EFFECTS OF *ZINGIBER OFFICINALE* AND *CURCUMA LONGA* ON THE LIVER FUNCTION OF ALBINO RATS INFECTED BY *TRYPANOSOMA BRUCEI BRUCEI*

NDINYELUM, Onyebuchi Miracle and UFELE-OBIESIE, Angela Nwogor
Department of Zoology, Faculty of Biosciences, Nnamdi Azikiwe University, Awka, Anambra State, Nigeria.

Corresponding Author: Ndinyelum, O. M. Department of Zoology, Nnamdi Azikiwe University, Awka, Anambra State, Nigeria. **Email:** miraclendinyelum@gmail.com **Phone:** +234 705 060 0072

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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-week-old 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 liver function tests were assayed using standard protocols and statistically analysed data. Results showed that AST showed no significant difference (p>0.05) in all the groups. ALT was normal in Group D, followed by Group B, then Groups C and E. There was no significant difference (p>0.05) between Groups B, C and D. The ALP result showed no significant difference (p>0.05) between Groups A, C and D. Still, it was significantly different for Groups B and E. At the levels administered, ginger and turmeric supplements had minimal to zero ameliorative hepatoprotective effects in Trypanosoma-infected 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 liver cells of rats.

Keywords: *Zingiber officinale, Curcuma longa*, Liver function, Albino rats, *Trypanosoma brucei brucei*

INTRODUCTION

A dietary supplement means a product (other than tobacco) intended to boost the diet by adding one or more of the following dietary ingredients: a vitamin, a mineral, a herb or other botanical, an amino acid, etc. It can be referred to as a dietary substance used by man to augment the diet by increasing the total dietary intake, or a concentrate, metabolite constituent, extract, or combination of any ingredient described in the abovementioned clauses (Udousoro *et al.,* 2017). Dietary supplements are generally marketed to consumers in tablets, capsules, powders, soft gels, gel caps, or liquid forms (NIH, 2018). The classes of nutrient compounds in supplements as mentioned in Udousoro *et al.* (2017) include minerals, fibre, fatty acids, amino acids, and collagen from chicken or fish. Dietary supplements are ingested purposely to supply nutrients that are lacking in the diet, strengthen body immunity, support subjects with genetic biochemical and physiological variations and fight disease conditions (Udousoro *et al.,* 2017). The formulations are often taken without a doctor's prescription or knowledge (Akabas *et al.,* 2016) and are available to the populace as over-the-counter medicines. In 2019, sales of herbal supplements just in the United States alone were $9.6 billion,

with the market growing at approximately 8.6% per year, with cannabidiol and mushroom product sales as the highest (Smith *et al.*, 2020). In 2023, sales of herbal supplements in the United States were $12.551 billion, with the market growing at 4.4% per year (Smith *et al.*, 2024). Italy, Germany, and Eastern European countries were leading consumers of botanical supplements in 2016, with the European Union market growth projected to be $8.7 billion as of 2020 (Becker, 2016; Agova *et al.*, 2020). In Nigeria, the government in response to the effects of undernourishment and micronutrient deficiencies on the populace, through the Standard Organization of Nigeria (SON) launched a food policy in 2001 intending to improve the nutritional status of all Nigerians (Zaruwa, 2016). In 2002, Nigeria started the implementation of mandatory supplementation of foods namely, wheat flour, maize flour, vegetable oil, sugar and confectionaries with vitamin A and by December 2004, 100% of wheat flour, 70% of sugar and 55% of vegetable oil sold in the market were fortified with vitamin A, all flour mills across Nigeria also fortified their products with vitamin A (Zaruwa, 2016). In recent times, Nigerians continue to be no strangers when it comes to food supplements, making use of black pepper and garlic meal to improve growth performance (Ufele *et al.,* 2020a), neem leaves to prevent, and in some cases treat malaria (Mbugi *et al.,* 2021; Sale *et al.*, 2023), and even *Moringa* (leaves and seeds) to improve growth, as well as many other physiological activities (Ufele *et al.,* 2020b). The study of the effect of dietary supplementation on organisms during physiological stress has helped scientists and researchers provide alternative herbal healthcare medication, as opposed to the contemporary use of synthesized drugs, which many believe to have incredible side effects (Moustafa *et al.,* 2024).

Trypanosomiasis, also known as sleeping sickness in humans, and nagana disease in farm animals and ungulates, is a tropical disease caused by parasitic protozoa of the genus *Trypanosoma*. Two main types of trypanosomiasis affect humans: African trypanosomiasis and American trypanosomiasis. The infectious disease (African Trypanosomiasis) is caused by the parasites *Trypanosoma brucei gambiense, Trypanosoma brucei brucei* or *Trypanosoma brucei rhodesiense*, among others, and the tsetse fly transmits the disease (Sutherland *et al.,* 2015). This disease is distinct from American trypanosomiasis or Chagas disease, which is caused by *Trypanosoma cruzi* and transmitted by kissing bugs (Hemiptera: Reduviidae). The most common genera responsible for the transmission of the disease are *Triatoma*, *Rhodnius*, and *Panstrongylus*. Infection usually occurs after bugs defecate on the bite site and are rubbed into the wound by the host scratching.

Both African and American trypanosomiasis have unique epidemiologic and clinical characteristics (Hollingshead and Bermudez, 2024). Clinically, the disease has two stages, characterized by an early/first haemolymphatic stage and a late/second meningoencephalitis stage with an invasion of the central nervous system (CNS) (Hollingshead and Bermudez, 2024). The earliest manifestation of the disease is a cutaneous chancre at the site of inoculation. This however only occurs rarely in patients with *T. brucei gambiense* and infrequently (19%) with those infected by *T. brucei rhodesiense.* Systemic symptoms develop after that with intermittent fever, headache, pruritus, and lymphadenopathy (Brun *et al.*, 2010). The lymphadenopathy may be particularly conspicuous in the posterior triangle of the neck and has received the eponym "winter bottom's sign" (Hollingshead and Bermudez, 2024). Fevers often persist from a day to a week and are separated by afebrile intervals of days to months. Undulating fevers reflect parasites multiplying within the blood. Less frequently hepato-splenomegaly may occur in the early stage. In the late/second stage, CNS symptoms manifest as sleep disturbances or neuropsychiatric disorders. A sleep disorder is the most common symptom of the second stage, and it is from this that the term "African sleeping sickness" was ascribed (Hollingshead and Bermudez, 2024).

Two important plants that may be of great importance to ameliorate the effects of trypanosomiasis on liver function are the ginger – *Zingiber officinale* Roscoe (Zingiberales: Zingiberaceae) and turmeric – *Curcuma longa* Linnaeus (Zingiberales: Zingiberaceae). Ginger is a flowering plant whose rhizome; ginger root or

ginger, is widely used as a spice and a folk medicine (Lim, 2016; Singletary, 2023). Turmeric is a perennial, rhizomatous, herbaceous flowering plant, native to the Indian subcontinent and Southeast Asia but with recent global distribution in the tropics. The rhizomes are used fresh or boiled in water and dried, after which they are ground into a deep orange-yellow powder commonly used as a colouring and flavouring agent in many Asian cuisines, especially for curries, as well as for the dyeing characteristics imparted by the principal turmeric constituent, curcumin (El-Saadony *et al.*, 2023). Turmeric and ginger are naturally loaded with antioxidants and nutrients that help in healing several ailments and common health issues (Dusabumuremyi *et al.*, 2022). 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). 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. 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; Jonah and Enoh, 2020; Adesola *et al.*, 2021). 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

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 cm^3 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 (0.9 g of NaCl per 100 ml of distilled water). The diluted blood containing approximately 1.25 x 10^6 parasites was used to inoculate the experimental animals from groups B – E via the intraperitoneal route (Dina *et al.*, 2002). Parasitaemia in the experimented animals was microscopically estimated on day three post-infection (PI) by making use of blood from the tail, and the test confirmed wriggling movement of approximately above 1.25 x 10^6 *Trypanosoma* parasites that indicated infection in the animals (Herbert and Lumsden, 1976; Quintana *et al.,* 2022).

Preparation of Ginger and Turmeric Supplements: One kilogram (1 Kg) each of fresh rhizomes of ginger and turmeric were washed with tap water and cut into tiny pieces. They were air-dried at room temperature (to avoid the reduction of the phytochemical components) to a constant weight (Lonkar *et al.,* 2013). The dried rhizomes were blended to powder making use of the Crown Star Blender (Model MC-B159) and sieved in super tiny mesh (80 mesh size – 0.177 mm), to remove debris.

Acute Toxicity and Phytochemical in Ginger and Turmeric Supplements: Following the OECD guideline for the testing of chemicals, acute oral toxicity studies of ginger and turmeric powders were adopted from Benny *et al.* (2021) and Aggarwal *et al.* (2016) respectively, while the qualitative phytochemical compositions of ginger and turmeric were adopted from Kela *et al.* (2021) and Arawande *et al.* (2018) 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 Department of Veterinary Medicine, University of Nigeria, Nsukka were used for the study. The rats were transported to the research station – the Zoology Research Centre in the Faculty of Biosciences, Nnamdi Azikiwe University, Awka in a transportation box measuring 40 x 20 x 20 cm^3 to ensure adequate ventilation.

The rats were acclimatized for two weeks before the experiment began. They were 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.

The experimental rats were of homogenous sizes and randomly stocked into three cages (24 x 24 cm^2) 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), 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

Liver Function Test: liver enzymes like alkaline phosphatase (ALP), alanine transaminase (ALT), and aspartate aminotransferase (AST) were assayed using samples of blood. Blood samples were collected from two rats per replicate through cardiac puncture and the pooled blood sample was centrifuged at 4000 rpm for 30 minutes, and the resultant sera was used for the liver enzyme assay.

Liver Function Test: All the liver function investigations were performed using Randox reagent kits. Alkaline phosphatase (ALP) was assayed using the optimized standard method according to the recommendations of Englehardt (1970) and the Deutsche Gesellschaft für Klinische Chemie (DGKC) (1972). Here, ALP hydrolyses p-nitrophenylphosphate (p-NPP) to p-nitrophenol and phosphate. The increase (change) in absorbance per minute is directly proportional to the enzyme activity at 405 nm.

Alanine aminotransaminase (ALT) was assayed using the optimized standard UV method according to the concentrations recommended by the International Federation of Clinical Chemistry (IFCC) (Bergmeyer and Hørder, 1980). ALT converts alpha-oxoglutarate and L-alanine to L-glutamate and pyruvate, and the cytosolic lactate dehydrogenase catalyzes the reversible reaction of pyruvate, NADH and H^+ to form lactate and NAD^+. The ALT activity was calculated using the following formulae: U/l = 1746 x ΔA 340 nm/minute.

The aspartate aminotransferase (AST) was assayed using the optimized standard UV method according to the concentrations recommended by the International Federation of Clinical Chemistry (IFCC) (Bergmeyer and Hørder, 1980). Alpha-oxoglutarate reacts with L-aspartate in the presence of AST to form L-glutamate plus oxaloacetate. The indicator reaction utilizes the oxaloacetate for a kinetic determination of NADH consumption. The AST activity was calculated using the following formulae: U/l = 1746 x ΔA 340 nm/minute.

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

RESULTS

Table 1 shows the mean effect of *Z. officinale* and *C. longa* on the liver function of *Trypanosoma*-infected rats. The negative control (Group A) had an ALP value of 147.51 ± 68.11, which was not significantly different ($p>0.05$) from the ALP values of Groups C and D, 108.87 ± 70.67 and 144.75 ± 80.29 respectively. The positive control (Group B) had an ALP value of 218.65 ± 9.40, which is significantly different from Groups A, C and D value, as well as Group E, having an ALP value of 297.62 ± 31.88.

Table 1: Effect of *Zingiber officinale* and *Curcuma longa* on the alkaline phosphatase levels in *Trypanosoma*-infected rats

Group	ALP (U/L)
Group A	147.51 ± 68.11[a]
Group B	218.65 ± 9.40[ab]
Group C	108.87 ± 70.67[a]
Group D	144.75 ± 80.29[a]
Group E	297.62 ± 31.88[b]

Table 2 shows the mean effect of *Z. officinale* and *C. longa* on the liver function of *Trypanosoma*-infected rats.

Table 2: Effect of *Zingiber officinale* and *Curcuma longa* on the alanine aminotransferase levels in *Trypanosoma*-infected rats

Group	ALT (U/L)
Group A	26.07 ± 15.03[a]
Group B	56.73 ± 32.67[ab]
Group C	78.93 ± 40.17[ab]
Group D	50.67 ± 32.02[ab]
Group E	91.9 ± 18.24[b]

The negative control, Group A has an ALT value of 26.07 ± 15.03, which is significantly different from all the other groups. The positive control Group B with a value of 56.73 ± 32.67 is not significantly different from Groups C and D which have values of 78.93 ± 40.17 and 50.67 ± 32.02 respectively. The combined group E with a value of 91.9 ± 18.24 is significantly different from all other groups.

Table 3 shows the mean effect of *Z. officinale* and *C. longa* on the liver function of *Trypanosoma*-infected rats with Table 3 showing the statistical margin of error. The negative control Group A has an AST value of 122 ± 36.19 which is not significantly different from any of the other groups; B, C, D and E, with values of 117 ± 28.62, 146.83 ± 9.41, 111.67 ± 18.94 and 173.75 ± 54.80 respectively.

DISCUSSION

On the toxicity of ginger and turmeric powders to the rats used, Benny *et al.* (2021) reported that no mortality or toxic signs and symptoms were observed in any of the animals in the first and subsequent treatments when higher dosages of ginger extract were administered.

Table 3: Effect of *Zingiber officinale* and *Curcuma longa* on the aspartate aminotransferase levels in *Trypanosoma*-infected rats

Group	AST (U/L)
Group A	122 ± 36.19[a]
Group B	117 ± 28.62[a]
Group C	146.83 ± 9.41[a]
Group D	111.67 ± 18.94[a]
Group E	173.75 ± 54.80[a]

Furthermore, Aggarwal *et al.* (2016) also reported that no mortality or toxic signs and symptoms were observed in any of the animals in the first and subsequent treatments when higher dosages of turmeric extract were administered. These findings inferred that both extracts were safe at the levels administered.

Qualitative phytochemical screening of ginger by Kela *et al.* (2021) following the standard method showed high levels of alkaloid and flavonoid, a moderate quantity of oxalate, phenolics and phytate, and a trace quantity of tannin, saponin, steroid and anthraquinone. A similar screening of turmeric by Arawande *et al.* (2018) showed high levels of volatile oil, balsam, ascorbic acid and flavonoid, a moderate quantity of phenol, reducing sugar, amino acid, chalcone, phlorotannin and anthraquinone; and little and traces of glycoside, saponin, steroid and acid.

Abubakar *et al.* (2022) reported that the anti-nutrients in turmeric extract include phytate, cyanide, nitrate, oxalate, tannins, and saponins. They reported very high concentrations of tannins and saponins compared to the traces of other anti-nutrients. Adanlawo and Dairo (2007) reported the anti-nutrients in ginger to include tannin, oxalate, and phytin, with very high levels of phytin, followed by oxalate, and tannin.

Investigation of the antitrypanosomal activity of traditional plants has been a major area of contemporary research focus. Phytochemicals such as flavonoid, tannin, oxalate, quinones, ascorbic acids and amino acids found in the *Zingiber* and *Curcuma* genera possess important health benefits ranging from antioxidative to immune potentials, the phytochemical properties of these plants possess chemicals of

health benefits, combating disease and supplementing the body for optimum physiological function.

The plants also have little anti-nutritional value, making them relatively safe. The liver function of the control and experimental rats was determined by using liver enzymes. The enzymes examined; ALP, ALT and AST were investigated once at the end of the experiment.

The mean ALP of Group A, although greater than the range, had an error margin that could make up for it, and is significantly lower than the ALP level discovered in Group B. The two main sources of the ALP in the body are the liver and bones. Although ALP cannot solely be used to diagnose liver or bone disease, a high level of ALP in the serum indicates liver disease or bone defect. This justifies the high level of ALP in the negative control rats since protozoan parasites are notorious for affecting the liver. Groups C and D treated with ginger and turmeric respectively recorded a marked normal range of the enzyme, with the *Z. officinale* Group C recording a very good level in the range, even when compared to the uninfected control. The *C. longa*-treated Group D also recorded a good level of ALP when compared to the uninfected group, but the group E; treated with the mixture of Z. *officinale* and *C. longa* recorded a very high ALP, a range even higher than the infected control group. This indicates that a combination of ginger and turmeric had no ameliorative effect on the *Trypanosoma*-induced liver damage. While the range of ALP in Groups C and D showed that ginger and turmeric relatively had minimal hepatoprotective effects on the liver of *Trypanosoma*-infected rats. These findings were in contrast with Hai (2024) who reported higher ALP levels in paracetamol-induced hepatotoxicity rats treated with *Z. officinale.* Group E is in complete agreement with very high levels of ALP.

The mean ALT level of Group A is observed to be well within the normal range of 10 – 40 U/L reported by Hasan *et al.* (2018). ALT is an enzyme produced when the liver is damaged; when this enzyme is high in the serum, it indicates greater damage in the liver. The negative control Group B recorded an ALT level which exceeded the normal range and is to be expected in trypanosomiasis. The treatment groups on the other hand recorded very high levels of ALT which indicated that ginger and turmeric have no ameliorative effect on the liver when infected with trypanosomes. This result is in agreement with Hai (2024) who discovered that the ALT of rats treated with *Z. officinale* when under paracetamol-induced hepatotoxicity was higher than the ALT levels in the negative control, albeit not significant. This result contradicted the report of El-Kady *et al.* (2024), where lower ALT levels were observed in rats infected with *Toxoplasma gondii* and treated with curcumin and ginger extract when compared to the negative and positive control.

The mean AST level of Group A is observed to be 122 ± 36.19 U/L which is well out of the normal range of 50 – 150 U/L Wistar rats reported by Hasan *et al.* (2018). AST is an enzyme produced when the liver is damaged; when the concentration of this enzyme is high in the serum, it indicates severe liver damage. The positive control Group B recorded an AST level of 117 ± 28.62 U/L. One treatment group (Group D) recorded an AST level closer to the positive control with 111.67 ± 18.94 U/L, and the other two experimental groups (C and E) recorded higher AST with 146.83 ± 9.41 U/L and 173.75 ± 54.80 U/L. It is important to note though, that there was no significant difference among the groups' AST levels, which indicates that ginger and turmeric had no hepatoprotective influence on the AST level, rather, the *Z. officinale* treated groups had higher AST levels than the other groups. This result disagrees with El-Kady *et al.* (2024) who discovered that the AST level of rats treated with ginger extract recorded a low AST level in the presence of *T. gondii* infection.

Conclusion: The results of the present study confirmed that although the use of medicinal plants in folk medicine contributes significantly to primary health care and that natural products are potential sources of new drugs for the treatment of a particular form of physiological stress; ginger and turmeric at the levels used in this study did not ameliorate physiological stress induced by trypanosomiasis on liver function of experimental rats. However, further studies need to be carried out on the plants in the same and other forms of physiological stress; since ginger and turmeric had a minimal impact on only ALP levels; these

plant products need to be studied again to confirm the result and get detailed information on their abilities and limitations.

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