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EVALUATING THE INFLUENCE OF TRYPANOSOMIASIS ON MURINE MODEL USING *Corchorus olitorius* LEAF EXTRACT AS A TRYPANOCIDAL AGENT

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ABSTRACT

Trypanosomiasis, a parasitic disease caused by trypanosomes, which are flagellate protozoa transmitted through the bite of the tsetse fly, manifests with symptoms including substantial weight loss, anemia, fever, edema, adenitis, dermatitis, and nervous disorders. This research investigated the impact of trypanosomiasis on a murine model while utilizing *Corchorus olitorius* leaf extract as a potential trypanocidal agent. An acute toxicity analysis was conducted following Lorke's method, and the antitrypanosomal efficacy was assessed in rats at doses of 100, 200, and 400 mg/kg over three weeks, monitoring changes in parasitemia count, body weight, and hematological parameters. Additionally, lipid profile, electrolyte concentration, and liver and kidney function were evaluated using standard techniques. The extract demonstrated potent antitrypanosomal activity at 400 mg/kg, significantly reducing the parasitemia count to 11.33±4.16 count/mL compared to the positive control at 2.5 mg/kg body weight doses. Furthermore, the 400 mg/kg dose notably increased packed cell volume and body weight in infected rats. Moreover, there were no significant discrepancies in numerous hematological parameters between the infected treated with diminazene aceturate and the extract's 400 mg/kg body weight. This study suggests that *Corchorus olitorius* extract exhibits significant antitrypanosomal, antilipidemic, and erythropoietic effects, mitigating parasitemia count, lipid levels, and oxidative damage by impeding the biochemical activities of trypanosomes through its active constituents. Thus, *Corchorus olitorius* extract may offer an alternative therapeutic approach for managing trypanosomal infections.

Keywords: *Corchorus olitorius*, Parasite, Phytochemical Analysis, *Trypanosoma brucei*, Trypanosomiasis.

INTRODUCTION

Trypanosomiasis , a persistent parasitic disease affecting both humans and animals (Mirshekar et al., 2019), stems from *Trypanosoma brucei*, a flagellate protozoan species within the *Trypanosomatidae* family and *Trypanosoma* genus (Sobhy et al., 2017; Ereqat et al., 2020). This single-celled parasite, transmitted by tsetse flies, is the causative agent of Human African Trypanosomiasis (HAT) (de Sousa et al., 2021). Globally, trypanosomiasis is prevalent in underprivileged and rural regions of sub-Saharan Africa, resulting in significant global losses and posing fatal risks if left undiagnosed and untreated (Ereqat et al., 2020). Manifesting through pronounced weight loss, intermittent fever, anemia, frequent diarrhea, adenitis, dermatitis, nervous disorders, and deteriorating health conditions, trypanosomiasis encompasses various diseases that often culminate in death (Field et al., 2017; Alanazi et al., 2018). Several ongoing studies are focused on the development of vaccines against Human African trypanosomiasis. Presently, chemotherapeutic agents like diminazene aceturate and isoethamidium chloride serve as common trypanocidal drugs for both preventive and curative purposes (WHO, 1998). Despite extensive disease control and prevention research, challenges such as inadequate clinical efficacy, drug toxicity, and resistance hinder progress (Fathabad et al., 2018). Trypanosomiasis compromises the immune system, rendering hosts incapable of eliminating the parasite (trypanosome) even after administering antitrypanosomal drugs (Zhou et al., 2017). Regrettably, these parasites have developed resistance to conventional treatment medications, underscoring the urgent need to explore highly effective and non-toxic remedies sourced from medicinal plants (Gao et al., 2019).

Corchorus olitorius, commonly known as 'Ewedu' in the Yoruba language, is a plant species belonging to the *Tiliaceae* family (Nasreen et al., 2022). This annual herb is valued for both its culinary and medicinal uses, with its leaves and roots being utilized in herbal medicine and consumed as vegetables (Becer et al., 2020). It is indigenous to regions such as East Malaysia, India, Egypt, and the Philippines, as well as various West African countries, notably Ghana, Nigeria, and Sierra Leone (Matsufuji et al., 2001). The leaves of *Corchorus olitorius* are particularly notable for their high antioxidant content, which has been documented to enhance metabolic and

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physiological processes within the human body (Airaodion et al., 2019). Additionally, they contain a diverse array of phytoconstituents, including flavonoids, phenols, fatty acids, minerals, vitamins, and mucilaginous polysaccharides, suggesting potential benefits such as anti-inflammatory, antimicrobial, antiplasmodial, and antitrypanosomal properties (Nasreen et al., 2022; Ogungbemi et al., 2019). Despite its various biological and medicinal attributes, research concerning its trypanocidal effects remains insufficient. Hence, this study aims to explore the antitrypanosomal activity of extracts derived from *Corchorus olitorius* against *Trypanosoma brucei* in male Wistar rats.

EXPERIMENTAL

Materials and methods

Chemicals

The chemicals used in this study are of analytical grade with 96% purity of the solvents.

Collection of plant materials

The fresh leaves of *Corchorus olitorius* were gathered from Kure Market in Minna, Nigeria, and subsequently verified and authenticated at the herbarium division of the Department of Biological Sciences, Federal University of Technology, Minna, Niger State, Nigeria.

Experimental animals

The experiment involved using thirty male Wistar rats weighing between 100-120 g. Male rats were used due to hormonal differences in female rats which might disrupt some of the biochemical parameters. These rats were sourced from the animal farm at the University of Jos in Plateau State, Nigeria. Upon acquisition, they underwent a two-week acclimatization period during which they were housed in well-ventilated plastic cages and provided with rat chow and water ad libitum. All procedures involving the animals adhered to stringent care protocols by established standards. The experimental protocols were conducted with utmost care, following established guidelines outlined in the Current Animal Care Regulations and Standards as approved by the Institute for Laboratory Animal Research (Guide for Care and Use of Laboratory Animals in Biomedical and Behavioral Research).

Trypanosome parasite

The *Trypanosoma brucei* parasite strain was procured from the National Institute for Research in Kaduna, Kaduna State, Nigeria, and then consistently preserved through asynchronous transfer within experimental rats in the Animal Housing Unit of the Department of Biochemistry.

Plant processing and extraction

The fresh leaves of *Corchorus olitorius* were detached from the stems, washed with distilled water, and subsequently air-dried to a consistent weight over seven (7) days at room temperature in the laboratory. Following this, the dried leaves were meticulously ground into a fine powder using an electric blender and then stored in an airtight container until the extraction process commenced (Gupta et al., 2015).

Preparation of Corchorus olitorius extract

The cold maceration extraction technique was employed to extract the plant sample. About 200 grams of the ground *Corchorus olitorius* sample were accurately weighed and immersed in 1600 mL of methanol (James & Dubery, 2011). The mixture was then placed in shade for 72 hours, undergoing constant agitation using a water bath shaker at the temperature of 37 degrees. Following this period, it was filtered using Whatman filter paper, with the resulting filtrate collected in a beaker. Subsequently, the water content was allowed to evaporate over a water bath (37 degrees), resulting in the concentration of the extract, as detailed by Abubakar & Haque, (2020).

Preparation of parasite

The *Trypanosoma brucei* parasite was sustained through asynchronous transfer within Wistar rats until required for experimentation. Blood samples were collected from the tail of a heavily infected rat using an EDTA-coated insulin syringe. Subsequently, 0.2 mL of blood from the infected Wistar rats was dissolved in 20 mL of 0.98% NaCl solution, and the resulting mixture was injected into healthy rats (Madaki et al., 2022).

Phytochemical Analysis

Preliminary quantitative phytochemical analysis of the *Corchorus olitorius* extract was conducted following standard methods utilized in comparable studies, as outlined by Abubakar & Haque, (2020) & Madhu et al., (2016).

Parasite inoculation

The parasites were intraperitoneally introduced into the acclimatized/healthy rats weighing 100-120 g, using 0.2 mL of infected blood containing *Trypanosoma brucei*, as described by (Madaki et al., 2016). This inoculum was prepared by diluting the blood with normal saline (20 mL 0.9% NaCl).

Parasitemia level determination

Blood obtained from the tail vein of the infected rat was examined under a microscope to assess the parasite load. A small volume of blood was applied to a slide using a ×40 objective lens. Parasitemia levels were evaluated 72 hours after parasite infection and subsequently monitored every

seven days until the conclusion of the treatment period, following the protocol outlined by (Madaki et al., 2016). The parasite count was measured in count/ml of blood sample.

In vivo antitrypanosomal activity

On the initial day (day 0), the parasites were intraperitoneally injected into the Wistar rats. After seventy-two hours post-infection, the rats were divided into five groups, each consisting of six rats. Groups, I-III received varying doses of *Corchorus olitorius* methanol extract orally (100, 200, and 400 mg/kg body weight), while group IV (the positive control) was administered 2.5 mg/kg body weight) of diminazene aceturate. Group V (the negative control) received an equivalent volume of distilled water. The administration of *Corchorus olitorius* and drugs began 72 hours post-parasite infection, with each administered at a single daily dose for three weeks, following the protocol outlined by (Madaki et al., 2016).

Biochemical/Hematological Analysis

The rats were weighed and euthanized through cervical dislocation 24 hours following the final administration. Each rat was secured on a dissecting board with twine, and blood samples were obtained for hematological analysis and assessment of biochemical markers. Hematological analysis, including the determination of red blood cell count (RBC) and white blood cell count (WBC), was conducted using the standard method outlined by Akhter et al., (2021) with an auto-hematological analyzer (Abacus Junior).

Biochemical parameters

ALT, AST, ALP, total protein, albumin, cholesterol, triglycerides, LDL, HDL, creatinine, urea, uric acid, sodium, potassium, and bicarbonate levels were determined using a commercial kit (AGAPE, Switzerland), and a UV-visible spectrophotometer at specified wavelengths, following established protocols (Nurudeen et al., 2023; Ojo et al., 2021). The multiplication factor was determined by the reagent manufacturer.

Alkaline phosphatase (ALP)

To each test tube, 1000 µL of reagent was dispensed, followed by the addition of 20 µL of each sample to its corresponding tube. The reaction mixture was thoroughly mixed and then incubated at 37 ºC for 1 minute. Subsequently, the absorbance (A) change per minute was assessed for 3 minutes at a wavelength of 405 nm.

$$ALP \text{ activity } (\text{U/L}) = (\text{change in absorbance x } 2750). \quad (1)$$

Alanine aminotransferase (ALT)

To each test tube, 1000 µL of reagent was dispensed, followed by the addition of 100 µL of each sample to its corresponding tube. The reaction mixture was thoroughly mixed and then incubated at 37 ºC for 1 minute. Subsequently, the absorbance change per minute was assessed for 3 minutes at a wavelength of 340 nm.

$$ALT \text{ activity } (\text{U/L}) = (\text{change in absorbance x } 1745). \quad (2)$$

Aspartate aminotransferase (AST)

An aliquot of the working reagent, measuring 1 µL, was dispensed into all test tubes, followed by the addition of 100 µL of each sample to their respective tubes. The reaction mixture was thoroughly mixed and then incubated at 37 ºC for 1 minute. Subsequently, the absorbance change per minute was assessed for 3 minutes at a wavelength of 340 nm.

$$AST \text{ activity } (\text{U/L}) = (\text{change in absorbance x } 1745). \quad (3)$$

Total protein

An aliquot of 1000 µL of reagent was added to blank, standard, and sample test tubes. Subsequently, 20 µL of standard reagent was added to the standard test tube, and 20 µL of each sample was added to their respective sample test tubes. The reaction mixture was thoroughly mixed and then incubated for 10 minutes at 37 ºC. Following incubation, the absorbance of the reaction mixtures was measured against a reagent blank at 546 nm.

$$Total \text{ Protein conc. } (\text{g/dL}) = \frac{\text{Absorbance of sample}}{\text{Absorbance of standard}} \text{ x } 3. \quad (4)$$

Albumin

To each of the blank, standard, and sample test tubes, 1000 µL of reagent was dispensed. Following this, 20 µL of standard reagent was added to the standard test tube, while 20 µL of each sample was added to their respective sample test tubes. The reaction mixture was thoroughly mixed and then incubated for 10 minutes at 37 ºC. Subsequently, the absorbance of the reaction mixtures was measured against a reagent blank at 546 nm.

$$Albumin \text{ conc. } (\text{g/dL}) = \frac{\text{Absorbance of sample}}{\text{Absorbance of standard}} \text{ x } 6. \quad (5)$$

Cholesterol

The test tubes were labeled to correspond with each sample, with two additional tubes designated as standard and blank, respectively. To each tube, 1000 µL of working reagent was dispensed. Following this, 10 µL of the standard reagent was added to the tube labeled as standard, while 10 µL of the serum sample was added to the tubes designated for serum. The mixture was thoroughly mixed and then incubated at 37 ºC for 5 minutes. Subsequently, the absorbance of the sample and standard against the reagent blank was measured at 505 nm.

$$\textit{Total cholesterol}\ \text{conc.}\ (\text{mg/dL}) = \frac{\text{Absorbance of sample}}{\text{Absorbance of standard}} \text{x}\, 200. \quad (6)$$

Triglycerides

The test tubes were labeled according to the identity of each serum sample, and two additional tubes were designated as standard and reagent blank, respectively. To each tube, 1000 µL of the working reagent was added. Subsequently, 10 µL of standard reagent was added to the tube labeled as standard, and 10 µL of the serum sample was added to the corresponding sample tube. The reaction mixtures were thoroughly mixed and then incubated at 37 ºC for 5 minutes. Following incubation, the absorbance of both the standard and sample against the reagent blank was measured at 546 nm.

$$\textit{Triglyceride}\ \text{conc.}\ (\text{mg/dL}) = \frac{\text{Absorbance of sample}}{\text{Absorbance of standard}} \text{x}\ 200. \quad (7)$$

Low-density lipoprotein (LDL)

The test tubes were labeled to correspond with the identity of each sample, with two additional tubes designated as calibrator and blank, respectively. To each tube, 270 µL of reagent R1 was dispensed. Subsequently, 3 µL of calibrator reagent was added to the tube labeled as the calibrator, and 3 µL of each serum sample was added to their corresponding sample tubes. The reaction mixtures were thoroughly mixed and then incubated at 37 ºC for 5 minutes. Absorbance (OD1) was measured at 546 nm/660 nm. Following this, 90 µL of reagent R2 was added to all the tubes. The reaction mixtures were once again incubated at 37 ºC for 5 minutes, and absorbance (OD2) was measured at 546 nm/660 nm.

$$\textit{LDL}\ \text{conc.}\ (\text{mg/dL}) = \frac{(\text{OD2-OD1})\text{sample}}{(\text{OD2-OD1})\text{calibrator}} \text{x C.C.} \quad (8)$$

Bicarbonate

To each of the blank, standard, and sample test tubes, 1000 µL of reagent was dispensed. Following this, 10 µL of standard reagent was added to the tube designated as the standard, while 10 µL of each sample was added to their respective sample test tubes. The reaction mixture was thoroughly mixed and then incubated for 1 minute at 37 ºC. Subsequently, the absorbance of the reaction mixtures was measured against a reagent blank at 505 nm.

$$\textit{Bicarbonate}\ (\text{mEq/L}) = \frac{\text{Absorbance of sample}}{\text{Absorbance of standard}} \text{x}\ 100. \quad (9)$$

Bilirubin

The test tubes were labeled according to the identity of each serum sample, with one additional tube designated as blank. To all tubes, 200 µL of R1 and 1000 µL of R3 were added. Subsequently, 200 µL of each sample was added to their corresponding tubes, excluding the blank tube. Additionally, 50 µL of R2 was added to each sample tube, while 50 µL of distilled water was added to the blank tube. The reaction mixtures were thoroughly mixed and then incubated at 25 ºC for 10 minutes. Following this incubation period, 1000 µL of R4 was added to all tubes, mixed, and further incubated for 30 minutes at 25 ºC. The absorbance of each sample against the blank was measured at 578 nm.

$$\text{Total}\ \textit{bilirubin}\ \text{conc.}\ (\text{mg/dL}) = 12.9 \text{x}\ \text{A}(\text{sample}). \quad (10)$$

Urea

In each of the sample, blank, and standard tubes, 10 µL of each sample, distilled water, and standard were dispensed, followed by the addition of 100 µl of R1 to each test tube. The mixtures were thoroughly mixed and then incubated at 37 °C for 10 minutes. Subsequently, 2.5 ml of both reagents R2 and R3 was added to the tubes. The resulting mixtures were further incubated at 37 °C for 15 minutes. The absorbance of the samples and standard against the blank was measured at 578 nm.

$$\textit{Urea}\ \text{conc.}\ (\text{mg/dL}) = \frac{\text{A}(\text{sample})}{\text{A}(\text{standard})} \text{x Standard curve.} \quad (11)$$

Creatinine

The test tubes were labeled to correspond with the identity of each serum sample, with two additional tubes designated as standard and blank, respectively. To all tubes, 1000 µL of working reagent was dispensed. Subsequently, 100 µL of standard reagent was added to the tube labeled as standard, and 100 µL of each serum sample was added to their corresponding tubes. The reaction mixtures were thoroughly mixed, and the absorbance (A1 and A2) of both the standard and serum samples against the blank was measured at 492 nm.

$$\textit{Creatinine}(\text{mg/dL}) = \frac{\Delta\text{A}(\text{sample})}{\Delta\text{A}(\text{standard})} \text{x Standard curve.} \quad (12)$$

Uric acid

The test tubes were labeled according to the identity of each sample, with two additional tubes designated as standard and blank, respectively. To all tubes, 1000 µL of uric acid reagent was added. Subsequently, 25 µL of the standard solution was added to the tube labeled as standard, while 25 µL of each sample was added to their corresponding tubes. The reaction mixtures were thoroughly mixed and then incubated at 37 ºC for 5 minutes. Following incubation, the absorbance of the standard and each sample against the reagent blank was measured at 546 nm.

$$\textit{Creatinine}(\text{mg/dL}) = \frac{\Delta\text{A}(\text{sample})}{\Delta\text{A}(\text{standard})} \text{x Standard.} \quad (13)$$

Sodium

To each of the blank, standard, and sample test tubes, 1000 µL of reagent was dispensed. Subsequently, 10 µL of standard reagent was added to the tube designated as the standard, while 10 µL of each sample was added to their respective sample test tubes. The reaction mixture was thoroughly mixed and then incubated for 5 minutes at 37 ºC. Following incubation, the absorbance of the reaction mixtures was measured against a reagent blank at 630 nm.

$$Na\ \text{conc.}(\text{mEq/L}) = \frac{\text{A}(\text{sample})}{\text{A}(\text{standard})} \text{x} 150. \quad (14)$$

Potassium

To each of the blank, standard, and sample (test) tubes, 1000 µL of R1 reagent was added. Following this, 25 µL of standard reagent was added to the tube designated as the standard, while 25 µL of each sample (test) was added to their respective sample test tubes. The reaction mixture was thoroughly mixed and then incubated for 5 minutes at 37 ºC. Subsequently, 250 µL of R2 reagent was added to all tubes. The reaction mixtures were further incubated for 1 minute at 37 ºC, and the absorbance (A1) was measured against the blank at 405 nm. Following this, the reaction mixtures were again incubated for 3 minutes at 37 ºC, and the absorbance (A2) was measured against the blank at 405 nm.

$$K\ \text{conc.}(\text{mEq/L}) = \frac{\text{A2-A1}(\text{sample})}{\text{A2-A1}(\text{standard})} \text{x} 150. \quad (15)$$

Glucose

To each of the blank, standard, and sample test tubes, 1000 µL of glucose reagent was dispensed. Following this, 10 µL of standard reagent was added to the tube designated as the standard, while 10 µL of each sample (test) was added to their respective sample test tubes. The reaction mixtures were then incubated at 37 ºC for 10 minutes, after which the absorbance was measured against the reagent blank.

$$Glucose\ \text{conc.}(\text{mg/dL}) = \frac{\text{A}(\text{sample})}{\text{A}(\text{standard})} \text{x Standard conc.} \quad (16)$$

Statistical Analysis

Statistical analysis was conducted using a statistical software package (IBM SPSS version 21.0), and the data are presented as mean ± standard error of the mean (SEM). The differences between groups were assessed using a one-way analysis of variance followed by Duncan's test. A significance level of $p \leq 0.05$ was considered statistically significant.

NUMERICAL RESULTS

Phytochemical analysis of Corchorus olitorius extract

Table 1 below presents the phytochemical constituents of the *Corchorus olitorius* extract. The findings indicate a notable disparity between phenol and the other constituents, with polyphenol exhibiting the highest value (540.60±0.24) and alkaloids showing the lowest value (12.50±0.16).

Table 1. Quantitative phytochemicals analysis of *Corchorus olitorius.*

Constituents (mg/g)					
Samples	**Polyphenol**	**Flavonoids**	**Tannins**	**Saponins**	**Alkaloids**
Amount (mg/g)	540.60±0.24^{e}	45.51± 0.17^{c}	89.85±0.18^{c}	27.71±0.17^{c}	12.50±0.16^{c}

*Values mean ± standard deviation of three replicates. Values with different superscripts (a, b, c, d, e…) are significant at $p<0.05$. The joined letter signifies how close the values are, while single letters show differences in the values.

Antitrypanosomal activities of the extract

Parasitemia level

The parasitemia levels of rats infected with *Trypanosoma brucei* were monitored across all experimental groups, as depicted in Figure 1. A notable rise in parasitemia was observed in infected rats that did not receive treatment ($P<0.05$). Among the studied groups, the 400 mg/kg group demonstrated a significant substantial reduction in parasitemia compared to others. The parasite was measured in count/ml of the sample collected.

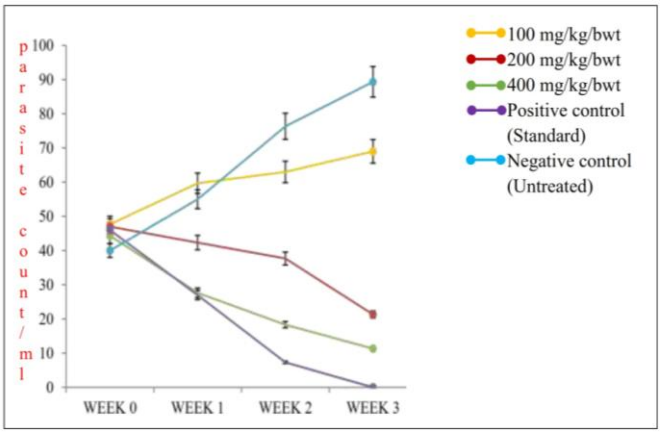

Figure 1. Effects of *Corchorus olitorius* methanolic extract on parasitemia in rats infected with *Trypanosoma brucei*.

Changes in packed cell volume

Figure 2 illustrates the packed cell volume (PCV) levels of infected rats. A decline in PCV levels was observed in the infected rats that did not receive treatment. Conversely, the groups treated with doses of 100, 200, and 400 mg/kg

exhibited a notable increase in their PCV levels compared to the negative control group.

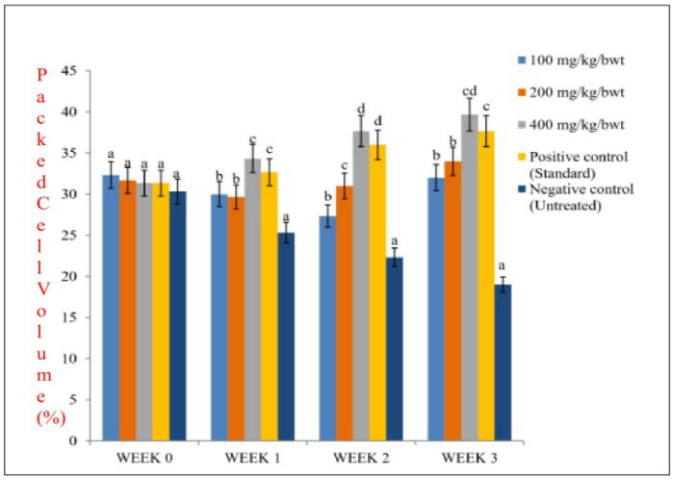

Figure 2. Effects of *Corchorus olitorius* methanolic extract on PCV levels in rats infected with *Trypanosoma brucei*.

Changes in body weight

Figure 3 depicts alterations in the body weight of rats infected with *Trypanosoma brucei*. A statistically significant decrease was observed in the body weights of the infected rats that did not receive treatment. Additionally, notable differences were observed in the body weights of the treated groups, with a significant increase noted in the rats treated with doses of 100, 200, and 400 mg/kg of the *Corchorus olitorius* methanolic extract compared to those in the negative control group (P<0.05).

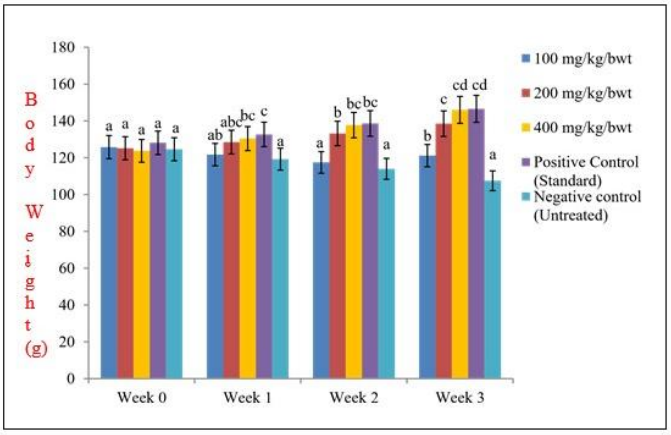

Figure 3. Effects of *Corchorus olitorius* methanolic extract on the body weight of rats infected with *Trypanosoma brucei*.

Hematological parameters

Table 2 below illustrates the impact of *Corchorus olitorius* extract on the hematological parameters of rats infected with *Trypanosoma brucei*. Administration of the extract to trypanosome-infected rats exhibited significant antitrypanosomal activity at the highest dose of 400 mg/kg, comparable to that observed in the positive control when compared to the infected but untreated group (P<0.05). Platelet count did not display significant differences (P>0.05) among rats treated with any extract concentrations. Similarly, no significant differences were observed in PCV and RBC at 200 mg/kg of the extract and in the positive control group. However, the PCV, mean corpuscular hemoglobin concentration (MCHC), platelet count (PLT), and lymphocyte levels were significantly higher in the positive control compared to those documented for the group administered 400 mg/kg of the extract (Table 2).

Table 2. Effect of *Corchorus olitorius* methanolic extract on hematological parameters.

PARAMETERS									
Sample	**Hb (g/dL)**	**PCV (%)**	**MCV (fi)**	**MCH (pg)**	**MCHC(g/dL)**	**PLT (10^6/L)**	**RBC (10^{12}/L)**	**TWBC (10^9/L)**	**L (10^9/L)**
100mg/kg bwt	3.98 ± 0.04^{b}	35.50 ± 1.50^{b}	42.00 ± 2.00^{abc}	36.00 ± 1.00^{bc}	43.50 ± 1.50^{c}	145.00 ± 2.00^{a}	5.25 ± 0.23^{b}	9.73 ± 0.61^{c}	30.00 ± 2.00^{c}
200 mg/kg bwt	5.09 ± 0.58^{c}	39.00 ± 1.00^{c}	43.50 ± 1.50^{bc}	34.00 ± 2.00^{b}	40.00 ± 2.00^{ab}	142.50 ± 2.50^{a}	7.49 ± 0.36^{c}	9.11 ± 0.36^{bc}	26.50 ± 1.50^{ab}
400 mg/kg bwt	7.96 ± 0.38^{d}	41.00 ± 1.00^{d}	44.50 ± 1.50^{c}	39.50 ± 0.50^{d}	37.50 ± 1.50^{a}	144.00 ± 2.00^{a}	9.00 ± 0.75^{d}	8.05 ± 0.69^{ab}	25.00 ± 1.00^{ab}
Positive control	8.93 ± 0.61^{d}	38.50 ± 0.50^{c}	39.50 ± 0.50^{a}	40.00 ± 2.00^{d}	41.00 ± 2.00^{bc}	150.50 ± 1.50^{b}	8.06 ± 0.24^{c}	6.53 ± 0.57^{a}	27.50 ± 1.50^{bc}
Negative control	2.92 ± 0.53^{a}	25.00 ± 1.00^{a}	47.50 ± 1.50^{d}	30.00 ± 2.00^{a}	47.00 ± 2.00^{d}	165.00 ± 2.00^{c}	3.17 ± 0.19^{a}	12.05 ± 1.81^{d}	33.50 ± 1.50^{d}

*Values mean ± standard deviation of three replicates. Values with different superscripts (a, b, c, d, e…) are significant at p<0.05. The joined letter signifies how close the values are, while single letters show differences in the values.

Biochemical parameters

The *Corchorus olitorius* extract, administered at concentrations of 100, 200, and 400 mg/kg, led to an increase in total serum proteins from 6.30±0.92 mg/dl (in the infected but untreated group) to 6.85±0.73, 6.73±1.12, and 11.55±0.79 mg/dl, respectively, in comparison with the positive control group (Table 3). Concurrently, at the same concentrations, the extract caused a decrease in albumin concentrations from 4.18±0.99 mg/dl (in the infected but untreated group) to 4.63±0.53, 3.79±0.61, and 8.29±1.06 mg/dl, respectively. Additionally, AST activities decreased from 45.06±1.77 U/l (in the infected but untreated group) to 35.25±1.34, 32.19±1.96, and 28.80±1.32 U/l in rats treated with 100, 200, and 400 mg/kg of the extract, respectively.

Table 3. Effect of *Corchorus olitorius* methanolic extract on Liver function and Lipid profile of rats infected with *Trypanosoma brucei*.

PARAMETERS										
Sample	***TP*** **(g/L)**	***ALB*** **(g/L)**	***ALP*** **(U/L)**	***ALT*** **(U/L)**	***AST*** **(U/L)**	***CHO*** **(mmol/L)**	***LDL*** **(mmol/L)**	***HDL*** **(mmol/L)**	***TRIG*** **(mmol/L)**	***TB*** **(mg/dL)**
100 mg/kg bwt	6.85±0.73^{a}	4.63±0.53^{a}	64.87±2.48^{c}	28.50±1.07^{b}	35.25±1.34^{d}	352.27±2.08^{d}	111.61±1.87^{c}	74.83±1.89^{b}	148.29±1.95^{b}	0.68±0.01^{d}
200 mg/kg bwt	6.73±1.12^{a}	3.79±0.61^{a}	56.14±1.80^{b}	25.23±1.74^{a}	32.19±1.96^{c}	350.08±1.86^{cd}	107.74±2.40^{c}	80.28±1.85^{c}	154.78±1.94^{c}	0.63±0.03^{bc}
400 mg/kg bwt	11.55±0.79^{b}	8.29±1.06^{bc}	47.59±1.65^{a}	26.08±1.13^{ab}	28.80±1.32^{b}	348.07±2.13^{c}	101.03±2.45^{b}	86.20±1.38^{d}	146.38±1.57^{b}	0.66±0.02^{cd}
Positive control	10.71±1.14^{b}	8.08± 0.35^{b}	50.36±2.04^{a}	24.55±1.45^{a}	34.23±1.38^{cd}	339.87±2.06^{b}	109.86±2.52^{c}	80.31±1.88^{c}	151.81±2.04^{c}	0.61±0.03^{b}
Negative control	6.30±0.92^{a}	4.18±0.99^{a}	81.34±1.89^{d}	32.76±1.53^{c}	45.06±1.77^{e}	370.78±1.94^{e}	127.32±1.91^{d}	59.10±1.33^{a}	166.07±1.78^{d}	0.89±0.02^{e}

*Different values (a, b, c, d, e…) on the same columns were significant at P< 0.05. The joined letter signifies how close the values are, while single letters show differences in the values.

Table 4. Effect of *Corchorus olitorius* methanolic extract on Kidney function and Electrolytes of rats infected with *Trypanosoma brucei.*

PARAMETERS						
Sample	***Creatinine*** **(mg/dL)**	***Urea*** **(mg/dL)**	***Uric acid*** **(mg/dL)**	***Na*** **(mEq/l)**	***K*** **(mEq/L)**	***Bicarbonate*** **(mg/dL)**
100 mg/kg bwt	7.76±0.64^{c}	25.04±1.55^{b}	6.20±0.52^{b}	146.92±1.76^{d}	4.69±0.46^{b}	21.90±1.95^{a}
200 mg/kg bwt	7.23±0.22^{c}	25.84±1.33^{bc}	6.48±0.45^{b}	139.74±2.20^{c}	6.34±0.37^{c}	24.59±1.10^{b}
400 mg/kg bwt	6.28±0.31^{b}	21.92±1.57^{a}	5.15±0.20^{b}	143.09±2.52^{c}	7.28±0.57^{d}	25.76±1.46^{b}
Positive control	7.41±0.46^{c}	28.10±1.52^{c}	6.25±0.53^{b}	146.76±1.59^{d}	6.18±0.54^{c}	26.40±0.95^{b}
Negative control	10.09±0.24^{d}	35.56±1.61^{d}	10.05±0.82^{c}	154.26±2.08^{e}	3.98±0.06^{a}	30.74±1.40^{c}

*Values mean ± standard deviation of three replicates. Values with different superscripts (a, b, c, d, e…) are significant at p<0.05. The joined letter signifies how close the values are, while single letters show differences in the values.

Trypanosoma brucei

All doses of the extract tested significantly increased the cholesterol and HDL levels in the treated rats compared to those in the infected but untreated group. Conversely, triglyceride and LDL concentrations significantly decreased among rats treated at doses of 100, 200, and 400 mg/kg of the extract compared to those for the infected but untreated group (Table 3). Moreover, at the same extract concentrations, total serum bilirubin, ALP, and ALT levels (Table 3), as well as creatinine, urea, and uric acid concentrations (Table 4), exhibited significant decreases in experimental rats from the control group to the 100, 200, and 400 mg/kg concentrations of the extract, respectively.

Furthermore, the extract at the same concentrations decreased the sodium concentrations from 154.26±2.08 mEq/l (in the infected but untreated group) to 146.92±1.76, 139.74±2.20, and 143.09±2.52 mEq/l, respectively. Additionally, potassium activities increased from 3.98±0.06 mEq/l (in the infected but untreated group) to 4.69±0.46, 6.34±0.37, and 7.28±0.57 mEq/l in rats treated with 100, 200, and 400 mg/kg of the extract, respectively. However, there

was no significant difference (P>0.05) in the bicarbonate concentration of the extract compared with the infected but untreated group (Table 4).

DISCUSSION

The quantitative phytochemical analysis of *Corchorus olitorius* extract revealed the presence of tannin, saponin, and flavonoids, with alkaloids exhibiting the lowest concentration at 45.67 mg/100 g, while polyphenol appeared to be the most concentrated among the other parameters. Significant differences (P<0.05) were observed in all analyzed parameters. This finding is consistent with the study by (Ogungbemi et al., 2019), which reported polyphenols as having the highest quantitative phytochemical concentration. Similarly, in research conducted by Waheed et al., (2021), flavonoids were found to have a high concentration (276.15±134.57 mg/100 g) in *Jatropha gossypifolia* extract which is in contrast with this study where the flavonoid concentration is 45.51 ± 0.17^{c} mg/g. The variation in these constituents could be attributed to factors such as the method of sample preparation, environmental conditions, and/or pest infestation affecting the plants (Ogungbemi et al., 2019).

The antitrypanosomal efficacy of the *Corchorus olitorius*methanol extract exhibited significant differences (p<0.05) in parasitemia counts between the treated groups and both control groups (positive and negative). Notably, the negative control group displayed a consistent increase in parasite levels until week 3, culminating in mortality, consistent with the findings of Obi et al., (2023), where all untreated rats succumbed to the infection. While complete parasite clearance was not achieved in the 400 mg/kg bw group and the standard group, both managed to substantially reduce parasite levels. Conversely, the 100 mg/kg-weight group experienced an initial increase in parasite levels until week 3; however, they successfully contained the parasites until the conclusion of the treatment period. This observation aligns with the findings of Madaki et al., (2022). who reported dose-dependent antitrypanosomal activity of *Allium sativum*. In a study conducted by Zhou et al., (2021). *G. Kobayashi* plant extract showed 100% antiparasitic activity at 10 mg/L and recorded a therapeutic index (TI, LC_{50}/EC_{50}) of 5.26. The incomplete clearance of parasites may be attributed to factors such as delayed initiation of treatment in infected rats and the development of resistance, possibly involving lipopolysaccharides, by the parasitic organisms against the activity of the extract and standard drugs (Madaki et al., 2022).

Hematological parameters, including RBC, PCV, Hb, MCH, MCHC, and RDW, serve as valuable indicators of circulatory erythrocyte levels, reflecting the bone marrow's capacity to produce RBCs in response to drug, toxin, or plant extract administration (Obi et al., 2023). The significant increases observed in hemoglobin, packed cell volume, red blood cells, and MCHC following treatment with 400 mg/kg of *Corchorus olitorius* methanolic extract suggest its potential to stimulate erythropoiesis. The extract likely enhances erythropoietin synthesis and release in the kidneys, the humoral regulator of RBC production (Abdeta et al., 2020). In this study, hematological analysis of surviving animals revealed significant differences (p<0.05) between the 100 and 200 mg/kg body weight groups compared to the positive control group in PCV, MCV, RBC, lymphocytes, and RDW. However, in the 400 mg/kg body weight group, most parameters analyzed did not show significant differences (p>0.05). The highest dose (400 mg/kg body weight) exhibited more promising values in hematological assays than the standard group. Notably, values for RBC and PCV at 400 mg/kg body weight fell within a more favorable range compared to those of the positive control group (Table 2), indicating the potential utility of the extract in managing anemia. Similar observations have been reported in rats treated with extracts of *T. occidentalis* (Obi et al., 2023). and *Allium sativum* (Madaki et al., 2022). The observed effects on white blood cell and lymphocyte counts suggest that the extract may exert leucopoietic and potentially immunomodulatory effects on treated animals (Madaki et al., 2022; Maikai et al., 2008). In another study by Akah et al., (2009), *Vernonia amygdalina* fraction at doses of 160 and 320 mg/kg significantly (p<0.05) increased lymphocyte levels from 50.4±4.69 in diabetic control rats to 60.1±2.05 and 60.6±3.42, respectively, in treated diabetic rats, while other hematological indices were not significantly altered.

Concentrations of total serum proteins, albumin, bilirubin, urea, creatinine, and electrolytes serve as informative markers of the liver and kidneys secretory, synthetic, and excretory functions (Madaki et al., 2022; Obi et al., 2023). The observed increases in total serum proteins and decreases in bilirubin suggest a compromised synthetic capacity of the liver due to *Corchorus olitorius* methanolic extract administration. The extract may have enhanced liver function by disturbing the equilibrium in synthesis and degradation, as well as removal or clearance of total proteins and bilirubin in the animals. This finding aligns with an earlier study on the hepatoprotective effects of leaf extracts of *V. amygdalina* in mice (Iwalokun et al., 2006). A similar outcome was previously reported in rats administered snail hemolymph (Obi et al., 2023). However, the elevation in total serum proteins could potentially lead to dehydration, adversely affecting cellular homeostasis and metabolic liver activities, thereby impacting animal health negatively.

Urea and creatinine tend to accumulate in urine when glomerular function is compromised. Hence, the preserved concentrations of urea and creatinine in rats treated with *Corchorus olitorius* methanolic extract indicate maintained glomerular function (Obi et al., 2023; Lorke, 1983).

Additionally, Akah et al., (2009) demonstrated that diabetic control rats exhibited elevated serum levels of urea (13.3±1.60 mmol/L) and creatinine (94.3±1.91 mmol/L), while 80 and 160 mg/kg of *Vernonia amygdalina* fraction significantly ($p<0.05$) reduced these indices. Literature suggests that ingestion of medicinal compounds or drugs can disrupt the normal range of hematological parameters (Ajagbona et al., 1999).

CONCLUSION

The extract derived from *Corchorus olitorius* showcases properties that are antitrypanosomal, antilipidemic, and erythropoietic. It effectively addresses parasitemia, extends the lifespan of infected rats, and mitigates trypanosomal infections, potentially due to its active metabolites disrupting metabolic pathways within trypanosomes. Furthermore, the extract demonstrates relative safety at concentrations of 400 mg/kg in rats, particularly concerning liver and kidney functions following three weeks of daily administration. Hence, it emerges as a promising option for managing trypanosomal infections, addressing challenges associated with current chemotherapeutic agents and drug resistance. There is encouragement for further exploration of the ethnobotanical aspects of *Corchorus olitorius*. Additionally, prompt adoption of early treatment strategies utilizing *Corchorus olitorius* extract is advocated to prevent the progression of trypanosome infections to chronic stages.

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LIST OF ABBREVIATIONS

HAT- Human African trypanosomiasis; EDTA- Ethylene diamine tetra acetic acid; RBC- Red blood cell; WBC- White blood cell; ALT- Alanine transaminase; AST- Aspartate transaminase; ALP- Alanine phosphatase; LDL- Low-density lipoprotein; HDL- High-density lipoprotein; UV- Ultraviolet; OD- Optical density; A- Absorbance; SEM- Standard error mean; PCV- Packed cell volume; PLT- platelet count; MCHC- Mean corpuscular hemoglobin concentration; MCH- Mean corpuscular hemoglobin; RDW- Red cell distribution width; TWBC- Total white blood cell count; ALB- Albumin; TP- Total protein; CHO- Carbohydrate; TRIG- Triglyceride; TB- Total bilirubin; K- Potassium; Na- Sodium; C.C-Calibration concentration.

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