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Some Haematological Changes in Trypanosoma-Infected Albino Rats When Treated with *Zingiber Officinale* and *Curcuma Longa*

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ABSTRACT

The study determined the effect of Zingiber officinale and Curcuma longa on the liver function of albino rats infected by Trypanosoma brucei brucei. Acclimated five to six-weekold male rats were divided into five groups (A – E), each with three replicates. Group A; positive control (uninfected and untreated), Group B; negative control (infected and untreated), Group C (treated with 10 g of ginger meal mixed with 1 kg of chick mash), Group D (treated with 10 g of turmeric meal mixed with 1 kg of chick mash) and Group E (treated with 5 g each of ginger and turmeric in 1 kg of chick mash). The data were subjected to one-way ANOVA, and tested at $p>0.05$. Results showed that while all the treatment groups (C – E) maintained a high PCV, there was no significant difference between the experimental groups and positive control; WBC showed significant difference from the other groups, with the ginger treated group C, showing no significant difference with the negative group B; while the combined group E and turmeric treated group D are significantly different from B and C. RBC results showed that ginger and turmeric influenced the experimental groups; with ginger and turmeric treated groups C and D showing great improvement; although there exist a significant different between them and the positive control. At the levels administered, ginger and turmeric supplements caused minimal changes in the haematological indices of rats. Based on these results, there is a need for further research; on higher dosages of ginger and turmeric supplements, and the use of other medicinal herbs that may ameliorate physiological stress caused by Trypanosoma infection in the haematological indices of rats.

Keywords: *Zingiber officinale*, *Curcuma longa*, haematological changes, albino rats, *Trypanosoma brucei brucei*

INTRODUCTION

The trypanosomes are slender cylindrical flagellates characterized by the possession of a flagellum that arises from a kinetoplastid, a unique DNA-rich organelle at the posterior end of the body, and runs the length of the cell body attached to it by an undulating membrane (Cox, 2004).[1] The name is derived from the *Greek trypano-* (borer) and *soma* (body) because of their corkscrew-like motion.

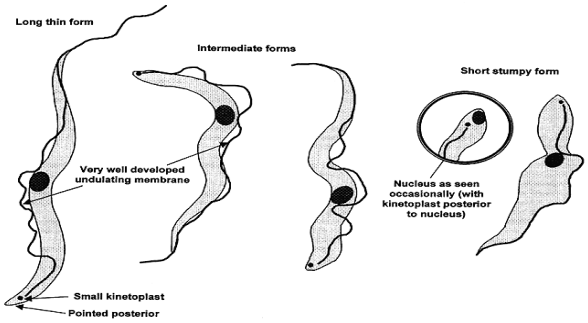

Fig. 1: Structure of *Trypanosoma brucei brucei.* **Source: Uilenberg (1998).[2]**

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Trypanosoma brucei is a species of parasitic *kinetoplastid* belonging to the genus *Trypanosoma* that is present in sub-Saharan Africa. Unlike other protozoan parasites that normally infect blood and tissue cells, it is exclusively extracellular and inhabits the blood plasma and body fluids (*Romera-Mesa* and *Mugnier*, 2020).[3] It is a species complex grouped into three subspecies: *T. b. brucei*, *T. b. gambiense and T. b. rhodesiense.* The first is a parasite of non-human mammals and causes nagana, while the latter two are *zoonotic* infecting both humans and animals and cause African *trypanosomiasis* (Baker, 1995).[4] *T. brucei* is transmitted between mammal hosts by an insect vector belonging to different species of tsetse fly (*Glossina*). The parasites undergo complex morphological changes as they move between insect and mammal over the course of their life cycle. The mammalian bloodstream forms are notable for their cell surface proteins, variant surface *glycoproteins*, which undergo remarkable antigenic variation, enabling persistent evasion of host adaptive immunity leading to chronic infection. *T. brucei* is one of only a few pathogens known to cross the blood brain barrier (Masocha, 2013).[5]

Phylogenetic reconstruction based on the genes coding for the small subunit ribosomal RNA suggested that all Salivarian trypanosomes (to which African trypanosomes belong) separated from other trypanosomes approximately 300 million years ago (Haag *et al*., 1998).[6] According to Steverding (2008),[7] soon after their emergence, Salivarian trypanosomes became gut parasites or commensals of early insects, which evolved around 380 million years ago, and with the appearance of tsetse flies (*Glossina spp*) some 35 million years ago, trypanosomes have been transmitted to mammals by these bloodsucking insects. While humans and wildlife animals are tolerant of trypanosomiasis to a reasonable extent, domestic animals are however, unable to develop any form of reasonable tolerance (Lambrecht, 1985)[8] – this was also evident in Uzu (2017),[9] as domesticated farm animals slaughtered in an abbatoir in Delta State were observed to be suffering from trypanosomiasis. The infectivity of the few species, e. g.; *T. b. rhodesiense* to humans is due to a serum-resistant-associated (*SRA*) gene (Rifkin *et al*., 1994) [10]. It seems that the SRA gene originated in a single event and then spread through *T. brucei* in East Africa by genetic exchange (Gibson *et al.,* 2017).[11]

Ginger and turmeric are flowering plants whose rhizomes are widely used as a spices and a folk medicine (Singletary, 2023).[12] Turmeric and ginger are naturally loaded with antioxidants and nutrients that help in healing several ailments and common health issues (Dusabumuremyi *et al.,* 2022).[13] They both possess anti-inflammatory properties, support the immune system, lower cholesterol, improve blood flow and digestion, as well as maintain a good body weight (Ajanaku *et al*., 2022).[14] Both plants are generally used as spices in cuisines from different parts of the world, yet others have used them medically based on their important medicinal properties (Ndinyelum and Ufele-Obiesie, 2024).[15] The effects of these spices on the blood; like supporting the immune system, and improving blood flow is a good indication of the effect they could have on blood protozoa disease. Information on the effect of these individual plants on trypanosomiasis has yielded results that allowed room for more discussion (Hussein *et al.,* 2017;[16] Jonah and Enoh, 2020).[17] This study was therefore carried out to determine the effect of *Z. officinale* and *C. longa* on the liver functions of albino rats infected with *T. brucei brucei*.

MATERIALS AND METHODS

Study Area

This research study was conducted at the Zoology Research Centre in the Faculty of Biosciences, Nnamdi Azikiwe University, Awka, Anambra State, and lasted for a period of five (5) weeks.

Ethical Clearance

The study adhered to ethical guidelines for the ethical treatment of animals during capture, handling, and sample collection. Necessary permits were obtained from the Animal Research Committee of Nnamdi Azikiwe University, Awka. The certificate with ref. no.; NAU/AREC/2024/010 5.

Trypanosome Parasite Procurement and Management: *T. brucei brucei* parasites were

obtained with permission from the trypanosome bank of NITR, head office, Kaduna. The parasites were then inoculated into two rats and transported down to Nnamdi Azikiwe University, Awka in a transportation box measuring 40 x 20 x 20 cm3 to ensure adequate ventilation.

Trypanosome Inoculation: One millilitre (1 ml) of *T. brucei brucei*-infected blood was taken from a donor rat and diluted with normal saline. The diluted blood containing approxi1*et al*. (2018)[25] respectively.

Animals Procurement and Experimental Design: A total of 75 male albino Wister rats at about 5 – 6 weeks old weighing between 60 – 70 grams obtained from the Faculty of Veterinary Sciences, University of Nigeria, Nsukka were used for the study. The rats were transported to the research station in a transportation box measuring 40 x 20 x 20 cm3 to ensure adequate ventilation. The rats were acclimatized for two weeks before the experiment began and randomly housed in 15 stainless steel metabolic cages laid out in a complete randomized design (CRD) of five treatments, replicated thrice with each replicate having five rats, and fed with commercial food (Vital Feed Broiler Starter, 18.00 ± 0.50 g/100 g crude protein, and 2106.00 kcal/kg metabolizable energy, Vital Feed, Grand Cereals Limited, Jos, Plateau State, Nigeria) and water ad libitum daily (Ndinyelum and Ufele-Obiesie, 2024).[15]

The experimental rats were of homogenous sizes and randomly stocked into three cages (24 x 24 cm2) per treatment at the rate of five rats per cage. The treatments were labelled A – E as follows; Group A, positive control (uninfected rats, fed 1000 g of chick mash), Group B, negative control (infected rats, fed 1000 g of chick mash), Group C (infected rats, fed with 10 g of *Z. officinale* mixed in 1000 g of chick mash), Group D (infected rats, fed with 10 g of *C. longa* mixed in 1000 g of chick mash), and Group E (infected rats, fed with 5 g of *Z. officinale* and 5 g of *C. longa* mixed in 1000 g of chick mash). The ratio of supplement to feed was according to Aniekwensi *et al*. (2024),[26] who reported that the inclusion of 2% ginger powder, a polyphenolic compound, specifically 6-gingerol, enhances iron absorption in humans (as confirmed in the experimental Wistar rats), mitigate iron deficiency anaemia, and protect against iron-induced oxidative damage in various tissues. 2% inclusion per feed translates to 20 g/1000 g; making 10 g/1000 g very attainable.

PCV Determination: The packed cell volume (PCV) was determined with the micro haematocrit centrifuge method as described by Dacie and Lewis (1999 [27] ;Bain *et al.,* 2016).[28]

White Blood Cell (WBC): The WBCs were counted using the principle of calibrated capillary tube for blood sampling with Haemocytometer (Nabi *et al.,* 2022) [29]. White blood cell diluting fluid (0.95ml) was dispensed into a test-tube using micropipette. The diluting fluid contained 1% glacial acetic acid (2 ml); which destroys the red blood cells, 1 ml of Gentian violet solution; which stains the white blood cells and Distilled water (98ml). The well mixed Ethylenediaminetetraacetic acid (EDTA) anticoagulated blood (0.05ml) was added to the diluting fluid in the test tube, and then mixed gently and properly. This gives a 1 in 20 dilution. The diluted sample was then loaded into the improved Neubauer counting chamber. The mixture was allowed to settle for a while before being viewed using the x40 objective lens and the cells contained in each of the four corner square millimeters were counted with a hand held tally counter. The counted White Blood Cells were recorded and calculated with the formula;

$$\text{Number of WBC in } 1\mu L = \frac{N \times 10 \times 20}{4}.$$

Source: Verso (1964).[30]

Red Blood Cell (RBC): The RBC count was determined using the principle of a calibrated tube for blood sampling with haemocytometer method (Nabi *et al.,* 2022).[29] Red blood cell diluting fluid (formol citrate) was drawn with a Pasteur pipette and then transferred into a test tube. The diluting fluid contained 3.0 g of sodium citrate, 1 cm^3 of formaldehyde solution and 100 cm3 of distilled water. 0.02ml of blood sample was drawn with a micropipette and added to the tube containing the diluting fluid to create a ratio of 1:200 dilution of blood in the dilution fluid, and the diluted blood was mixed gently and properly. The diluted blood sample was loaded into an Improved Neubauer counting chamber and when the cells settled, all red blood cells in

the five groups of 16 small squares (0,04mm^2) in the central area of the Neubauer chamber were counted using light microscope at x10 eyepiece and x40 objective lens. The counted RBCs was recorded and calculated with the formula;

$$\text{Number of RBC in 1µL} = \frac{N \times 10 \times 200}{5}.$$

Source: Verso (1964).[30]

Data Analysis: The data collected on the haematological indices of albino rats were subjected to analysis of variance (ANOVA) using SPSS version 25 (IBM SPSS, 2017).[31] The least significant difference (LSD) was used to separate significant differences between treatment means at a 5% significant level.

RESULTS

Table 1 showed the effect of *Zingiber officinale* and *Curcuma longa* on the packed cell volume (PCV) of Trypanosoma-infected rats with the groups having PCV values of 38.37%, 33.27%, 35.78%, 35.17% and 34.69% from group A – E respectively. Only rats from group A maintained a high PCV throughout the experiment, as they were not infected. They were easily followed by rats in the experimental groups C and D; with PCV values in normal range. Finally group E; which is a combination of treatment C and D followed with a value only about 1% off of groups C and D, before the untreated group B followed, also with a slightly lesser value. There was no significant difference between the groups (p>0.05).

Table 1: Effect of *Zingiber officinale* and *Curcuma longa* on the packed cell volume (PCV) of Trypanosoma infected rats

Groups	PCV
Group A	38.37a ±10.984
Group B	33.27a ±3.399
Group C	35.78a ±4.943
Group D	35.17a±5.277
Group E	34.69a ±4.54

Similar superscripts are not significantly different at p>0.05

Table 2 shows the effect of *Zingiber officinale* and *Curcuma longa* o n the white blood cell (WBC) count of Trypanosoma infected rats with their statistical margin of error. Only rats from group A maintained a normal margin of WBC, with 5.52 × 10^3. This is significantly different (p<0.05) from all the other groups. The experimental groups; C, D and E are all significantly different (p<0.05) from each other; recording a value of 24.54 × 10^3, 11.12 × 10^3 and 16.88 × 10^3 respectively; but, there was no significant difference (p>0.05) between group C and the negative control group B, with a value of 20.34 × 10^3, where group B recorded a lower WBC to group C.

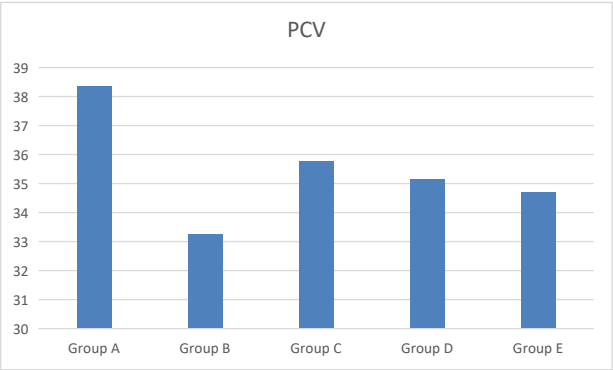

Fig. 1: Effect of *Zingiber officinale* and *Curcuma longa* on the packed cell volume (PCV) of Trypanosoma infected rats

Table 2: Effect of *Zingiber officinale* and *Curcuma longa* on the white blood cell counts of Trypanosoma infected rats

Group	WBC (cells/µL)
Group A	$5.52^{a} \pm 1.43 \times 10^3$
Group B	$20.34^{c} \pm 8.16 \times 10^3$
Group C	$24.54^{c} \pm 12.03 \times 10^3$
Group D	$11.12^{ab} \pm 7.08 \times 10^3$
Group E	$16.88^{bc} \pm 8.05 \times 10^3$

Similar superscripts are not significantly different at p>0.05

Table 3 showed the effect of *Zingiber officinale* and *Curcuma longa* on the red blood cell (RBC) count of Trypanosoma infected rats with their statistical margin of error. Rats from the positive control group A recorded a relatively healthy number of RBCs, 9.41 × 10^6; which is significantly different (p>0.05) from all the other groups. Groups C and D are also significantly different (p>0.05) from the negative control group B; with values of 8.65 × 10^6 and 8.04 × 10^6 respectively,

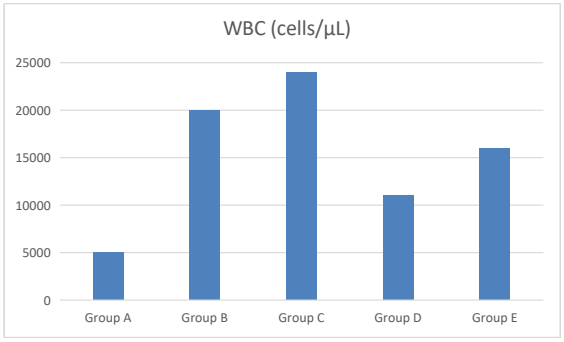

Fig. 2: Effect of *Zingiber officinale* and *Curcuma longa* on the white blood cell counts of Trypanosoma infected rats

but there was no significant difference (p>0.05) between the ginger and turmeric combined group E and negative control group B with values of 6.62 × 10^6 and 6.56 × 10^6 respectively.

Table 3: Effect of *Zingiber officinale* and *Curcuma longa* on the red blood cell counts of Trypanosoma infected rats

Group	RBC (cells/µL)
Group A	9.41b ± 1.39 × 10^6
Group B	6.56a ± 0.90 × 10^6
Group C	8.65ab ± 3.07 × 10^6
Group D	8.04ab ± 2.00 × 10^6
Group E	6.62a ± 2.19 × 10^6

Similar superscripts are not significantly different at p>0.05

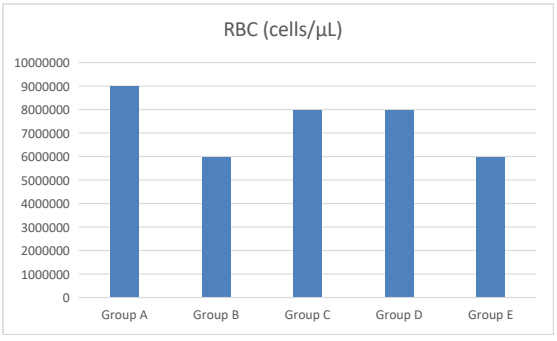

Fig. 3: Effect of *Zingiber officinale* and *Curcuma longa* on the red blood cell counts of Trypanosoma infected rats

DISCUSSION

The result of the packed cell volume following the experiment showed that group A and its replicates containing the positive control organisms recorded the highest mean PCV value. The PCV value of group A was closely followed by the values from the experimental groups C and D respectively. Group E then immediately followed group D with a value only slightly below group D, and finally group B of the infected untreated rats followed with the lowest PCV value; although, there was no significant difference (p>0.05) between the values recorded by the groups. Albeit no significant difference, all the experimental groups recorded a greater PCV value than the infected untreated group. Yet the ginger group performed the best, followed by the turmeric group, before the combined group; this agrees with Kaufman (2016),[32] who reported that ginger is used for medicinal purposes in Asia. Kaufman (2016)[32] further mentioned that in Asia, the rhizome is also considered to have diaphoretic, diuretic, anti-inflammatory, anti-emetic and sialagogic properties, and it is used as an emmenagogue, abortifacient and vermifuge, whereas it also had a reputation as an aphrodisiac; but these activities of the plant played only a little role to curtail parasitic effects in the rats. Although, no infection was involved, Ahmad *et al*. (2024),[33] also recorded that both ginger and turmeric caused an increased in the PCV value of birds and rats respectively. Which is also in line with Kujero *et al*. (2021),[34] with an improved general haematological parameter when treated with ginger and turmeric. The result although disagrees with Aljedaie and Al-Malki (2020)[35] who discovered decreased PCV in birds when treated with ginger and turmeric in the presence of another protozoan parasite Coccidiosis; as they recorded similar PCV in the untreated and experimental groups of ginger and turmeric. This may be as a result of species difference, both in the experimental organisms and the infection they are under. Fahmy *et al.* (2024)[36] is also in some agreement with this result as their study found that *Z. officinale* reduced the effect of chronic cerebral toxoplasmosis in mice when subjected to it. This result also throws light on Ufele and Njoku (2009)[37] who reported that another dietary supplement; vitamin C, inhibited the effect of Trypanosoma brucei in rats they are fed, when compared to the rats not fed with vitamin C.

The infected untreated group B recorded a PCV value that is not significantly different

(p>0.05) from any of the other groups. This result agrees with Aljedaie and Al-Malki (2020)[35] who recorded low PCV value in infected untreated birds when they are infected with another protozoan parasite Coccidiosis. It also agrees with Ufele and Njoku (2009)[37] who found that rats suffering from Trypanosomiasis recorded a lower PCV value when they are not treated. While the untreated group recorded a low PCV, the lack of significant difference may be as a result of the chick mash fed the animals, as its content boost immune response (Vital Feed Manufacturers, 2024).[38] This may have created an immune response that slowed down the effect of the parasites in the infected organisms.

Group A containing the positive control organisms recorded a mean WBC count that remained relatively stable throughout the experiment. The white cells recorded by the group A was significantly different (p>0.05) from any other group. The group that closely followed group A is the group D of turmeric treatment; the white blood cell count of the group D rats indicates amelioration in the immune system of the rats, as the white blood cell count continued to normalize through the testing. This is in agreement with Ahmad *et al.* (2024),[33] who discovered the anti-infectious properties of turmeric after studying the leucocytes of rats under infection in their work. The result also agrees with Maksudi *et al.* (2020) [39] who found that the inclusion of turmeric lowered leukocytosis in broiler chicken. Kujero *et al.* (2021) [34], also discovered an incredible immune response in birds, when turmeric was included in their meal. The control of leukocytosis in the experimental animals as a result off the inclusion of turmeric further solidifies the discoveries of Sivakumar *et al.* (2022),[40] who stated that among values attributed to turmeric are anti-inflammatory properties (making it useful in treatment of arthritis), prevention of or treatment of gallstones, enhancement of the flow of bile, reduction of serum cholesterol levels, and anti-bacterial and anti-fungal properties. Now, also showing anti-parasitic properties. The result of group D was further augmented by group E; where though a significant difference (p>0.05) exist, recorded a leucocyte count closest to group D. The difference could result from smaller dosage inclusion of turmeric. The group C on the other hand, recorded greater number of leucocytes than the infected and untreated group B; although there was no significant difference (p>0.05), the value of WBC of the group C definitely indicates that ginger has no positive effect on the leucocyte count of group C. This result is in disagreement with Kobo *et al.* (2014)[41] who discovered that *Z. officinale* facilitates slight amelioration in Trypanosoma infected mice. The result also disagrees with Maksudi *et al*. (2020),[39] who found that inclusion of ginger lowered leucocyte count in broiler chicken infected by Coccidiosis, another protozoan parasite.

The red blood cell count of group A containing the positive control rats recorded a mean RBC count that is relatively normal. The rats in group B recorded the lowest number of RBC, but there was no significant difference (p>0.05) between groups B and E. This shows that as physiological stress ensued by the prolonging of the disease; trypanosomiasis, the erythrocyte levels decreased. This is to be expected, as trypanosome, just like many other protozoa parasites attack the red blood cells. The RBC count in all the treatment groups (C, D and E) recorded lower RBC than the uninfected control, with group C recording RBC almost as high as group A; albeit a significant difference (p>0.05). The high number of RBC in the group C indicates the positive effect of ginger on the erythrocytes, preventing anemia in the infected animals. This is in line with the anti-anemic discovery of ginger by Ahmad *et al.* (2020).[33] It also agrees with Aljadae and Al-Malki (2020)[35] with their confirmation of decrease in RBC after treated with ginger in chicken Coccidiosis. The result also throws more light to Fahmy *et al.* (2024)[36] who reported that both ginger and turmeric can inhibit the effect of parasites. This is considered valid as the turmeric treated group D recorded an erythrocyte value very similar to the one recorded by group C, and there was no significant difference (p>0.05) between them, thus turmeric has almost similar effect to ginger on the red blood cells of the infected rat. This also agrees with both Ahmad *et al.* (2020)[33] and Aljadae and Al-Malki (2020)[35] in their discoveries. The ginger and turmeric combined

group E was the exception in the effects of both treatment on RBC, the value only slightly greater than the negative control group, and significantly different from the other experimental groups showed that the halved quantity of both plant combined could not repeat the effect provided by the individual plants. This most likely indicate that the inclusion at the quantities of the plants used by the researcher could not produce a positive result.

CONCLUSION

The ability of the ginger and turmeric rhizomes to increase the life span of infected rats can be attributed to high phytochemical contents of the plants. The plants produced an improved PCV result in infected rats and even improved RBC count, but ginger and turmeric cannot be said to have complete ameliorative effect on physiological stress induced by *Trypanosoma brucei brucei*.

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