Journal of Practical Medicine and Medical Science
Volume 2(1), pages 1-10, October 2023
Article Number: 3F793BCE1
https://doi.org/10.31248/JPMMS2023.016
https://integrityresjournals.org/journal/JPMMS

Full Length Research

Ameliorative effects of methanol leaf extract of *Andrographis paniculata* against *Trypanosoma brucei* induced alteration in packed cell volume and in liver biomarkers of mice

Shariff, M. A.[1], Agboola, A. R.[2], Adeniyi, K. A.[3,4]*, Ibeh, E. O.[5], Oyibo-Usman, K. A.[6], Ado, I. M.[3] and Salami, K. D.[7]

[1]Department of Hospital Service, Jigawa State Ministry of Health, Jigawa State, Nigeria.
[2]Department of Biochemistry, College of Medicine, University of Calabar, Cross River State, Nigeria.
[3]Department of Biological Sciences, Federal University Dutse, Jigawa State, Nigeria.
[4]Parasitology and Public Health Unit, Center for Arid Zone Ecology, Federal University Dutse, Jigawa State, Nigeria.
[5]Department of Biotechnology and Environmental Biology, Veritas University Abuja.
[6]Zoology Unit. Department of Biological Sciences, Federal University Gusau, Zamfara, Nigeria.
[7]Department of Forestry and Wildlife Management, Federal University Dutse, Jigawa State, Nigeria.

*Corresponding author. Email: adeniyi.k@fud.edu.ng; Tel: +2347032985563.

Received 11th August 2023; Accepted 26th August 2023

ABSTRACT: The goal of the study was to ascertain the *in vivo* anti-trypanosomal activity of methanol extract from leaves of *Andrographis paniculata* and its impact on a number of biomarkers in *Trypanosoma brucei*-infected mice. Six groups of three mice each were created at random from a total of eighteen mice. As negative and positive control, Groups I and II received normal saline (2 ml/kg body weight) and diminazene aceturate (3.5 mg/kg body weight), respectively. Groups III-V were treated with leaf extract at doses of 150, 300, and 600 mg/kg body weight, respectively. The oral route was used for all treatments. The average body weight, survival time, and daily parasitemia level were assessed. Alkaline phosphatase (ALP), aspartate aminotransferase (AST), alanine aminotransferase (ALT), and total protein concentration in mouse serum and liver were also assessed. Phytochemical screening was done. Flavonoids, saponins, glycosides, alkaloids, tannins, and phenols were all present in the leaves of *Andrographis paniculata.* The extract was relatively safe according to acute toxicity tests. When compared to untreated mice, the extract at 600 mg/kg body weight considerably increased mice body weight and survival rates while showing negligible trypanostatic potential. The serum, liver ALP and AST activities of infected increased significantly ($p < 0.05$) compared to untreated mice. The activities of these enzymes were significantly ($p < 0.05$) recovered after treatment with methanol extract of leaves from *Andrographis paniculata*. The serum AST and liver ALT activities, however, did not significantly change. Although *Andrographis paniculata* showed little trypanostatic efficacy, it considerably ameliorated the metabolic changes in mice caused by *T. brucei.*

Keywords: *Andrographis paniculata,* biochemical parameters, parasitaemia, trypanosomiasis.

INTRODUCTION

Human African trypanosomiasis is a vector-borne parasitic disease. It is caused by protozoans of the genus *Trypanosoma,* transmitted to humans by bites of tsetse flies (glossina) which have acquired the parasites from infected humans or animals (Adeyemi *et al.,* 2009).

African trypanosomiasis, also known as sleeping sickness, is a type of parasitic disease that is contracted by both humans and animals. *Trypanosoma* species, a protozoan parasite that is spread by tsetse flies (*Glossina genus*), is the causative agent of this disease. Even though there were less than 10,000 documented cases in 2009 and 2010, the illness continues to be a serious health concern, particularly in sub-Saharan Africa (WHO, 2012; Holanda-Freitas *et al*., 2020). The efficiency and safety of the traditional chemotherapies and vector control systems used to tackle the disease are still questionable because of the negative effects of the medications and the drug resistance that has been demonstrated by the parasite (De Rycker *et al*., 2023). Due to these problems, efforts must be made in order to find novel chemical principles for the treatment of human African trypanosomiasis (HAT) (De Koning, 2020). Interest in natural products has increased dramatically over the past 20 years, from the widespread usage of herbal remedies for self-care and beauty purposes to the scientific study of plants to determine how they affect human biology (Dias *et al*., 2012).

King of bitters, also known as *Andrographis paniculata* (Burm.f.) Wall.ex Nees is an annual herbaceous plant that is widely cultivated in Southern Asia and China. However, it is currently grown in Southwestern Nigeria (Ullah and Hassan, 2021). *A. paniculata* is frequently used in traditional medicine to relieve body heat, eliminate toxins from the body, prevent common cold, upper respiratory tract infections, including sinusitis and fever, and serve as an antidote for insect and snake venoms (Hossain *et al.,* 2014). The plant has been shown to have a variety of biological activities both *in vivo* and *in vitro,* including antibacterial, antiviral, anti-inflammatory, anti-HIV (Human Immunodeficiency Virus), immunomodulating/immunostimulatory, and anticancer properties (Intharuksa *et al*., 2022). The plant demonstrated potential medicinal efficacy in treating human colds, common coughs, and liver diseases (Akbar, 2011). This plant's significance in the field of medicinal plants has been greatly increased by the distinctive secondary metabolites found in it (Hossain *et al*., 2014). *A. paniculata* extract's anti-trypanosomal action is still poorly understood. In order to determine the *in vivo* antitrypanosomal action of methanolic extract of *A. paniculata* in mice infected with *T. brucei brucei*, the current study was conducted.

MATERIALS AND METHODS

Samples collection

Fresh samples of *Andrographis paniculata* (king of bitters) leaves were obtained from Ondo State, Nigeria. Taxonomic authentication of the plant was carried out at the Department of Plant Biology, Federal University of Technology, Minna, Niger State, Nigeria. A voucher specimen was deposited in the department for future reference.

Experimental animals

Healthy albino mice weighing 22–25 g on average were bought from the Animal Holding Facility of the School of Life Sciences, Federal University of Technology, Minna, Niger State, Nigeria. The rodents were kept in a typical setting and fed with commercially available pelletized feed with free access to water. The animals were kept under environmental conditions of 70% relative humidity level, temperature of 27± 20°C, and 12-hour night/day cycles.

Ethical approval

All experimental procedures involving animals were conducted in accordance with the Canadian Council on Animal Care Guidelines and Protocol Review and approved by the Federal University of Technology, Minna Committee on Ethics for Medical and Scientific Research (ILAS. 1997).

Reagent and assay kits

Total protein, AST, ALT, ALP, and albumin assay kits were made by Randox Laboratories Ltd. in the United Kingdom. The preparation of all other reagents was done with distilled water and they were all of analytical grade.

Plant sample preparation and extraction

The freshly acquired sample of leaves was cleaned with clean water, allowed to dry at room temperature, then ground in a grinder mill and allowed to dry after previous drying. The extraction of plant materials (leaves) was done by weighing 50 g of the powdered sample into a thimble and placed inside a Soxhlet extractor with 200 mL of methanol. The resultant extract was concentrated in a water bath.

Phytochemical screening

A standard screening test was used to identify the main classes of bioactive compounds in the plant extract. These include the detection of saponins, glycosides, alkaloids, tannins, phenol anthraquinone, phlobatannins, flavonoids and steroids (Sofowora, 2008).

Acute toxicity (LD50)

The acute toxicity study was conducted in accordance with Lorke’s method (Lorke, 1983). The study was conducted in two phases. In phase I, extract doses of 10,500 and

1000 mg/kg body weight were respectively administered to three groups of three mice each. In the second phase, the experiment was set up like the first phase but with the oral administration of 1600, 2900 and 5000 mg/kg body weight doses of the extract to another set of three groups of three mice each. Observations were made for seven days after extraction administration.

Inoculation of mice with parasite

Trypanosoma brucei brucei was obtained from the Nigerian Institute for Trypanosomiasis Research (NITR), Kaduna, Kaduna State, Nigeria. Parasite infected blood was obtained from the tail of infected rats with high parasitemia and was suspended in 0.90% saline solution. The solution containing approximately 3 to 4 trypanosomes was then used to inoculate the peritoneal cavity of the uninfected mice.

Treatment of animals

The animals were divided into the following groups and administered appropriate dosages of the drug, extract or distilled water (vehicle for drug administration) corresponding to their body weight.

Group 1 – infected and received no treatment (negative control)
Group 2 – infected and treated with 5 mg/kg of diminazeneaceturate (berenil),
Group 3 – infected and treated with 150 mg/kg methanol extract of *A. paniculata* extract
Group 4 – infected and treated with 300 mg/kg methanol extract of *A. paniculata* extract
Group 5 – infected and treated with 600 mg/kg methanol extract of *A. paniculata* extract

Parasitemia counts

Parasitaemia was monitored on a daily basis as described by Adeyemi *et al.* (2009). A thin film of blood was obtained from the animal's tail and viewed under a light microscope at x100 magnification.

Determination of packed cell volume (PCV)

The technique as outlined by Dacie and Lewis (1991) was adopted for the determination of packed cell volume (PCV) of treated mice. To get blood, the tail ends of the mice were chopped off. Blood was drawn into heparinized capillary tubes, which were then sealed using a sealing agent (plastacin). The capillary tubes were centrifuged for 5 minutes at 11,000 rpm (revolutions per minute) in a micro-haematocrit centrifuge after which the packed cell volume was read with a micro Hematocrit reader.

Serum and liver collection

Mice were anaesthetized with mild chloroform and blood was collected through cardiac puncture. Blood was drawn into a centrifuge tube, which was subsequently centrifuged at 1000 rpm for 15 minutes after standing for 10 minutes. The serum was carefully separated from the supernatant and set aside for later examination. The liver was removed and placed in a 0.25 M sucrose solution that was extremely cold. The supernatant from this was employed for further research after it had been homogenized in an ice-cold 0.25 M sucrose solution [1:5w/v].

Serum and liver biochemical assay

Using established protocols, the activities of the enzymes alkaline phosphatase (ALP), aspartate aminotransferase (AST), and alanine aminotransferase (ALT) were measured (Reitman and Frankel, 1957). Utilizing the Biuret method as reported by Reitman and Frankel (1957), total protein concentration was measured. UV Spectrophotometer was used for all measurements (Adeyemi *et al*., 2015).

Statistical analysis

The Statistical Package for Social Science (SPSS) version 21 was used to analyze the data, and the results are shown as ±SE of the mean. One-way analysis of variance (ANOVA) and Duncan's Multiple Regression were used to compare data from various groups.

RESULTS

Phytochemical components and acute oral toxicity

The phytochemical analysis revealed the presence of anthraquinone, phlobatannins, and steroids in the plant extract. Flavonoids, saponins, glycosides, alkaloids, tannins, and phenol were absent (Table 1). The results of the acute oral toxicity are detailed in Table 2. The animals showed no sign of toxicity up to the dose of 1000 mg/kg body weight. Although no mortality was recorded, toxicity signs including weakness, recklessness and erythremia were observed among the animals from a dose of 1600 to 5000 mg/kg body weight. The LD_{50} of the plant extract was therefore extrapolated to above 5000 mg/kg body weight.

Table 1. Phytochemical compositions of methanol leaf extract of *Andrographis paniculate*.

No.	Phytochemicals	Inference
1	Alkaloids	+
2	Glycosides	+
3	Flavonoids	+
4	Steroid	-
5	Tannins	+
6	Saponins	+
7	Phenol	+
8	Anthraquinone	_
9	Phlobatannins	-

Key: (-) absent, (+) present.

Table 2. Acute oral toxicity profile of methanol leaf extract of *Andrographis paniculata* in mice.

Group	Dosage(mg/kg)	Mortality/Toxicity
1	10	ND, NST
2	100	ND, NST
3	1000	ND, NST
4	1600	No death, animals were restless for some minute
5	2900	Animals were restless/hypercardia
6	5000	No death, animals were very week/erythremia

ND= No death, NST = No sign of mortality.

Parasitemia count

The average parasitemia test findings revealed that both mice treated with 150 and 300 mg/kg body weight of *Andrographis paniculata* and infected untreated mice had progressively higher parasite loads (Figure 1). The mice which were given doses of *Andrographis paniculata* at 150, 300, and 600 mg/kg survived as well as the control group. However, compared to mice that were not treated and those who were treated with 150 and 300 mg/kg body weight from the leave of *Andrographis paniculata*, mice treated with 600 mg/kg body weight of the herb demonstrated a considerably lower parasite burden and extended survival (6 days). However, when compared to the infected untreated and all other extract treated groups, the infected mice treated with the standard demonstrated a progressive reduction in the parasitemia count of the infected mice and prolonged the life of the infected mice.

Body weight

In Figure 2, the body weight changes in *T. brucei*-infected mice treated with methanol extract of *Andrographis paniculata* are presented. On the third day after infection, the body weight of all experimental groups decreased, and the infected mice treated with 150 and 300 mg/kg methanol extract of *Andrographis paniculata* as well as the infected mice that were not treated both showed further weight loss. Mice treated with the standard medication gained weight whereas those infected with 600 mg/kg showed stabilized body weight.

Packed cell volume

Effects of methanol extract of *Andrographis paniculata* on packed cell volume of *T. brucei*-infected mice are presented in Figure 3. On the third day of infection, the packed cell volume of every experimental group decreased. The infected animals treated with 150, 300, and 600 mg/kg methanol extract of *Andrographis paniculata* showed a continued decline in PCV until the animals died. This PCV decline is equivalent to the PCV decline seen in infected, untreated mice. However, infected mice treated with normal medications showed an increase in packed cell volume.

Biochemical parameters

Effects of methanol extract from the leave of *Andrographis*

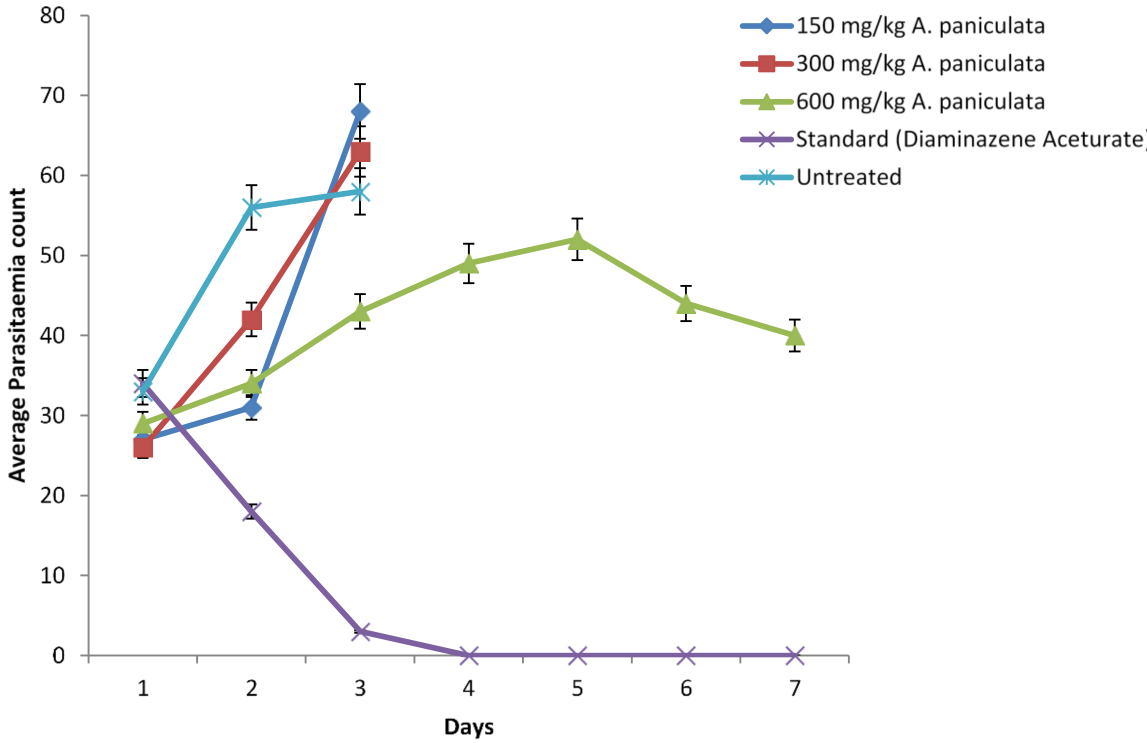

Figure 1. Parasitemia counts of *T. brucei infected* mice treated with methanol extract of *Andrographis paniculata.*

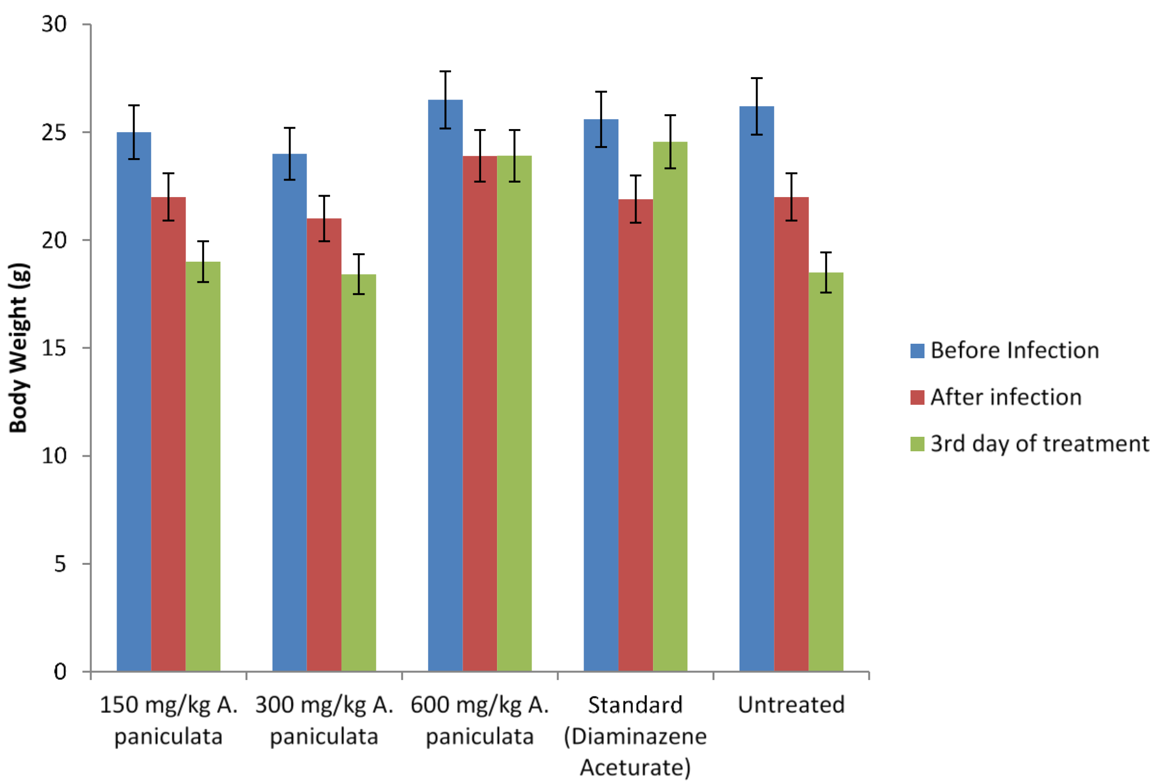

Figure 2. Effects of methanol extract of *Andrographis paniculata* on body weight in *T. brucei* infected mice.

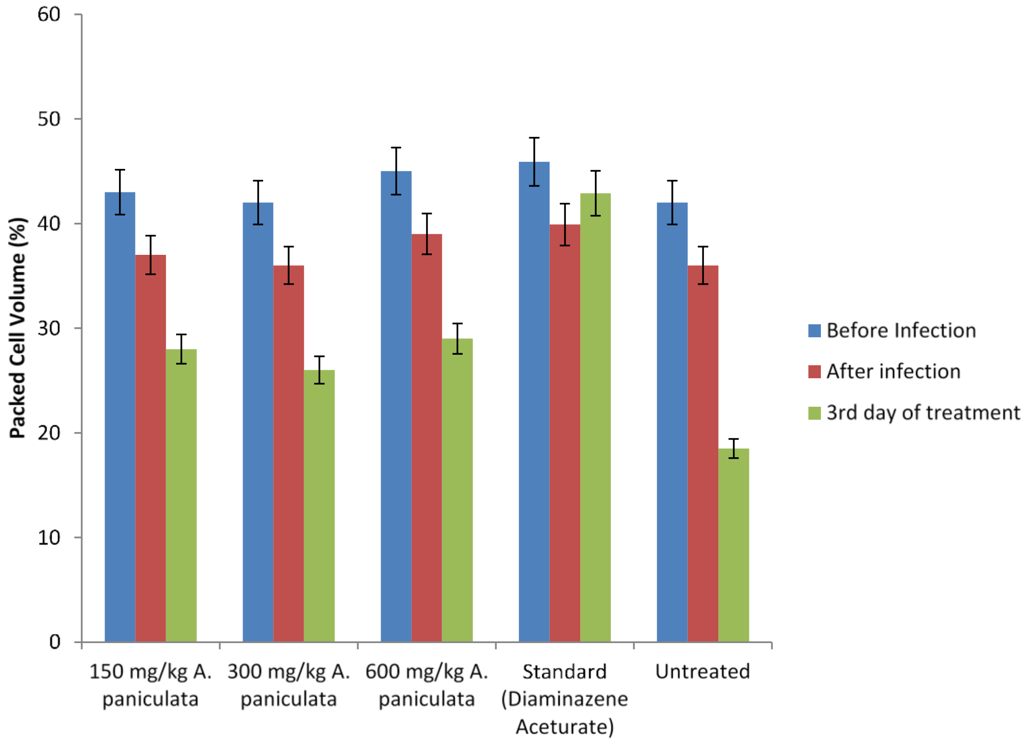

Figure 3. Effects of methanol extract of *Andrographis paniculata* on PCV in *T. brucei infected* mice.

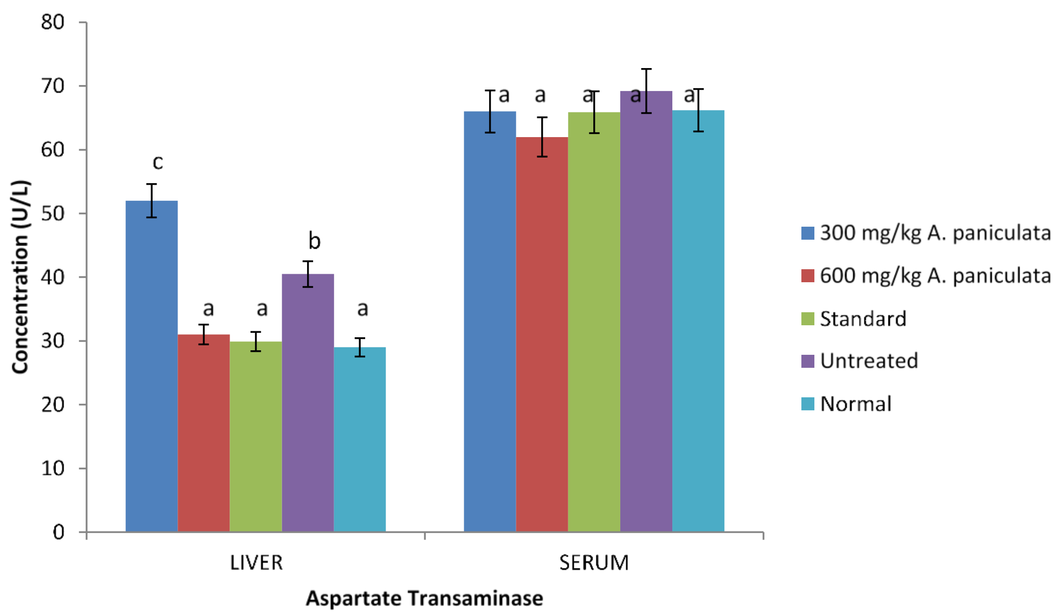

Figure 4. Effects of methanol extract of *Andrographis paniculata* on serum and liver AST activities in *T. brucei* infected mice.

paniculata on serum and liver biochemical parameters are shown in Figures 4 to 7. When compared to the infected treated group, the liver AST activities of infected untreated mice increased significantly ($p > 0.05$). However, none of the experimental groups showed significantly different blood AST and liver ALT activities ($p < 0.05$). *T. brucei*-

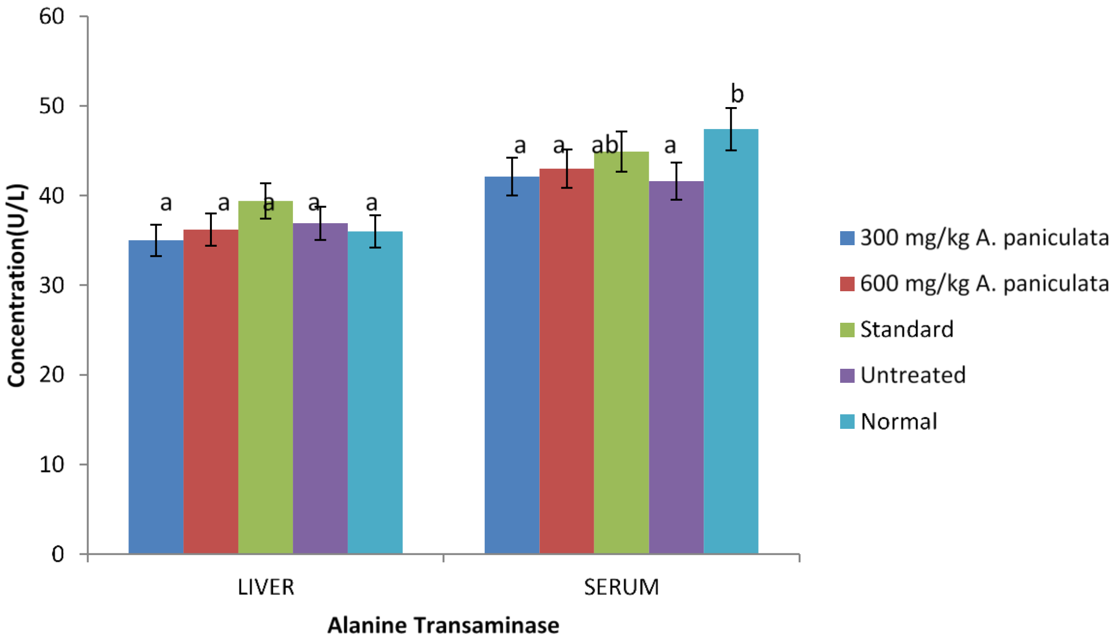

Figure 5. Effects of methanol extract of *Andrographis paniculata* on serum and liver ALT activities in *T. brucei* infected mice.

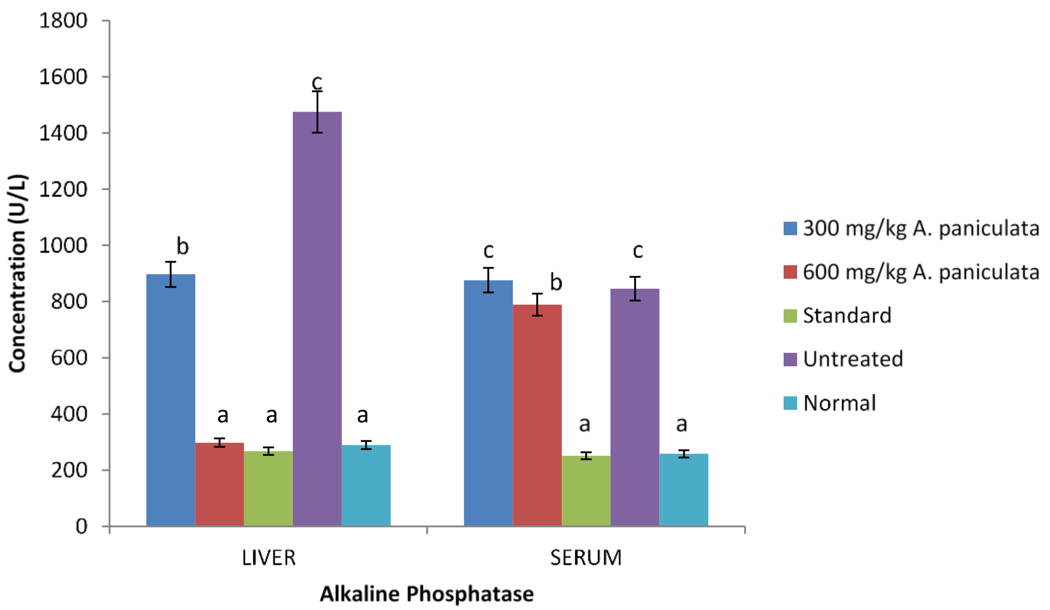

Figure 6. Effects of methanol extract of *Andrographis paniculata* on serum and liver ALP activities in *T. brucei infected* mice.

infected mice that were left untreated showed a substantial rise ($p > 0.05$) in serum and liver ALP activity compared to the healthy control. However, there was no statistically significant ($p > 0.05$) difference in the liver ALP activity of mice treated with a conventional medication and those treated with a 600 mg/kg methanol extract of *Andrographis paniculata.*

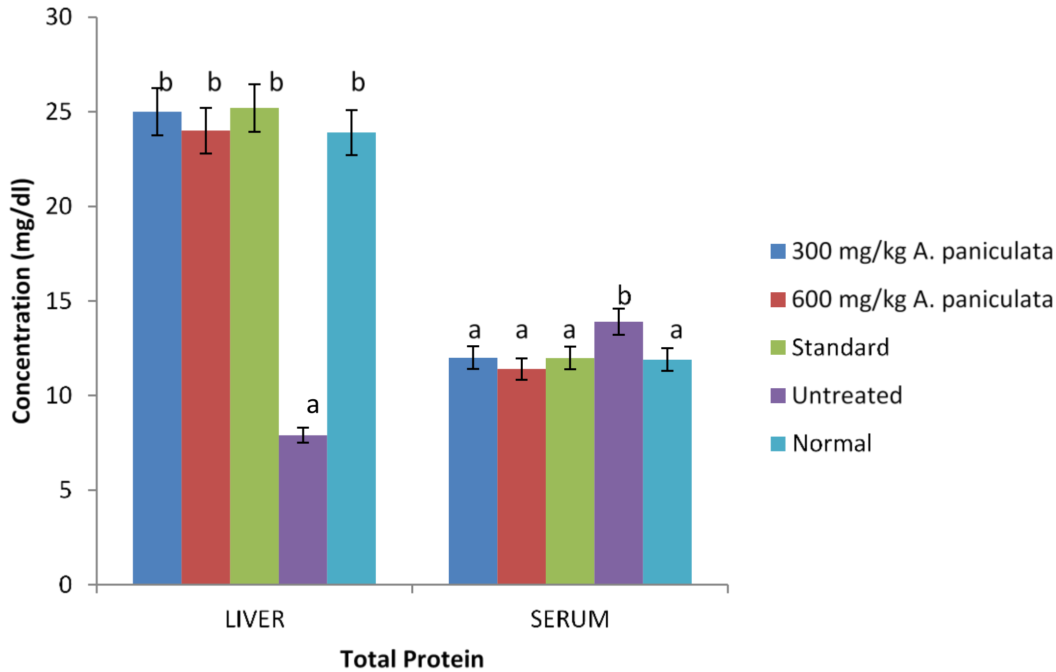

Figure 7. Effects of methanol extract of *Andrographis paniculata* on serum and liver total protein concentration in *T. brucei* infected mice.

DISCUSSION

According to certain theories, plants employed in illness treatment contain active substances called phytochemicals, some of which are responsible for their distinctive therapeutic (Prakash, 2020). This study found several significant therapeutic compounds in the *Andrographis paniculata* methanol leaf extract. The presence of these phytochemicals in the plant has been previously reported (Pandey *et al*., 2019). According to study reports, saponins possess anti-inflammatory, cardiac-depressing, and hypercholesterolemic properties (Trease and Evans, 1985). The most varied classes of phenolic compounds found in plants are flavonoids and among its several biological effects are antiparasitic, antibacterial, anti-inflammatory, and anticancer (Ullah *et al*., 2020). The most effective plant ingredient for therapeutic significance is alkaloids. Due to their analgesic, antimicrobial, antisplasmodic, and anti-trypanosomal effects, pure separated alkaloids and their synthetic derivatives are utilized as the foundation of basic therapeutic agents (Osorio *et al*., 2008). Therefore, the phytochemical components suggest that the *Andrographis paniculata* methanol leaf extract may have potential as an anti-trypanosomal agent. This presence of significant phytochemicals in this plant is a hint that it has the potential to produce a pharmaceutically significant medication when thoroughly evaluated. But the absence of phlobatannins, anthraquinone, and steroids is consistent with earlier research that discovered that not all phytochemicals are present in all plants, and those that are present vary depending on the section of the plant and the extraction solvent utilized (Lawal *et al.,* 2016; Busari *et al*., 2021).

Trypanosomiasis is a complicated, crippling, and frequently fatal illness. Trypanosomes multiply rapidly after entering the mammalian system to establish their population in the infected host and emit toxins (Shittu *et al*., 2017). Because the parasite can create a wide variety of antigens, the antibodies made by the host in response to the parasite are ineffective. In the current study, *Andrographis paniculata* methanol leaf extract only exhibits some trypanostatic activity by inhibiting parasite multiplication and also stops further loss of body weight in mice at high a dose (600 mg/kg body weight). The plant extract did not, however, stop the mice's packed cell volume from gradually declining. Anaemia is a typical issue associated with trypanosome infection. The hemolysis may be caused by the parasite that is consuming and degrading intracellular proteins, primarily haemoglobin, as it grows **(**Gavigan *et al.,* 2001; Balogun *et al*., 2009). However, it has been claimed that unpurified bioactive chemicals may need to undergo initial conversions, and this time lag allows for parasite development, hence, it has been claimed that crude plant extracts typically have lesser anti-trypanosomal properties (Feyera *et al*., 2014).

Assessment of serum enzymes has been shown to be crucial in the diagnosis of various disorders as well as in evaluating the severity of parasite- or disease-induced liver damage (Ansar *et al*., 2016). Alkaline phosphatase is a “marker” enzyme for the plasma membrane, liver and endoplasmic reticulum (Bachs *et al*., 1985). The significant increase ($p > 0.05$) in serum and liver ALP activity in *T. brucei* infected untreated mice compared to the healthy control is evidence that trypanosome infection has damaged the integrity of the plasma membrane and endoplasmic reticulum. However, there was no statistically significant ($p < 0.05$) difference in the liver ALP activity of mice treated with a conventional medication and those treated with a 600 mg/kg methanol extract of *Andrographis paniculata.*

The significant increase in liver AST activity of the infected untreated group when compared to the infected treated group confirms a previous report that disease condition can gradually affect enzyme activity, even though there were no significant differences in serum AST and liver ALT activities among all experimental groups ($p < 0.05$) (Lawal *et al*., 2015). However, administration of an *Andrographis paniculata* methanol extract at 600 mg/kg resulted in a reduction of the high AST activity. This demonstrates how well the extract (at 600 mg/kg) reduces the negative effects of *T. brucei* infection. However, *Andrographis paniculata* methanol extract demonstrated no beneficial effects on the parasite-induced rise of serum ALT activity.

Conclusion

It can be deduced from the study that, *Andrographis paniculata* contain important phytochemicals with minimal trypanostatic activity and ameliorative effects on *T. brucei* induced mice. The minimal ameliorative effects are achieved when high doses (> 600 mg/kg) of methanol extract of *Andrographis paniculata* are used. The animals showed no sign of toxicity up to the dose of 1000 mg/kg body weight while doses from 1600 to 5000 mg/kg body weight had a response. The LD_{50} of the plant extract was therefore extrapolated to above 5000 mg/kg body weight.

CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

REFERENCES

Adeyemi, O. S., Akanji, M. A., & Oguntoye, S. (2009). Ethanolic leaf extract of *Psidium guajava*: Phytochemical and trypanocidal activity in rats infected with *Trypanosoma brucei brucei*, Journal of Medicinal Plants Research, *3*(5), 420-423.

Adeyemi, O. T., Osilesi, O., Adebawo, O. O., Onajobi, F. D., Oyedemi, S. O., & Afolayan, A. J. (2015). Alkaline phosphatase (ALP), aspartate aminotransferase (AST) and alanine aminotransferase (ALT) Activities in selected tissues of rats fed on processed atlantic horse mackerel (*Trachurus trachurus*). *Advances in Bioscience and Biotechnology*, *6*(03), 139-152.

Akbar, S. (2011). *Andrographis paniculata*: a review of pharmacological activities and clinical effects. *Alternative Medicine Review*, *16*(1), 66-77.

Ansar, W., Ghosh, S., Ansar, W., & Ghosh, S. (2016). Inflammation and inflammatory diseases, markers, and mediators: Role of CRP in some inflammatory diseases. In: Biology of C Reactive Protein in Health and Disease. Springer, New Delhi. Pp. 67-107.

Bachs, O., Enrich, C., Soriano, M., Piñol, R., & Domingo, J. (1985). Induction of plasma membrane alkaline phosphatase in rat liver. *Cell Biochemistry and Function*, *3*(2), 95-100.

Balogun, E. A., Adebayo, J. O., Zailani, A. H., Kolawole, O. M., & Ademowo, O. G. (2009). Activity of ethanolic extract of *Clerodendrum violaceum* leaves against Plasmodium berghei in mice. *Agriculture and Biology Journal of North America*, *1*(3), 307-312.

Busari, I. O., Soetan, K. O., Aiyelaagbe, O. O., & Babayemi, O. J. (2021). Phytochemical screening and in vitro anthelmintic activity of methanolic extract of *Terminalia glaucescens* leaf on *Haemonchus contortus* eggs. *Acta Tropica*, *223*, 106091.

Dacie, J. V., & Lewis, S. M. (1991). *Practical Haematology*, 7th edition. London. Churchill Livingston. Edingburgh. Pp. 12-17.

De Koning, H. P. (2020). The drugs of sleeping sickness: their mechanisms of action and resistance, and a brief history. *Tropical Medicine and Infectious Disease*, *5*(1), Article number 14.

De Rycker, M., Wyllie, S., Horn, D., Read, K. D., & Gilbert, I. H. (2023). Anti-trypanosomatid drug discovery: progress and challenges. *Nature Reviews Microbiology*, *21*(1), 35-50.

Dias, D. A., Urban, S., & Roessner, U. (2012). A historical overview of natural products in drug discovery. *Metabolites*, *2*(2), 303-336.

Feyera, T., Terefe, G., & Shibeshi, W. (2014). Evaluation of in vivo antitrypanosomal activity of crude extracts of *Artemisia abyssinica* against a *Trypanosoma congolense* isolate. *BMC Complementary and Alternative Medicine*, *14*(1), 1-9.

Gavigan, C. S., Dalton, J. P., & Bell, A. (2001) The role of aminopeptidases in haemoglobin degradation in *Plasmodium falciparum*-infected erythrocytes. *Molecular Biochemistry Parasitology, 117*(1), 37-48.

Holanda-Freitas, I. T., do Carmo Cupertino, M., dos Santos, E. C., Oliveira, L., Geller, M., & Siqueira-Batista, R. (2020). Human African trypanosomiasis: current standing and challenges. *Revista de Patologia Tropical/Journal of Tropical Pathology*, *49*(3), 133-148.

Hossain, M. S., Urbi, Z., Sule, A., & Rahman, K. M. (2014). *Andrographis paniculata* (Burm. f.) Wall. ex Nees: A review of ethnobotany, phytochemistry, and pharmacology. *The Scientific World Journal*, Volume 2014, Article ID 274905, 28 pages.

Intharuksa, A., Arunotayanun, W., Yooin, W., & Sirisa-Ard, P. (2022). A comprehensive review of *Andrographis paniculata* (Burm. f.) Nees and its constituents as potential lead compounds for COVID-19 drug discovery. *Molecules*, *27*(14), 4479.

Lawal, B., Shittu, O. K., Abubakar, A. N., Umar, M. B., Ibrahim, A. M. & Haruna, G. M. (2015). Biochemical evaluation in Wister rats (*Rattus novergicus*) following chronic exposure of methanol leaf extract of *Telfairia occidentalis*. *Journal of Pharmaceutical and Biomedical Science*, *5*(9), 740-744.

Lawal, B., Shittu, O. K., Oibiokpa, I. F., Mohammed, H., Umar, S. I., & Haruna, G. M. (2016). Antimicrobial evaluation, acute and sub-acute toxicity studies of *Allium sativum*. *Journal of Acute Disease*, *5*(4), 296-301.

Lorke, D. (1983). A new approach to practical acute toxicity testing. *Archives of Toxicology*, *54*(4), 275-289.

Osorio, E. J., Robledo, S. M., & Bastida, J. (2008). Alkaloids with antiprotozoal activity. *The Alkaloids: Chemistry and Biology*, *66*, 113-190.

Pandey, J., Saini, V. K., & Raja, W. (2019). Evaluation of phytochemical analysis of *Andrographis paniculata* leaf and stem extract. *World Journal of Pharmaceutical and Life Sciences*, *5*(2), 188-190.

Prakash, B. (Ed.). (2020). *Functional and preservative properties of phytochemicals*. Academic Press.

Reitman, S., & Frankel, S. A. (1957). Colorimetric method for the determination of serum glutamic oxalacetic and glutamic pyruvic transaminases. *American Journal of Clinical Pathology, 28*, 56-63.

Shittu, O. K., Lawal, B., Adeniyi, A. K., Kilani, L. T., & Saka, R. B. (2017). Effect of methanol extract of *Musca domestica* larva on some enzymes and haematological parameters in *Trypanosoma brucei brucei*-infected rats. *Nigerian Journal of Basic and Applied Sciences*, *25*(2), 66-74.

Sofowora, A. (2008). *Medicinal plants and traditional medicine in Africa* (3rd edition). Spectrum Books, Ibadan.

Trease, G. E., & Evans, W. C. (1989). *Pharmacognosy* (13th edition). Baillere. Tindall London pp176-180.

Ullah, A., Munir, S., Badshah, S. L., Khan, N., Ghani, L., Poulson, B. G., Emwas, A. H., & Jaremko, M. (2020). Important flavonoids and their role as a therapeutic agent. *Molecules*, *25*(22), 5243.

Ullah, M. A., & Hassan, A. (2021). Green chiretta (*Andrographis paniculata*) as medicinal herb. *International Journal of Phytology Research*, *1*(2), 15-18.

World Health Organization (WHO) (2012). Accelerating work to overcome the global impact of neglected tropical diseases – A roadmap for implementation. Geneva, Switzerland. World Health Organization. Retrieved from https://www.who.int/publications/i/item/WHO-HTM-NTD-2012.1.