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Hematology Parameters Modulation by Blends of *Zingiber Officinale* and *Allium Sativum* Ethanol Extracts in Female *Rattus Norvegicus* Models.

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A B S T R A C T

The blood circulatory system is made up of the heart, the blood, and the blood vessels. Blood transports oxygen, nutrients, waste products, and various molecules around the body. It is the central compartment that interacts with every living cell. It is also an indicator of the health of an organism. This study evaluated the modulation of hematology parameters by blends of Zingiber officinale and Allium sativum using female Rattus norvegicus. A total of 40 female R. norvegicus were used for the study. They were divided into 8 groups of 5 animals each. Group 1 served as the normal control and received distilled water. Groups 2 and 3 were treated with Z. officinale and A. sativum ethanol extracts, respectively. Different proportions of the blends of the two herbs (2:8, 4:6, 5:5, 6:4, and 8:2 of Z. officinale:A. sativum) were given to groups 4, 5, 6, 7, and 8 respectively. The duration of treatment was 90 days. On the 90th day, blood samples were collected from all the R. norvegicus and used for analysis. A. sativum increased PCV, HB, RBC, and WBC, and decreased platelet counts significantly ($P < 0.05$). Overall, A. sativum and Z. officinale monotherapies were better than the blends in any proportion in enhancing all the tested parameters. However, A. sativum had the best activity. It modulated all the parameters towards physiologically desired serum levels. The blends in various proportions showed some activities which might be attributed to the interactions between the two herbs.

Keywords: *Allium sativum*, hematology parameters, packed cell volume, *Zingiber officinal*.

1. Introduction

The blood circulatory system is made up of the heart, the blood, and the blood vessels (InformedHealth.org, 2023). Blood transports oxygen, nutrients, waste products and several other molecules around the body (Dean L., 2005). It represents the central compartment that interacts with every living cell (Wallace, M. A., Kormos, T. M., & Pleil, J. D. (2016).). It is also a good indicator that determines the health of an organism (Ariel M G W, Tzipporah M K, and Joachim D P. (2016). Cellular components of blood, such as red blood cell (RBC), white blood cell (WBC), hemoglobin, among others, are very important in immunotoxicology because changes in these cellular components can reflect the body's response to toxic substances and help determine a compound's immunotoxic potential (Ladokun, O., Ojezele, M., & Arojojoye, O. (2015). This implies that hematology parameters are important in determining the influence of toxicants, including herbal drugs, on the body. The acute effects of extracts of mistletoe on hematology indices of Wister albino rats caused reductions in platelet count, hemoglobin concentration, packed cell volume (PCV) and red blood cell (RBC) and increases in white blood cells (WBC) (Ladokun, O., Ojezele, M., & Arojojoye, O. (2015). Also, *Salvadora persica* extract showed substantial decreases in several hematological parameters, including WBC, RBC, and platelet counts (Kholoud S. R. and Salha A. A (2015). In a more recent study, leaf extract of *Stachytarpheta cayennensis* in rats following acute and 28-day repeated doses in male and female rats revealed varying degrees of significant ($p < 0.05$) changes in hematological indices (Oladotun A. O, Michael O D, and Gbola O. (2020). In this present study, the influence of sub-chronic administration of a blend of *Zingiber officinale* and *Allium sativum* ethanol extracts on hematology parameters including PCV, RBC, WBC, hemoglobin (HB), and platelets were evaluated using female *Rattus norvegicus* models.

Z. officinale, which is a member of the zingiberaceae family and its edible underground rhizome, is used globally both as a spice and as a herbal medicine (Sharifi-Rad, M., Varoni, E. M., Salehi, B., Sharifi-Rad, J., Matthews, K. R., Ayatollahi, S. A., et al., (2017). *Z. officinale* contains numerous phytocomponents whose medicinal values have been employed in the treatment of various health conditions including gastrointestinal disorders, oxidative stress, inflammatory responses, cancers, diabetes, emesis, cardiovascular effects, among others. (Bera, K., Nosalova, G., Sivova, V., & Ray, B. (2016).

The rhizome of *Z. officinale* contains a wide variety of biologically active compounds such as phenolic and terpene compounds, mainly gingerols, shogaols, and paradols (Ayustaningwarno, F., Anjani, G., Ayu, A. M., & Fogliano, V. (2024). One of the factors that determines the presence of these constituents is time after harvesting. In newly harvested *Z. officinale*, gingerols, the major polyphenols are 6-gingerol, 8-gingerol, and 10-gingerol, while heat treatment, long-time storage and hydrogenation facilitates the transformation of gingerols into corresponding shogaols and paradols (Maghraby, Y. R., Labib, R. M., Sobeh, M., & Farag, M. A. (2023). Other phenolic compounds in *Z. officinale* include quercetin, zingerone, gingerenone-A, and 6-dehydrogingerdione, while terpene components include β-bisabolene, α-curcumene, zingiberene, α-farnesene, and β-sesquiphellandrene, which are considered to be the main constituents of *Z. officinale* essential oils (Mao, Q. Q., Xu, X. Y., Cao, S. Y., Gan, R. Y., Corke, H., Beta, T., et al. (2019). Besides these, polysaccharides, lipids, organic acids, volatile oils, and raw fibers are also present in *Z. officinale* (Varsha M, Mennaallah E, Raafat E, Ekram S. (2021). The volatile oils consist mainly of sesquiterpene hydrocarbons, mainly zingiberol which gives rise to the characteristic aroma of ginger (Shaukat, M. N., Nazir, A., & Fallico, B. (2023). Both the aqueous and petroleum ether extract of *Z. officinale* contain alkaloids, saponins, flavornoids, polyphenols, cardiac glycosides and reducing sugars and mineral elements including iron (Fe), chromium (Cr), copper (Cu), nickel (Ni), zinc (Zn), and cadmium (Cd) (Aschalew G, Atnafu G, and Molla T∗ (2021). The wide variety of bioactive compounds in *Z. officinale* enables it to exhibit a lot of pharmacological activities. This study was therefore intended to investigate the effects of a blend of *Z officinale* and *A. sativum* on hematology parameters using female *Rattus norvegicus.*

A. sativum is an herb growing from a strongly aromatic, rounded bulb comprising around 10 to 20 cloves covered in a papery coat with long sword-shaped leaves which are attached to an underground stem (Khorshed A, Shahab U, Shahab M , Md. SU. (2016). It has greenish-white or pinkish flowers which grow in dense, spherical clusters on top of a flower stalk (Khorshed A, Shahab U, Shahab M , Md. SU. (2016). *A. sativum* has been used since ancient times for its medicinal properties and its bulbs are found in many traditional medicines (Tesfaye A. (2021). An example is the traditional Indian Medicine (TIM), in which Ayurveda, a Sanskrit Language word signifying “true knowledge of life”, is recognized as one of the oldest traditional health care systems of the world by World Health Organization (WHO) (Kumar Joshi V, Joshi A. (2021). It was actually a major health care system in India until the introduction of orthodox medicine and the sources of medicines used in Ayurveda include plant, animal, and natural substances of mineral origin which are used for health and healing (Pandey, M. M., Rastogi, S., & Rawat, A. K. (2013). *A. sativum* is a medicinal plant recommended for physical strength, intellect promotion, as an aphrodisiac, and to maintain healthy state of life, among other uses. (Kumar J V and Joshi A. (2021). Extracts from dried *A. sativum* bulbs have been used in Unani medicine to regulate menstruation, treat digestive problems, and fever. (Peter C I, Rose N A, Sonne I M, Daniel I O,* and Daniel L A. (2023). Hot water extract from *A. sativum* bulbs mixed with honey was a folk remedy for whooping cough and intestinal worms. (Peter C I, Rose N A, Sonne I M, Daniel I O,* and Daniel L A. (2023). In Pakistan, an *A. sativum* extract is traditionally taken orally to settle the stomach, treat coughs and reduce fever; where as in Nepal, East Asia and the Middle East, it has been used to treat fevers, digestive and lung problems, and high blood pressure, among other illnesses (Tudu C K, Dutta T G M, Biswas P, Samanta D, Oleksak P, Jha N K, *et al*. (2022). Some phytochemicals present in *A. sativum*, such as allicin, contain sulphur, which enables them to exhibit anti-bacterial, anti-fungal, anti-viral, and antioxidant potentials. (Sahidur M.R., Islam S., and Jahurul M.H.A. (2023). These compounds may also provide pain relief, support immune function, treat diabetes, and reduce blood pressure. (Sahidur M.R., Islam S., and Jahurul M.H.A. (2023). *A. sativum* compounds were recorded as promising immune-boosters and they help in the treatment of cardiovascular ailments, neoplastic growth, rheumatism, diabetes, intestinal worms, flatulence, colic, dysentery, liver diseases, facial paralysis, tuberculosis, bronchitis, high blood pressure, and several other diseases **(**Arreola, R., Quintero-Fabián, S., López-Roa, R. I., Flores-Gutiérrez, E. O., Reyes-Grajeda, J. P., Carrera-Quintanar, L., *et al*., (2015).). *A. sativum* extracts showed good antibacterial activity against selected pathogenic bacterial cultures including Pseudomonas, Bacillus, Shigella and Salmonella and this was attributed to the presence of principal phytochemicals that belong to the oil-soluble organosulfur compounds and include allicin, ajoenes, and allyl sulfides (Gemilang L, Utama, Z, Rahmi, M, Puspita S, In-in H. (2024). The organosulfur compounds of *A. sativum* exhibited a range of antibacterial properties such as bactericidal, antibiofilm, antitoxin, and anti-quorum sensing activities against a wide range of bacteria including multi-drug resistant (MDR) strains (Bhatwalkar, S. B., Mondal, R., Krishna, S. B. N., Adam, J. K., Govender, P., & Anupam, R. (2021). The practice of complementary and alternative medicine is now on the increase in developing countries in response to World Health Organization’s directives and this has culminated in several pre-clinical and clinical studies that have provided the scientific basis for the efficacy of many plants used in folk medicine (Mortada E. M. (2024). Due to its biologically active components that contribute to its pharmacological properties, *A. sativum* is used in the drug development for various human diseases. This study therefore evaluated the influence of a blend of *A. sativum* and *Z. officinale* ethanol extracts on hematology parameters using female *Rattus norvegicus* models.

2. Materials and methods

2.1 Animals

R. norvegicus were purchased from the animal house of Nnamdi Azikiwe University. The animals were allowed 2 weeks to acclimatize under standard conditions of temperature and light. They were fed with pellets and given free access to drinking water. Nnamdi Azikiwe University, Animal Research and Ethics Committee approved the study and the approval number is NAU/AREC/2023/00021.

2.2 Plant materials

The plants used for this research were *Z. officinale* and *A. sativum*. The two herbs were washed, dried, and pulverized. After grinding, 200 g of each were soaked in one litre of ethanol for 48 hours. They were then sieved to obtain the filtrates which were concentrated in water bath at 50 °C. The extracts were then stored in a refrigerator until they were used.

2.3 Phytochemical analysis of Z. officinale and A. sativum separately

The qualitative phytochemical analysis of the extracts were carried out using standard methods described by Odoh, U. E., Obi, P. E., Ezea, C. C., and Anwuchaepe, A. U. (2019).

Test for alkaloids

The plant extract (0.2 g) was heated in 20 mL of 2% acid solution (HCL) individually in a water bath for about 2 minutes. The resulting solutions were allowed to cool and then filtered. Then 5 mL of the filtrates were used for the following tests:

Dragendorff's test: The test tubes were labelled and to each test tube, 5 mL of the sample was added. This was followed by 1 mL of Dragendorff's reagent. Alkaloids were confirmed by the formation of orange or red precipitates.

Hager's test

Measures of 5 ml of each sample were poured into labelled test tubes. A few drops of Hager's reagent were added to each tube. Alkaloids were indicated by the formation of a yellow precipitate. Two alternative tests that could have been used for this are Wagner's and Mayer's tests. In these tests, alkaloids would be indicated by reddish brown and cream coloured precipitates respectively.

Test for glycosides

The samples were subjected to extraction with 1% sulphuric acid (H_2SO_4) solution in hot water bath for about 2 minutes. The resulting solution was filtered and made alkaline by adding 4 drops of 20% KOH. The pH was confirmed with litmus paper. One millilitre of Fehling's solution (equal volume of A and B) was added to the filtrates and heated on hot water bath for 2 minutes. Brick red precipitate showed the presence of glycosides.

Test for saponins

The herbal extracts (0.2 g) were dissolved in methanol individually and the resulting solutions were used to run the following analysis:

Frothing test: The samples (5 mL) were poured into labelled test tubes and 5 mL of distilled water was added and the mixtures shaken energetically. The test tubes were observed for the occurrence of persistent froth.

Test for tannins

The plant extracts (0.2 g) were dissolved in methanol separately and the resulting solutions were used for the experiment. A few drops of 1% Ferric chloride was added to 3 mL of each of the samples and the contents were observed for brownish green or a blue-black colouration.

Test for flavonoids

Methanol was used to dissolve 0.2 g of the herbal extracts individually and the resulting solutions were used to carry out the following assessments:

Ammonium hydroxide test: A measure of 2 mL of 10% ammonia solution was added to a portion of each of the samples and allowed to stand for 2 minutes. Yellow coloration in the lower ammoniacal layer indicated the presence of flavonoid.

Sodium hydroxide solution test: in another test for flavonoids, 10 mL of 10% sodium hydroxide solution was\ added to a portion of each of the samples and observed for color changes in the lower alkaline layer. The yellow color showed flavones, the blue to violet color was an indication of anthocyanin, and the yellow to orange color signified flavones.

Concentrated sulfuric acid test: A portion of each of the samples was mixed gently with conc. Sulfuric acid and observed for color change, yellowish orange color showed anthocyanin, yellow to orange color indicated flavones, and orange to crimson denoted flavones.

Test for steroids and terpenoids

Salkowski test: The plant extracts were liquefied in methanol independently. The resulting solutions were then used for the investigations. A 5 ml of each of the samples was mixed in 2 ml of chloroform and concentrated H_2SO_4 was carefully poured into each mixture to form layers. Reddish-brown coloration at the interfaces indicates the presence of steroids and terpenoids. The Liebermann-Burchard test is an alternative to this experiment. Here, a color change from violet to blue or green would indicate the presence of steroids and terpenoids.

2.4 ***Acute toxicity studies (LD50) of Zingiber officinale ethanol extract***

Evaluations of the actual median lethal doses of *Z. officinale* and *A. sativum* ethanol extracts were conducted using Lorke's method (1983). This method was modified before use. (Peter C I, Rose N A, Sonne I M, Daniel I O *, and Daniel L A. (2023).

2.5 Experimental design

A total of 40 female *R. norvegicus* were used for this study. They were divided into 8 groups. Each group was made up of 5 animals. Group 1 served as the normal control and received distilled water. Groups 2 and 3 were treated with *Z. officinale* and *A. sativum* ethanol extracts, respectively. Different proportions of the blends of the two herbs, including 2:8, 4:6, 5:5, 6:4, and 8:2 were given to groups 4, 5, 6, 7, and 8 respectively. The duration of

treatment was 90 days. On the 90th day, blood samples were collected from all the *R. norvegicus* by ocular puncture and used for hematology parameters analysis.

2.6 Hematological analysis

Quantitative determination of packed cell volume (PCV)

PCV was determined by the Microhaematocrit method as described by Thrall MA, Weiser MG, Allison R, and Campbell TW. (2012). A microcapillary tube was nearly filled with the blood samples and sealed at one end. It was centrifuged at 10,000 revolutions per minute for 5 minutes using a micro-haematocrit centrifuge. After centrifugation, the PCV was read using a microhaematocrit reader.

Hemoglobin quantification (Hb)

The hemoglobin content was estimated using Drabkin's reagent. This reagent comprised a mixture of $K_3Fe(CN)_6$ 200 mg/l, KCN 50 mg/l, and $NaHCO_3$ 1 g/l, at an alkaline pH of 8.6. A measure of 5 ml of the Drabkin's solution was added to all the tubes. Also, 20 µl of blood was added to the tubes which contained the samples. Distilled water was poured into the tube that contained the blank. All the tubes were mixed thoroughly and allowed to stand at room temperature for 15 minutes. Absorbances were read at 540 nm. Total Hb concentrations (g/dl) were extrapolated from the calibration curve of cyanmethemoglobin.

Quantitative determination of total red blood cell count (RBC)

RBC was determined by the Hemocytometer method as described by Thrall MA, Weiser MG, Allison R, and Campbell TW. (2012). A 0.02 ml of blood was piped from the blood samples and added to 4 ml of the red blood cell diluting fluid in clean test tubes to make a 1:200 dilution of the blood sample. The diluted blood sample was loaded onto a Neubauer counting chamber and all red blood cells in the five groups of 16 small squares in the central area of the Neubauer chamber were counted using a light microscope at X40 objective. The number of cells counted for each sample was multiplied by 10,000 to obtain the red blood cell count per microliter of blood.

Quantitative determination of total white blood cell count (WBC)

White blood cell assay was done using a standard method (Thrall MA, Weiser MG, Allison R, and Campbell TW. (2012). A 0.38 ml of WBC diluting fluid was poured into a test tube. Afterward, 0.02 ml of blood was put into the tube. This gives rise to a 1:20 dilution. The diluted sample was introduced into the Neubauer counting chamber. The WBCs were counted with a light microscope at a magnification of X10. The number of WBCs was multiplied by 50 to get the total count per microliter of blood.

Quantitative determination of platelet count

Platelet counting was done by the Rees and Ecker method of direct counting of platelets as described by Brown, B.A. (1976). A 4 ml of the platelet diluting fluid was added to a clean test tube using an automatic pipette. To the 4 ml, platelet diluting fluid, 0.02 ml of blood samples were added to make a 1:200 dilution. The diluted blood was shaken gently for 3–5 minutes. The Neubauer chamber was thoroughly cleaned and filled with the diluted blood. The filled Neubauer chamber was placed in a moist chamber created using moist filter paper and Petri dish and allowed to stay there for 15 minutes to permit the platelets to settle. After 15 minutes, the Neubauer chamber was placed on the microscope stage and the platelets were counted at the high dry objective (X45). Five primary squares were counted on the red cell counting area of the Neubauer chamber – 4 from the four edges of the Neubauer chamber and one from the centre. The platelets appear as light bluish-coloured, round, oval or elongated refractive particles which are much smaller than the red blood cells. The total blood platelet counting was calculated by multiplying the total number counted on the 5 squares by 10,000 to give the number of platelets per microliter of blood.

3. Results

3.1 Phytochemical analysis of Z. officinale and A. sativum ethanol leaf extracts

Phytocompounds in *Z. officinale* were: Alkaloids, Tannins, Flavonoids, Steroids and terpenoids, while those in *A. sativum* were Alkaloids, Saponins, Flavonoids, and Glycosides (Peter C I, Rose N A, Sonne I M, Daniel I O, * and Daniel L A. (2023).

3.2 Acute toxicity studies

The actual lethal doses of *Z. officinale*, *A. sativum* and a combination of the two were 8,660, 4,472, and 5,477 mg/kg body weight respectively (Peter C I, Rose N A, Sonne I M, Daniel I O, * and Daniel L A. (2023).

3.3 Hematology assays

Table 1: The 91st day PCV assay

Groups	Treatments given/kg body weight	Mean PCV ± SEM (%)	P-Value
1	Distilled water 10 ml	42.00 ± 0.27	-
2	*Zingiber officinale* 530 mg	47.83 ± 0.25	0.0002
3	*Allium sativum* 530 mg	48.50 ± 0.26	0.0001
4	Ratio of Z.:A. 2:8 (106 mg:424 mg)	46.33 ± 0.51	0.022
5	Ratio of Z.:A. 4:6 (212 mg:318 mg)	45.67 ± 0.34	0.012
6	Ratio of Z.:A. 5:5 (265 mg:265 mg)	50.50 ± 0.59	0.001
7	Ratio of Z.:A. 6:4 (318 mg:212 mg)	47.50 ± 0.56	0.01
8	Ratio of Z.:A. 8:2 (424 mg:106 mg)	46.00 ± 0.32	0.006

Table 2: The 91st day hemoglobin serum concentration assay

Groups	Treatments given/kg body weight	Mean Hb ± SEM (g/dl)	P-Value
1	Distilled water 10 ml	14.10 ± 0.14	-
2	*Zingiber officinale* 530 mg	16.03 ± 0.12	5.3E-05
3	*Allium sativum* 530 mg	16.37 ± 0.15	4.5E-05
4	Ratio of Z.:A. 2:8 (106 mg:424 mg)	15.48 ± 0.30	0.025
5	Ratio of Z.:A. 4:6 (212 mg:318 mg)	15.38 ± 0.17	0.004
6	Ratio of Z.:A. 5:5 (265 mg:265 mg)	17.1 ± 0.31	0.0003
7	Ratio of Z.:A. 6:4 (318 mg:212 mg)	16.13 ± 0.34	0.007
8	Ratio of Z.:A. 8:2 (424 mg:106 mg)	15.37 ± 0.15	0.003

Table 3: The 91st day RBC serum concentration assay

Groups	Treatments given/kg body weight	Mean RBC ± SEM ($x10^6$/ul)	P-Value
1	Distilled water 10 ml	6.24 ± 0.04	-
2	*Zingiber officinale* 530 mg	8.04 ± 0.05	2.14E-10
3	*Allium sativum* 530 mg	8.84 ± 0.07	1.14E-10
4	Ratio of Z.:A. 2:8 (106 mg:424 mg)	8.58 ± 0.09	3.52E-09
5	Ratio of Z.:A. 4:6 (212 mg:318 mg)	8.71 ± 0.16	1.72E-07
6	Ratio of Z.:A. 5:5 (265 mg:265 mg)	9.01 ± 0.11	2.86E-09
7	Ratio of Z.:A. 6:4 (318 mg:212 mg)	8.82 ± 0.15	8.94E-08
8	Ratio of Z.:A. 8:2 (424 mg:106 mg)	8.71 ± 0.18	4.47E-07

Table 4: The 91st day WBC serum concentration assay

Groups	Treatments given/kg body weight	Mean WBC ± SEM ($x10^3$/ul)	P-Value
1	Distilled water 10 ml	25.91 ± 0.05	-
2	*Zingiber officinale* 530 mg	26.42 ± 0.07	0.022
3	*Allium sativum* 530 mg	29.88 ± 0.67	0.021
4	Ratio of Z.:A. 2:8 (106 mg:424 mg)	28.58 ± 0.09	3.52E-09
5	Ratio of Z.:A. 4:6 (212 mg:318 mg)	27.80 ± 0.51	0.112

6	Ratio of Z.:A. 5:5 (265 mg:265 mg)	27.25 ± 0.45	0.173
7	Ratio of Z.:A. 6:4 (318 mg:212 mg)	26.67 ± 0.16	0.060
8	Ratio of Z.:A. 8:2 (424 mg:106 mg)	24.72 ± 0.30	0.081

Table 5: The 91st day platelet serum concentration assay

Groups	Treatments given/kg body weight	Mean platelet ± SEM ($x10^3$/ul)	P-Value
1	Distilled water 10 ml	883 ± 0.53	-
2	*Zingiber officinale* 530 mg	812 ± 0.66	3.7E-05
3	*Allium sativum* 530 mg	796 ± 0.95	4.66E-05
4	Ratio of Z.:A. 2:8 (106 mg:424 mg)	824 ± 0.60	9.74E-05
5	Ratio of Z.:A. 4:6 (212 mg:318 mg)	824 ± 1.27	4.57E-05
6	Ratio of Z.:A. 5:5 (265 mg:265 mg)	823 ± 0.67	0.0002
7	Ratio of Z.:A. 6:4 (318 mg:212 mg)	861 ± 1.03	5.46E-04
8	Ratio of Z.:A. 8:2 (424 mg:106 mg)	821 ± 0.95	7.27E-04

4. Discussion

The results of the 91st-day packed cell volume (PCV) assay showed that all tested groups increased the PCV significantly ($P < 0.05$) when compared with the control group 1 that recorded a PCV of 43.00 ± 0.27%. However, *A. sativum* monotherapy had a greater PCV increment (48.50 ± 0.26%) than *Z. officinale* given alone. The blends of the two herbs also increased the PCV, but group 6, which was treated with an equal proportion of the two herbs, indicated the greatest increase in PCV of 50.50 ± 0.59% ($P = 0.001$). Low PCV is a sign of anemia which implies that both *Z. officinale* and *A. sativum* have the capability to prevent/treat anemia; *A. sativum* being more effective. This might be attributed to either an increase in iron (Fe) concentration or absorption. Iron therapy in pregnancy has been noted to be helpful in maintaining the hemoglobin percent and packed cell volume nearer to that of non-pregnant normal women (Wahed, F., Latif, S., Uddin, M., & Mahmud, M. (2008). This observation was similar to what was detected in the case of hemoglobin (Hb) and red blood cell assays.

In the case of Hb, the blend of *Z. officinale* and *A. sativun* in equal proportion had the greatest increment of 17.10 ± 0.31 mg/dL ($P = 0.0003$) when compared to the control group that had 14.10 ± 0.14 mg/dL. *A. sativum* alone exhibited greater increment than *Z. officinale* alone which are 16.37 ± 0.15 and 16.03 ± 0.12 mg/dL respectively. The same was applicable to the red blood cell (RBC) count where 5:5 blend, *A. sativum* alone and *Z. officinale* alone recorded 9.61 ± 0.04, 8.84 ± 0.07 and 8.04 ± 0.05 mg/dl respectively. These findings support an earlier study which reported that herbal medicines are commonly being used in the treatment of anemia traditionally; and *Jatropha tanjorensis* Ellis & Saroja, *Vernonia amygdalina* Delile, *Manihot esculenta* Crantz, *Megaskepasma erythrochlamys* Lindau, *Solanum macrocarpon* L. are local shrubs mostly employed (Obika O I and Ochekwu E B. (2021). This was attributed to the iron composition of these herbs. In rural Mkuranga district of Tanzania, some of the plant species used for treatment of anemia include: *Hibiscus sabdariff*, *Lawsonia inermis*, Aloe sp, *Uvaria acuminata*, *Parinari curatellifolia*, *Ozoroa reticulata*, *Manihot esculenta*, Canthium sp and *Afzelia quanzensis* (Emanuel L. P, Susan F. R, Kijakazi O. M, Hamisi M. M, (2014).

In the case of white blood cells (WBCs), group 3, which was treated with *A. sativum* alone, had the greatest and significant increase in WBC count of 29.88 ± 0.67 x103/µl ($P = 0.021$) when compared with the control group 1 which had WBC count of 25.91 ± 0.05 x103/µl. *Z. officinale* monotherapy also recorded an increase in WBC count (26.42 ± 0.07 x103/µl) ($P = 0.022$), which was not as much as the increment observed for *A. sativum* alone. The blends of the two herbs increased the WBC count less than the increment by *A. sativum* alone. These increments by the combinations yet became smaller as the proportion of *A. sativum* reduced; and in group 8, when the proportion of *A. sativum* became much smaller than that of *Z. officinale* (ratio Z.O:A.S = 8:2), none significant decrease in WBC count was recorded. (24.72 ± 0.30 x103/µl) ($P = 0.081$). WBCs are part of the immune system, and they play major roles in both the innate and humoral immune responses. (Iara M O V, Sabrina R, Joan D, Eliana M L, Frédéric B, and Nicolas B. (2021). They circulate in the blood and support inflammatory and cellular responses to injury or pathogens (Tigner A, Ibrahim SA, and Murray IV. (2024). Deficiency of WBC is an indication of neutropenia or leukopenia (Rout P, Reynolds S. B, Zito P. M., (2024). WBCs primarily help the body in fighting infections and diseases; and in situations where they are low, there are higher risks of infections (Blumenreich MS., (1990). Leukopenia may be as a result of reduced production of white blood cells, increased utilization or destruction, or both (Britannica, (2015). Infection, drugs, malignancy, megaloblastosis, hypersplenism, and immuno-neutropenia are responsible for most cases of neutropenia (Ing V. W., (1984). Therefore, *A. sativum* monotherapy, which had the greatest increment of WBC, may have the best potential to enhance immunity, and to protect the body against infections. *Z. officinale* monotherapy also enhanced WBC count, but not as much as *A. sativum*. The herbal blend should only be considered for immune boosting only when the proportion of *A. sativum* is very much larger than that of *Z. officinale*.

There was a reversal in trend when platelet serum count was analyzed. Instead of increasing, there were decreases in the platelet count in all the groups. This was as expected because platelets cause coagulation and any abnormal increase can result in clotting of blood with consequent cardiovascular diseases such as stroke and myocardial infarction, among others. When compared with group 1 (control group), which had a platelet count of 883.00 ± 0.53 x103/ µl, group 3, which was treated with *A. sativum* alone, recorded the greatest and significant decrease in platelet count and had 796.00 ± 0.95 x103/µl) (P = 4.66 x 10-5). This was less than the value obtained for *Z. officinale* administered alone (812.00 ± 0.66) (P = 3.7 x 10-5). The blend of the two herbs also decreased platelet count, but not as much as *A. sativum* or *Z. officinale* monotherapy. The decrease in platelet count was a good attribute considering the fact that excess platelets can result in thrombosis, which might lead to cardiovascular complications. Essential thrombocythaemia (ET) was associated with a broad spectrum of microvascular circulation disturbances including erythromelalgia and its ischemic complications, episodic neurological symptoms of atypical and typical transient ischemic attacks (TIAs), transient ocular ischemic attacks, acute coronary syndromes, and superficial 'thrombophlebitis' (Michiels, J. J., Berneman, Z. N., Schroyens, W., & Van Vliet, H. H. (2004). The microvascular circulation disturbances were caused by spontaneous activation and aggregation of hypersensitive thrombocythaemic platelets at high shear stress in the endarterial microcirculation involving the peripheral, cerebral and coronary circulation (Michiels, J. J., Berneman, Z. N., Schroyens, W., & Van Vliet, H. H. (2004). This implies that *A. sativum* given alone exhibited the best properties capable of preventing various cardiovascular complications. *Z. officinale* alone could also lower platelet count, but not as much as *A. sativum*. The blend of the two herbs reduced the platelet count also significantly ($P < 0.05$), but the reduction by either of *A. sativum* or *Z. officinale* was more profound.

5. Conclusion

Overall, the separate administration of *A. sativum* and *Z. officinale* was better than the combinations of the two herbs given in any proportion, in enhancing all the tested hematology parameters (PCV, HB, RBC, WBC and Platelet). However, *A. sativum* had the best activities and modulated all the parameters towards the physiologically desired serum level. The blends in various proportions showed some activities which were attributable to the interaction between the two herbs. This outcome is relevant to the area of herbal drug formulation use, which is more empirical than evidence-based. This research has brought to the fore the fact that blending herbs together can have interactions which may be synergistic or antagonistic. This counteracts the popular idea of indiscriminate combinations of herbs in poly-herbal drug formulations.

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7. Statement of ethical approval

Maintenance of all the animals used for this research, and all experiments followed EU Directives (2010/63/EU). Guides on how to care for and use Laboratory Animals (DHHS Publ. # (NIH 86-123) were also adhered to. Approval was gotten from Nnamdi Azikiwe University's Animal Research Ethics Committee, and the approval number is: NAU/AREC/2023/00021.

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