Original article

Effect of Extracts of *Senna occidentalis* on Biochemical Parameters of Wistar rats Infected with *Trypanosoma congolense*

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ABSTRACT

Animal Trypanosomiasis has remained a great threat to livestock in tropical Africa. Chemotherapy of trypanosomiasis is faced with lots of problems. Fexinidazole recently discovered in 2018 is not easily available and can only be used for the treatment of the first stage of the disease, hence the need for cheaper drugs which will be easily available and assessable. The study aimed to determine the effect of *Senna occidentalis* extracts on some biochemical parameters of wistar rats infected with *Trypanosoma congolense*. Phytochemical screening revealed the presence of alkaloids, flavonoids, steroids, terpenoids phenolic compounds and carbohydrates in both the ethanol and aqueous extracts. Acute toxicity studies, determined by administering 1500, 3000 and 5000 mg/kg body weight (b. wt) of extracts to wistar rats revealed significant ($P<0.05$) increase in AST and ALP in group receiving 3000mg/kgbwt and 5000mg/kg b. wt of the extracts. There was no significant difference in ALT in group receiving 1500 mg/kg b. wt and 3000mg/kg b. wt. of both ethanol and aqueous extracts compared with the control. This showed that the plant is relatively non-toxic. Post infection treatment of animals stirred the emergence of pasitemia by Day 5. However, only animals receiving 250mg/kg b. wt of ethanol extract and 500mg/kg b. wt of aqueous extract survived till day 13th post infection. A significant ($P<0.05$) decrease in AST and ALP was discovered in all the test groups except the uninfected/untreated group. For group receiving 500mg/kgbwt of ethanol extract, there was no significant difference in the ALT and T.bil. No significant difference was observed in potassium, sodium, chloride, urea and creatinine concentrations of group receiving 500mg/kg b. wt of ethanol extracts compared with the uninfected/untreated rat. These results thereby demonstrate ameliorative potentials of *Senna occidentalis* leaves on hepatic and renal functions of wistar rats infected with *Trypanosoma congolense*. Findings from the study recommend bioassay guided fractionation to characterize the active components of the plant.

Keywords: Trypanosomiasis, *Senna occidentalis*, Parasitemia, Phytochemicals, Toxicity

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INTRODUCTION

Trypanosomiasis is a protozoan disease of zoonotic importance that affects both animals and human. It is a neglected tropical disease that poses a huge hindrance to livestock production in many parts of Africa where the disease occurs [1]. Trypanosome, the causative agent of this infection belongs to the genus *Trypanosoma*. The parasites are transmitted to their hosts via tsetse fly bites which have acquired their infection from human or animals harboring the pathogenic parasite [1]. In tropical Africa, a range of trypanosome species are transmitted through the saliva of tsetse flies, of which *Trypanosoma brucei* sub species cause Human African Trypanosomiasis (HAT) and a wider range of species cause Animal African Trypanosomiasis [2]. *Trypanosoma vivax* and *Trypanosoma congolense* are regarded as major pathogens of cattle and other ruminants [3,4]. *Trypanosoma congolense* is probably the most prevalent and widespread pathogenic trypanosome of livestock in tropical Africa, being found in ruminants, pigs, dogs and other domestic animals throughout the tsetse belt [5,6]. The three main species of flies responsible for the transmission of trypanosomes are *Glossina morsitans,* which favours the open woodland of the savannah, *Glossina palpalis*, which prefers the shaded habitat immediately adjacent to rivers and lakes, and *Glossina fusca*, which are found in the high dense forest areas [7,8].

Medicinal plants have been used for centuries as remedies for human diseases and other animals because they contain certain components of therapeutic value [9]. There are more than 35, 000 plant species being used in various human cultures around the world for medicinal purposes. About 80% of the world's population still depends solely on traditional or herbal medicine for treatment of disease, mostly in Africa and other developing nations. Natural products (plants and animals) are important sources of new drugs because their derivatives are extremely useful as lead structures for synthetic modification and optimization of bioactivity [9,10]. Thus, traditional medicine remains popular for both historical and cultural reasons. Like other parts of sub-Saharan countries, 70% of human and 90% of livestock population of Ethiopia rely on traditional medicine for primary health care [11]. In Nigeria, *Senna occidentalis* has been reported traditionally for the treatment of some bacteria and protozoan diseases such as dysentery and malaria [12].

According to World Health Organization, medicinal plants will be the best choice to obtain a variety of drugs [9]. About 80% of people rom developing countries use traditional medicine [13]. Therefore, such plants should be investigated to better understand their properties, safety and efficiency. *Senna occidentalis* is a weed of the leguminosae family, and is distributed throughout the tropical and subtropical regions of the world. It can be found in open pastures and in fields cultivated with cereals such as soybean, corn, sorghum and others; thus, during the harvest it is almost impossible to prevent this plant from mixing with the cultivated crops. The leaf is used as a remedy for renal calculi. Leaves are made into a tea for treating afterbirth problem, fever, coughs and cold, headache, hemorrhage and thrush [14]. With this knowledge about the plant, it became very necessary to subject it to detailed scientific screening and also validate its efficacy in the treatment of

some of important diseases such as trypanosomiasis.

MATERIALS AND METHODS

Collection and Authentication of Plant Materials

The leaves of *Senna occidentalis* plant was harvested from Kaduna Township, Kaduna state with the coordinate ($10^0$31'39.263'N; $7^0$27'49.435'). It was authenticated by a botanist in the herbarium of the Department of Biological Sciences, Kaduna State University with a voucher number 1831. The leaves were dried at 25 ^{0}C (room temperature), pulverized in a mortar and sieved to obtain a fine powder and then stored in an air tight container with proper labeling for future use.

Extraction of plant material

Hot maceration method using Soxhlet apparatus was used for plant extraction. One thousand grams (1000 g of powdered) plant material was weighed and extracted using Hexane (solvent). The Hexane portion was concentrated *in vacuo*, to obtain a product referred to as Hexane extract (HE). The marc (Hexane defatted residue) was extracted with ethanol and the solvent recovered and concentrated *in vacuo*, to obtain ethanol fraction (EF). One hundred gram (100g) of the EF was then dissolved in water and partitioned four times with equal portion of water in one thousand millilitre (1000ml) separatory funnel. The water portion was pooled and concentrated *in vacuo*, to obtain a product referred to as the aqueous fraction (AF). The remaining portion of the reconstituted aqueous fraction portion was concentrated *in vacuo* and subsequently referred to as aqueous residue (AR).

Phytochemical Screening of Crude Extract of *Senna occidentalis*

Method of Sofowora as described in the work done by [13] was used to detect the presence of flavonoids, carbohydrate, alkaloids, saponin, glycosides, tannins, triterpenoids, phenolic compounds, steroid and terpenoids.

Test for alkaloids

To 1ml of the test solution, 1ml of HCL was added and 3 drops of Wegner's reagent was also added. The formation of a brown precipitate indicates the presence of alkaloids.

Test for Glycosides

To 1ml of the test solution, 2 drops of Conc. Sulphuric acid was added and placed in water bath for about 15 minutes. 20% KOH was added to make the solution alkaline. To this solution, few drops of FeCl2 was added. The formation of brick red precipitate indicates the presence of glycosides.

Test for Saponins

To 1ml of dissolve solution, 3ml of water was added, shaken and allowed to stand for about 3 minutes. The presence of persistent froth denotes the presence of saponins.

Test for phenolic compounds

To 2ml of 5% neutral ferric chloride solution 1ml of extract was added, the dark blue colour indicated the presence of phenolic compounds.

Test for Tannins

To 1ml of the test solution, 5ml of 1% gelatin containing NaCl was added. Formation of a yellow precipitate denotes the presence of tannins.

Test for triterpenoids

Five milligram (5mg) of the plant extract was dissolved in 2ml of chloroform and then 1ml of acetic anhydride was added to it. 1ml of concentrated sulphuric acid was added to the solution. Formation of reddish violet colour indicated the presence of triterpenoids

Test for flavonoids

To 1ml of the test diluted plant extract, 3mls of 10% sodium hydroxide was added followed by 3mls of 10% HCL. The formation of a yellow colour on addition of sodium hydroxide, which disappeared on addition of the HCL, indicated the presence of flavonoids.

Test for Carbohydrates

Molish test: Aqueous solution of extract carbohydrate mixed with few drops of Molish reagent (alpha naphthol) and Concentrated H2SO4 was added from side wall of test tube.Formation of purple coloured ring at junction indicates presence of carbohydrates.

Test for Terpenoids

Exactly (5ml) of the plant extract was mixed with (2ml) of chloroform and concentrated sulphuric acid (3mL) was carefully added to form a layer. A reddish brown coloration of the interface was formed to show presence of terpenoids

Experimental Animals

Sixty-one wistar rats of same sex were purchased from the animal house of Nigerian Institute for Trypanosomiasis Research, Kaduna. The animals were housed in rat cages, maintained on pellet diet and water *ad libitum*. Wistar rats were kept in a group of five rats per cage in an environment with adequate light and were allowed to acclimatized before the commencement of the experiment.

Acute Toxicity Test of the Crude Extract

Determination of acute toxicity test of the extract was undertaken according to the method described by [15]. Twenty wistar rats were used for the study. The feed was withdrawn for 24hrs. Fifteen animals were divided into three groups of five animals each and administered orally with the doses of 1500, 3000 and 5000 mg /kg b. wt of the extract with the aid of an oral gavage; another five animals served as control group. Animals were observed for signs of toxicity which includes but not limited to mortality, paw licking, sneezing, salivation, and paralysis, dilation of the pupil and reduced motor activity for the period of 24 hours.

Test organisms: *Trypansoma congolense*

Cryopreserved stabilate of *Trypansoma congolense* was obtained from the cryobank maintained at the Vector and Parasitology Study Department of the Nigerian Institute for Trypanosomiasis Research, Kaduna, Nigeria. After thawing the stabilate at 37 °C, trypanosomes were screened for viability by examining wet smears prepared from the stabilate in the light microscope at ×40 magnification. The presence of motile trypanosomes was taken as indication of trypanosome viability [16].

Determination of parasitemia

The trypanosome count in the infected rats was estimated by the rapid matching method of [17]. Briefly, a drop of whole blood collected by tail snip was placed on a clean grease free glass slide and a cover slip placed over it. The slide was then placed under the light microscope and examined at ×40 magnification. The distribution of trypanosomes among the red blood cells (RBCs) was matched against the Lumsden's chart and the approximate number of trypanosomes per milliliter of blood estimated [16].

Collection of parasitized blood

Blood containing trypanosomes was collected into a 5 ml syringe from the donor rats by the cardiac puncture technique after chloroform anesthesia. The blood was dispensed into a plane sample bottle for biochemical test [16].

Animal grouping

The *in vivo* antitrypanosomal activity was carried out as described by [18]. Thirty-five wistar rats grouped into seven groups of five rats each in each cage were used for the study. The animals were inoculated with approximately 10^4 Trypanosomes/ml and infection stirred the emergence of parasitemia by Day 5. Four groups served as extract test (Ethanol extract A, ethanol extract B, aqueous extract A and aqueous extract B). A positive control group (i.e. reference drug Diminazene aceturate). A sixth group served as negative control (untreated) and seventh as uninfected and untreated. The extracts were administered orally. Treatment with extract and reference drug commenced on 5^{th} day post infection.

Collection of blood sample for analysis

At the end of the acute toxicity studies and *in vivo* screening, two animals were randomly selected from each group and anaesthized in a desiccator with cotton-wool soaked in chloroform and sacrificed. The blood was collected through cardiac puncture and blood serum was collected into dry tubes for biochemical analysis. The blood was centrifuged at 3000 rpm for ten minutes. The serum was collected and stored in dry test tubes.

Therapeutic Monitoring of Experimental Wistar rats

Parasitemia of each rat was monitored every twenty-four hours during administration of the extracts and reference drug. The levels were estimated by Rapid Matching Method of Herbert and Lumsden as described by [19]. Parasitemia levels were compared with untreated control animals.

Determination of Biochemical indices

Alanine Aminotransferase (ALT) and Aspartate Aminotransferase (AST)

Activities of aspartate aminotransferase (AST) and alanine aminotransferase (ALT) were assayed for by the method of Reitman and Frankel using Randox assay kits as described in [20].

Alanine Aminotransferase (ALT)

Procedure: Exactly, 0.5ml of reagent 1 which is made up of phosphate buffer, L-alanine and α-Oxoglutarate was added into two clean test tubes, one containing 0.1 ml of serum and the other containing 0.1ml distilled water (blank). The content in each test tube was mixed, incubated for exactly 30 minutes at 37^0C. 0.5 ml of reagent 2 which is made up of 2, 4-

dinitrophenylhydrazine was added to each of the test tubes, mixed and allowed to stand for exactly twenty minutes at 20-25 ^{0}C. Then 0.5 ml of sodium hydroxide solution was added to each of the test tubes, the content in each of test tubes was mixed and absorbance was read against the blank at 540nm after 5 minutes. The ALT activity (U/I) was determined from the standard calibration table provided in the manual of Randox Lab. Ltd, UK Reagent Kit.

Aspartate Aminotransferase (AST)

Procedure: 0.5mL of reagent 1 which is made up of phosphate buffer, L-aspartate and α-Oxoglutarate was added into two clean test tubes labelled as test sample and reagent blank, containing 0.1 ml of serum and 0.1 ml of distilled water, the content in each test tubes was mixed and incubated for exactly thirty minutes at 37 ^{0}C. 0.5 ml of reagent 2 which is made up of 2, 4-dinitrophenylhydrazine was added to each of the test tubes, the content of each of the test tubes was mixed and allowed to stand for exactly twenty minutes at 20-25 ^{0}C. To each of the test tubes, 0.5ml of sodium hydroxide solution was added, mixed and absorbance was read against the blank at 540nm after five minutes. The AST activity (U/l) was determined from the standard calibration table provided in the manual of Randox Lab. Ltd, UK Reagent Kit.

Alkaline Phosphatase (ALP)

The serum level of alkaline phosphatase was quantified by optimized standard method described by Haussament using Randox assay kits as described in a work done by [20]. Procedure: Exactly 1ml of reagent 1 containing Diethanolamine buffer, magnesium chloride and substrate (P-nitrophenylphosphate) was added into a clean test tube containing 0.02ml of serum. This was mixed, initial absorbance was read and timer was set simultaneously, absorbance was read again after 1, 2 and 3 min at 405nm.

Determination of serum total bilirubin (TB) concentration

The serum TB concentration was determined using Randox Kit (Randox laboratories limited UK) based on the method of Jendrassik and Grof as described in a work done by [21]. Procedure: Two hundred mililitres(200ml) of reagent 1 (sulphanillic acid) was dispensed each into two different test tubes labeled 'sample blank' and 'sample' followed by the addition of one drop (50µl) of reagent 2 (nitrite) and one thousandµl of reagent 3 (caffeine). Two hundred µl of the test serum was then dispensed into each of the test tubes and the mixtures incubated in a water bath for ten minutes at 25^{0}C. This was followed by the addition of one hundred µl of reagent 4 (tartrate) and the mixture incubated again at 25^{0}C for ten minutes. The absorbance of the sample (A_{TB}) was then read against the sample blank using a colorimeter at 578nm wavelength. The total bilirubin concentration was then calculated using the formula provided by the manufacturers.

Determination of serum unconjugated bilirubin concentration

The serum unconjugated bilirubin concentration was determined using the Randox Kit (Randox laboratories limited UK) based on the method described by Jendrassik and Grof as described in a work done by [20]. Procedure: Two hundred meals (200ml) of reagent 1 (sulphanillic acid) was dispensed each into two

different test tubes labeled 'sample blank' and 'sample' followed by the addition of one drop (50µl) of reagent 2 (nitrite) and two thousand µl of 0.9 % NaCl. Two hundred micro litres (200µl) of the test serum was dispensed into each of the test tubes and the mixtures incubated in a water bath for ten minutes at 25^0C. The absorbance of the sample (A_{TB}) was then read against the sample blank using a colorimeter at 546 nm wavelength. The direct bilirubin concentration was then calculated.

Determination of Serum Creatinine Concentration

The colourimetric method was used to determined serum creatinine concentration according to [20] using Randox assay kits.

Procedure: One meal (1ml) of working reagent containing picric acid and sodium hydroxide was added into two clean test tubes labelled sample test and standard, containing 0.1ml of sample and 0.1ml of standard solution. The content in each test tube was mixed and after thirty seconds, the absorbance A_1 of the standard and sample were read. Two minutes later, absorbance A_2 of the standard and sample were read at 490nm.

Determination of sodium, potassium and chloride ions

The concentration of Sodium, Chloride and potassium ions were determined using the method of Trinder using Elyte kits [22].

Sodium Procedure Precipitation

The following were Pipetted into a clean dry test tubes labelled as Standard (S) and Test (T).

Addition Sequence	Blank (ml)	Standard (ml)
Precipitating Reagent (L1)	1.0	1.0
Na+/K+ Standard (S)	0.02	-
Sample	-	0.02

The solutions were mixed well and let to stand at room temperature for 5 minutes, shaking well intermittently. The solution was centrifuged at 2500 rpm to obtain clear supernatant

Colour development:

The following were pipetted into clean dry test tubes labeled as Blank (B), Standard (S), and Test (T)

Addition Sequence	Blank (ml)	Standard (ml)	Test (ml)
Acid Reagent (L2)	1.0	1.0	1.0
Supernatant from Step 1.	0.02	0.02	0.02
Precipitating Reagent (L1)	-	-	-
Colour Reagent (L3)	0.1	0.1	0.1

The solution was mixed well and incubated at room temperature for 5 min. The absorbance of the Blank (Abs.B)

Standard (Abs.S), and Test Sample (Abs.T) were measured, against distilled water within 15 min at 530nm.

Calculation

Sodium concentration (mmol/l) = $\frac{Abs.B - Abs.T}{Abs.B - Abs.S} X\ 150$

Potassium

Procedure

The following were pipetted into clean dry test tubes labeled as Blank (B), Standard (S), and Test (T) as follows

Addition Sequence	**Blank** (ml)	**Standard** (ml)	**Test** (ml)
Potassium Reagent (L1)	1.0	1.0	1.0
Deionised water	0.02	-	-
Na+/K+ Standard (S)	-	0.02	-
Sample	-	-	0.02

The solutions were mixed well and incubated at room temperature for 5 min. The absorbance of the Standard (Abs.S), and Test Sample (Abs.T) were measured against Blank, at 630nm, within 15 min.

Calculation

Potassium concentration (mmol/l) = $\frac{Abs.T}{Abs.S} X\ 5$

Chloride Procedure

The following were pipetted into clean dry test tubes labeled as Blank (B), Standard (S), and Test (T).

Addition Sequence	**Blank** (ml)	**Standard** (ml)	**Test** (ml)
Chloride Reagent (L1)	1.0	1.0	1.0
Deionised water	0.01	-	-
Chloride Standard (S)	-	0.01	-
Sample	-	-	0.01

The solution was mixed well and incubated at R.T. for 2 minutes. The absorbance of the Standard (Abs.S) and Test Sample (Abs.T) were measured against Blank, within 60 min at 505nm.

Calculations

Chloride concentration (mmol/l) = $\frac{Abs.T}{Abs.S} X\ 100$

Determination of Bicarbonate Concentration

The bicarbonate reagent utilizes the enzymatic method developed by (Forrester *et al.* 1976) as described in a work done by Woyesa [20].

Principle

Bicarbonate (HCO_3^-) and phosphoenolpyruvate (PEP) are converted to oxaloacetate and phosphate in the reaction catalyzed by phosphoenolpyruvate carboxylase (PEPC). Malate dehydrogenase (MD) catalyzes the reduction of oxaloacetate to malate with the concomitant oxidation of reduced nicotinamide adeninedinucleotide (NADH). This oxidation of NADH results in

a decrease in absorbance of the reaction mixture measured bichromatically at 380/410 nm proportional to the Bicarbonate content of the sample.

PEPC
PEP + HCO_3^- ⟶
Oxaloacetate + H2PO4
Magnesium
MD
Oxaloacetate + NADH + H^+ ⟶
Malate + NAD+

Reagents

Final concentration of reactive ingredients:
MD (microbial)
2,000 U/L
PEPC (microbial)
572 U/L
Magnesium
2.8mmol/L
PEP
8.2mmol/L
NADH
1.6mmol/L

Ethical Approval

The ethical approval for the use of laboratory animals was obtained from Animal committee of Kaduna State Ministry of Agriculture and Forestry.

Data Analysis

Data obtained from the study was summarized and expressed as mean ± standard deviation. Data analysis was performed using Statistical Package for Social Science (SPSS), version 20.0. To compare the results obtained from different groups, one way ANOVA followed by Duncan multiple comparison tests was performed to determine statistical significance. P values less than 0.05 was considered significant.

RESULTS

Phytochemical screening of the n-hexane, ethanol and aqueous leaf extracts of *Senna occidentalis*

The results of the phytochemical screening indicated that saponins and tannins were the phyto-constituents identified in the n-hexane extract of *Senna occidentalis*, while in the ethanol extract alkaloids, flavonoids, steroids, terpenoids, phenolics, triterpenoids, and carbohydrates were detected; tannins, and glycosides were not detected in the ethanol extract. Detected in the extract of Alkaloids, flavonoids, steroids, terpenoids, phenolic compound, and carbohydrates were detected in the aqueous extract of *S. occidentalis*. The flavonoids content detected in the aqueous extract was higher than that observed in the ethanol extract. Significant quantities of phenols were detected in both the ethanol and aqueous extract. Triterpenoids were seen in the ethanol extract, while only trace amounts of carbohydrates were detected in both the ethanol and aqueous extract of *Senna occidentalis* (Table 1).

Table 1: Phytochemical screening of *Senna occidentalis* leaf extracts

S/N	TEST	Solvent extracts	
		Ethanol	Aqeous
1	Alkaloids	++	++
2	Flavonoids	+	++
3	Saponins	++	-
4	Tannins	-	-
5	Steroids	+	++
6	Terpenoids	++	+++
7	Glycosides	-	-
8	Phenolic compounds	+++	+++
9	Triterpenoids	++	-
10	Carbohydrates	+	+

n-Hx: n-Hexane leaf extract of *Senna occidentalis*; EtOH: ethanol leaf extract of *Senna occidentalis*; Aq: aqueous leaf extract of *Senna occidentalis*.
"-": Not detected; "+": Detected

4.2. Acute effects of ethanol and aqueous extracts of *S. occidentalis* on liver function parameters of Wistar rats

Administration of single high doses of either the aqueous or ethanol leaf extracts of *S. occidentalis* resulted in significantly higher serum aspartate transaminase activity in all the groups when compared with the normal control. In the control group, the mean AST level was 49.3±11.0mm/l, while in groups that received either 1500, 3000, or 5000 mg/kg of the aqueous extract, the AST levels were 58.9±12.1, 78.2±0.21, and 82.8±6.15 mm/l, respectively, as the values indicate increase in serum AST activity with increasing dose of the aqueous extract. The AST level in ethanol leaf extract did not show dose related elevations: in the 1500 mg/kg group, the value for AST was 71.0±1.13mm/l, while in the 3000 and 5000 mg/kg dose groups, the respective values were 65.9±0.14 and 77.2±6.71mm/l (Table 2).

Alanine transaminase activity recorded in the control group was 33.6±10.4 mm/l. In groups that receive either the aqueous or ethanol leaf extracts of *S. occidentalis*, the ALT levels recorded exhibited a dose dependent pattern as increasing levels of ALT activity were seen with increase in dose of the extracts. ALT levels recorded in groups that received 1500 mg/kg of the aqueous extract was 43.0±14.4 mm/l, while in groups that receive 3000 or 5000 mg/kg of the aqueous extract, the ALT levels were 52.6±22.3 and 71.5±25.4 mm/l, respectively. The respective ALT activity recorded in rats administered 1500, 3000, or 5000 mg/kg of the ethanol leaf extract of *S. occidentalis* were 31.3±4.10, 35.8±0.14, and 62.8±3.67 mm/l (Table 2).

At 1500 mg/kg of the ethanol leaf extract, alkaline phosphate (ALP) levels in rats averaged 88.8±3.89 mm/l, while at 3000 and 5000 mg/kg the respective values for ALP were 86.4±4.17 and 98.1±12.7 mm/l. In rats that received the aqueous leaf extract of *S. occidentalis*, the mean values for ALP were 62.6±10.4, 74.4±5.51, and 102.3±8.91 mm/l at 1500, 3000, and 5000 mg/kg dose, respectively, while in the control, a mean of 64.7±6.01 mg/dlwas recorded (Table 2).

Total bilirubin in the control group was 6.72±0.08 mg/dl at 1500 mg/kg of the aqueous leaf extract it was 6.59±0.93 mg/dl while at 3000 and 5000 mg/kg of

the aqueous extract, the mean values were 7.19±0.37 and 6.76±0.13 mg/dl, respectively. In rats that received the ethanol leaf extract, the respective values for total bilirubin were 7.84±1.18, 5.92±1.30, and 7.57±1.23 mg/dlat 1500, 3000, and 5000 mg/kg body weight (Table 2).

Conjugated bilirubin was significantly lower in the control group than in groups that were administered the extract. In the control, the conjugated bilirubin was 3.73±0.93 mg/dl while in the extract treated groups, the values ranged between 4.41 and 4.90 mg/dl. The level of conjugated bilirubin in rats that were administered 1500 mg/kg of the aqueous extract was 4.66±1.04 mg/dl while in groups that received 3000 or 5000 mg/kg of the aqueous extracts the values of conjugated bilirubin were 4.69±1.92 and 4.51±0.44 mg/dl, respectively. In rats that received that ethanol leaf extracts, the values for conjugated bilirubin were 4.61±2.17, 4.90±0.35, and 4.41±0.53 mg/dl, respectively (Table 2).

Table 2: Acute toxicity effects of ethanol and aqueous extracts of *S. occidentalis* on liver function parameters of Wistar rats

Groups	AST(mm/l)	ALT(mm/l)	ALP(mm/l)	T bil. (mg/dl)	C. bil.(mg/dl)
Control	49.3±11.00^{a}	33.6±10.04^{a}	64.7±60.01^{a}	6.72±00.08^{a}	3.73±00.93^{a}
A	58.9±12.01^{ab}	43.0±14.04^{ab}	62.6±10.04^{a}	6.59±00.93^{a}	4.66±10.04^{a}
B	78.2±00.21^{c}	52.6±22.03^{ab}	74.4±500.51^{ab}	7.19±00.37^{a}	4.69±100.92^{a}
C	82.8±60.15^{c}	71.5±25.04^{b}	102.3±80.91^{c}	6.76±00.13^{a}	4.51±0.44^{a}
D	71.0±10.13^{bc}	31.3±4.10^{a}	88.8±3.89^{bc}	7.84±10.18^{a}	4.61±20.17^{a}
E	65.9±00.14^{abc}	35.8±00.14^{ab}	86.4±4.17^{bc}	5.92±10.30^{a}	4.90±00.35^{a}
F	77.2±60.71^{c}	62.8±03.67^{ab}	98.1±12.07^{c}	7.57±10.23^{a}	4.41±00.53^{a}
p value	0.020	0.154	0.008	0.473	0.974

Values are given as mean ± standard deviation of mean. In each column, mean values with different superscripts have statistically significant difference ($p < 0.05$).
Group A =1500 mg/kg aqueous extract; Group B = 3000 mg/kg aqueous extract; Group C = 5000 mg/kg aqueous extract; Group D =1500 mg/kg ethanol extract; Group E = 3000 mg/kg ethanol extract; Group F= 5000 mg/kg ethanol extract. AST = Aspartate transaminase; ALT = Alanine transaminase; ALP = Alkaline phosphatase; T. bil. = Total bilirubin; C. bil. = Conjugated bilirubin

4.3 Acute effects of ethanol and aqueous extracts of *S. occidentalis* on kidney function parameters of Wistar rats

Sodium ion concentration: There were elevations in the concentrations of sodium ion in rats that received either the aqueous or ethanol extract of *S. occidentalis* than in rats that served as control, but the difference was not statistically significant. Sodium ion concentration in the control group was 124.5±4.95mm/l, while in the extract treated groups the values ranged between 127 and 145. In rats that received the aqueous extract, there was a dose dependent increase in the sodium ion concentration: at 1500 gm/kg, the mean [Na^+] was 127.0±2.83mm/l while at 3000 and 5000 mg/kg they were 138.5±2.12 and 144.0±1.41mm/l, respectively. In rats that received the ethanol extract, the mean [Na^+] were 130.0±12.7, 145.5±9.19, and 142.0±7.07mm/l at 1500, 3000, and 5000 mg/kg dose, respectively (Table 3).

There were significant differences in the potassium ion concentration [K^+] in the

experimental rats used in the acute toxicity study with the least value of 4.50±0.28mm/l recorded in the control group. At 1500 and 3000 mg/kg of the aqueous extract, the mean [K^+] were 5.30±4.60 and 4.60±0.84mm/l, respectively, while at 5000 mg/kg dose the mean was 6.40±0.00mm/l. Dose associated increases in [K^+] were seen in rats that received the ethanol leaf extract: at 1500, 3000, and 5000 mg/kg dose, the respective [K^+] were 4.90±0.56, 5.95±0.07 and 6.25±0.07mm/l (Table 3).

Chloride ion concentration: the concentrations of chloride ion recorded in rats during the toxicity study did not vary significantly from each other. The [Cl^-] was recorded in the control group with a mean of 94.0±2.83mm/l. At 1500, 3000 and 5000 mg/kg dose of the aqueous extract, the Cl^- concentrations were 100.0±2.82, 97.5±0.71, and 99.5±0.71mm/l. In rats that received the ethanol leaf extract, the mean [Cl^-] were 96.0±2.82, 95.0±4.24, and 104.0±2.82mm/l, respectively (Table 3).

In all groups treated with the extracts, the bicarbonate ion concentrations were higher than in the control group, however, the difference was not statistically significant. The [HCO_3^-] in the control group of rats was 17.5±0.71mm/l; in groups that received the aqueous extract at 1500, 3000, and 5000 mg/kg dose the respective concentrations for bicarbonate ions were 18.5±0.71, 18.0±1.41, and 21.0±2.82mm/l. The corresponding values for [HCO_3^-] in rats that were administered that ethanol leaf extract were 19.5±0.71, 20.5±0.71, and 21.0±1.41mm/l (Table 3).

Urea concentrations did not vary significantly between groups with values varying between 4.00 and 5.95. In the control group, the average concentration of urea in blood was 4.65±0.91mm/l, while in groups that received 1500, 3000 or 5000 mg/kg of the aqueous leaf extract of *S. occidentalis* the blood urea concentrations were 4.50±0.71, 4.05±0.35, and 5.95±0.92mm/l, respectively; in groups that were administered same doses of the aqueous leaf extracts the urea concentrations in blood were 4.20±0.14, 4.00±0.28, and 5.40±0.42mm/l, respectively (Table 3).

Creatinine concentration showed significant difference between the experimental groups with significantly lower value recorded in the control group (0.35±0.07mm/l). Dose-dependent increases in blood creatinine were observed in rats that were administered either the aqueous or ethanol leaf extract of *S. occidentalis*. In rats given 1500, 3000, or 5000 mg/kg dose of the aqueous extract, the mean concentrations of creatinine in the blood were 0.75±0.07, 0.90±0.00, and 0.95±0.07mm/l, respectively, while in those that received the same doses of the ethanol extract the mean creatinine concentrations were 0.60±0.00, 0.80±0.00, and 0.95±0.07mm/l, respectively (Table 3).

Table 3: Acute toxicity effects of ethanol and aqueous extracts of *S. occidentalis* on kidney function parameters of Wistar rats

Groups	Na^+ (mm/l)	K^+(mm/l)	Cl^-(mm/l)	HCO_3^-(mm/l)	Urea(mm/l)	Cr(mm/l)
Control	124.5±4.95^a	4.50±0.28^a	94.0±2.83^a	17.5±0.71^a	4.65±0.91^a	0.35±0.07^a
A	127.0±2.83^a	5.30±4.60^{abc}	100.0±2.82^a	18.5±0.71^a	4.50±0.71^a	0.75±0.07^c
B	138.5±2.12^a	4.60±0.84^a	97.5±0.71^a	18.0±1.41^a	4.05±0.35^a	0.90±0.00^{de}
C	144.0±1.41^a	6.40±0.00^c	99.5±0.71^a	21.0±2.82^a	5.95±0.92^a	0.95±0.07^d
D	130.0±12.7^a	4.90±0.56^{ab}	96.0±2.82^a	19.5±0.71^a	4.20±0.14^a	0.60±0.00^b
E	145.5±9.19^a	5.95±0.07^a	95.0±4.24^a	20.5±0.71^a	4.00±0.28^a	0.80±0.00^{cd}
F	142.0±7.07^a	6.25±0.07^c	104.0±2.82^a	21.0±1.41^a	5.40±0.42^a	0.95±0.07^d
p value	0.082	0.016	0.073	0.174	0.093	0.000

Values are given as mean ± standard deviation of mean. In each column, mean values with different superscripts have statistically significant difference ($p < 0.05$).
Na^+ = Sodium ion; K^+ = Potassium ion; Cl^- = Chloride ion; HCO_3^- = Bicarbonate ion; Cr = Creatinine; Group A =1500 mg/kg aqueous extract; Group B = 3000 mg/kg aqueous extract; Group C = 5000 mg/kg aqueous extract; Group D =1500 mg/kg ethanol extract; Group E = 3000 mg/kg ethanol extract; Group F = 5000 mg/kg ethanol extract.

4.7 Effect of Ethanol and aqueous extracts of *S. occidentalis* on some liver function parameters of wistar rats infected with *Trypanosoma congolense*

Aspartate transaminase (AST): Significant increase in Aspartate activity was recorded in *T. congolense* infected rats when compared with uninfected rats. The mean AST level recorded in the untreated control was 78.6±1.04 mm/l U, while in the uninfected rats the corresponding value was 55.9±1.77 mm/l . AST levels in Drug treated group was further elevated above that of the untreated (negative) control group with a mean value of 89.1±0.35 mm/l . In the ethanol leaf extract treated groups, there was a dose dependent decrease in AST levels when compared with the untreated control group: in 250 mg/kg ethanol leaf extract treated group, the AST level was seen to decrease relative to the untreated control with a mean value of 76.1±0.93 mm/l U, however, the decrease was not statistically significant ($p > 0.05$); in the 500 mg/kg ethanol leaf extract treated group, the AST level was significantly lower than that of the untreated control with a mean AST value of 68.7±1.25 mm/l U. Significant decreases in AST levels were recorded in *T. congolense* infected rats that were treated with the aqueous leaf extracts of *S. occidentalis* with marked decline observed with lower dose of the extract: at 250 mg/kg dose, the AST was 60.5±1.65 mm/l U, while at 500 mg/kg of the aqueous extract, AST was 65.6±1.07 mm/l U (Table 4).

Alanine transaminase: Untreated *T. congolense* infected was associated with significant elevations in ALT activity in the infected rats when compare with the uninfected control: the mean ALT levels for the untreated and the uninfected groups of rats were 68.7±1.03 and 31.3±7.14 mm/l U, respectively. Relative to the untreated control, the non-significant decline in ALT activity was recorded in infected rats treated with Diminazine aceturate with a mean ALT value of 63.9±0.86 mm/l U. In both the ethanol and aqueous extracts treated groups, a significant dose dependent decrease in ALT level, relative to the

untreated control, were observed. In rats treated with 250 or 500 mg/kg of the ethanol leaf extract, the mean ALT levels were 54.1±0.13 and 34.8±0.85 mm/l U, respectively, while ALT levels in groups treated with 250 or 500 mg/kg of the aqueous leaf extracts of *S. occidentalis* were 55.1±1.06 and 51.9±1.34 mm/l U, respectively (Table 4).

Alkaline phosphatase (ALP): Phosphatase activity in the uninfected rats was 60.8±0.57 mm/l U, with a significant increase recorded in the *T. congolense* infected group with a mean value of 120.9±1.46 mm/l U. A significant decrease in ALP value, compared with the untreated control, was recorded in group that received Diminazine aceturate with a mean value of 102.2±1.09 mm/l U; however, the value was significantly higher than that recorded in uninfected rats. In all *S. occidentalis* treated groups of rats, the ALP activity was significantly lower than the value recorded in the untreated control; however, they were all significantly higher than the value recorded in the uninfected control: in the groups treated with 250 or 500 mg/kg of the ethanol leaf extract, the mean values for ALP were 83.7±0.65 and 92.0±0.34 mm/l U, respectively; in groups that received 250 or 500 mg/kg of the aqueous leaf extract, the mean ALP levels were 81.9±0.57 and 92.7±1.27 mm/l U, respectively (Table 4).

Total bilirubin: total bilirubin values in uninfected and infected-untreated rats were 6.91±0.18 and 7.28±0.06 mg/dl, respectively ($p > 0.05$). In the drug treated group, the value for total bilirubin was lower (7.08±0.18mg/dl) than that recorded in the untreated control, but the difference was not statistically significant ($p > 0.05$). in the extract treated group, there were significant dose-dependent decreases in the levels of total bilirubin in the blood of *T. congolense* infected rats: in groups treated with either 250 or 500 mg/kg of the ethanol extract, the mean total bilirubin contents were 7.70±0.03 and 4.83±0.24 mg/dl, respectively; while in groups treated with 250 or 500 mg/kg of the aqueous leaf extract, the respective mean values for total bilirubin were 6.61±0.14 and 5.24±0.14 mg/dl (Table 4).

Conjugated bilirubin: In uninfected control, the level of conjugated bilirubin was 4.77±0.54 mg/dl. A significantly higher level of conjugated bilirubin was however recorded in untreated rats infected with *T. congolense* with a mean value of 6.11±0.09 mg/dl. Drug or extract treatment of the *T. congolense* infected rats was associated with significant decreases in the level of conjugated bilirubin when compared with the value recorded in the untreated control: in group treated with Diminazine, the conjugated bilirubin level was 4.24±0.07 mg/dl, while in groups that received 250 or 500 mg/kg of the ethanol leaf extract conjugated bilirubin levels were 4.07±0.06 and 4.62±0.04 mg/dl, respectively. In groups that received 250 or 500 mg/kg of the aqueous extract the conjugated bilirubin levels were 4.36±0.04 and 3.59±0.42 mg/dl, respectively (Table 4).

Table 4: Effect of Ethanol and aqueous extracts of *S. occidentalis* on some liver function parameters of wistar rats infected with *Trypanosoma congolense*

Groups	AST(mm/l)	ALT(mm/l)	ALPmm/l)	T bil.(mg/dl)	C. bil.(mg/dl)
A	55.9±1.77^{a}	31.3±7.14^{a}	60.8±0.57^{a}	6.91±0.18^{cd}	4.77±0.54^{c}
B	89.1±0.35^{f}	63.9±0.86^{c}	102.2±1.09^{d}	7.08±0.18^{d}	4.24±0.07^{bc}
C	78.6±1.04^{e}	68.7±1.03^{c}	120.9±1.46^{e}	7.28±0.06^{d}	6.11±0.09^{d}
D	60.5±1.65^{b}	55.1±1.06^{b}	81.9±0.57^{b}	6.61±0.14^{c}	4.36±0.04^{bc}
E	65.6±1.07^{c}	51.9±1.34^{b}	92.7±1.27^{c}	5.24±0.14^{b}	3.59±0.42^{a}
F	76.1±0.93^{e}	54.1±0.13^{b}	83.7±0.65^{b}	7.70±0.03^{e}	4.07±0.06^{ab}
G	68.7±1.25^{d}	34.8±0.85^{a}	92.0±0.34^{c}	4.83±0.24^{a}	4.62±0.04^{bc}

AST = Aspartate transaminase; ALT = Alanine transaminase; ALP = Alkaline phosphatase; T. bil. = Total bilirubin; C. bil. = Conjugated bilirubin.
Group A = uninfected-untreated control; Group B = infected and treated with Diminazine aceturate; Group C = Negative control; D = 250 mg/kg aqueous extract; E =500 mg/kg aqueous extract; F = 250 mg/kg ethanol extract; G =500 mg/kg ethanol extract. Values are given as mean ± standard deviation of mean. In each column, mean values with different superscripts have statistically significant difference ($p < 0.05$).

Table 4. Effect of Ethanol and aqueous extracts of *S. occidentalis* on kidney function parameters of wistar rats infected with *Trypanosoma congolense*

Sodium ion (Na^+): There was significant difference in the concentration of sodium ions in the treatment groups. The highest level of sodium ion was recorded in infected-untreated group with a mean value 147.2±1.20mm/l while the least was recorded in group that received 250 mg/kg of the aqueous extract with a mean value of 100.9±0.11 mm/l. In group of rats treated with Diminazine aceturate, the sodium ion concentration was 141.4±0.85. The sodium ion concentrations in *T. congolense* infected rats treated with 250 or 500 mg/kg of the ethanol extract of *S. occidentalis* were 107.9±1.52 and 118.6±0.48 mm/l, respectively, while in group treated with 500 mg/kg of the aqueous extract, the mean value was 112.6±3.79 (Table 5).

Potassium ion concentration (K^+): Potassium ion concentration in uninfected control group was 4.60±0.14 mm/l. However, in infected-untreated control group, a significantly higher value of 6.17±0.47 mm/l for K^+ was recorded. In the drug treated group, the mean value for K^+ was 7.35±0.21 mm/l, which was higher than that recorded in the untreated control. In all the groups of infected rats treated with either the ethanol or aqueous extract of *S. occidentalis*, the potassium ion concentrations were significantly lower than the value recorded in the untreated control group: K^+ concentrations in rats treated with 250 or 500 mg/kg of the ethanol extract were 3.63±0.04 and 4.62±0.02 mm/l, respectively, while in those that received 250 or 500 mg/kg of the aqueous extract, the mean concentrations of potassium ions were 3.28±0.11 and 3.72±0.03 mm/l, respectively (Table 5).

Chloride ion concentration (Cl^-): Chloride ion concentration was significantly higher in untreated-infected rats than in uninfected rats with mean values of 106.4±0.84 and 96.2±0.21 mm/l, respectively. In the drug treated group, the value was significantly lower compared to the untreated control with a mean value of

97.8±0.28 mm/l. The mean values for chloride ions in rats treated with either 250 or 500 mg/kg of the ethanol leaf extract were 86.9±1.43 and 98.0±0.06 mm/l, respectively, while groups that received 250 or 500 mg/kg of the aqueous extract, the respective values were 95.7±0.40 and 100.3±1.84 mm/l (Table 5).

Bicarbonate ion concentration (HCO_3^-): The least concentration of serum HCO_3^- was recorded in the uninfected control with a mean value of 17.6±0.58 mm/l, while the highest was recorded in the infected-untreated control with a mean value of 23.9±0.07 mm/l. In the Diminazine aceturate treated group, the bicarbonate ion concentration was 20.7±0.42 mm/l, while in groups treated with 250 or 500 mg/kg of the aqueous extract the bicarbonate ion concentrations were 19.3±0.45 and 18.7±0.37 mm/l, respectively (Table 5).

Urea concentration: The level of urate ion in the uninfected rats was significantly lower than in the infected-untreated group with mean values of 3.90±0.14 and 6.03±0.66 mm/l, respectively, while in the drug treated group, the urea concentration was 5.72±0.16 mm/l. Urea concentrations in groups treated with either 250 or 500 mg/kg of the ethanol extract were 3.88±0.11 and 4.33±0.04 mm/l, respectively, while in groups treated with corresponding doses of the aqueous extract the respective urea concentrations were 4.73±0.18 and 4.38±0.03 mm/l (Table 5).

Creatinine concentration: serum creatinine concentrations were elevated in the untreated control and in rats that received 250 mg/kg of the ethanol leaf extract of *S. occidentalis* with respective mean vales of 0.79±0.01 and 1.11±0.15 mm/l; however, only in the latter group was the difference significant compared to the uninfected group which had a mean urea concentration of 0.67±0.19 mm/l. In the drug treated group, the urate concentration was 0.63±0.04 mm/l, while in groups that received 250 or 500 mg/kg of the aqueous extract, the mean urea concentrations were 0.69±0.01 and 0.57±0.09 mm/l. A 0.68±0.02 mm/l of urea was recorded in rats that received 500 mg/kg of the ethanol leaf extract (Table 5).

Table 4: Effect of Ethanol and aqueous extracts of *S. occidentalis* on kidney function parameters of wistar rats infected with *Trypanosoma congolense*

Groups	Na^+(mm/l)	K^+(mm/l)	Cl^-(mm/l)	HCO_3^-(mm/l)	Urea(mm/l)	Cr(mm/l)
A	120.5±0.74^d	4.60±0.14^b	96.2±0.21^b	17.6±0.58^a	3.90±0.14^a	0.67±0.19^a
B	141.4±0.85^e	6.17±0.47^c	97.8±0.28^b	20.7±0.42^d	5.72±0.16^c	0.63±0.04^a
C	147.2±1.20^f	7.35±0.21^d	106.4±0.84^d	23.9±0.07^e	6.03±0.66^c	0.79±0.01^a
D	100.9±0.11^a	3.28±0.11^a	95.7±0.40^b	19.3±0.45^{bc}	4.73±0.18^b	0.69±0.01^a
E	112.6±3.79^c	3.72±0.03^a	100.3±1.84^c	18.7±0.37^b	4.38±0.03^{ab}	0.57±0.09^a
F	107.9±1.52^b	3.63±0.04^a	86.9±1.43^a	20.1±0.17^{cd}	3.88±0.11^a	1.11±0.15^b
G	118.6±0.48^d	4.62±0.02^b	98.0±0.06^{bc}	18.9±0.11^b	4.33±0.04^{ab}	0.68±0.02^a

Na^+ = Sodium ion; K^+ = Potassium ion; Cl^- = Chloride ion; HCO_3^-= Bicarbonate; Cr. = Creatinine. Group A = uninfected-untreated control; Group B = infected and treated with Diminazine aceturate; Group C = Negative control; D = 250 mg/kg aqueous extract; E =500 mg/kg aqueous extract; F = 250 mg/kg ethanol extract; G =500 mg/kg ethanol extract. Values are given as mean ± standard deviation of mean. In each column, mean values with different superscripts have statistically significant difference ($p < 0.05$).

Effect of Ethanol and aqueous extracts of *S. occidentalis* on parasitemia progression in *T. congolense* infected wistar rats

In the uninfected untreated group (group A), no infection with trypanosome was detected throughout the duration of the study. Trypanosomes were detected in all infected rats on the 5th day after inoculation with mean values ranging between 1.00±0.71 and 1.80±0.45 trypanosomes per field. On the 8th day post-inoculation, parasitemia was observed to significantly increase in all treatment groups with values ranging between 10.6±1.16 and 15.7±4.04 trypanosomes per field. By the 11th day, no trypanosome was detected in previous *T. congolense* infected that were treated with the standard drug (group B). The results further showed that while there was an increase in parasite count in all groups of infected rats by the 11th day of observation (except the drug treated group), the increase recorded in the untreated control(group C) was significantly higher than those recorded in the groups treated with either the aqueous or ethanol extracts of *S. occidentalis*: in the untreated control, the parasite count was 125.0±35.4 trypanosomes per field, while in the extract treated groups, the counts were between 66.8±23.4 and 96.7±5.77 trypanosomes per field. In addition, there was no significant dose dependent effect in activity of both the ethanol and aqueous extracts of *S. occidentalis*; however, the daily trypanosome counts in rats treated with 500 mg/kg ethanol extract of *S. occidentalis* were lower than those recorded in rats treated with 250 mg/kg of the ethanol extract. Similarly, the aqueous extract of *S. occidentalis* did not exhibit dose dependent effect on trypanosome counts in the treated rats. However, the counts recorded in rats treated with 250 mg/kg of the extract were lower than those recorded in rats treated at 500 mg/kg.

The mean survival times of the experimental rats differ significantly between the treatment groups. In group A, the mean survival times was greater than 28 days. Similarly, in the group of infected rats treated with the standard drug (group B), the mean survival time was also greater than 28 days. However, the mean

survival time in the infected-untreated group(C) was significantly lower with a value of 12.6±0.89 days. Similarly, the mean survival times in the *S. occidentalis* extracts treated groups were significantly lower than in group A and the standard control groups. In rats treated with either the 250 or 500 mg/kg of the ethanol extract of *S. occidentalis*, the mean survival times were 12.5±1.00 and 14.3±3.94 days, respectively; in rats treated with 250 or 500 mg/kg of the aqueous extract of *S. occidentalis*, the mean survival times were 14.8±3.50 and 12.5±1.00 days, respectively (Table 6).

Table 6: Effect of Ethanol and aqueous extracts of *S. occidentalis* on parasitemia progression in *T. congolense* infected wistar rats

Groups	Day 5	Day 8	Day 11	Day 13	Day 15	MST (days)
A	0.00±0.00[a]	0.00±0.00[a]	0.00±0.00[a]	0.00±0.00[a]	0.00±0.00[a]	> 28
B	1.80±0.45[b]	15.0±5.43[b]	0.00±0.00[a]	0.00±0.00[a]	0.00±0.00[a]	> 28
C	1.60±0.55[b]	13.3±4.16[b]	125.0±35.4[b]	-	-	12.6±0.89
D	1.60±0.55[b]	11.7±2.88[b]	66.8±23.4[b]	200[b]	-	14.8±3.50
E	1.80±0.45[b]	15.7±4.04[b]	93.3±11.5[b]	-	-	12.5±1.00
F	1.00±0.71[b]	13.3±5.77[b]	96.7±5.77[b]	-	-	12.5±1.00
G	1.20±0.84[b]	10.6±1.16[b]	87.3±21.9[b]	200[b]	-	14.3±3.94

Group A = uninfected-untreated control; Group B = infected and treated with Diminazine aceturate; Group C = Negative control; D = 250 mg/kg aqueous extract; E =500 mg/kg aqueous extract; F = 250 mg/kg ethanol extract; G =500 mg/kg ethanol extract. Values are given as mean ± standard deviation of mean. In each **column**, mean values with different superscripts have statistically significant difference ($p < 0.05$).

DISCUSSION

The varieties of phytochemical components present in the n-hexane, ethanol and aqueous extracts of *Senna occidentalis* could account for its medicinal values. This is in line with the findings of [23,24] who revealed that there are many different secondary metabolites such as alkaloids, anthraquinones, saponins, tannins, terpenes, steroids, flavonoids, carbohydrates found in different parts of *Senna* spp. Different solvents have different solubility capacities for different phyto-constituents, hence the differences in the activities of the various extracts ranging from non-polar to polar solvents [25]. This explains why some phytochemicals were absent in some extracts and present in others. Findings of previous studies by [26] indicated the presence of alkaloids, anthraquinones, carbohydrates, flavonoids, saponins, tannins, terpenes and steroids in the crude extract of *S. occidentalis* L. stem bark. These secondary metabolites exert antimicrobial activity through different mechanisms [27] and

may be responsible for the antitrypanosomal effects.

A number of studies have documented the toxic effects of medicinal plants [28,29]. Acute toxicity test gives clues on the doses of ranges that could be safe to the animal; it could also be used to estimate the therapeutic index (LD50/ED/50)/of drugs and Xenobiotics [30]. The Organization for Economic Corporation and Development document on Acute Toxicity Testing recommended that the maximum dose levels for any chemical compounds should not exceed 5000 mg/kg of the animal body weight. The results of the present study suggest that the ethanol and aqueous extracts of *Senna occidentalis* are not toxic to the wistar rats thereby providing a support to the use of the leaves indigenously. Generally, extracts of *Senna occidentalis* can be considered safe for use and this may also give credence to its domestic use by traditional healers. Infection with *Trypanosoma congolense* was established in all the experimental animals, five days' post-inoculation.

The *in vivo* studies revealed an ameliorative effect of *Senna occidentalis* against *Trypanosoma congolense* infected wistar rats. It is also possible that this extract possesses some antioxidant activities that could scavenge *Trypanosoma congolense* generated free radicals which are implicated in the development of anemia and are capable of causing oxidative stress during trypanosomiasis infection [31].

The *in vivo* evaluation of the ethanol and aqueous extracts of *Senna occidentalis* plant revealed ethanol extract at 500 mg/kg b. wt and aqueous extract at 250mg/kg b. wt to be the most potent by inhibiting rise in parasitemia most. This is in agreement with the result of Ene *et al.* (2009) who evaluated the petroleum ether, chloroform and methanol extracts of the whole plant of *Artemisia maciverae* for *in vivo* antitrypanosomal effects at 100 mg/kg body weight. The mechanism of action of the plant *in vivo* is unknown but previous reports attributed the trypanocidal activity of a number of tropical plants to the flavonoids (azaanthraquinone), highly aromatic planar quaternary alkaloids, barbarine and harmaine [32]. It was also suggested that many natural products exhibit their trypanocidal activity through interference with redox balance of the parasites acting either on the respiratory chain or on the cellular defenses against oxidative stress. This is because natural products possess structures capable of generating radicals that may cause peroxidative damage to trypanothione reductase that is very sensitive to alterations in redox balance. It is also known that some agents act by binding with the kinetoplast DNA of the parasite [33].

A significant increase in ALT, AST and ALP in the infected not treated rats compared to the normal control is an indication of hepatocellular injury. It has been reported that hepatic abnormalities such as elevations of transaminases and alkaline phosphatase (ALP) are common in diabetes mellitus. The rise in levels of ALT is always accompanied by elevation in the level of AST, which play a role in the conversion of amino acid to keto acid. Both AST and ALT are excellent marker of liver damage caused by exposure to toxic substances [36]. Administration of extract caused a significant reduction in ALT, AST and ALP of all induced treated groups compared to the normal control.

Creatinine is synthesized in the liver, passes into the circulation and is taken up almost entirely by skeletal muscle. Bilirubin is transported to the liver bound

to albumin. High plasma conjugated bilirubin concentration indicates impaired hepatic excretory function [37]. Trypanosomiasis has been shown to cause a perturbation in the electrolyte homeostasis [38]. The elevated serum potassium level after infection may be due to the damaging effect of *T. congolense* on the kidney and the heart myocardium. The results indicated that *T. congolense* infection generated increased serum potassium which has been reported in myocardial ischemia and renal damage could cause a reduction in the resting membrane potential and quicken depolarization [39].

Ethics Approval and Informed Consent

The ethical approval for the use of laboratory animals was obtained from Animal committee of Kaduna State Ministry of Agriculture and Forestry.

Authors Contribution

SHM and DAJ conceptualized the study. SHM, AAM and DAJ designed the study. SHM and AAM participated in laboratory work and data collection. SHM performed the data analysis; SHM, AAM and DAJ interpreted the data. SHM prepared the first draft of the manuscript, reviewed by AAM and DAJ. All authors contributed to the development of the final manuscript and approved it.

Declaration

None

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