



E-ISSN: 2278-4136
P-ISSN: 2349-8234
JPP 2017; 6(5): 1217-1224
Received: 03-07-2017
Accepted: 04-08-2017

Muhammad Y

School of Agriculture,
Department of Animal Health
and Production, Binyaminu
Usman Polytechnic Hadejia
Jigawa State, Nigeria

Suleiman MM

Faculty of Veterinary Medicine,
Department of Veterinary
Pharmacology and Toxicology,
A.B.U Zaria, Kaduna State
Nigeria

Uma,r AM

School of Agriculture,
Department of Animal Health
and Production, Binyaminu
Usman Polytechnic Hadejia
Jigawa State, Nigeria

Ahmed G

School of Science, Department of
Science Laboratory Technology,
Binyaminu Usman Polytechnic
Hadejia Jigawa State, Nigeria

Gumel MA

School of Agriculture,
Department of Animal Health
and Production, Binyaminu
Usman Polytechnic Hadejia
Jigawa State, Nigeria

Mohammed H

School of Agriculture,
Department of Agricultural
Extension and Management,
Binyaminu Usman Polytechnic
Hadejia Jigawa State, Nigeria

Alhassan M

Faculty of Medical Laboratory
Sciences, Department of
Haematology, Usmanu
Danfodiyo University Sokoto,
Sokoto State, Nigeria

Correspondence**Muhammad Y**

School of Agriculture,
Department of Animal Health
and Production, Binyaminu
Usman Polytechnic Hadejia
Jigawa State, Nigeria

Effect of crude methanol extract of *Senna occidentalis* on haematological parameters of wistar rats experimentally infected with *Trypanosoma congolense*

Muhammad Y, Suleiman MM, Uma,r AM, Ahmed G, Gumel MA, Mohammed H and Alhassan M

Abstract

The study was aimed at investigating the effect of crude methanol leaves extract of *Senna occidentalis* on haematological parameters of wistar rats with experimental *Trypanosoma congolense* infection. The phytochemical screening test was conducted on the extract using standard procedure. Acute toxicity study was carried out and the LD₅₀ was found to be ≥ 5000 mg/kg. Thirty wistar rats were used in this study, and they were divided into six groups of five rats each. Rats in group 1 were untreated uninfected (neutral control). Groups 2-6 received intraperitoneal injection of *Trypanosoma congolense* (10^5 trypanosomes/ml of blood). Parasitaemia was established 8 days post infection. Rats in groups 2, 3 and 4 were orally treated with 100, 400 and 600 mg/kg body weight of the extract respectively for ten days. Rats in group 5 were treated with diminazene aceturates (3.5 mg/kg, i.p) once. Rats in group 6 were treated with normal saline for ten days. The level of parasitaemia and haematological parameters were determined. At doses 100, 400 and 600 mg/kg body weight the extract showed significant ($P < 0.05$) reduction in the level of parasitaemia in wistar rats when compared to rats infected and treated with normal saline (group 6). The parasites were completely cleared from the blood stream of rats treated with diminazene aceturates (3.5 mg/kg) 24 hours following treatment, and the rats remain aparasitaemic. Packed Cell Volume (PCV), Haemoglobin (Hb) concentration and Red Blood Cell (RBC) count significantly ($P < 0.05$) decreased in rats treated with normal saline (group 6) when compared to extract treated groups. Total white blood cell count significantly ($P < 0.05$) decreased in rats treated with normal saline (group 6). In general, the results obtained suggest ethno-pharmacological usefulness of the plant and necessitate further studies to be carried on the isolated active substances from the plant.

Keywords: Crude Methanol, Haematology, *Senna occidentalis* and *Trypanosoma congolense*.

Introduction

Trypanosomes are extracellular haemoprotozoan parasites that survived in the blood stream of the host by complex evasion mechanism, including antigenic variation of the variant surface glycoprotein (VCG) (Adamu *et al.*, 2009) [1], immunosuppression (Bissalla *et al.*, 2007) The resultant effects are inability of the host to clear the trypanosomes from its body even after administration of trypanocides (Gow *et al.*, 2007) [14] as well as making the host more susceptible to secondary infections (Ford, 2013) [12]. Trypanosomosis of domestic animals (cattle), sometimes called *nagana*, cause the death of about 3 million cattle a year. Trypanosomosis has played an important role in tropical Africa, hence the World Health Organisation listed major problem affecting man and animals (WHO, 1975) [41]. In spite of the existence of trypanotolerant cattle in West Africa and the discovery of the trypanocide suramin since 1921, trypanosomosis still continues to wreak havoc in the livestock industry (Nok *et al.*, 2001) [26]. The phenomenon of antigenic variation has rendered the prospect of vaccination hopeless, and the emergence of drug resistant strains is gradually incapacitating current trypanocidal drugs, hence there is need to seek new chemotherapeutic and chemoprophylactic agents for combating trypanosomosis (Nok *et al.*, 2001) [26]. Before the discovery of synthetic drugs, local herdsmen were controlling trypanosomosis through different ethnoveterinary practices. Knowledge of these practices may provide the chemical lead for the discovery of a new generation of trypanocides that are more potent and less toxic. Unfortunately, however, apart from few reports (Talakai *et al.*, 1975; Nok *et al.*, 1994; Frieburghaus *et al.*, 1996) [35, 13], not much has been done to document the ethnoveterinary practices among the local herdsmen. Hence this work was designed to obtain the necessary information on traditional treatments of trypanosomosis.

Plants are important source of drugs, especially in traditional medicine (Bako *et al.*, 2005) [4]. It is a common practice in Nigeria and other parts of the world to use plant in the form of crude

extracts, decoction, infusion or tincture to treat common infections and chronic conditions. According to WHO (2008)^[42] over 70% of the world populations rely on medicinal plants for primary health care and there are reports from various researchers on natural substances of plant origin which are biologically active, with desirable antitrypanosomal, antimicrobial and antioxidant properties (Mahesh and Satish, 2008; Hamid *et al.*, 2010)^[24, 15].

Senna occidentalis (Linn.) (formerly *Cassia 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 (Igbokwe, 1994; Umar *et al.*, 2008)^[18-19, 39]. It is a shrub that grows between 5 to 8 cm in height and commonly found in the tropics (Kaey, 2000). The leaves of the plant are used in the treatment of yaws, scabies, itches and ringworm among the Yoruba tribe of southwestern Nigeria (Umar *et al.*, 2008)^[39]. Infusion of *C. occidentalis* leaves is used as an effective treatment for hepatitis among the rural dwellers in northern part of Nigeria (Nuhu and Aliyu, 2008)^[27]. *Senna occidentalis* is used as a diuretic and in the treatment of snake-bite (Yadava and Satnami, 2011)^[43]. Different parts of this plant have been reported to possess anti-inflammatory and antiplasmodial activities (Kuo *et al.*, 1996; Tona *et al.*, 2004)^[21, 36].

In the present study, the antitrypanosomal activity of crude methanol leaves extract of *Senna occidentalis* was investigated in wistar rats.

Material and methods

Plant Collection, Identification and Extraction

Fresh leaves of *Senna occidentalis* was collected around Hadejia, Jigawa State, Nigeria. The plant was identified at the Herbarium, Department of Forestry Technology Binyaminu Usman Polytechnic Hadejia, Jigawa State, with a voucher specimen number A002. Fresh leaves of *Senna occidentalis* was air-dried and made into powdered form using pestle and mortar. About 500g of the powdered plant material was extracted in a percolator using methanol as a solvent and was mixed in the ratio of 1:5 of plant material and solvent, respectively. The mixture was allowed to stand for 48 hours following which the tap of percolator was opened to obtain liquid extract. The whole extraction process was repeated 3 times. The extract obtained during the extraction processes were pooled out together and concentrated *in vacuo* at 50 °C. The dark brown dried extract was dissolved in freshly prepared normal saline solution at concentration of 50 mg/ml and refrigerated at 4 °C until used.

Experimental Animals

Thirty wistar rats of either sexes, weighing between 200 and 220 grams were used. They were purchased from the Animal House, Department of Veterinary Pharmacology and Toxicology, Faculty of Veterinary Medicine, Ahmadu Bello University Zaria. The animals were kept in a locally constructed rat cages and pre-conditioned for two weeks in the laboratory (25 ± 2 °C and 12 hours light/ dark cycle). Wood shavings were used as beddings and changed once every week throughout the period of the experiment. Animals were fed with rat chow and allowed free access to water.

Phytochemical screening

The methanol leaves extract of the *Senna occidentalis* was subjected to phytochemical screening tests for the presence of

carbohydrates, glycosides, flavonoids, tannins, alkaloids, saponins, steroids and triterpenes using standard techniques (Brain and Turner, 1975)^[16].

Acute Toxicity Study

Median lethal dose (LD₅₀) as a measure of establishing the safety of the extract was determined as described by Lorke (1983)^[23]. Briefly, 12 wistar rats were deprived of feed and water for 24 and 12 hours, respectively and randomly divided into 4 groups of 3 rats each. Animals in groups 1, 2 and 3 received the extract orally at the doses of 10mg/kg, 100mg/kg and 1000mg/kg, respectively, while animals in group 4 were given the diluent (normal saline) at 5 ml/kg (maximum convenient volume). Animals were observed continuously for 1 hour after the treatment, intermittently for 4 hours, and thereafter over a period of 24 hours. Rats were further observed for up to 14 days for any signs of toxicity.

In the second phase of the trial, 3 rats were randomly divided into 3 groups of 1 rat each. Rats in groups 1, 2, 3 were given the extract at 1600 mg/kg, 2900 mg/kg and 5000 mg/kg, respectively. All treatments were given through the oral route, and then observed as described earlier.

Parasites

The parasites, *Trypanosoma congolense*, were obtained from National Veterinary Research Institute (N.V.R.I) Vom. The parasites were maintained by continuous passage in a donor rats. Parasitaemia was monitored by wet mount and viewed under × 40 magnification. Parasites were harvested from the blood of a donor rat at peak parasitemia (10⁹parasites/ml) and were diluted with phosphate buffered saline. The preparation was used for *in vivo* (infection of experimental animals) studies (Herbert and Lumsden, 1976)^[16].

Experimental Infection of the Rats

Trypanosomes infected blood was obtained from the tail of the infected donor rats at peak of parasitaemia (10⁹) and used to maintain parasite suspension in phosphate buffer saline glucose solution, which was inoculated into peritoneal cavity of uninfected rats. The suspension contained 3 or 4 trypanosomes per microscopic field at × 40 magnification (approximately 10⁶ trypanosomes per ml) as described by Ekanem and Yusuf, 2008)^[10].

Determination of Parasitaemia in Rats

Parasitaemia was monitored in rats from blood obtained from the tail vein. The number of parasites were determined microscopically at × 40 magnification using rapid matching method (Herbert and Lumsden, 1976)^[16]. The microscopic field was compared with a range of standard logarithmic values. To count the number of parasites in the blood, a drop of the collected blood was placed on a glass slide and covered with coverslip. The wet mount on the slide was microscopically examined at × 40 magnification. The number of parasites were compared with the table of logarithmic values. The logarithmic values which matched the microscopic observation was therefore converted to antilogarithm where the absolute number of trypanosome per milliliter (ml) of blood was obtained.

Drug Preparation

The dark brown dried extract and diminazene aceturate were dissolved in freshly prepared normal saline solution. The concentrations of the extract and diminazene aceturate used were 50 mg/ml and 3 mg/12.5 ml respectively.

Treatment of Experimental Animals

Thirty wistar rats were randomly divided into six groups of 5 rats each and were treated as described below:

Group I- The rats in this group were neither infected nor treated with any substance and served as negative control group.

Group II- Each rat in this group was infected with *Trypanosoma congolense* (10^6 trypanosomes/ml), after the establishment of infection, they were treated with 100 mg/kg body weight of the extract orally for 10 days.

Group III- Rats in this group were infected with *Trypanosoma congolense* (10^6 trypanosomes/ml), after infection has been established, each rat was treated with 400 mg/kg body weight of the extract orally for 10 days.

Group IV- Rats in this group were infected with *Trypanosoma congolense* (10^6 trypanosomes/ml), after infection has been established, all rats were treated with 600 mg/kg body weight the extract orally for 10 days.

Group V- Rats in this group were infected with *Trypanosoma congolense* (10^6 trypanosomes/ml), after infection has been established, each rat was treated with diminazene aceturate (3.5 mg/kg) I.P, once.

Group VI- Rats in this group were infected with *Trypanosoma congolense* (10^6 trypanosomes/ml) and then treated with distilled water at (5 ml/kg) for 10 days, served as positive control.

Haematological Analysis

At the end of experiment, rats from each group were euthanized by severing the jugular vein. About 1 ml of blood was collected from each rat in a 20 ml vial containing EDTA as an anti-coagulant and was used to determine the haematological parameters. The packed cell volume (PCV), total erythrocyte count and haemoglobin concentration were determined using methods described by Dacie and Lewis (1991) [9].

Evaluation of Packed Cell Volume (PCV)

Packed Cell Volume (PCV) was determined using the microhaematocrit method as described by Coles (1986) [8]. Briefly, heparinized capillary tubes were filled with blood samples to 75% ($\frac{3}{4}$) their length by capillary action. Piece of gauze was carefully used to dry the outside of the tubes while the opposite end of the tubes were sealed with flame. The tubes were then loaded onto a microhematocrit centrifuge (Hawksley® England) and centrifuged at $3000 \times g$ for ten minutes. The PCV values was measured using hematocrit reader (Hawksley® England).

Evaluation of Haemoglobin (Hb) Concentration

Cyanmethaemoglobin method described by Coles (1986) [8] was used to determine haemoglobin concentration. About 0.02 ml of blood was pipetted into a clean cuvette and diluted with 5 ml of cyanmethemoglobin reagent (Modified Drabkin Fluid). Following addition of blood, the cuvette was inverted 2-3 times and allowed to stand for 10 minutes for maximum conversion of haemoglobin to cyanmethaemoglobin. The absorbance of the resulting mixture was taken using a UV-spectrophotometer (Beckham Coulter, Model B U520, Austria) at 540 nm against blank. The percent transmission or optical density at 540 nm was recorded and compared with

the reading obtained using standard solution of cyanmethaemoglobin.

Evaluation of Total Erythrocyte count

Haemocytometer method described by Coles (1986) [8] was used to determine total erythrocyte count. Blood sample was drawn carefully by aspiration to 0.5 mark of the pipette and transferred into a clean cuvette containing diluent (0.9% of normal saline). With another pipette the dilution fluid was drawn to 101 mark and mixed gently, the diluted blood was then discharged into haemocytometer counting chamber and allow to settle. The Total erythrocyte count was determined by viewing under microscope at $40 \times$ magnification. The total number of cells in five squares in the centre of the counting chamber was calculated and multiplied by 10,000. The value obtained represented the total number of the erythrocyte per microliter (μL).

Evaluation of Total White Blood Cell Counts

The total leucocytes count was determined using haemocytometer method as described by Coles (1986) [8]. The clean micropipette was used to draw blood by aspiration up 0.5 mark using rubber mouth piece attached to the stem end of the micropipette above the bulb. Cotton wool was used to take off the excess blood from the stem of the pipette. Dilution fluid (2% glacial acetic acid in 100 ml of distilled water to which 1 ml of a 1% aqueous solution of gentian violet has been added) was drawn to the 11.0 mark. Blood and dilution fluid were then mixed gently and properly by inversion (up and down) for 2-3 minutes, six drops of the mixture was removed by releasing the thumb immediately into haemocytometer counting chamber. The haemocytometer was filled with blood by capillary action. This was examined under microscope at low power objectives $\times 40$ magnification. The leucocytes in 4 squares within the larger area of the corner of the haemocytometer were counted and the value obtained was multiplied by factor 50 so as to obtain total leucocyte counts.

Statistical analysis

Data obtained was expressed as mean \pm S.E.M and then analysed by one-way analysis of variance (ANOVA) followed by Tukey's post-hoc test. The analyses were done using Graph pad Prism Version 5.0 for windows from Graph pad Software, San Diego, California, USA. The level of significance was set at $p < 0.05$.

Results

Extract Yield and Phytochemical Test

The percentage yield of the dried methanol leaves extract of *Senna occidentalis* was 19.86 %. Reducing sugar, glycosides, flavonoids, tannins, alkaloids, saponins, steroids and triterpenes were detected in the extract of *Senna occidentalis*.

Acute toxicity studies

The extract at doses of 10, 100, 1000, 1600, 2900 and 5000 mg/kg did not produce any sign of toxicity or mortality, therefore the median lethal dose (LD_{50}) is equal to or above 5000 mg/kg (Lorke, 1983) [23].

Effect of Treatment on the Level of Parasitaemia in Infected rats

Figure 1 shows the effect of the treatment with Crude methanol leaves extract of *Senna occidentalis* on the level of parasitaemia in rats infected with *Trypanosoma congolense*.

The parasitaemia increased progressively in rats treated with extract at doses; 100 mg/kg (group 2), 400 mg/kg (group 3), and 600 mg/kg (group 4) in a dose dependent manner, and also in those rats that were treated with normal saline (5 ml/kg) until when they were sacrificed on day 10 post-infection. The parasites were cleared completely from the blood stream of rats treated with diminazene aceturate (group 5) 24 post treatment, and the rats remain aparasitaemic. Although the onset of parasitaemia was not different from

other groups, there were significant ($P < 0.05$) reduction in the level of parasitaemia in rats treated with diminazene aceturate (group 5) when compared to other groups. Similarly, a significant ($P < 0.05$) reduction in the level of parasitaemia in rats treated with extract at doses; 100 mg/kg (group 2), 400 mg/kg (group 3), and 600 mg/kg (group 4) were recorded when compared to those that were treated with normal saline (group 6).

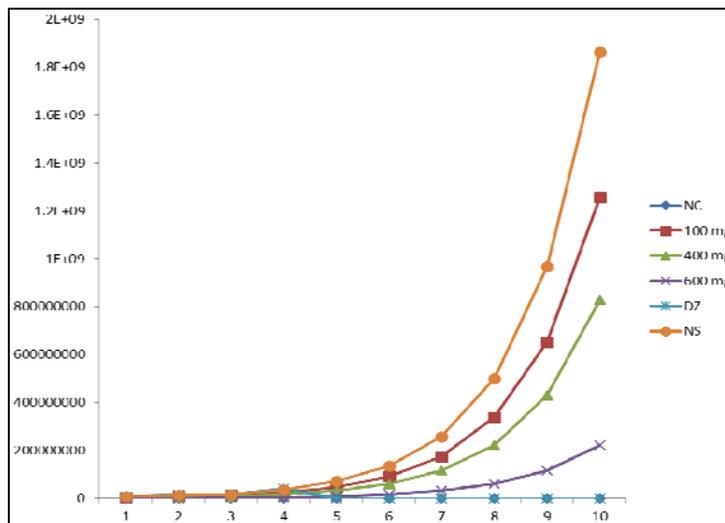


Fig 1: Effect of the treatments with crude methanol leaves extract of *Senna occidentalis* on the level of parasitaemia of rats experimentally infected with *Trypanosoma congolense*

Keys

- NC= uninfected untreated;
- 100= infected and treated with extract (100 mg/kg)
- 400= infected and treated with extract (400 mg/kg)
- 600= infected and treated with extract (600 mg/kg)
- DZ= infected and treated with diminazene aceturate (3.5 mg/kg)
- NS= infected and treated with normal saline (5ml/kg)

Effect of treatment on haematological parameters

Effect of Treatment on Packed Cell Volume

Figure 2 shows the effect of the treatment with crude methanol leaves extract of *Senna occidentalis* on packed cell

volume of rats infected with *Trypanosome congolense*. The packed cell volume significantly ($P < 0.05$) increased in rats treated with extract at doses; 100 mg/kg (group 2), 400 mg/kg (group 3), and 600 mg/kg (group 4) in a dose dependent manner when compared to those that were treated with normal saline (group 6). There were also significant ($P < 0.05$) reduction in the packed cell volume of rats treated with extract at doses; 100 mg/kg (group 2) and 400 mg/kg (group 3), when compared to those that were treated with diminazene aceturate (3.5 mg/kg). Packed cell volume was slightly higher in rats treated with extract, 600 mg/kg (group 4) when compared to those that were treated with diminazene aceturate

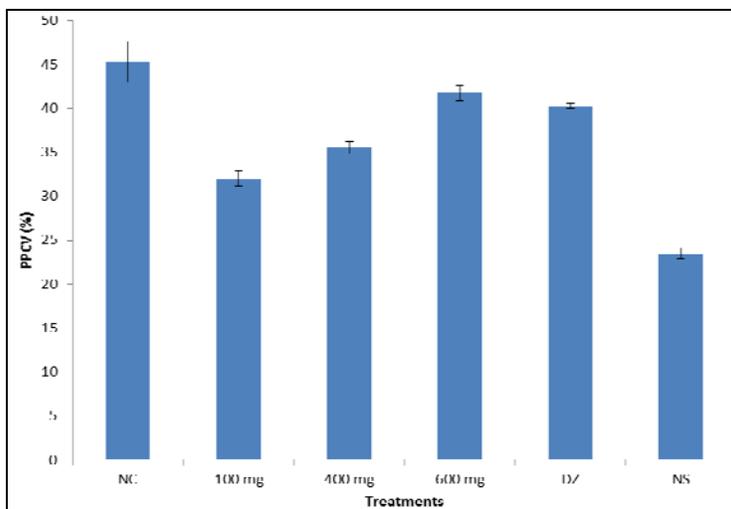


Fig 2: Effect of the treatments with crude methanol leaves extract of *Senna occidentalis* on packed cell volume of rats experimentally infected with *Trypanosoma congolense*.

Keys

NC= uninfected untreated;
 100= infected and treated with extract (100 mg/kg)
 400= infected and treated with extract (400 mg/kg)
 600= infected and treated with extract (600 mg/kg)
 DZ= infected and treated with diminazene aceturate (3.5 mg/kg)
 NS= infected and treated with normal saline (5ml/kg)

Effect of Treatment on Haemoglobin concentration

Figure 3 shows the effect of the treatment with crude methanol leaves extract of *Senna occidentalis* on haemoglobin concentration of rats infected with *Trypanosome*

congolense. Haemoglobin concentration significantly ($P < 0.05$) increased in rats treated with extract at doses; 100 mg/kg (group 2), 400 mg/kg (group 3), and 600 mg/kg (group 4) in a dose dependent manner when compared to those that were treated with normal saline (group 6). There were also significant ($P < 0.05$) reduction in the haemoglobin concentration of rats treated with extract at doses; 100 mg/kg (group 2) and 400 mg/kg (group 3), when compared to those that were treated with diminazene aceturate (3.5 mg/kg). Haemoglobin concentration was slightly higher in rats treated with extract, 600 mg/kg (group 4) when compared to those that were treated with diminazene aceturate (3.5 mg/kg).

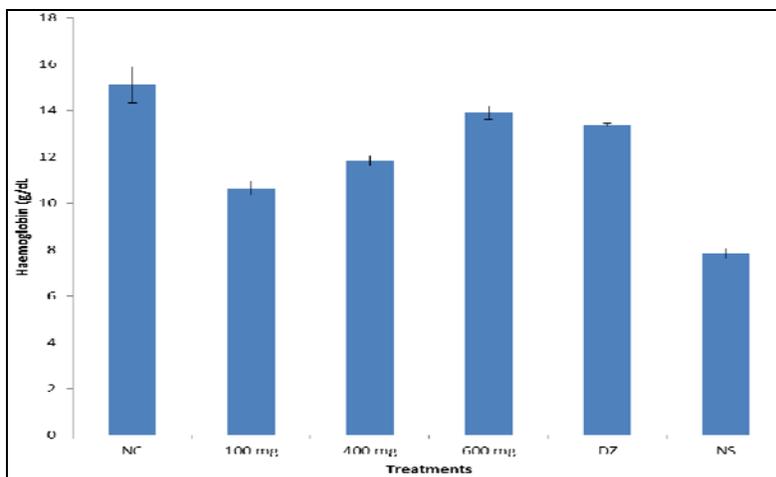


Fig 3: Effect of the treatments with crude methanol leaves extract of *Senna occidentalis* on The haemoglobin concentration of rats experimentally infected with *Trypanosoma congolense*.

Keys

NC= uninfected untreated
 100=infected and treated with extract (100 mg/kg)
 400= infected and treated with extract (400 mg/kg)
 600= infected and treated with extract (600 mg/kg)
 DZ= infected and treated with diminazene aceturate (3.5 mg/kg)
 NS= infected and treated with normal saline (5ml/kg)

Effect of Treatment on red Blood Cell Count

Figure 4 shows the effect of the treatment with crude methanol leaves extract of *Senna occidentalis* on haemoglobin concentration of rats infected with *Trypanosome*

congolense. The red blood cell count significantly ($P < 0.05$) increased in rats treated with extract at doses; 100 mg/kg (group 2), 400 mg/kg (group 3), and 600 mg/kg (group 4) in a dose dependent manner when compared to those that were treated with normal saline (group 6). There were also significant ($P < 0.05$) reduction in the red blood cell count of rats treated with extract at doses; 100 mg/kg (group 2) and 400 mg/kg (group 3), when compared to those that were treated with diminazene aceturate (3.5 mg/kg). Red blood cell count was slightly higher in rats treated with extract, 600 mg/kg (group 4) when compared to those that were treated with diminazene aceturate (3.5 mg/kg).

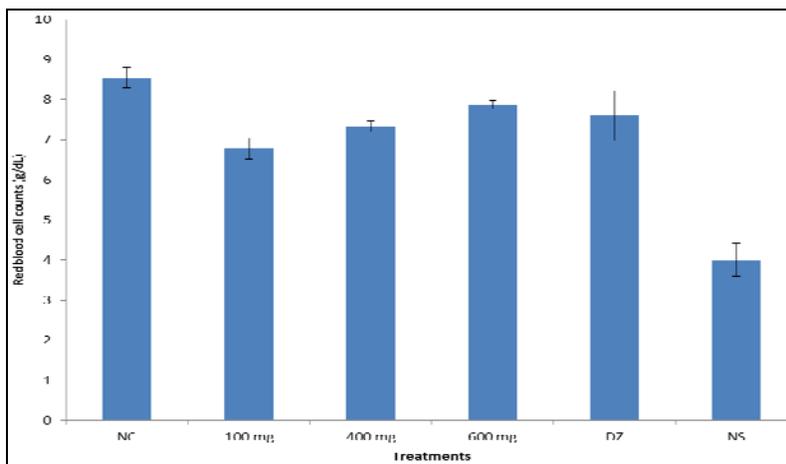


Fig 4: Effect of the treatments with crude methanol leaves extract of *Senna occidentalis* on red blood cell counts of rats experimentally infected with *Trypanosoma congolense*

Keys

NC= uninfected untreated

100=infected and treated with extract (100 mg/kg)

400= infected and treated with extract (400 mg/kg)

600= infected and treated with extract (600 mg/kg)

DZ= infected and treated with diminazene aceturate (3.5 mg/kg)

NS= infected and treated with normal saline (5ml/kg)

Effect of Treatment on Total White Blood Cell Counts

Figure 5 shows the effect of the treatment with crude methanol leaves extract of *Senna occidentalis* on total white

blood cell count of rats infected with *Trypanosoma congolense*. The total white blood cell count significantly ($P < 0.05$) progressively increased in rats treated with extract at doses; 100 mg/kg (group 2), 400 mg/kg (group 3), and 600 mg/kg (group 4) in a dose dependent manner when compared to those that were treated with normal saline (group 6). There were also significant ($P < 0.05$) reduction in total white blood cell counts of rats in treated with extract at 100 mg/kg (group 2) and normal saline treated group (group 6), when compared to those that were treated with diminazene aceturate (3.5 mg/kg) and 600 mg/kg of the extract.

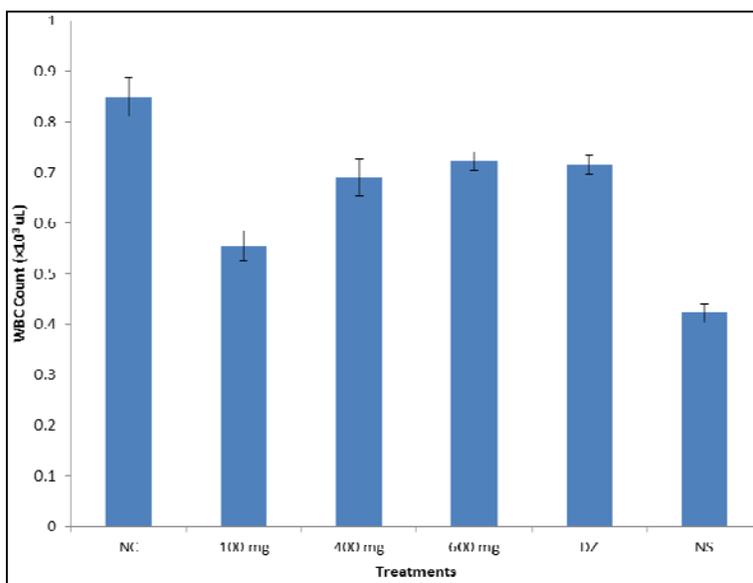


Fig 5: Effect of the treatments with crude methanol leaves extract of *Senna occidentalis* on total white blood cell count of rats experimentally infected with *Trypanosoma congolense*

Keys

NC= uninfected untreated

100=infected and treated with extract (100 mg/kg)

400= infected and treated with extract (400 mg/kg)

600= infected and treated with extract (600 mg/kg)

DZ= infected and treated with diminazene aceturate (3.5 mg/kg)

NS= infected and treated with normal saline (5ml/kg)

Discussion

There are widespread use and few scientific findings to ascertain the safety and efficacy of traditional remedies (Tahraoui *et al.*, 2010) [33]. To determine the safety of drugs and plant products for human and animal use, toxicological study is first done on any potential drug candidate in order to establish its safety. In the present study, the crude methanol extract of *Senna occidentalis* at doses of 10, 100, 1000, 1600, 2900 and 5000 mg/kg body weight did not produce any sign of toxicity or mortality, therefore the LD₅₀ is either equal to or above 5000 mg/kg (Lorke, 1983) [23].

The methanol extract of the stem bark of *Senna occidentalis* contains flavonoids, tannins, saponins, sterols, cardiac glycoside, alkaloid, triterpenes and other secondary chemical metabolites. Flavonoids are substances that delay or prevent the oxidation of cellular oxidizable substrates. The various flavonoids exerts their effect by scavenging free radicals generated during the course of infection or by activating of a battery of detoxifying/defensive proteins (Cao *et al.*, 1997) [7]. The prevention of oxidation is an essential process in all the

aerobic organisms, as decreased antioxidant protection may lead to cytotoxicity (Fonseca-Silva *et al.*, 2011) [11]. Although, the precise molecular mechanism of action of flavonoid have not yet been demonstrated (Fonseca-Silva *et al.*, 2011) [11], but there are various ways in which Reactive Oxygen Species (ROS) are produced. ROS are generated in cells infected by pathogens in an attempt to combat infection (Fonseca-Silva *et al.*, 2011) [11].

The clinical signs observed were more obvious in group six, which includes; anorexia, loss of body weight, pale ocular mucous membrane and weakness, these signs were in agreement with the findings of Samuel *et al* (2016) [28] in Donkeys with experimental *Trypanosoma congolense* infection. Variable disorders occur sequel to trypanosome infection in animals (Adamu *et al.*, 2009) [1], depending on the virulence of the infecting trypanosome, the infective dose and the immune status of the host, the symptoms usually associated with trypanosomiasis includes; pallor of the mucous membranes, enlargement of lymph nodes, anoraexia and emaciation (Shimelis *et al.*, 2015) [32].

The mean PCV, Hb concentration and RBC count reduced significantly ($P < 0.05$) in rats treated with normal saline (group 6) when compared to untreated uninfected rats (group 1), indicating anaemia, this agrees with the work of Ukpai and Nwabuko (2014) [38] who reported the Effects of *Trypanosoma brucei brucei* on haematological parameters and pathology of internal organs in albino rats. Anaemia is regarded as the most consistent pathology in trypanosomiasis of man and domesticated animal, it has been reported in *T.*

vivax infected cattle and goats (Saror, 1980) [29], *T. congolense* infected sheep (Bisalla *et al.*, 2007), *T. congolense* infected dogs (Gow *et al.*, 2007) [14] and *T. brucei brucei* infected goats, sheep and rabbits (Seed, 1969; Taiwo *et al.*, 2003) [30, 34]. The pathophysiology of anaemia in *Trypanosomiasis* is complex and multi factorial in origin (Naessens *et al.*, 2005) [25]. Trypanosomes initiates a cascade of events in the animal host leading to anaemia and cardiovascular collapse (Anosa, 1988) [3]. This anaemia might be due to haemolysis induced by the trypanosomes (Soulsby, 1982). The anaemia may also be caused by mechanical injury to erythrocyte via the lashing action of the powerful locomotory flagella and microtubule reinforced bodies of the millions of the organisms during high parasitaemia (Vickerman and Tetley, 1978) [40]. Erythrocyte membrane damage has also been associated with adhesion of erythrocytes, platelets and reticulocytes to trypanosome surfaces via sialic acid receptors leading to damages to erythrocyte cell membranes (Shehu *et al.*, 2006) [31].

The decreased total white blood cell count also agrees with the work of Longdet *et al* (2014) [22] and Samuel *et al* (2016) [28] in rats and Donkeys with experimental *Trypanosoma congolense* infection respectively. The decrease could be attributed to the immunosuppressive actions of trypanosomes in the body. Leukopenia after an initial period of leucocytosis is a common finding in trypanosomes infection in rats and rabbits and other animals (Ibrahim *et al.*, 2016) [17]. Leukopenia in animal trypanosomiasis has been reported to be due largely to ineffective or depressed granulopoiesis in the bone marrow (Anosa *et al.*, 1997) [2]. The immunosuppression may result in free radicals accumulation, therefore, in trypanosomes infection with increased free radicals production, the immune cells are vulnerable to oxidative damage, which may be responsible, among other factors, for the leukopenia observed in the infected normal saline treated group.

In conclusion, the experimental findings of this study suggest that the crude methanol extract of *Senna occidentalis* possess antitrypanosomal activity. The results obtained further justify of the traditional used of plant in the treatment of trypanosomiasis. Further work to isolate, identify, characterize and elucidate the structure of the phytoconstituents responsible for the observed pharmacological activities in this study are ongoing.

Acknowledgement: The authors are thankful to all technical staff of the departments of Animal Health and Production, Binyaminu Usman Polytechnic Hadejia, Jigawa State and Veterinary Parasitology, Faculty of Veterinary Medicine Ahmadu Bello University Zaria Kaduna State.

References

- Adamu S, Barde N, Abenga JN, Useh NM, Ibrahim NDG, Esievo KAN. Experimental *Trypanosoma brucei* infection-induced changes in the serum profiles of lipids and cholesterol and the clinical implications in pigs. *Journal of Cell and Animal Biology*. 2009; 3(2):015-020.
- Anosa VO, Logan-Henfrey LL, Wells CW. The haematology of *T. congolense* infection in cattle 11: Macrophages structure and function in adult Boran cattle. *International Journal of Comparative Haematology*. 1997a; 7:23-29.
- Anosa VO. Haematological and biochemical changes in human and animal trypanosomiasis, Parts I & II, *Revue d' Elevage et de Medicine Ve'terinaire des pays Tropicaux*. 1988; 41(2):65-78.
- Bako SP, Bakfur MJ, John I, Bala EI. Ethnomedicinal and phytochemical profile of some savanna plant species in Nigeria. *International Journal Botany*. 2005; 1(2):147-150.
- Bisalla M, Ibrahim NDG, Lawal IA, Esievo KAN. Serum total protein, albumin and albumin globulin ratio in Yankassa sheep experimentally infected with *Trypanosoma congolense* and immunomodulated with levamisole. *Journal of Protozoology Research*. 2007; 17:39-43.
- Brain KR, Turner TD. The practical evaluation of phytopharmaceuticals. Wright-scientifica, Briotol, 1975, 10-30.
- Cao G, Sofic E, Prior RL. Antioxidant and prooxidant behavior of flavonoids: structure-activity relationships. *Free Radicals Biology and Medicine*. 1997; 22:749-760.
- Coles EH. *Veterinary Clinical Pathology* 4th edition, W.B Saunders, Philadelphia, London, 1986, 1-42.
- Dacie JV, Lewis SM. *Practical Haematology*, 7th Edn, Churchill Livingstone, London, 1991, 659-661.
- Ekanem JT, Yusuf OK. Some biochemical and haematological effects of black seed (*Nigella sativa*) oil on *T. brucei*-infected rats. *African Journal of Biomedical Research*. 2008; 11:79-85.
- Fonseca-Silva F, Job DF, Inacio MM, Canto-Cavalheiro, Elmo Eduardo A. Reactive Oxygen Species Production and Mitochondrial Dysfunction Contribute to Quercetin Induced Death in *Leishmania amazonensis*. *PLoS One*. 2011; 6(2):e14666.
- Ford J. Red blood cell morphology. *International Journal of Laboratory Hematology*. 2013; 35(3):351-357.
- Frieburghaus F, Kaminsky R, Nkanya MHN, Brun R. Evaluation of African medical plants for their invitro trypanocidal activity Y. *Ethnopharmacology*. 1996; 55(1):1-11.
- Gow AG, Simpson JW, Picozzi K. First report of canine Africa trypanosomiasis in the UK. *Journal of small animal practice*. 2007; 48:658-661.
- Hamid AA, Aiyelaagbe OO, Usman LA, Ameen OM, Lawal A. Antioxidants: Its medicinal and pharmacological applications. *African Journal of Pure and Applied Chemistry*. 2010; 4(1):7-10.
- Herbert WJ, Lumsden WH. *Trypanosoma brucei*: A rapid matching method for estimating the host's parasitaemia. *Experimental Parasitology*. 1976; 40:427-31.
- Ibrahim A, Mbaya AW, Anene BM, Luka J. Comparative parasitaemia and haematology of mice, rats and rabbits experimentally infected with *Trypanosoma brucei brucei* and their responses to diminazene diaceturate (Veriben®) therapy. *Asian Pacific Journal of Tropical Disease*. 2016; 6(7):527-532.
- Igbokwe IO. Mechanisms of cellular injury in African trypanosomiasis, *Veterinary Bulletin*. 1994; 64(7):611-620.
- Igbokwe IO. Mechanism of cellular injury in African Trypanosomiasis. *Annals Tropical Medicine and Parasitology*. 1994; 64:611-5.
- Keay KA, Clement CL, Bandler R. The neuroanatomy of cardiac nociceptive pathway. *Nigerian Journal of Pharmaceutical Sciences*. 2000; 5:221-229.
- Kuo SC, Chen SC, La CF, Teng CM, Wang JP. Studies on the anti-inflammatory and antiplatelet activities of constituents isolated from the roots and stem of *Cassia occidentalis* L. *Journal of Pharmaceutical Chemistry*. 1996; 48:291-302.

22. Longdet IY, Achemu HU, Okanlawon CN. Potentials of Methanolic Extract of *N. latifolia* stem bark against *T. congolense* infection in Experimental, Rats. Journal of Agricultural Sciences and Policy Research. 2014; 4:29-41.
23. Lorke D. A new approach to acute toxicity testing. Archive of toxicology. 1983; 54:275-287.
24. Mahesh B, Satish S. Antimicrobial activity of some important medicinal plant against plant and human pathogens. World Journal of Agricultural Science. 2008; 4:839-843.
25. Naessens J, Kitani H, Yagi Y, Sekikawa K, Iraqqi F. TNF- α mediates the development of anaemia in a murine *Trypanosoma brucei* rhodesiense infection, but not the anaemia associated with a murine *T. congolense* infection, Clinical and Experimental Immunology. 2005; 139(3):403-410.
26. Nok AJ. Azaanthraquinone inhibits respiration and *in vitro* growth of long slender blood stream forms of *T. congolense*. Cell Biochemical Function. 2001; 20:205-12.
27. Nuhu AA, Aliyu R. Effects of *Cassia occidentalis* aqueous leaf extract on biochemical markers of tissue damage in rats. Tropical Journal of Pharmaceutical Research. 2008; 7(4):1137-1142.
28. Samuel FU, Adamu S, Bisalla M, Chiezey NP, Mohammed AK, Bello TK *et al.* Effect of *T. congolense* on Haematological Parameters in Experimentally Infected donkeys. Journal of Animal Production Research. 2016; 28(1):14-24.
29. Saror DI. Observation on the course and pathology of *Trypanosoma vivax* in red Sokoto goats. Research in Veterinary Science. 1980; 28:36-38.
30. Seed JR. *Trypanosoma gambiense* and *Trypanosoma lewisi*: Increase vascular permeability and skin lesion in rabbits. Experimental Parasitology. 1969; 26:214-223.
31. Shehu SA, Ibrahim NDG, Esievo KAN, Mohammed G. Role of erythrocyte surface sialic acid inducing anaemia in Savannah Brown bucks experimentally infected with *Trypanosoma evansi*, Veterenarski Arhive. 2006; 26(6):521-530.
32. Shimelis D, Melkamu B, Getachew T, Getachew A, David B, Bruno MG. Comparative clinico-haematological analysis in young Zebu cattle experimentally infected with *Trypanosoma vivax* isolates from tsetse infested and non-tsetse infested areas of Northwest Ethiopia. Acta Veterinaria Scandinavica, 2015, 57:24.
33. Tahraoui A, Israili ZH, Lyoussia B. Acute and sub-chronic toxicity of a lyophilised aqueous extract of *Centaurium erythraea* in rodents. Journal of Ethnopharmacology. 2010; 132:48-55.
34. Taiwo VO, Olaniyi MO, Ogunsanmi AO. Comparative plasma biochemical changes and susceptibility of erythrocytes to *in vitro* peroxidation during experimental *Trypanosoma congolense* and *Trypanosoma brucei* infection in sheep. Israel Journal of Veterinary Medicine. 2003; 58(4):435-443.
35. Talakal TS, Dwivedi SK, Shama SR. *In vitro* and *in vivo* antitrypanosomal activity of *Xanthium strumarium* leaves. Journal of Ethnopharmacology. 1975; 49(3):141-145.
36. Tona L, Cimanga RK, Mesia K, Musuamba CT, De Bruyne T, Apers S. *In vitro* antiplasmodial activity of extracts and fractions from seven medicinal plants used in the Democratic Republic of Congo. Journal of Ethnopharmacology. 2004; 93:27-32.
37. Trease GE, Evans WC. Textbook of Pharmacognosy, 12th Edt, (Balliere, Tindall, London), 1983; 57-59, 343-383.
38. Ukpai OM, Nwabuko OP. Effects on haematological parameters and pathology of internal organs of *Trypanosoma brucei brucei* infected albino rats. Nigerian Journal of Biotechnology. 2014; 27: 8-13.
39. Umar IA, Rumah BL, Bulus SL, Kamla AA, Jobin A, Suleiman BI. Effects of intraperitoneal administration of vitamins C and E or A and E combinations on the severity of *Trypanosoma brucei brucei* infection in rats. African Journal of Biochemistry. 2008; 2:88-91.
40. Vickerman K, Tetley L. Biology and ultra structure of trypanosomes in relation to pathogenesis. In: *Pathogenecity of trypanosomes*, Proceedings of a workshop, pp. 231- 31, ISBN 0- 88936-214-9, Nairobi, Kenya, November, 1978, 20-23.
41. World Health Organisation. Tropical Diseases Today: The Challenges and Opportunities WHO, Switzerland, 1975.
42. World Health Organization. Traditional Medicine. Media Centre Fact Sheet No 134. Accessed from <http://www.who.int/mediacentre/factsheets/fs134/en/> 2008.
43. Yadava RN, Satnami DK. Chemical constituents from *Cassia occidentalis* Linn. Indian Journal of Chemistry. 2011; 50B:1112-1118.