a beverage is 1-2 teaspoonful 3 times daily (i.e., 5 g - 15.0 g daily). The phytochemical components of UNCP are likely to play a major role, and since UNCP is a non-alkalized powder, it is likely to have a greater percentage of total polyphenols, increased epicatechin and proanthocyanidins, as compared to alkalinized cocoa powder. Though comparatively this was not part of this study, other studies have proved these differences in phytochemical composition [3-7].

This study contributes much to the hematoprotective potential of UNCP during HD A-L administration, since in the pathogenesis of malarial infection, hematological values are also paramount. It is important to determine the mechanism of this activity and the quantitative components of UNCP in further studies. Besides, it would be more expedient to conduct further studies in malarious guinea pigs much more, as UNCP has been found to possess antimalarial activity [13,14,41]. Thus, this study may have a beneficial impact on therapeutic strategies.

CONCLUSION

UNCP restored some hematological disorders induced by A-L in guinea pigs by causing a significant increase in lymphocyte and PLT levels at a dose of 1500 mg/kg that was otherwise decreased by the administration of HD A-L. There was also an increase in NO with different doses of UNCP administration as a sequel to A-L dosing, which makes them a safe and advantageous combination. This research indicates the potential of daily ingestion of UNCP to prevent deleterious effects of A-L for the management of malaria.

RECOMMENDATION

Further studies to study into the quantitative analysis of UNCP and its mechanism behind this hematological effect are recommended.

LIMITATIONS

Quantitative components of this cocoa powder would have to be analyzed in subsequent studies and to explore mechanisms of this action. Further, this effect of cocoa should be studied in malarious guinea pigs.

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