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COMPARATIVE HEMATOLOGICAL EFFECTS OF CIMETIDINE, ASCORBIC ACID, *CITRUS AURANTIFOLIA* AND *TETRACARPIDIUM CONOPHORIUM* IN ADULT MALE ALBINO WISTAR RATS

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ABSTRACT

The day-to-day use of Cimetidine, Vitamin C, consumption of Lime and Walnuts increases globally with its rapid social and medicinal acceptance by both genders. In view of their wide usage especially in Africa, it becomes necessary to ascertain its comparative effects on haematological parameters of male albino wistar rats. Thus to study the effects of cimetidine, ascorbic acid (Vit. C), *Citrus aurantifolia* (lime) and *Tetracarpidium conophorium* (walnut) on haematological parameters, ninety six male albino wistar rats weighing 154 g - 281 g were obtained and divided into six groups. Groups A-D had 18 animals per group while Groups E and F had 12 animals each. Groups A-D were further divided into three sub-groups each of six animals per group with sub-group$_1$ as control group, sub-group$_2$ and $_3$ as experimental groups treated with medium and high doses of cimetidine, Vitamin C, Lime and walnut respectively. Group E and F were divided into two sub-groups of six animals each with sub-group$_1$ as control and sub-group$_2$ as experimental group treated with combined medium doses of cimetidine + Vitamin C and lime + walnut respectively. Administration was done twice daily for 21 days. At the end of the treatment period, blood samples were obtained and analysed for haematological investigation. The results revealed that Cimetidine significantly reduced ($p<0.05$) WBC, RBC and PCV in medium dose groups compared to control. Lime significant decreased ($p<0.05$) WBC in medium dose group and Hb in medium and high dose groups compared to control. Combination treatment of Cimetidine and Vitamin C significantly decreased ($p<0.05$) WBC compared to control. Conclusively, cimetidine and lime interferes with haematopoiesis, Vitamin C has no effect on blood meanwhile Walnut plays a role in blood pressure regulation.

KEYWORDS: Cimetidine Hematological indices, *Citrus aurantifolia,* haematopoiesis, *Tetracarpidium conophorium.*

INTRODUCTION

Cimetidine is a histamine H2-receptor antagonist that inhibits stomach acid production, marketed by GlaxoSmithKline under the trade name Tagamet, it is used to alleviate the symptoms of peptic ulcer disease, erosive gastroesophageal reflux disease, and hypersecretory conditions including Zollinger-Ellison syndrome and multiple endocrine adenomas. It is available over the counter and by prescription.[1] Studies show that Cimetidine affects the metabolism of methadone, sometimes resulting in higher blood levels and a higher incidence of side effects, and may interact with the antimalarial medication hydroxychloroquine.[2] Also Cimetidine is known to potentiate the effects of several opioids which are partially metabolized via the cytochrome P450 pathway via inhibiting their metabolism and a temporary decrease of liver function due to reduced hepatic blood flow.[3]

Vitamin C is an important antioxidant substance in biological systems.[4] It is a water-soluble micronutrient, well absorbed by the gastrointestinal tract and required for multiple biological functions and biochemical reactions in humans and animals.[5] Low levels of vitamin C are known to occur in several pathologies which cause increased oxidative stress, such as diabetes mellitus, cancer, cataract, HIV infection, and smoking habits.[4]

Citrus aurantifolia (lime) is a polyembryonic species with greenish yellow smooth surfaced, thin-skinned fruits, and solid core at maturity with high acidic juice.[6] Lime is believed to be native of South East Asia, and was carried by Arab traders to the Middle East and eventually came to Europe during the crusades, and are today cultivated in many countries of the world.[7] The principal use is for food, refreshing drinks, tasty desserts and for seasoning meats, vegetables, salads, sauces,

marmalade and casserole.[8,9] Citrus is a source of flavonoids and are a large class of low molecular weight polyphenolic compounds. Consumption of food rich in flavonoids prevents several degenerative pathologies, including cardiovascular diseases, atherosclerosis, cataract and several forms of cancer.[10]

The plant *Tetracarpidium conophorium* commonly called African Walnut belongs to the family Euphorbiaceae.[11] The fruits are edible, leaves, bark, and fruit of *T. conophorum* are used medicinally, and their uses include masticatory, giddiness, thrush, anti-helminthic, toothache, syphilis, dysentery, and as an antidote to snakebite.[12] Phytochemical analysis of *Tetracarpidium conophorium* indicates that it contains ingredients such as Omega-3 fatty acids and phytosterols that may reduce the risk of cardiovascular diseases.[13] The presence of oxalates, phytates and tannins in the raw walnut has been reported and also investigations has shown presence of amino acid and fatty acid components in the nut and on its leaf juice.[14,15]

Blood is a bodily fluid in animals that delivers necessary substances such as nutrients and oxygen to the cells and transports metabolic waste products away from those same cells. In vertebrates, blood is composed of blood cells suspended in blood plasma. Plasma, which constitutes 55 percent of blood fluid, is mostly water (92 percent by volume), and contains dissipated proteins, glucose, mineral ions, hormones, carbon dioxide (plasma being the main medium for excretory product transportation), and blood cells.[16]

Day after day, the use of Cimetidine (most common/relatively cheap antiulcerative drugs), and Vitamin C as drugs (for cold, scurvy and some minor injuries), and the consumption of Lime (a major source of food ingredients, drinks and medication) and Walnuts (as snacks) increases globally with its rapid social and medicinal acceptance by both men and women. In view of its uses, especially in Nigeria this study was planned to ascertain their effects on haematological parameters.

MATERIALS AND METHODS
Collection and identification of drug and fruits
The Cimetidine and Vitamin C tablets were purchased from Top Care Pharmaceutical Store, Nwaniba road, Uyo, Akwa-Ibom State, Nigeria. The brand of cimetidine drug used in the cause of this research work had identification records as follows.

CIMEC 400mg: cimetidine tablets B.P. 400mg is its composition. It's a pack of 20 tablets. Manufactured by ZIM LABORATORIES LTD. B-21/22 MIDC area KalmesKalmeshwar, Nagpur 441-501. Manufacturing license no. 1224.Sole agent – Climax Pharmchem Ltd, Nigeria.

Fresh lime fruits were obtained from an early morning market in Uyo metropolis, Akwa Ibom State, Nigeria and

were identified at the department of Botany and Ecological studies of the University of Uyo, Nigeria.

Tetracarpidium conophorum seeds (walnuts) were obtained in large quantities, fresh and uncooked from the cementary market in Aba, Abia State of Nigeria. The seeds were identified and authenticated by a senior technologist in the Department of Botany and Ecological Studies of the University of Uyo, Akwa Ibom State, Nigeria.

Extraction of plant material
The fruits of *Citrus aurantifolia* (Lime) were carefully washed to remove sand/dirt, sliced into two halves, gently squeezed into a container. The obtained lime juice was filtered through a filter paper; the seeds and residual pulp were discarded. Fifty-four lime fruits were processed in like manner, collected into a clean plastic vessel, covered and stored in the refrigerator. The pH of the lime juice was 1.7. Fruits of *Tetracarpidium conophorum* (Walnut) were washed, cooked for about two and a half hours, after which they were removed from the water, allowed to cool for about thirty minutes. Boiling was done to reduce its toxic effect as fresh walnut can be corrosive to the mouth. A light hammer was then used to break up the shell of the fruits. An electric blender was used to blend the edible part of the nut into powder. 200 ml of distilled water was added to it and allowed for 24 hours. After 24 hours it was filtered and the filtrate was obtained, stored in a cork sealed container and put into the refrigerator for use.

Acute Toxicity Study
Toxicity test for lime juice
Nine adult male mice weighing between 15 – 21 g were randomly placed in 3 cages, containing equal number of 3 mice per cage. The animals were fasted for 24 hours before administration of the lime juice. The nine mice were treated in aqueous extract of *Citrus aurantifolia* at dosages 3000 mg/kg, 4000 mg/kg and 5000 mg/kg depending on their body weight. They were observed for 24 hours for signs of toxicity. All nine mice were alive.

Dosage design
LD 50 values greater than 5000 mg/kg are of no practical interest.[17] Absence of lethality at such a higher value indicates that the substance is relatively non toxic. Therefore, using Miller and Tainter's method.[18] of 10 % low dose, 20 % medium dose and 30 % high dose, the dosage for the experiment was designed.

Toxicity test for walnut
The medium lethal dose (LD50) of the plant extraction was also determined by using Lorke's method.[17] The LD50 was done on 2 phase. The 1st phase was done using 21 mice and 3 mice in each group. The animals were treated with aqueous extract of *Tetracarpidium conophorium* at dosages 5000 mg/kg, 4500 mg/kg, 4000 mg/kg, 3500 mg/kg, 3000 mg/kg, 2000 mg/kg and 1000 mg/kg depending on their body weight and the

administration was intraperitoneal. They were observed for 24 hours for signs of toxicity. After 24 hrs all the 21 mice were dead and others signs of toxicity were observed 2-4 hours after extract administration. There was decreased locomotion, weight loss and decreased feed intake after 15 hours of extract administration.

The second phase, dosages of 100 mg/kg, 200 mg/kg, 300 mg/kg, 400 mg/kg, and 500 mg/kg for 10 mice, 2 per group. They were well labelled and administered intraperitoneal, allowed for 24 hrs for toxicity. All 10 mice survived and the median lethal dose was calculated to be 70.7 mg/kg body weight. **All** procedures involving animals in this study conformed to the guiding principles for research involving animals as recommended by the guiding principles in the care and use of animals laid down in the European Community guidelines.[19]

Experimental animals
Mature albino rats of Wistar strain were obtained from the animal house of the Faculty of Basic Medical Sciences, University of Calabar, Nigeria and were kept for 2 weeks in a well ventilated section in the animal house of the Department of Pharmacology and Toxicology, Faculty of Pharmacy, University of Uyo, Akwa-Ibom State, for acclimatization. The rats were weighed before the commencement of experimental treatment and their weights fall between the weights of 154g -281g, kept in wooden cages of 50 x 30cm dimension. The animals were fed and had free access to drinking water while the experiment lasted. All procedures involving animals in this study conformed to the guiding principles for research involving animals as recommended by the guiding principles in the care and use of animals laid down in the European Community guidelines.[19]

Experimental procedure
Total of ninety-six (96) adult male albino wistar rats were randomly designed into six (6) groups. Group A – D had eighteen (18) animals per group while Group E and F had twelve (12) animals each. Groups A - D were further divided into 3 Sub - Groups of 6 animals each, with sub-group $_1$ as control group, sub-group $_2$ and $_3$ as experimental groups. Sub - Groups $_2$ and $_3$ were treated with medium and high doses respectively. Group E and F were divided into two Sub - Groups of six animals each with sub-group$_1$ as control and sub-group$_2$ as experimental group.

Group A – Administered Cimetidine
Sub-group A$_1$: this group was treated with 10 ml/kg of distilled water
Sub-group A$_2$: this group was treated orally with a medium dose of cimetidine (475 mg/kg)
Sub-group A$_3$: sthis group was treated orally with a high dose of cimetidine (950 mg/kg).

Group B – Administered Ascorbic Acid
Sub-group B$_1$: this group was treated with 10 ml/kg of distilled water
Sub-group B$_2$: this group was treated orally with a medium dose of ascorbic acid (250 mg/kg)
Sub-group B$_3$: this group of animals was treated orally with a high dose of ascorbic acid (400 mg/kg).

Group C – Administered *Citrus aurantifolia* (Lime Juice)
Sub-group C$_1$: this group was treated with 10 ml/kg of distilled water
Sub-group C$_2$: this group received 1000 mg/kg lime juice (medium dose)
Sub-group C$_3$: this group received 1500 mg/kg lime juice (high dose)

Group D – Administered *Tetracarpidium conophorium* (Walnut)
Sub-group D$_1$: this group was treated with 10 ml/kg of distilled water
Sub-group D$_2$: this group received 14.14 mg/kg *Tetracarpidium conophorium* (medium dose)
Sub-group D$_3$: this group received 21.21 mg/kg of *Tetracarpidium conophorium* (high dose)

Group E – Administered Cimetidine + Ascorbic acid
Sub-group E$_1$: this group was treated with 10 ml/kg of distilled water
Sub-group E$_2$: test group treated with Cimetidine (Medium dose) + Ascorbic acid (medium dose)

Group F – Administered Lime Juice + Walnut
Sub-group F$_1$: this group was treated with 10 ml/kg of distilled water
Sub-group F$_2$: treated with Lime Juice (medium dose) + Walnut (medium dose)

All procedures involving animals in this study conformed to the guiding principles for research involving animals as recommended by the guiding principles in the care and use of animals.

Sample collection
After 21 days of oral administration of cimetidine, ascorbic acid, *Citrus aurantifolia* juice and *Tetracarpidium conophorium extract*, the rats in the different groups were anesthetized using chloroform. Skin incisions were made underneath the thoracic region using a sterile pair of scissors to expose the heart and then a 5 ml syringe fitted with a needle was used to aspirate blood from the right ventricle of the heart of each of the rats a procedure called "cardiac puncture". The blood gotten was stored in properly labelled plain capped sample bottles containing ethylenediaminetetraacetate (EDTA) by a modified method of Ohwada.[20] The samples were then used immediately for the estimation of the various haematological parameters.

Blood analysis
Blood samples were analysed using an automated cell counter (Coulter electronics, Bedfordshire, UK) with standard calibration, according to the manufacturer's instructions for analysis of human blood.[21] and accurately programmed for the analysis of the following Haematological parameters; Red blood cell count (RBC), total white blood cell count (WBC) and differential counts, haemoglobin (Hb), packed cell volume (PCV), mean corpuscular volume (MCV), mean corpuscular haemoglobin concentration (MCHC), mean corpuscular haemoglobin (MCH) and total platelet count (PLT).

Statistical analysis
4.1.1 The results were analysed for statistical significance by Student T-TEST method using the SPSS statistical program and Post Hoc Test (LSD) between groups using Microsoft excel program, all data are expressed as mean SEM. P values < 0.05 are considered significant.[22]

RESULTS
Haematological Parameters and Differential WBC Count Results of Cimetidine Treated Group, Group A.
Haematological parameters for cimetidine treated group shows there was a significant (p<0.05) decrease in WBC of medium dose group compared to control, a significant (p<0.05) decrease in RBC of medium dose group compared to control and a significant (p<0.05) decrease in PCV of medium dose compared to control group (Table 1). While the differential WBC count showed no significant decrease compared to the control (Table 2).

Haematological Parameters and Differential WBC Count of Ascorbic Acid Treated Group, Group B.
The haematological parameters and differential WBC count of Vitamin C treated group showed no significant difference (Tables 3 and 4).

Haematological Parameters and Differential WBC Count of *Citrus aurantifolia* Treated Group, Group C
There was a significant (p<0.05) decrease observed for WBC at medium dose group compared to control, a significant (p<0.05) decrease for haemoglobin (Hb) at medium and high dose groups compared to control (Table 5). While the differential WBC count showed no significant, decrease compared to the control (Table 6).

Haematological Parameters and Differential WBC Count of *Tetracarpidium conophorium* Treated Group, Group D
A significant (p<0.05) decrease was observed in RBC at medium dose group compared to control. Also a significant (p<0.05) decrease was observed for lymphocytes at medium dose treatment group compared to control, a significant (p<0.05) increase of lymphocytes at high dose group compared to medium dose and a significant (p<0.05) decrease in neutrophil count at high doses compared to medium dose (Tables 7 and 8).

Haematological Parameters and Differential WBC Count of Cimetidine and Ascorbic Acid Treated Group, Group E
A significant (p<0.05) decrease in WBC was observed for the test group compared to control (Table 9). While the differential WBC count showed no significant change compared to the control (Table 10).

Haematological Parameters and Differential WBC Count of *Citrus aurantifola* and *Tetracarpidium conophorium* Treated Group, Group F
There was a significant (p<0.05) decrease in RBC for the test group compared to the control (Tables 11). While the differential WBC count showed no significant, change compared to the control (Table 12).

Table 1: Haematological Parameters of Cimetidine Treated Group

Dose (mg/kg)	WBC (10^3/µl)	RBC (10^6/µl)	Hb (mmol/l)	PCV (%)	MCV (µm^3)	MCH (pg/cell)	MCHC (g/dl)	PLT (10^3/µl)
Control Distilled water 10ml/kg	20.12±1.61	8.40±0.25	14.16±0.28	44.92±1.09	53.56±0.77	16.90±0.31	31.54±0.30	851.40±68.91
475	15.52±3.17*	7.02±0.56*	10.54±2.16	37.46±3.38*	53.14±0.68	14.50±2.46	27.24±4.56	852.80±43.53
950	12.56±1.50	7.44±0.18	13.24±0.19	41.08±0.84	55.28±0.69	17.82±0.32	32.26±0.26	821.20±30.75

Values are expressed as mean ± SEM. *significantly different from control p<0.05, n=6.

Table 2: Differential White Blood Cell Count of Cimetidine Treated Group

	Lymphocytes (%)	Monocytes (%)	Neutrophils (%)	Eosinophils (%)	Basophils (%)
Control Distilled water 10ml/kg	79.80±3.40	1.20±0.58	17.20±3.40	1.80±0.58	0.00±0.00
475	74.00±3.96	3.20±0.66	20.00±4.18	2.80±0.97	0.00±0.00
950	8±3.40±1.03	3.00±0.95	12.00±1.30	1.60±0.68	0.00±0.00

Values are expressed as mean ± SEM. *significantly different from control p<0.05, n=6.

Table 3: Haematological Parameters of Vitamin C Treated Group

Dose (mg/kg)	WBC (10^3/µl)	RBC (10^6/µl)	Hb (mmol/l)	PCV (%)	MCV (µm^3)	MCH (pg/cell)	MCHC (g/dl)	PLT (10^3/µl)
Control Distilled water (10ml/kg)	20.12±1.61	8.40±0.25	14.16±0.28	44.92±1.09	53.56±0.77	16.90±0.31	31.54±0.30	842.80±64.04
250	16.64±2.28	7.94±0.21	13.60±0.28	42.72±1.22	53.84±0.97	17.16±0.27	32.06±0.27	771.60±32.32
400	16.10±1.19	8.13±0.16	13.64±0.23	43.38±0.89	53.36±0.36	16.78±0.17	31.44±0.28	863.80±38.78

Values are expressed as mean ± SEM. n=6

Table 4: Differential White Blood Cell Count of Vitamin C Treated Group

Dose (mg/kg)	Lymphocytes (%)	Monocytes (%)	Neutrophils (%)	Eosinophils (%)	Basophils (%)
Control Distilled water (10ml/kg)	79.80±3.40	1.20±0.58	17.20±3.40	1.80±0.58	0.00±0.00
250	81.00±1.58	1.80±0.66	14.00±1.14	2.60±0.51	0.20±0.20
400	86.80±2.96	1.20±0.73	9.60±2.36	2.40±0.68	0.00±0.00

Values are expressed as mean ± SEM. n=6

Table 5: Haematological Parameters of *Citrus aurantifolia* Treated Group

Dose (mg/kg)	WBC (10^3/µl)	RBC (10^6/µl)	Hb (mmol/l)	PCV (%)	MCV (µm^3)	MCH (pg/cell)	MCHC (g/dl)	PLT (10^3/µl)
Control Distilled water (10ml/kg)	20.12±1.61	8.40±0.25	14.16±0.28	44.92±1.09	53.56±0.77	16.90±0.31	31.54±0.30	851.40±68.91
1000	12.82±0.85*	8.00±0.13	13.42±0.14*	42.62±0.53	53.30±0.66	16.80±0.16	31.50±0.17	802.20±47.19
1500	16.36±1.47	8.06±0.25	13.50±0.16*	43.82±1.03	54.24±0.73	16.80±0.36	30.98±0.40	879.20±68.24

Values are expressed as mean ± SEM. *significantly different from control p<0.05, n=6

Table 6: Differential White Blood Cell Count of *Citrus aurantifolia* Treated Group

Dose(mg/kg)	Lymphocytes (%)	Monocytes (%)	Neutrophils (%)	Eosinophils (%)	Basophils (%)
Control Distilled water (10ml/kg)	79.80±3.40	1.20 ±0.58	17.20±3.40	1.80±0.58	0.00±0.00
1000	82.60±4.01	2.40±1.17	13.40±2.93	1.60±0.51	0.00±0.00
1500	78.40±3.36	2.60±0.68	16.40±4.07	2.00±0.95	0.00±0.00

Values are expressed as mean ± SEM. n=6

Table 7: Haematological Parameters of *Tetracarpidium conophorium* Treated Group

Dose(mg/kg)	WBC (10^3/µl)	RBC (10^6/µl)	Hb (mmol/l)	PCV (%)	MCV (µm^3)	MCH (pg/cell)	MCHC (g/dl)	PLT (10^3/µl)
Control Distilled water (10ml/kg)	20.12±1.61	8.40±0.25	14.16±0.28	44.92±1.09	53.56±0.77	16.90±0.31	31.54±0.30	851.40±68.91
14.14	18.18±1.24	7.92±0.32*	13.36±0.48	41.88±1.63	53.38±0.64	16.90±0.19	31.62±0.26	914.20±51.83
21.21	17.80±2.50	7.62±0.16	13.18±0.21	42.32±0.93	55.60±0.56	17.32±0.16	31.18±0.33	979.20±94.40

Values are expressed as mean ± SEM. *significantly different from control p<0.05, n=6

Table 8: Differential White Blood Cell Count of *Tetracarpidium conophorium* Treated Group

Dose(mg/kg)	Lymphocytes (%)	Monocytes (%)	Neutrophils (%)	Eosinophils (%)	Basophils (%)
Control Distilled water (10ml/kg)	79.80±3.40	1.20±0.58	17.20±3.40	1.80±0.58	0.00±0.00
14.14	69.40±2.82*	3.20±1.02	24.20±1.24	3.00±1.14	0.20±0.20
21.21	83.40±2.42[a]	2.20±0.73	12.00±2.53[a]	2.40±0.24	0.00±0.00

Values are expressed as mean ± SEM. *significantly different control p<0.05
a = significantly different from MD p<0.05, n=6

Table 9: Haematological Parameters of Cimetidine and Vitamin C Treated Group

Dose(mg/kg)	WBC ($10^3/\mu l$)	RBC ($10^6/\mu l$)	Hb (mmol/l)	PCV (%)	MCV (μm^3)	MCH (pg/cell)	MCHC (g/dl)	PLT ($10^3/\mu l$)
Control Distilled water (10ml/kg)	20.12±1.61	8.56±0.25	14.16±0.28	44.92±1.09	53.56±0.77	16.90±0.31	31.54±0.30	851.40±68.91
475+250	10.23±0.62*	7.96±0.10	13.66±0.29	43.10±1.20	55.04±0.71	17.22±0.22	31.30±0.25	846.00±36.90

Values are expressed as mean ± SEM. *significantly different from control $p<0.05$, n=6

Table 10: Differential White Blood Cell Count of Cimetidine and Vitamin C Treated Group

Dose(mg/kg)	Lymphocytes (%)	Monocytes (%)	Neutrophils (%)	Eosinophils (%)	Basophils (%)
Control Distilled water (10ml/kg)	79.80±3.40	1.20±0.58	17.20±3.40	1.80±0.58	0.00±0.00
475+250	79.00±3.61	2.60±0.60	14.80±3.60	3.60±1.03	0.00±0.00

Values are expressed as mean ± SEM. n=6

Table 11: Haematological Parameters of *Citrus aurantifolia* and *Tetracarpidium conophorium* Treated Group

Dose (mg/kg)	WBC ($10^3/\mu l$)	RBC ($10^6/\mu l$)	Hb (mmol/l)	PCV (%)	MCV (μm^3)	MCH (pg/cell)	MCHC (g/dl)	PLT ($10^3/\mu l$)
Control Distilled water (10ml/kg)	20.12±1.61	8.56±0.25	14.16±0.28	44.92±1.09	53.56±0.77	16.90±0.31	31.54±0.30	851.40±68.91
1000 +14.14	17.22±1.52	7.68±0.24*	13.46±0.37	41.98±1.01	55.56±0.42	17.82±0.08	32.06±0.22	876.80±39.06

Values are expressed as mean ± SEM. *significantly different from control $p<0.05$, n=6

Table 12: Differential White Blood Cell Count of *Citrus aurantifolia* and *Tetracarpidium conophorium* Treated Group

Dose (mg/kg)	Lymphocytes (%)	Monocytes (%)	Neutrophils (%)	Eosinophils (%)	Basophils (%)
Distilled water 10ml/kg	79.80±3.40	1.20±0.58	17.20±3.40	1.80±0.58	0.00±0.00
1000+14.14	83.20±2.67	2.20±1.24	12.60±2.91	2.00±1.05	0.00±0.00

Values are expressed as mean ± SEM, n=6

DISCUSSION

Blood is the most important body fluid that regulates various vital functions of the body including transport of metabolic substances and defence against foreign substance, among others. Nutritional, environmental and bacterial infection are among several other factors which have been shown to have adverse effects on the haematological indices of most organism.[23,24,25] Hematocrit measures the percentage of red blood cells of total body blood while the total white blood count and the differential count helps to determine the state of the immune system of an organism.

It was observed in the cimetidine treated group that RBC, PCV and WBC counts were significantly decreased at medium doses and non-significantly decreased at high doses when compared to that of control. The report shows that the metabolites of these chemicals can interact with the red blood cell membrane proteins to increase the rate of red blood cell destruction. Therefore, the decrease in RBC counts, Hb and PCV observed in this study were due to retarded haematopoiesis, destruction and shrinkage of RBC. It can be said that the decreased RBC counts observed may be due to haemolysis mediated via the chemical components of cimetidine, or caused by failure of erythropoietin production. The observed result is an indication of anaemic condition. The slight non-significant increase in

Hb concentration at high doses of cimetidine may therefore result due to the increase in haemolysis of RBC. It is well known that PCV otherwise called haematocrit represents the percentage of RBC in blood. There is a direct relationship between erythrocytes, PCV and haemoglobin concentration[26] hence, an alteration in one parameter, alternately alters another. As such, a probable reason for the decreased PCV.

The decrease in total WBC counts following oral administration of cimetidine is not in line with the normal physiological response following perception of a foreign attack by body defence mechanism. The decrease observed may have resulted from suppression of leucocytosis by the drug and also from the suppression of their production in the bone marrow.

Histamine is produced by basophils and mast cells in nearby connective tissues. Histamine increases the permeability of the capillaries to white blood cells and some proteins, to allow them to engage pathogens in the infected tissues.[27] Cimetidine being a histamine H$_2$-receptor antagonist might have an effect on blood thus resulting in decreased WBC. Decreased white blood cell counts in cimetidine-treated patients (approximately 1 per 100,000 patients), including agranulocytosis (approximately 3 per million patients), have been reported, including a few reports of recurrence on re-

challenge. Most of these reports were in patients who had serious concomitant illnesses and received drugs and/or treatment known to produce neutropenia. Thrombocytopenia (approximately 3 per million patients) and, very rarely, cases of pancytopenia or aplastic anaemia have also been reported. As with some other H$_2$-receptor antagonists, there have been extremely rare reports of immune haemolytic anaemia. However, its mechanism of action is not established.

It is also observed that lime significantly decreased WBC at medium dose and a slight but significant decrease in the concentration of haemoglobin (Hb) cells was observed at both medium and high doses. Hb is the pigment part of the erythrocyte, and the oxygen-carrying part of the blood. The reduction in haemoglobin level can be due to suppressive effect of cimetidine on erythropoiesis or a result of haemolysis caused by excessive destruction of erythrocytes. Any test substance that affects the bone marrow could inhibit certain enzyme activities involved in the production of haemoglobin in red blood cells, and then reduce the ability of the blood to distribute oxygen through- out the body, a condition known as anaemia.[28]

Lime is a natural alkalizing agent, gastric antiacid, antiseptic, bactericide, activator of white blood cells (immune defences), mild anti-infiammatory, tonic for the sympathetic and nervous system, cardiac tonic, detoxicant, depurative and diuretic, antirheumatic, antigout, antiarthritic, antischlerotic (combats ageing), anti scorbutic and antivenom, blood-fluidizer, hypotensive (lowers blood pressure), remineralizer, antianemic, gastro-hepatic-pancreatic stimulator, haemostat, carminative and vermifuge, antipruritic, tonic and antidiarrhoeal.[29,30]

The wide range of bioactive compounds from the citrus species has been found to possess anti-infection and anti-inflammatory activities.[31,32] Guthrie and Carroll.[33] Hollman, Hertog and Katan[34], Kawaii, Tomono, Katase, Ogawa and Yano[35] and Lam and Hasegawa[36] showed that the flavonoids, limonoids, and ascorbic acid are groups of citrus phytochemicals and micronutrients which are responsible for the anti-inflammatory and antitumor activities. However, results from this study did not support some of the previous report. For example, C*itrus aurantifolia* previously reported to be an activator of WBC, rather it was seen in this research to decrease WBC.

In this study, treatment with walnut shows a significantly decreased RBC count and in percentage lymphocytes at medium doses when compared to the control. At high doses, a significantly increased lymphocyte and a significantly decreased neutrophil is observed when compared to medium dose.

The observed decrease in RBC counts may have been due to the suppressive effect of some components of the extract on the bone marrow. These components such as alkaloids, saponins, flavonoids, tannis and phenols may have suppressed the growth and differentiation factors in the bone marrow. Which is in tandem with the fact that walnut helps to prevent and control high blood pressure.

As already known that there is a direct relationship between erythrocytes, PCV and haemoglobin concentration[26] thus, an alteration in one parameter, alternately alters another. There was no significant change in PCV observed in this study, and this is not in agreement with the decrease in RBC count observed.

However, there was a non-significant increase in percentage neutrophil count low dose group compared to control, while there was a significant decrease in percentage neutrophil count at high doses compared to its medium dose. The observed increase in neutrophil proliferation for those that received a low dose of the extract may be related to the chemical composition of cimetidine, which later had a suppressing effect on rats that received a high dose of the drug.

There was a significant decrease in percentage lymphocytes count at medium dose compared to the control group while a significant increase in percentage lymphocytes count was observed at high doses when compared to medium dose group. The observed increase in lymphocyte proliferation at high doses may also be related to the chemical composition of the drug. Lymphocyte proliferation is a common parameter which has been measured in several studies investigating the immunomodulatory effects of metabolites.[37]

More than a decade of scientific evidence shows that incorporating walnuts (*Tetracarpidium conophorum*) in a healthy diet reduces the risk of heart disease by improving blood vessel elasticity and plaque accumulation.[38] It is also said by Ganiyu and Mofoluso.[39] that walnut helps to prevent and control high blood pressure. From this study it shows a significant decrease in RBC and lymphocytes at medium doses and decrease in neutrophils at high doses, as such not conforming to the above report. However, walnut extract increases lymphocytes at high doses thus promoting the body immune system.

In the combined group of cimetidine and vitamin C, it can be said that taking both can help to stabilize RBC level since Vitamin C at both low and high doses promotes RBC production. Most histamine in the body is produced in granules of mast cells, basophils and eosinophils. Also, Cimetidine being a strong H$_2$ receptor antagonist seems to have an effect on WBC. While the combined group of lime and walnut showed do not promote RBC production as speculated. The decrease in RBC in this group can be suggested to come from an erythropoietin deficiency or nutritional deficiencies. However, the treatment of lime and walnut can be administered to persons suffering from hypertension.

CONCLUSION

The present study did reveal that Cimetidine significantly reduced WBC, RBC and PCV. Vitamin C had no effect on blood. Lime significantly reduced WBC count HB. Walnut significantly decreased RBC count at medium dose, neutrophils at high dose compared to its medium dose, lymphocytes at medium dose, and significantly increased lymphocytes at high dose compared to its medium dose. Combination treatment of Cimetidine and Vitamin C significantly decreased WBC. Combination treatment of lime and walnut significantly decreased RBC count.

Therefore the following conclusions can be drawn from the observations made in the present study

  I.   Cimetidine interferes with haematopoiesis thus reducing RBC count
 II.   Vitamin C has no effect on blood.
III.   Lime has a negative effect on total WBC and Hemoglobin concentration.
 IV.   Walnut can be used to control or regulate high blood pressure.
  V.   Combination of cimetidine and Vitamin C cannot completely remedy the effect of cimetidine on WBC.
 VI.   Combination of both lime and walnut shows it does not promote RBC production.

Based on this study, it is observed that these drugs and plants interfere with the body regulatory mechanism and haematological parameters. As such it is recommended that caution be taken during cimetidine therapy. Since Vitamin C proves to promote RBC; it is advisable that it should be taken more regularly. Lime juice has demonstrated to decrease WBC; it is recommended it should be consumed with caution. Walnut consumption should be reduced as it tends to decrease RBC and WBC.

The information reported in this study may enhance efforts to promote wider use of these plants and drugs aimed at educating local populations on the benefits of the plants existing in their environment and the common drugs sold over the counter.

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