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Effect of Crude Methanol Extract of *Senna occidentalis* on Biomarkers of Oxidative Stress of wistar Rats with Experimental *Trypanosoma congolense* Infection

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Abstract

The experiment was carried out to evaluate the effect of crude methanol leaves extract of *Senna occidentalis* on biomarkers of oxidative stress 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 randomly divided into six groups of five rats each. Rats in group one were untreated uninfected (neutral control). Groups two to six received intraperitoneal injection of *Trypanosoma congolense* (10^6 trypanosomes/ml of blood). Parasitaemia was established 8 days post infection. Rats in groups two, three, and four were orally treated with 100, 400 and 600 mg/kg body weight of the extract respectively for ten days. Rats in group five were treated with diminazene aceturates (3.5 mg/kg, i.p) once. Rats in group six were treated with normal saline for ten days. At doses 100, 400 and 600 mg/kg body weight the extract showed significant ($P < 0.05$) progressive increase in the serum level of glutathione peroxidase (GPx), catalase (CAT) and superoxide dismutase (SOD). Serum level of GPx, CAT and SOD were slightly higher in untreated uninfected rats (group 1) when compared to rats treated with 600 mg/kg body weight of the extract (group 4) and diminazene aceturates (3.5 mg/kg). So also, a significant ($P < 0.001$) increase in the serum level of malondialdehyde concentration in infected untreated rats (group 6) when compared to the remaining groups. Treatment with crude methanolic leaves extract of *Senna occidentalis* significantly reduced oxidative stress induced by trypanosomes, suggesting possible antioxidant properties of the extract and its trypanosuppressive activity in trypanosomosis.

Keywords: Crude Methanol, Oxidative stress, *Senna occidentalis* and *Trypanosoma congolense*

1. Introduction

Trypanosomosis is a vector-borne parasitic disease which causes major health and economic problems in rural sub-Saharan Africa affecting livestock (Mortelmans, 1986) [25]. The disease is known to render approximately a quarter of African arable land mass unsuitable for profitable livestock farming (Molyneux, 1997) [24] and is responsible for causing death of well over 3 million cattle annually with an estimated lost potential of \$ 6-12 billion US dollars (Mortelmans, 1986; ILRAD, 1994) [25, 17]. The prevalence rate is in excess of 70% in some villages in the Democratic Republic of the Congo according to WHO (2000).

The mechanism of anaemia in trypanosomosis is greatly associated with the generation of free radicals and super oxides following lipid peroxidation. These oxidative products generally attack the cellular integrity of erythrocytes during trypanosomosis (Anosa and Kaneko, 1983; Igbokwe, 1994; Umar *et al.*, 2007) [4, 16]. They also particularly attack erythrocyte membrane polyunsaturated fatty acids and proteins (Slater, 1984; Mbaya *et al.*, 2012) [22] or red blood cells directly leading to oxidative haemolysis (Ameh, 1984; Igbokwe, *et al.*, 1989; Umar *et al.*, 2007) [3, 15]. Sialic acids consist of about four derivatives of nine-carbon sugar neuraminic acids (Schauer and Kamerling, 1997; Mbaya *et al.*, 2012) [29, 22]. It was therefore concluded that anaemia in trypanosomosis might occur due to erythrophagocytosis (Holmes and Jennings, 1976) [14] and may be associated with the formation of antigen-antibody complexes with sialic acids (Audu *et al.*, 1999) [5].

An antioxidant can be broadly defined as any substance that delays or inhibits oxidative damage to a target molecule (Yamagishi and Matsui, 2011) [34]. The main characteristic of an antioxidant is its ability to trap free radicals. Antioxidant compounds like phenolic acids, polyphenols and flavonoids scavenge free radicals such as peroxide, hydroperoxide or lipid peroxy and thus inhibit the oxidative mechanisms that lead to degenerative diseases (Wu *et al.*, 2011) [33]. Herbal plants considered as good antioxidant since ancient times.

Before the discovery of synthetic drugs, local herdsmen were controlling trypanosomiasis through different ethnoveterinary practices (Nok *et al.*, 2001) [26]. 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 (Nok *et al.*, 1994). However, not much has been done to document the ethnoveterinary practices among the local herdsmen (Frieburghaus *et al.*, 1996) [11].

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 (Umar *et al.*, 2008; Igbokwe, 1994) [32, 16]. It is a shrub that grows between 5 to 8 cm in height and commonly found in the tropics (Kaey, 2000) [18]. 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) [32]. 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]. *C. occidentalis* is used as a diuretic and in the treatment of snake-bite (Yadava and Satnami, 2011). Different parts of this plant have been reported to possess anti-inflammatory and antiplasmodial activities (Kuo *et al.*, 1996; Tona *et al.*, 2004) [20, 31].

In the present study, the effect of crude methanol leaves extract of *Senna occidentalis* on biomarkers of oxidative stress of wistar rats with experimental *Trypanosoma congolense* infection was evaluated.

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, and a voucher specimen number A002 was deposited. Fresh leaves of *Senna occidentalis* was air-dried and made into powdered form using pestle and mortar. Five hundred grams 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) [7].

Acute Toxicity Study

Median lethal dose (LD₅₀) as a measure of establishing the safety of the extract was determined as described by Lorke (1983) [21]. Briefly, 12 wistar rats 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 following 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 1600mg/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 × 400 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) [13].

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 × 100 magnification (approximately 10⁶ trypanosomes per ml) as described by Ekanem and Yusuf, (2005).

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 be infected nor treated with any substance and served as neutral control group.

Group II: Each rat in this group was infected with *Trypanosoma congolense* (10⁶ 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, all rats were 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.

Evaluation of Biomarkers of Oxidative Stress

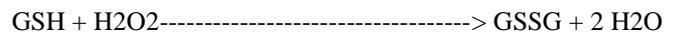
One hundred microliters of fresh serum sample obtained from each rat was used to analyse for the levels of malondialdehyde concentration and activities of antioxidant enzymes; glutathione peroxidase, catalase and superoxide dismutase.

Determination of Serum Glutathine Peroxidase (GPx) Specific activity

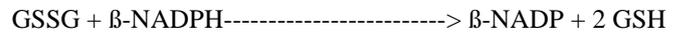
Modified method of Paglia and Valentine (1967) was used to determine glutathione peroxidase (GPx) activity spectrophotometrically by coupling the oxidation of glutathione and beta nicotinamide adenine dinucleotide phosphate (NADPH) using GP and glutathione reductase (GR). Briefly, 1 mL of assay mixture containing an optimized concentrations of the following chemicals: 0,5 M K_2HPO_4 (pH 7.0), 2,5 mM EDTA, 0,18 U/mL GR, 100 mM glutathione and 10 mM reduced NADPH and plasma (0,5

mL) was added into the spectrophotometer cuvette along with 0,1 mL of 60 mM cumene hydroperoxide, a suitable substrate for GP. The mixture was placed into a 1 mL cuvette and read with Shimadzu UVPC 2100 spectrophotometer set at 366 nm at 37 °C.

Glutathione Peroxidase



Glutathione Reductase



Abbreviations used:

GSH = Glutathione, Reduced Form

GSSG = Glutathione, Oxidized Form

β -NADPH = β -Nicotinamide Adenine Dinucleotide Phosphate, Reduced Form

β -NADP = β -Nicotinamide Adenine Dinucleotide Phosphate, Oxidized Form.

Determination of Serum Catalase (CAT) activity

The specific activity of catalase in the serum collected from each rat was determined using method described by Beers and Sizer (1952) [6]. Catalase is an enzyme that scavenges H_2O_2 and convert it to water and molecular oxygen. By measuring the rate of breakdown of H_2O_2 spectrophotometrically, the specific activity of CAT in each sample was measured. Briefly, 167 μ L of substrate (0.053 M H_2O_2) in 0.05 M potassium phosphate buffer (pH 7.0, 25 °C) and 25 μ L of the serum from each mouse was added to a 0.5 ml quartz cuvette and brought to final volume of 0.5 ml at 25 °C, using 0.05 M potassium phosphate buffer, (pH 7.0). The rate of decrease in absorbance at 240 nm was measured with UV spectrophotometer. The catalase activity was calculated using the co-efficient of H_2O_2 $43.6 M^{-1} Cm^{-1}$ as follow:

$$\text{Unit/mg protein} = \frac{\Delta A \text{ at } 240 \text{ nm} \times \text{cuvette volume (ml)} \times \text{Dilution factor (1000)} \times 1 \text{ cm light}}{43.6 M^{-1} Cm^{-1} \times \text{mg protein}}$$

Determination of Serum Superoxide Dismutase (SOD) Specific Activity

The specific activity of SOD was measured using the method described by Misra and Fridovich (1972) [23] as modified by Rabideau (2001). The method is an indirect method of inhibiting auto-oxidation of adrenaline to its adrenochrome using SOD activity in the serum sample. By measuring the rate at which SOD decreases oxygen-dependent auto-oxidation reaction, the specific activity of SOD in each sample was calculated. One hundred a microlitres (0.1 ml) of

serum was diluted with 0.9 ml of distilled water to make 1:10 dilution of microsome. Twenty microlitres of the diluted microsome were added to two hundred of and fifty microlitres of 0.05 M carbonate buffer. The reaction started by addition of thirty microlitres of 0.3 mM adrenaline. The reference mixture contain two hundred and fifty microlitres of 0.05 M of carbonate buffer, thirty microliters of 0.3mM adrenaline and twenty microlitres of distilled water. Thereafter, absorbance was measured for 30 seconds to 150 seconds at 480 nm. The percentatge inhibition was calculated as:

$$\% \text{ inhibition} = 100 - \left(\frac{\text{Increase in absorbance for sample}}{\text{Increase in absorbance of blank}} \times 100 \right)$$

Determination of Serum Malondialdehyde Concentration

Fifty microlitres of serum from each animal were used to determine the level of serum malondialdehyde by double heating method as described by Draper and Hadley (1990) [8] and modified by Altuntas *et al* (2002) [2]. The principle will be based on spectrophotometric measurement of the colour produced during the reaction of thiobarbituric acid (TBA) with the serum malondialdehyde. Two hundred and fifty microlitres of 100 g/L of trichloro acetic acid solution was added to fifty microliters of serum in a centrifuge tube and placed in a boiling water bath for 15 minutes. After cooling in tap water, the mixture was centrifuged at $1000 \times g$ for 10 minutes and two hundred microlitres of the supernatant was

added to one hundred microlitres 6.7 g/L of TBA in a test tube, then placed in a boiling water bath for 15 minutes. The solution was cooled in tap water and the absorbance was measured using UV-spectrophotometer (Jenway, 6405 Model, Japan) set at 532 nm.

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

The percentage yield of the dried methanol leaves extract of *Senna occidentalis* was 19.86 %.

Phytochemical Test

The results of phytochemical test are presented in Table 1.

Table 1: Phytochemical screening results of crude Methanol Leaves Extract of *Cassia tora*

Compounds	Inference
Carbohydrate	+++
Glycosides	+
Anthraquinones	+++
Cardiac glycoside	+
Saponins	-
Steroids	+
Triterpenes	++
Flavonoid	+++
Tannins	++
Alkaloids	-

Key: +++=Highly positive, ++=moderately positive, +=faintly positive, -=not present

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 (LD₅₀) is equal to or above 5000 mg/kg (Lorke, 1983) [21].

Effect of the treatments on antioxidant enzymes

Effect of the Treatments on Serum Glutathione Peroxidase Specific Activity.

Figure 1 shows the effect of the treatment with methanol leaves extract of *Senna occidentalis* on serum level of glutathione peroxidase activity of rats infected with *Trypanosoma congolense*. Serum level of glutathione peroxidase activity 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). Similarly, serum glutathione peroxidase activity were significantly ($P < 0.001$) higher in untreated uninfected rats (group 1) when compared to those that were treated with normal saline (group 6).

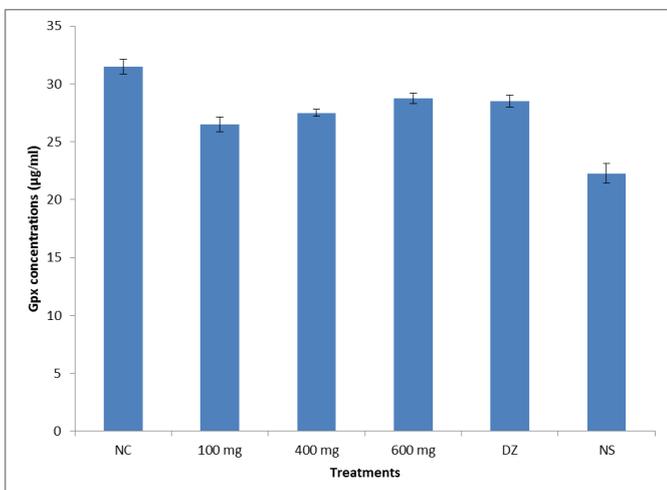


Fig 1: Effect of the treatments with crude methanol leaves extract of *Senna occidentalis* on serum glutathione peroxidase activity of rats experimentally infected with *Trypanosoma congolense*.

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 the Treatments on Serum Catalase Specific Activity

Figure 2 shows the effect of the treatment with methanol leaves extract of *Senna occidentalis* on serum level of catalase activity of rats infected with *Trypanosoma congolense*. Serum level of catalase activity were 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). Similarly, serum level of catalase activity were significantly ($P < 0.001$) higher in untreated uninfected rats (group 1) when compared to those that were treated with normal saline (group 6).

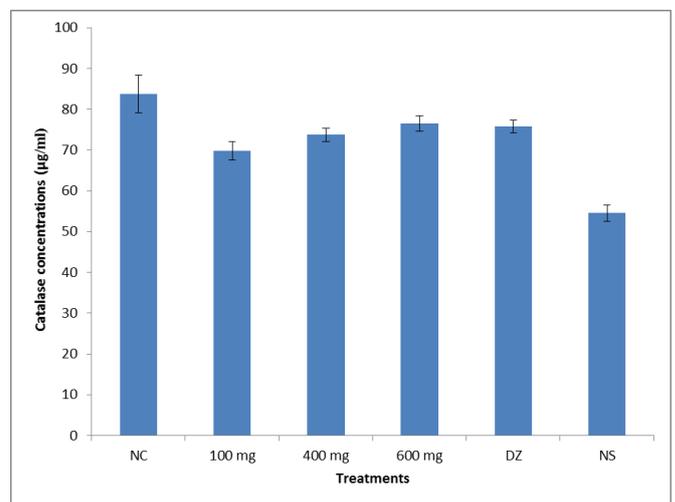


Fig 2: Effect of the treatments with crude methanol leaves extract of *Senna occidentalis* on serum catalase concentration of rats experimentally infected with *Trypanosoma congolense*.

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 the Treatments on Superoxide Dismutase (SOD) Specific Activity

Figure 3 shows the effect of the treatment with methanol leaves extract of *Senna occidentalis* on serum level of SOD activity of rats infected with *Trypanosoma congolense*. Serum level of SOD activity were 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). Similarly, serum level of SOD activity were significantly ($P < 0.001$) higher in untreated uninfected rats (group 1) when compared to those that were treated with normal saline (group 6).

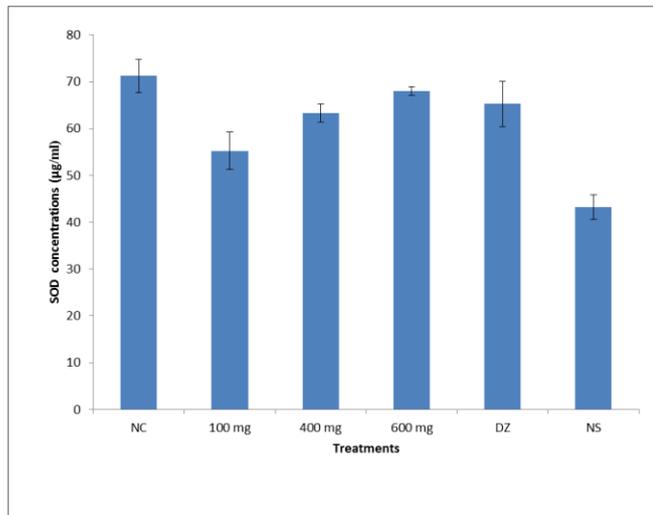


Fig 3: Effect of the treatments with crude methanol leaves extract of *Senna occidentalis* on serum SOD concentration of rats experimentally infected with *Trypanosoma congolense*.

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 the Treatments on the Serum Level of Malondialdehyde (MDA) Concentration

Figure 3 shows the effect of the treatment with methanol leaves extract of *Senna occidentalis* on serum level of MDA concentration of rats infected with *Trypanosoma congolense*. Serum level of MDA concentration were significantly ($P < 0.05$) decreased 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). Similarly, serum level of MDA concentration were significantly ($P < 0.001$) lower in untreated uninfected rats (group 1) when compared to those that were treated with normal saline (group 6).

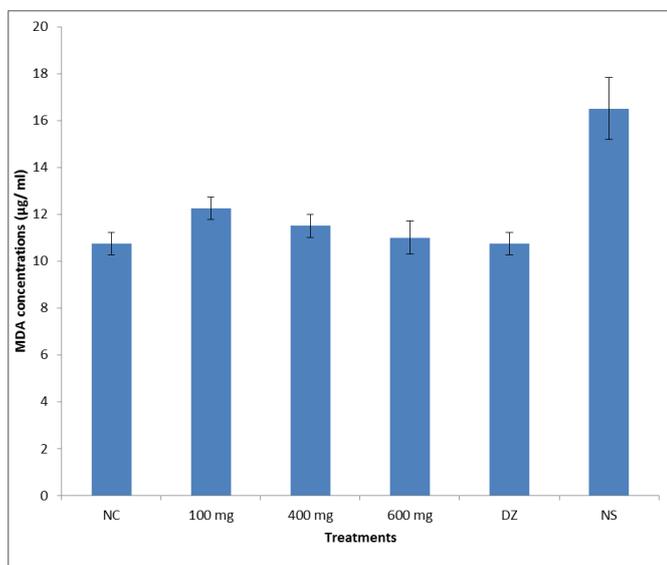


Fig 4: Effect of the treatments with crude methanol leaves extract of *Senna occidentalis* on serum MDA concentration of rats experimentally infected with *Trypanosoma congolense*.

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)

Discussion

Anorexia, loss of body weight, pale ocular mucous membrane and weakness were the clinical signs observed in this study, these signs were more obvious in rats treated with normal saline. These findings were similar to that of Gow *et al* (2007) [12] in dogs infected with *Trypanosoma congolense*, in sheep infected with *Trypanosoma congolense* (Bisalla *et al.*, 2007), 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 these includes; pallor of the mucous membranes, enlargement of lymph nodes, anorexia and emaciation (Shimelis *et al.*, 2015) [30].

The extract at doses of 10, 100, 1000, 1600, 2900 and 5000 mg/kg did not produced any sign of toxicity nor mortality. Lorke, (1983) [21] reported that if no toxicity and mortality is observed in two phases of acute toxicity test, it means the LD₅₀ is equal or above 5000 mg/kg.

The *S. occidentalis* methanolic leaves extract possess trypanosuppressive activity in a dose-dependent, this supports earlier reports that some plant extracts contain potent trypanocides (Igbokwe, 1994) [16], although the exact mechanism for the observed *in vivo* trypanosuppressive effect is not known, it is obvious that the extract contains some phytochemicals that could interfere with the survival of the parasites *in vivo*. However, the presence of flavonoids have been attributed to trypanosuppressive activity of some plant extracts (Ogunkunle and Ladejobi, 2006), but some previous reports attributed the antitrypanosomal activity of a number of tropical plants to the flavonoids (azaanthraquinone), highly aromatic planar quaternary alkaloids, barbarine and harmaine (Igbokwe, 1994) [16]. It is thus possible that the observed trypanosuppressive action of *S. occidentalis* methanolic leaves extract could be due to the presence of one or more of phytoconstituent.

A significant ($P < 0.05$) increase in the serum level of specific antioxidants enzymes (SOD, CAT and GPX) were recorded in rats treated with extracts at doses; 100 mg/kg (groups 2), 400 mg/kg (groups 3) and 600 mg/kg (groups 1) and those that were treated with diminazene aceturate 3.5 mg/kg (groups 5) when compared to those that were treated with normal saline (group 6) these agrees with the findings of Kobo *et al* (2014) [19] in rats with trypanosomiasis and treated with a mixture of flavonoids. SOD, CAT and GPX are enzymatic antioxidant that mop-up free radicals and ROS. SOD is the first line of defense against oxidative stress. It dismutates superoxide anion to H₂O₂ and water. The H₂O₂ generated from the activity of SOD will be detoxified to water and oxygen by CAT and GPX. Hence, CAT and GPX are positioned in the second line of defence to scavenge free radical and ROS (Eghianruwa, 2012) [9]. Trypanosomiasis was reported to induce oxidative stress and deplete antioxidants whereas exogenous antioxidants supplement, such as Vitamin C and dimethyl sulfoxide gave a better clinical improvement and survival than diminazene (a known chemopreventive drug) in *T. brucei* infection in rats (Eghianruwa, 2012) [9].

In conclusion, the experimental findings of this study suggest that the crude methanol extract of *Senna occidentalis* possess phytoconstituents with antioxidant effect. Further work to determine the antioxidant activity of each phytoconstituent present in the crude methanol extract of *Senna occidentalis* would be worthwhile.

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