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Full Length Research Paper

Effects of methanol extract of *Vernonia amygdalina* leaf on survival and some biochemical parameters in acute *Trypanosoma brucei brucei* infection

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The effects of methanol extract of *Vernonia amygdalina* leaf (chosen from several extracts tested) on survival, parasitaemia, packed cell volume and some biochemical parameters were investigated in acute *Trypanosoma brucei brucei* infection. Results showed that the extract was relatively safe with median lethal dose, LD_{50} ≥5000 mg/kg/day. Although none of the extracts tested cured infected mice, the methanol extract of *V. amygdalina* leaf at a dose of 300 mg/kg/day increased the maximum survival days of the mice to 24 days compared to 8 days for infected control, and also temporarily cleared the parasites from circulation for up to 72 h before relapse occurred. The active methanol extract significantly ($p<0.05$) reduced the parasitaemia and improved the packed cell volume of the extract-treated rats. Similarly, it significantly decreased ($p < 0.05$) the activities of alanine and aspartate aminotransferases, urea, triglycerides and malondialdehyde levels; increased the levels of reduced glutathione ($p< 0.05$), superoxide dismutase ($p < 0.05$) and catalase ($p > 0.05$) activities in serum, liver and kidney; but caused no change in total protein, albumin and creatinine levels. These suggest that the methanol extract of *V. amygdalina* leaf has ameliorative effects and may therefore be useful in the management of animal trypanosomiasis.

Key words: *Vernonia amygdalina*, *Trypanosoma brucei brucei*, oxidative stress, anti-trypanosomal, toxicity.

INTRODUCTION

The chemotherapy of African trypanosomiasis remains unsatisfactory and is besieged with numerous problems as the few registered trypanocides are frequently toxic, require lengthy parenteral administration, lack efficacy and are unaffordable for most of the patients (Fairlamb, 1990; Legros et al., 2002). In addition, the prospect of vaccine development is still far (Nok, 2005). The need for alternative new molecules that are safe, effective and affordable is therefore urgent. Emphasis is now shifting to medicinal plants and other natural products in search of such molecules that could be further developed into new antitrypanosomal agents (Nok et al., 1993; Freiburghaus et al., 1996; Merschjohann et al., 2001; Atawodi et al., 2003; Newman et al., 2003; Hoet et al., 2004; Umar et al., 2010).

The implications of biochemical changes to the disease pathogenesis have been reported by several investigators. Taiwo et al. (2003) reported elevated levels of total protein, globulin and decrease in cholesterol and glucose levels in sheep experimentally infected with *T. b. brucei* and *Trypanosoma congolense.* Abenga and Anosa (2005) reported increased protein, creatinine and globulin levels in monkeys experimentally infected with *Trypanosoma brucei gambiense*. Vickerman and Tetley (1979) had also reported hypo-albuminaemia which may occur because of the uptake of albumin-bound fatty acid and lipoproteins and haemodilution (Katunguka-Rwakishaya et al., 1992b) in trypanosome infected animals. Awobode (2006) reported hypoalbuminaemia,

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normal total protein, decreased urea level and increased creatinine level in natural human trypanosomal infections. Furthermore, increasing evidence indicates that free radical-induced oxidative stress plays an important role in the pathogenesis of African trypanosomiasis (Igbokwe, 1994; Ogunsanmi and Taiwo, 2007; Umar et al., 2007; Akanji et al., 2009). Large amounts of reactive oxygen species and hydrogen peroxide are produced by *Trypanosoma brucei brucei* group (Meshnick et al., 1977) and host animal activated macrophages and monocytes (Schwacha and Loegering, 1992). Foods and plants rich in antioxidants play an essential role in the prevention or amelioration of free radicals-related diseases such as cancers (Kris-Etherton et al., 2002), neurodegenerative diseases (Di Matteo and Esposito, 2003) and trypanosomiasis (Akanji et al., 2009; Umar et al., 2010). Thus, with no prospect on new drugs and vaccines in the near future, couple with the role these biochemical changes play in the disease pathogenesis, it becomes imperative to find agents that could ameliorate the trypanosomiasis-induced biochemical changes and thus improve the conditions of infected animals.

Vernonia amygdalina, a very popular plant used in Africa and commonly known in Nigeria as "bitter leaf" has been reported to have multiple health benefits such as anticancer (Sweeney et al., 2005); anti-diabetic (Nwanjo, 2005), antioxidant (Erasto et al., 2007) and anti-trypanosomal (Wurochekke and Nok, 2004). Accordingly, in the present study, we aimed to investigate the *in vivo* anti-trypanosomal activity and ameliorative effects of the methanolic extract of *V. amygdalina* leaf in mice and rats experimentally infected with *T. brucei brucei*.

MATERIALS AND METHODS

Chemicals/reagents

All chemicals and reagents were purchased either from Fluka or Sigma-Aldrich Ltd and all are of Analar grade.

Plant materials

The candidate plants were selected on an ethnopharmacological basis with assistance of a traditional herbalist. *V. amygdalina* (leaf) and *Hymenocardia acida* (leaf, stem-bark and root-bark) were collected in Vom, and Zaria, North central Nigeria, respectively. They were identified at the herbarium of the Department of Biological Sciences, Ahmadu Bello University, Zaria. Voucher numbers 675 and 900719 for *V. amygdalina* and *H. acida*, respectively, were deposited at the herbarium. All plant parts were collected in February.

Preparation of crude plant extracts

About 40 g of each dried and powdered part was extracted by reflux with 400 ml of methanol and dichloromethane separately for 3 h. Aqueous extract was prepared by soaking 40 g of dried parts in distilled water and gently heating at 50°C for 3 h. The liquid extracts obtained were then filtered through muslin cloth and filter paper; concentrated in a rotary evaporator and then gently and carefully open-dried over water bath at 40°C. The solid extracts were then kept in a refrigerator at 4°C until required. A stock solution of 500 mg/ml was prepared for each extract by dissolving 5 g/10 ml in normal saline or normal saline in 10% dimethyl sulfoxide (DMSO) (methanol extracts only). By serial dilutions, different extract solutions of 50, 100, 200, 300 mg/ml were then prepared from the stock solution.

Trypanosomes isolates

T. brucei brucei (Federe strain) was obtained from the Parasitology Section of Nigerian Institute for Trypanosomiasis Research (NITR), Vom, Nigeria. The parasites were maintained by serial passage in donor mice. Characteristically, this strain produces a severe acute infection in all small laboratory animals and if not treated all animals rarely survived more than 7 days corresponding to the first wave of parasitaemia.

Experimental animals

Forty-five adult male and female albino mice (30 – 35 g) and thirty-two rats (160 – 225 g) were purchased from the National Veterinary Research Institute (NVRI), Vom, and the Department of Biochemistry, Bayero University Kano, Nigeria, respectively. All animals were fed on standard animal feeds (Vital feeds, Nigeria Ltd) and water *ad libitum.* For studies on antitrypanosomal activity and survival, 45 mice were divided into 3 groups of 15 mice and each group is made up of 5 sub-groups of 3 mice. For studies on biochemical parameters, 32 rats were divided into four Groups (A, B, C and D) of 8 rats each.

Determination of acute toxicity (LD_{50}) of crude extracts

The median lethal dose (LD_{50}) in rats and mice was determined according to the method of Lorke (1983).

Assay for anti-trypanosomal activity (phase I)

V. amygdalina and *H. acida* extracts were assayed for anti-trypanosomal activity in *T. brucei brucei* infected mice. Briefly, 45 mice inoculated intraperitoneally with 10^5 parasites were divided into 3 groups of 15 mice and each group is made up of 5 sub-groups of 3 mice. At 48 h post-inoculation, each sub-group was treated intraperitoneally for 7 days with extract at dosages of 50, 100, 200, 300 and 500 mg/kg/day. Another group of 3 mice served as control. Parasitaemia was monitored and checked daily according to the method of Herbert and Lumsden (1976). Mice that survived more than 30 days beyond the death of untreated controls, with no parasites in their blood, were considered cured (Cyrus et al., 1998).

Effect of extract on biochemical parameters (phase II)

Thirty-two rats divided into four Groups (A, B, C and D) of eight rats each were inoculated (A and B only) intraperitoneally with 10^5 parasites. At 48 h post-inoculation, when parasites begin to appear in the circulation, intraperitoneal treatment with the methanol extract of *V. amygdalina* leaf commenced for Group A at 300 mg/kg/day. Groups C and D received extract at 300 mg/kg/day and normal

Table 1. Anti-trypanosomal activity of *Vernonia amygdalina* and *Hymenocardia acida* extracts against *Trypanosoma brucei brucei* infection in mice.

Extract	Dosage (mg/kg/day)	Number of animals	Treatment duration (days)	Maximum survival time (days)	Number cured
Hymenocardia acida Root bark (dichloromethane)	50	3	7	10	0
	100	3	7	9	0
	200	3	7	9	0
	300	3	7	9	0
	500	3	7	9	0
Vernonia amygdalina Leaf (aqueous)	50	3	7	11	0
	100	3	7	12	0
	200	3	7	12	0
	300	3	7	12	0
	500	3	7	11	0
Vernonia amygdalina Leaf (methanol)	50	3	7	13	0
	100	3	7	16	0
	200	3	7	22	0
	300	3	7	24	0
	500	3	7	14	0
Control	-	3	-	8	-

Groups that did not survive more than 8 days are not shown.

saline, respectively, while Group B was infected control. Parasitaemia was monitored and checked daily, while packed cell volume (PCV) and animal's weight were taken every 48 h and daily, respectively.

Sample collection and preparation

All the animals in Groups A to D were anaesthetized in slight chloroform and then decapitated 4 days post treatment (day 6 pi). Plasma and serum samples were prepared in duplicate and frozen. The tissues (liver and kidney) were excised, rinsed with cold water, blotted with filter paper and weighed. One part was homogenized in ice-cold 0.25 M sucrose solution and the other part homogenized in ice-cold phosphate-saline (50 mM sodium phosphate and 150 mM NaCl, pH 7.4) and kept frozen until required.

Biochemical assays

Randox assay kits (Antrim, UK) were used to assay for alanine aminotransferase (ALT) and aspartate aminotransferase (AST) activities by Reitman and Frankel (1957); total protein by Weichselbaum (1957); urea by Fawcett and Scott (1960); creatinine by Bartels and Bohmer (1972); and triglycerides by Jacobs and Van Demark (1960). Lipid peroxidation as evidenced by the level of malondialdehyde (MDA) was assayed according to the method described by Akanji et al. (2009).

Reduced glutathione (GSH) was assayed as described by Rajagopalan et al. (2004). Catalase (CAT) and superoxide dismutase (SOD) activities were assayed according to the methods of Beers and Sizer (1952) and Winterbourne et al. (1975), respectively.

Statistical analysis

All data were analyzed by analysis of variance (ANOVA) to test for statistical difference within all the groups and Duncan's test for significant difference between two groups' means. Significance was determined at 95% confidence limit and all results expressed as mean ± SD.

RESULTS

Acute toxicity of the extract

The LD_{50} of methanol extract of *V. amygdalina* leaf in mice and rats was found to be ≥ 5000 mg/kg. Thus, according to Lorke (1983), it is considered safe.

Effect on survival (phase I)

Table 1 gives a summary of the anti-trypanosomal activity of the extracts tested. None of the extracts cured infected mice as they all eventually died. However, the methanol extract of *V. amygdalina* leaf significantly increased the maximum survival days of mice to 24 days compared to 12, 10 and 8 days for aqueous extract *of V. amygdalina* leaf, dichloromethane extract of *H. acida* root bark, and untreated infected control, respectively. Hence, only the active methanol extract of *V. amygdalina* leaf was used in subsequent studies.

Table 2. Effect of methanol extract of *Vernonia amygdalina* leaf on some serum biochemical parameters in acute *T. b. brucei* Infection in rats.

Parameter	Infected treated (A)	Infected Control (B)	Uninfected treated (C)	Uninfected-untreated (D)
ALT activity (U/l)	54.8 ±2.61^{ac}	69.2 ±6.32^{a}	27.6 ±7.13^{b}	40.53 ± 3.72^{bc}
AST activity (U/l)	322.2 ± 53.06^{a}	463.0 ± 43.93^{b}	222.71 ±39.09^{c}	232.71 ± 40.77^{c}
Total Protein (g/dl)	5.76 ± 0.61^{a}	6.83 ± 0.22^{a}	7.61 ± 1.00^{a}	6.83 ± 0.84^{a}
Albumin (g/dl)	2.48 ± 0.56^{a}	3.27 ± 0.79^{a}	2.89 ± 0.59^{a}	3.03 ± 0.59^{a}
Creatinine (µmol/l)	174.0 ± 71.03^{a}	108.75 ± 43.5^{a}	139.2± 47.65^{a}	188.5 ± 101.7^{a}
Urea (mmol/l)	3.2 ±0.52^{ac}	4.16 ± 0.82^{a}	2.71 ± 0.29^{bc}	4.09 ± 0.42^{a}
Triglycerides (mg/dl)	169.57 ± 64.34^{bc}	632.91 ±125.89^{a}	294.2 ± 122.16^{bc}	406.57 ± 58.57^{b}
MDA (mmol/l)	0.15±0.13^{a}	0.20 ±0.02^{b}	0.17± 0.03^{ac}	0.17± 0.02^{ac}
SOD activity (U/mg protein)	81.67±3.73^{a}	68.3±5.89^{a}	75.00±20.71^{a}	65.00±6.97^{a}
Catalase activity(U/mg protein)	0.99± 0.67^{a}	0.62± 0.50^{a}	0.73 ±0.31^{a}	0.44± 0.69^{a}

All values are mean ± SD of five replicate determinations. Same superscripts indicates values are not significantly different at $p > 0.05$, while different superscripts indicates values are significantly different at $p<0.05$. ALT, alanine aminotransferase; AST, aspartate aminotransferase; SOD, superoxide dismutase, MDA, malondialdehyde.

Table 3. Effect of methanol extract of *Vernonia amygdalina* leaf on some biochemical indices of oxidative stress in organs of *T. b. brucei*-infected rats.

Parameter	Infected treated (A)	Infected control (B)	Uninfected treated (C)	Uninfected-untreated (D)
Liver SOD activity (U/mg protein)	76.05 ± 11.46^{a}	50 ± 16.67^{b}	91.7 ± 8.33^{c}	54.2 ± 29.17^{b}
Kidney SOD activity (U/mg protein)	42.00 ± 13.50^{a}	37.67 ± 19.67^{ac}	33.14 ± 13.27^{bc}	24.71 ± 21.96^{b}
Liver Catalase activity (U/mg protein)	1.39 ± 0.51^{a}	1.28 ± 0.96^{a}	1.22 ± 0.63^{a}	1.08 ± 0.27^{a}
Kidney Catalase activity (U/mg protein)	1.74 ± 0.80^{a}	1.21 ± 1.08^{a}	1.23 ± 0.28^{a}	0.57 ± 0.34^{a}
Liver GSH (mmol/g tissue)	0.64 ± 0.22^{a}	0.52 ± 0.26^{a}	0.42 ± 0.12^{b}	0.38 ± 0.10^{b}
Kidney GSH (mmol/g tissue)	0.89 ± 0.41^{a}	0.54 ± 0.20^{b}	0.46 ± 0.12^{b}	0.40 ± 0.13^{b}
Liver MDA (mmol/g tissue)	0.27 ± 0.06^{a}	0.31 ± 0.09^{a}	0.28 ± 0.07^{a}	0.30 ± 0.07^{a}
Kidney MDA (mmol/g tissue)	0.21± 0.03^{a}	0.25± 0.03^{b}	0.21± 0.02^{a}	0.25± 0.02^{b}

All values are mean ± SD of five replicate determinations. Same superscripts indicates values are not significantly different at $p > 0.05$, while different superscripts indicates values are significantly different at $p<0.0$. SOD (superoxide dismutase); GSH (glutathione reduced); MDA (malondialdehyde).

Effect on biochemical changes (phase II)

Table 2 presents the effects of methanol extract of *V. amygdalina* leaf on some biochemical parameters in *T. b. brucei* infected rats. Infection (Group B) significantly increased ($p < 0.05$) ALT, AST, CAT and SOD activities, as well as MDA and triglycerides serum levels compared to normal controls. Treatment (Group A) significantly decreased ($p < 0.05$) ALT, and AST activities, and MDA and triglycerides levels, while it increased insignificantly ($p> 0.05$) the CAT and SOD activities. However, no significant differences ($p > 0.05$) observed in total protein, albumin and creatinine in all the groups.

Table 3 shows the effect of the extract on some biochemical indices of oxidative stress in liver and kidney of *T. b. brucei* infected rats. No significant difference ($p > 0.05$) was observed in liver MDA levels in all the groups. MDA levels in kidney was lower ($p < 0.05$) in infected-treated rats compared to infected control.

Groups A and C both have higher SOD activities in both Kidney and liver compared to Groups B and normal control. Catalase activity was not significantly affected by the extract. Reduced glutathione levels in liver and kidney were increased by infection and treatment of infected rats with the extract caused further increase. Figure 1 shows the effect of the methanol extract of *V. amygdalina* leaf on parasitaemia in acute *T. b. brucei* infection of rats. The parasitaemia of treated rats (Group A) was significantly ($p < 0.05$) lower than untreated rats (Group B).

In addition, Figure 2 shows the effect of the extract on the PCV of *T. b. brucei* infected rats. The PCV of infected treated rats (Group A), although decreased, was still significantly ($P<0.05$) higher than infected control.

Table 4 shows the effect of the extract on relative organ

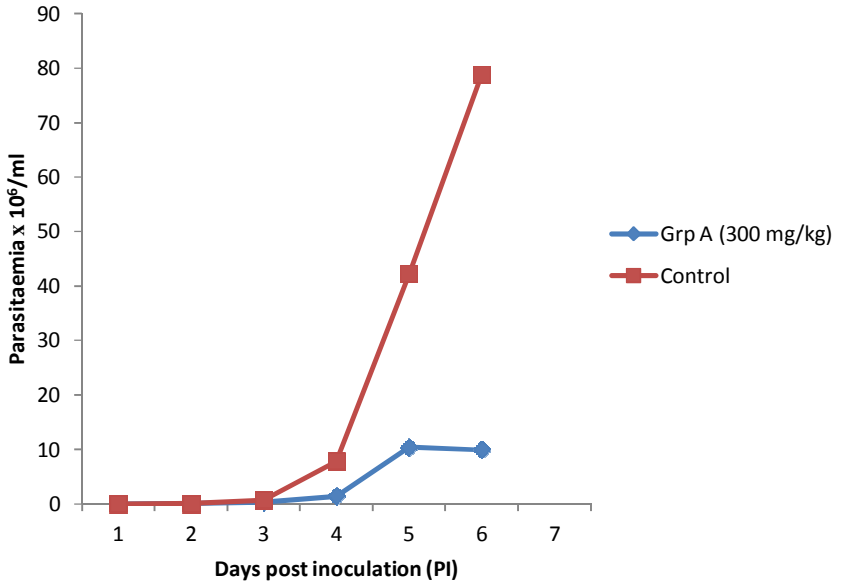

Figure 1. Effect of methanol extract of *Vernonia amygdalina* leaf on parasitaemia in acute *T. b. brucei* infection in rats.

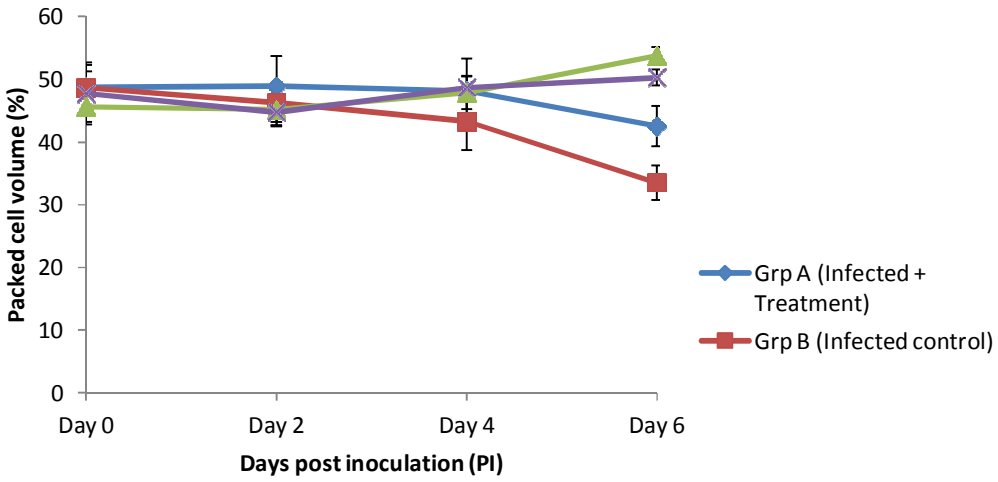

Figure 2. Effect of methanol extract of *Vernonia amygdalina* leaf on packed cell volume in acute *T. b. brucei* Infection of rats.

Table 4. Effect of methanol extract of *V. amygdalina* Leaf on relative organ weight in acute *T. b. brucei* Infection in rats.

Parameter	Infected Treated (A)	Infected Control (B)	Uninfected Treated (C)	Uninfected- untreated (D)
Liver (%)	4.20 ± 0.43^{a}	4.30 ± 0.82^{a}	3.60 ± 0.69^{b}	3.60 ± 0.17^{b}
Kidney (% x 10^{-1})	8.30 ± 0.50^{a}	7.80 ± 1.30^{a}	8.00 ± 0.90^{a}	7.60 ± 0.90^{a}

All values are mean ± SD of five replicate determinations. Same superscripts indicates values are not significantly different at $p > 0.05$, while different superscripts indicates values are significantly different at $p<0.05$.

weight as an indicator of organ inflammation. Groups A and C showed slightly higher but insignificant ($p > 0.05$) relative kidney weight compared to Groups B and D. Also, Groups A and B showed higher and significant ($p <$

0.05) relative liver weight compared to Groups C and D.

DISCUSSION

Acute toxicity study of methanol extract of *V. amygdalina* leaf showed this extract to have a median lethal dose (LD_{50}) ≥ 5000 mg/kg/day. Therefore, it can be considered safe of acute toxicity. This comes as no surprise at all, since *V. amygdalina* is a well known and common vegetable used and eaten by animals and many people. In fact, it is a delicacy in many homes in Nigeria, and other African countries (Bonsi et al., 1995a).

Results on the antitrypanosomal activity show that among the 12 extracts tested, only the methanol extract of *V. amygdalina* leaf possessed potential activity against *T. brucei brucei.* All the untreated control mice died within eight days post-inoculation. Mice treated with 50, 100, 200, 300 and 500 mg/kg/day of dichloromethane extract of *H. acida* root bark, and aqueous extract of *V. amygdalina* leaf, all died within 10 and 12 days post-inoculation, respectively. However, mice treated with methanol extract of V*. amygdalina* leaf survived longest with significant survival days of 22 and 24 at 200 and 300 mg/kg/day, respectively. This may be due to the ability of the methanol extract of *V. amygdalina* leaf to keep the parasitaemia of treated mice low compared to other extracts and thus made the mice able to survive the infection longer. It is pertinent to mention here that four doses of the methanol extract of *V. amygdalina* leaf (100, 200, 300 and 500 g/kg/day) temporarily cleared the parasites from circulation for 24 to 72 h, before relapse occurred and the mice eventually died. The promising antitrypanosomal potential shown by methanol extract of *V. amygdalina* leaf is further supported by similar results obtained in other studies on parasitic protozoans. Anthonia and Benjamin (2003) reported that *V. amygdalina* leaf extract suppressed parasitaemia by 67% in mice infected with *Plasmodium berghei*. Similarly, we have earlier observed methanol extract of *V. amygdalina* leaf to have strong *in vitro* antitrypanosomal activity (Yusuf et al., 2012).

The parasite *T. brucei brucei* (Federe strain) produced a very severe acute infection in inoculated rats. The parasitaemia of untreated infected rats continued to rise steadily and three rats died on day 6 post-inoculation. However, the methanol extract of *V. amygdalina* leaf was able to significantly keep the parasitaemia of treated rats very low and none of these rats died day 6 post-inoculation. Indeed, three rats in this Group A did not show parasites in their circulation for 48 h corresponding to day 3 to 5 of treatment (day 5 to 6 post-inoculation). This does not mean the three rats were cured since the experiment was terminated on day 6 post-inoculation, but it was a good indication of the potential *in vivo* antitrypanosomal activity of the extract.

The packed cell volume (PCV) of Group B rats continued to decrease and the animals developed early signs of anaemia with eventual death of three rats on day 6 pi. Decrease in PCV is an early sign of anaemia, which is a cardinal symptom associated with animal trypanosomiasis (Esievo and Saror, 1991). Expectedly, the drop in PCV corresponds with the period of progressive increased in parasitaemia. The PCV of infected treated rats also decreased, but it was still significantly higher than that of the infected control rats at day 6 pi. The extract also improved the PCV of treated uninfected rats (Group C) compared to normal healthy rats (Group D).

The results on organ/body weight ratio showed that *T. brucei brucei* infection had caused significant ($p < 0.05$) inflammation of liver in both infected treated (Group A) and infected control (Group B) compared to normal control (Group D) and treated control (Group C). No significant difference ($p > 0.05$) was observed in the kidney. Umar et al. (2007) had also reported hepatomegaly in trypanosomal infection.

Triglycerides levels in infected controls significantly increased and methanol extract of *V. amygdalina* leaf decreased the triglycerides levels significantly in infected treated and uninfected treated control. The increased triglycerides in infected control may be due to mobilization of lipid from adipose tissue for energy production especially as this group had lost appetite, which is a cardinal symptom of animal trypanosomiasis. In fact this group (infected control) had started emaciating probably due this lipid mobilization coupled with loss of appetite. On the hand, methanol extract of *V. amygdalina* leaf treated groups appeared as physically good as the normal control.

Alanine and aspartate aminotransferases (ALT and AST) are marker enzymes for liver function. In this study, the ALT activity significantly ($p < 0.05$) increased in infected control group compared to normal healthy group. The ALT activity in treated infected group also increased but insignificantly ($p > 0.05$) compared to the normal healthy group. Similar pattern was seen for AST activity although the difference between infected treated group and infected control was significant. The increase in ALT and AST activities may be related to the observed liver inflammation and is an indication of abnormal function of the liver. The elevation of these enzyme levels recorded here is in agreement with earlier reports from natural and experimental infected animals (Singh and Gaur, 1983; Awobode, 2006; Umar et al., 2007). The results suggest probable infiltration of vital body organs and inflammation particularly of liver, muscles, and kidneys by *T. b. brucei.* Elevated enzyme levels may also result from effect of trypanosome lyses resulting from the host's defense mechanisms (Kennedy, 2004).

Data in the present investigation showed that the infected treated group had lower MDA levels compared to infected control group. The lower MDA levels in the infected treated group suggest that the extract was able

to protect these animals against lipid peroxidation. The extract may have also prevented or delayed the on-set of oxidative stress which arises when there is an imbalance between radical-generating and radical-scavenging activity. Ogunsanmi and Taiwo (2007) have earlier demonstrated that oxidative stress plays an important etiologic role in the pathogenesis of trypanosomiasis. Similarly, it has also been shown that infections by the *T. brucei* group of parasites may alter the host's antioxidant defense against free radicals (Igbokwe et al., 1996; Omer et al., 2007).

Aleksandro et al. (2009) reported that plasma MDA levels were significantly higher in the infected group than in the uninfected group, and in animals with acute (day 21) and chronic (day 49) trypanosomiasis, MDA levels were found to be proportional to the time of infection. Increase in MDA concentrations have been related to the amount of stress and are well correlated with lipid membrane damage and deterioration of membrane integrity. Increased generation of MDA in plasma, tissues and erythrocytes was also reported in murine models and humans infected with *Trypanosoma cruzi* (Malvezi et al., 2004).

The results of the effect of the extract on endogenous antioxidants show that the infected control had higher GSH than normal control. This may be because their antioxidant defense system, which included GSH, was mobilized to fight the presence of the parasites. It appeared that the antioxidant defense system of these animals was not yet suppressed or exhausted at the very early stage of infection, which in this case is a 6 days sub-acute infection. Importantly, the extract was able to increased GSH levels of both infected treated and uninfected treated groups compared to infected and normal control groups. Perhaps the extract was able to stimulate *de novo* synthesis of glutathione or spared endogenous GSH, to fight trypanosomes-generated free radicals, which could also explain the observed lower MDA levels in treated groups.

Previous studies showed a decrease in erythrocytic and hepatic glutathione concentrations in rats infected with *T. brucei* (Ameh, 1984). Significant decrease in GSH concentrations in the tissues and serum of infected rats compared to other groups might be attributed not only to the oxidation of GSH to GSSH by activated oxygen produced as a result of trypanosome infection (Igbokwe et al., 1996; Ogunsanmi and Taiwo, 2007), but also to high increase in the glutathione peroxidase activity (Omer et al., 2007) since the reaction catalyzed by this enzyme consumes GSH.

For the endogenous antioxidant enzymes, no change in the catalase (CAT) activity was observed in all the groups in serum, kidney and liver. In liver, superoxide dismutase (SOD) activity decreased in infected control compared to normal control, but increased in treated groups. However, in both kidney and serum, SOD activity increased in infected control compared to normal control. Some investigators have reported decrease of SOD activity in infected animals (Omer et al., 2007) as observed in liver in the present study. However, others reported increased SOD activity (Ogunsanmi and Taiwo, 2007) as seen in the serum and kidney in the present work. Ataley et al. (2000) also reported that under condition of oxidative stress, activities of antioxidant enzymes such as SOD, CAT and glutathione peroxidase increases. In all the tissues, the extract caused increased SOD activity compared to normal control. This is beneficial since in an environment of high oxidants such as trypanosomal infections, the need to mop up the oxidants is important in order to prevent oxidative stress.

Taken together, it will appear that tissue susceptibility and intensity of lipid peroxidation and oxidative stress depends on many factors which includes; lipid content of the tissues, the specie of trypanosomes, strain difference within same species, host animal, and importantly the duration of the infection. In addition, the levels of MDA and antioxidants also appear to be affected by sample preparation and the time it takes during storage. It is advisable to analyze samples within 24 to 48 h.

Erasto et al. (2007) had reported the antioxidant activity of *V. amygdalina*. Similarly, we have also found this extract to contain significant amount of antioxidant vitamins: C and E, and also phenols and flavonoids (unpublished). The antioxidative effect of many medicinal plants is mainly due to phenolic components, such as flavonoids, phenolic acids, and phenolic diterpenes (Shahidi et al., 1992). The ability of vitamin C and E to reduce the severity of anemia in *T. b. brucei* infected animals have all been reported (Umar et al., 2007).

Conclusion

Our findings support earlier reports on biochemical changes induced by trypanosomal infections. Methanol extract of *V. amygdalina* leaf showed no sign of acute toxicity. Indeed, it is beneficial to the animals as it lowered parasitaemia and improves their PCV as well as improved the oxidative status of the infected animals; it also protected them against liver damage by significantly decreasing ALT and AST activities. Further works are on-going to fully exploit the beneficial effects of this extract on different trypanosomal infections.

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