STM JOURNALS

Research & Reviews: Journal of
Veterinary Science and Technology

ISSN: 2319-3441 (Online)
ISSN: 2349-3690 (Print)
Volume 14, Issue 1, 2025
January–April
DOI (Journal): 10.37591/RRJoVST

https://journals.stmjournals.com/rrjovst

Research RRJoVST

Assay of the Antitrypanosomal Activity of *Ziingiber Officinale* Extract in Wister Rats

Rufa'I, F.A.[1,*], Zakari, A.I.[2], Sani, A.A.[3], Umar, A.[4], Shu'aibu, M.[5]

Abstract

This research investigated the antitrypanosomal effects of Zingiber officinale in rats infected with Trypanosoma brucei brucei. Twelve adult rats, 7 weeks old and of both sexes, were randomly divided into six groups (1 through 6), with each group consisting of two rats. All six groups (1–6) were intraperitoneally injected with 0.1 ml of blood containing 10^6 Trypanosomes/ml. Groups 1–4 received daily intraperitoneal doses of the plant extract at concentrations of 0.01, 0.1, 1.0, and 10 mg/ml/kg body weight, respectively. Group 5 was administered suramin, a standard anti-trypanosomal drug, at a dose of 7.86 mg/kg, serving as the positive control, while Group 6 remained untreated, acting as the negative control. Treatments were initiated four days after infection. The study evaluated physical and behavioral changes, along with hematological parameters. Phytochemical analysis revealed that Zingiber officinale contains saponins, tannins, glycosides, terpenoids, steroids, alkaloids, and flavonoids as major components. Acute toxicity tests suggested the plant is relatively safe. Rats treated with the plant extract displayed mild mucous membrane pallor, poor body condition, fever, lacrimation, aggression, and increased food intake. Furthermore, a significant reduction in mean PCV, Hgb, RBC, MCV, MCH, and MCHC values was observed in all groups treated with the extract compared to the suramin-treated group ($P < 0.05$).The study concluded that Zingiber officinale contains essential phytochemicals and bioactive compounds that are relatively safe and possess potent trypanocidal properties, making it a potential candidate for the treatment of trypanosomiasis

Keywords: Zingiber Officinale, antitrypanosmal, hematology, suramin, lacrimation

INTRODUCTION

Trypanosomiasis is a chronic protozoal disease that impacts both domestic and wild animals, with a high morbidity rate ranging from 50% to 100% within months of exposure, especially when factors, such as poor nutrition or other debilitating conditions are present. The disease is marked by severe anemia, poor body condition, fever, pale mucous membranes, weight loss, infertility, and abortion, ultimately resulting in death during its acute phase. Trypanosome infections are known to suppress the immune system, hindering the host's ability to eliminate the parasites and leading to severe illness in humans, as well as significant losses in meat and milk production in animals. Efforts to control trypanosomiasis are estimated to cost between $600 million and $1.2 billion annually [1]. Over 60 million people are estimated to be at risk of trypanosomiasis, with only 3.5 million under surveillance in endemic regions. The management of the disease primarily relies on vector control, vaccine development, and drug treatments [2]. However, vaccine production is largely ineffective due to the parasite's antigenic variation, vector control strategies face numerous challenges, and drug therapies often result in significant side effects. These include direct toxicity to the liver and kidneys, fatal loss of consciousness during initial administration, heavy albuminuria, oral

***Author for Correspondence**
Rufa'i, F. A.
E-mail: fatihurufai@gmail.com

[1–5]Researchers, Nigerian institute for Trypanosomiasis (and onchocerciasis) Research, Kano Liasion office, Bayero University Kano, Gwarzo Road, Kano State, Nigeria

Received Date: January 18, 2025
Accepted Date: February 03, 2025
Published Date: February 12, 2025

Citation: Rufa'I, F.A., Zakari, A.I., Sani, A.A., Umar, A., Shu'aibu, M.. Assay of the Antitrypanosomal Activity of *Ziingiber Officinale* Extract in Wister Rats. Research & Reviews: Journal of Veterinary Science and Technology. 2025; 14(1): 32–37p.

ulcers, exfoliative dermatitis, severe diarrhea, and fever [3].

These challenges underscore the urgent need for alternative treatments, particularly those derived from plants with antitrypanosomal potential [4]. Zingiber officinale has been widely used in traditional medicine, attributed to its rich bioactive compounds, including saponins, tannins, alkaloids, diisooctyl adipate, and benzene. These compounds are increasingly valued for their potency and diverse pharmacological properties, especially given the high cost and limited availability of conventional drugs in rural areas, as well as the growing issue of drug resistance. This study aims to identify the bioactive compounds in Zingiber officinale and evaluate their antitrypanosomal activity [5].

MATERIALS AND METHODS

Ethical Approval

All experimental procedures were carried out in full accordance with the guidelines set by the Ethical Committee for Animal Use at Bayero University, Kano, Nigeria [6].

Plant Material

The rhizome of Zingiber officinale was authenticated at the Herbarium of the Department of Plant Science, Bayero University, Kano, Nigeria. The plant material was carefully cleaned, ground, and air-dried at room temperature for seven days. It was then stored in a clean, dry container to protect it from contamination by environmental pathogens [7, 8].

Extraction and Phytochemical Screenings

The plant powder was extracted using the cold maceration technique with methanol. A total of 300 g of the powdered plant material was measured and mixed with 900 ml of methanol, maintaining a 1:3 ratio of plant powder to solvent. The mixture was allowed to stand for 48 hours with intermittent shaking. It was then filtered using muslin cloth and the filtrate was evaporated in a water bath set at 40°C. Phytochemical screening was performed using standard methods to identify the presence of saponins, tannins, glycosides, terpenoids, steroids, alkaloids, and flavonoids [9, 10].

Determination of Acute Lethal Toxicity (LD_{50})

The Acute Lethal Toxicity (LD50) was assessed using a specific protocol involving 12 rats across two phases. During the first phase, nine rats were randomly divided into three groups, each consisting of 3 animals, and administered the plant extract orally at doses of 10 mg/kg, 100 mg/kg, and 1000 mg/kg, respectively. In the second phase, three additional rats were randomly assigned to three groups, each with a single animal, and received oral doses of 1600 mg/kg, 2900 mg/kg, and 5000 mg/kg, respectively [11, 12].

Parasite Inoculation

Each experimental rat was intraperitonially infected with 0.1 ml of blood containing approximately 10^6 Trypanosomes/ml [13].

Experimental Design

This study utilized 12 adult rats, approximately 7 weeks old, weighing between 140 and 200 g. The animals were obtained from the Animal House at the Department of Human Anatomy, Bayero University Kano, and allowed a 14-day acclimatization period in the research laboratory. Trypanosoma brucei brucei was sourced from the Nigerian Institute for Trypanosomiasis and Onchocerciasis Research. The rats were randomly divided into six groups (1 through 6), with each group consisting of two animals [14]. All groups were intraperitoneally infected with 0.1 ml of blood containing approximately 10^6 Trypanosomes/ml. Treatment commenced on the fourth day, following the detection of parasites in the bloodstream via microscopy, and continued until the 14th day, by which time all animals in the untreated control group had succumbed. Groups 1–4 received daily intraperitoneal doses of the plant extract at concentrations of 0.01, 0.1, 1.0, and 10 mg/ml/kg body

weight, respectively. Group 5 was treated with a standard trypanosomal drug, suramin (7.86 mg/kg), as positive control, while Group 6, the negative control, was infected but received no treatment [15, 16].

Monitoring Physical and Behavioral Changes and Determination of Hematological Parameters

Physical and behavioral changes, including the condition of the mucous membranes, loss of condition, and aggression, were observed daily. Blood parameters, such as Packed Cell Volume (PCV), Red Blood Cell (RBC) count, Hemoglobin Concentration (Hgb), Mean Corpuscular Volume (MCV), Mean Corpuscular Hemoglobin (MCH), and Mean Corpuscular Hemoglobin Concentration (MCHC) were assessed using standard procedures [17, 18].

RESULTS

Phytochemical Screening

The phytochemical screening revealed the presence of saponins, Tanins, Glycosides, Terpenoids, Steroids, Alkaloids, and Flavonoids (Table 1).

Table 1. Phytochemical constituents of Zingiber officinale.

Indication	Bioactive Compounds
Saponins	Presence
Tanins	Presence
Glycosides	Presence
Terpenoids	Presence
Steroids	Presence
Alkaloids	Presence
Flavonoids	Presence

Acute Toxicity Test

The overall acute toxicity outcome (LD_{50}) is shown in Table 2. No animal died within 24 hours after treatment with the plant extract at dose levels up to 5000 mg/kg.

Table 2. Acute toxicity effect of the experimental rats.

Extract	Experiment	Dose (mg/kg	Number of Animals Used	Number of Animals Dead
CME	Phase 1	10	3	0
		100	3	0
		1000	3	0
CME	Phase 2	1600	1	0
		2900	1	0
		5000	1	0

Note: CME = Crude methanol extract.

Physical and Behavioral Changes

The physical and behavioral changes observed included low pallor of the mucous membranes, loss of condition, pyrexia, lacrimation, aggression, and increased food consumption in Groups 1–5 (Table 3). In contrast, the opposite of these symptoms was observed in Group 6. No signs of eye redness, alopecia, ringtail, or gangrene were noted in any of the experimental groups [19, 20].

Hematological Parameters

The result of the hematological Indices indicated a significant decrease in mean PCV, Hgb, RBC, MCV, MCH, and MCHC in group 1, group 2, group 3, group 4, and group 6, when comparing with group 5 P-value <0.05 (Table 4) [21].

Table 3. Physical and behavioral changes of experimental animals.

Parameters	G1	G2	G3	G4	G5	G6
Pallor of Mucous membrane	+	–	–	–	–	+++
Loss of conditions	–	–	–	–	–	+++
Pyrexia	–	–	+	–	–	+++
Lacrimation	–	+	–	–	–	+++
Eye redness	+	–	–	+	+	+
Aggression	–	–	–	++	++	–
Alopecia	–	–	–	–	–	–
Ringtail	–	+	–	–	–	–
Gangrene	–	–	–	–	–	–
Food consumption	++	++	+++	+++	+++	+

Note: +++ = High, ++ = Moderate, + = Low, – = Absent.

Table 4. Mean ± Standard error hematological indices of experimental animals.

Parameters	G1	G2	G3	G4	G5	G6	P-value
PCV (%)	28.5 ± 1.56	30.0 ± 0.0	32.75 ± 0.95	33.75 ± 0.25	36.0 ± 0.58	14.5 ± 0.29	<0.001
Hgb (g/dL)	9.65 ± 0.59	12.9 ± 2.44	11.05 ± 0.29	11.15 ± 0.05	12.15 ± 0.14	4.7 ± 0.06	<0.001
RBC (10^6/µL)	4.73 ± 0.11	4.85 ± 0.05	5.98 ± 0.1	5.18 ± 0.03	5.4 ± 0.14	2.85 ± 0.03	<0.001
MCV (fl)	60.2 ± 1.89	62.89 ± 0.38	64.5 ± 0.69	63.72 ± 0.38	65.45 ± 0.38	50.8 ± 0.46	<0.001
MCH (pg)	20.38 ± 0.75	21.48 ± 0.18	21.75 ± 0.15	21.25 ± 0.05	22.05 ± 0.03	16.5 ± 0.06	<0.001
MCHC (g/dL)	33.83 ± 0.24	34.1 ± 0.1	33.73 ± 0.13	33.75 ± 0.15	33.73 ± 0.14	32.4 ± 0.23	<0.001

Means are significantly different when P-value <0.05.

DISCUSSIONS

Phytochemical analysis of Zingiber officinale confirmed the presence of saponins, tannins, glycosides, terpenoids, steroids, alkaloids, and flavonoids. These findings are consistent with earlier studies that have reported similar results. Furthermore, research has attributed the trypanocidal activity of Zingiber officinale to the presence of tannins and steroids. This observation is consistent with other studies that identified and isolated tannins and steroids from Zingiber officinale extracts, further supporting their role in the plant's biological activity. The findings from this study suggest that the presence of these phytochemicals may contribute to the trypanocidal activity of Zingiber officinale. It has been established that phytochemicals, such as tannins, saponins, and alkaloids, among others, are vital components of natural products with well-documented bioactive properties. The presence of these compounds strongly indicates the plant's potential trypanocidal effects. Research has demonstrated that *Zingiber officinale* contains bioactive compounds, such as alkaloids, flavonoids, saponins, and tannins, which contribute to its trypanocidal properties. These compounds play a vital role in providing the plant with defense mechanisms against various parasites, including trypanosomes and other microorganisms. For instance, tannins coagulate cell wall proteins, saponins enhance the entry of toxic substances or cause leakage of essential cellular components, flavonoids inhibit enzyme activity by forming complexes with extracellular materials and soluble proteins, and alkaloids disrupt nucleic acid synthesis, interfere with DNA interactions, and inhibit protein biosynthesis. These mechanisms collectively explain the demonstrated trypanocidal effects of the plant. The results of the acute toxicity test (LD50) revealed that the *Zingiber officinale* extract did not cause any mortality when administered orally at doses of up to 5000 mg/kg. This indicates that the extract is relatively non-toxic to rats. These findings are consistent with previous studies, which have characterized the extract as a safe and effective herbal remedy, exhibiting minimal and insignificant side effects [22].

The observed physical and behavioral changes align with previous reports that identified symptoms of trypanosomiasis, including weight loss, fever, lacrimation, and pallor of the mucous membranes.

The trypanocidal activity of Zingiber officinale extract may be attributed to the action of specific compounds isolated from the extract or the synergistic effects of various classes of compounds contributing to its biological activity. For instance, tannins coagulate the wall proteins of trypanosomes, saponins enable the entry of toxic substances or leakage of vital cell components, flavonoids inhibit enzymatic activity by forming complexes with extracellular materials and soluble proteins, and alkaloids disrupt nucleic acid synthesis, DNA interaction, and protein biosynthesis. Similarly, compounds, like hexadecanoic acid, dodecanoic acid, benzene, cycloheptatriene, and isopropanol, have been reported to exhibit trypanocidal effects against various trypanosome species [23].

Additionally, anemia serves as a common and reliable indicator of trypanosomiasis, with its severity reflecting the extent of infection. Groups 1 through 5 showed no signs of anemia, as their red blood cell parameters (PCV, Hgb, RBC, MCV, MCH, and MCHC) remained within the normal range. In contrast, Group 6 exhibited significantly lower red cell parameters, as these animals were untreated. The absence of treatment in this group led to continuous oxidative damage to red blood cells, parasite movement in the bloodstream, obstruction of blood flow, and the removal of red blood cells by the expanding mononuclear phagocytic system, resulting in anemia – a critical indicator of trypanosomiasis severity.

CONCLUSIONS

Zingiber officinale contains a wide range of essential phytochemical components, including tannins, saponins, flavonoids, and alkaloids, as well as other bioactive compounds, which collectively contribute to its trypanocidal properties. These compounds exhibit various mechanisms of action, such as disrupting parasite cell structures, inhibiting enzyme activity, and interfering with nucleic acid synthesis, making the plant an effective agent against trypanosomes. Furthermore, studies have demonstrated that the extract is relatively safe, with minimal toxicity, and can be considered a potent natural remedy for the treatment of trypanosomiasis. Its bioactive properties and safety profile highlight its potential for therapeutic applications in combating parasitic infections.

REFERENCES

1. Abenga JN, Enwezor FNC, Lawani FAG, Ezebuiro C, Sule J, David KM. Prevalence of trypanosomiasis in trade cattle at slaughter in Kaduna, Nigeria. **Niger J Parasitol**. 2017;23:107–110.
2. Ajakaiye JJ, Muhammad AA, Mazadu MR, Shuaibu Y, Kugu BA, Mohammad B, et al. Trypadim®, Trypamidium® and Novidium® can eliminate the negative effects on the body temperature and serum chemistry in Wistar rats infected with Trypanosoma brucei brucei (Federe strain). Int Res J Biochem Bioinform. 2014;4(4):37–41. doi: 10.14303/irjbb.2014.072.
3. Aparna V, Dileep KV, Mandal PK, Karthe P, Sadavisan C, Haridas M. Anti-inflammatory property of n-hexadecanoic acid: Structural evidence and kinetic assessment. Chem Biol Drug Des. 2012;80(3):434–439. doi: 10.1111/j.1747-0285.2012.01418.x.
4. Balogun JB, Abubakar Z, Ibrahim TB, Balogun SU, Sadiq IS, Dogara MM, et al. In vivo anti-trypanosomal potential of methanol root extract of Terminalia macroptera (Guill. And Perr.) in Trypanosoma brucei brucei infected Wistar rat. IOSR J Pharm Biol Sci. 2017;2(5):12–17.
5. Chanie M, Dulecha A, Basaznew B. Socio-economic assessment of the impacts of trypanosomiasis on cattle in Girja District, Southern Oromia Region, Southern Ethiopia. Acta Parasitologica Globalis 2013;4(3):80–85. doi: 10.5829/idosi.apg.2013.4.3.7523.
6. Deitsch KW, Lukehart SA, Stringer JR. Common strategies for antigenic variation by bacterial, fungal and protozoan pathogens. Nat Rev Microbiol. 2009;7(7):493–503. doi: 10.1038/nrmicro2145.
7. Ejikeugwu C, Umeokoli B, Iroha I, Ugwu M, Esimone C. Phytochemical and antibacterial screening of crude extracts from leaves of wonderful kola. Am J Life Sci. 2015;3(2):5–8. doi: 10.11648/j.ajls.s.2014020603.12.

8. Erhirhei EO, Moke GE, Prabhu KS. A review of its ethnomedicinal, chemical and pharmacological properties. AJPTR. 2014;4(6):22–37.
9. Erhirhie EO, Ben-AzuBenne A, Moke GE, Chinwub P, Omonjiahio I. Ethno-pharmacological review of *Buchhoiziacoriacea* (Wonderful Kola). Int J Adv Pharm, Biol Chem. 2015;4(1).
10. Fatope A, Coleman CT, Phumg OJ. Extraction of plant materials. Ann Fam Med. 2001;(5):432–439. doi: 10.123/afm.1517.
11. Herbert WJ, Lumsden WA. Trypanosome brucei: A rapid matching method for estimating the host's parasitaemia. Exp Parasitol. 1976;40(3):427–431. doi: 10.1016/0014-4894(76)90110-7.
12. John L, Ogle GD, Scianna J, Winslow S, Holzworth K. Plant materials collection guide. 4 th ed. New York: USAID Inc.; 2003.
13. Kumar PP, Kumaravel S, Lalitha C. Screening of antioxidant activity, total phenolics and GC-MS Study of *Vitex negundo.* Afr J Biochem Res. 2010;4(2):191–195.
14. Lorke D. A new approach to practical acute toxicity testing. Arch Toxicol. 1983;54:275–285.
15. Maigari AK, Liman SB, Musa AM, Sani A, Jarmai Abubakar S, et al. Mixed occurrence of trypanosomes in trade cattle slaughtered at Kano Abattoir, Northwestern Nigeria. Int J Curr Res. 2015;7(9):20916–20919.
16. Maigari AK, Dabo NT. Prevalence and source distribution of *Trypanosoma evansi* in trade camels in Kano state. Fudma J Microbiol. 2018;1(1):234–239.
17. Mbaya A, Kumshe H, Nwosu CO. The Mechanisms of Anaemia in Trypanosomosis: A Review. In: Silverberg D, editor. Anemia. Available at http://www.intechopen.com/books/anemia/the–mechanisms–of Anemia–in tryp
18. Mugasa CM, Katiti D, Boobo A, Lubega GW, Schallig HD, Matoru E. Comparison of nucleic acid sequence based amplification and loop – mediated isothermal amplification for diagnosis of human African trypanosomiasis. Diagn Microbol Infect Dis. 2014;78(2):144–148. doi: 10.1016/j.diagmicrobio.2013.06.025.
19. Nakayima J. Diagnostic methods for African trypanosomiasis. Available at https://www.researchgate.net/publication/292616299 [Accessed on September 2016].
20. Rufa'i, FA, Baecker D, Mukhtar MD. Phytochemical screening, GC-MS analysis, and evaluating in vivo antitrypanosomal effects of a methanolic extract of *Garcinia kola* nuts on rats. Antibiotics (Basel). 2023;12:713. doi: 10.3390/antibiotics12040713.
21. Silva GL, Lee I, Kinghorn AD. Special problems with the extraction of plants. In: Cannel RJP, editor. Natural Products Isolation. Totowa: Human Press; 1998. pp. 354–360.
22. World Health Organization. (2023). Human Africantrypanosomiasis (sleeping sickness). URL: Fact sheet N°259: African trypanosomiasis or sleeping sickness. [Accessed on August 2013].
23. Woo PTK. The haematocrit centrifuge for the detection of trypanosomes in blood. Can J Zool. 1969;47:921–923. doi: 10.1139/z69-150.

