Investigation on the trypanocidal effects of aqueous extracts of *Vernonia amygdalina* and *Nauclea latifolia* in albino rats

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Abstract: An evaluation on the efficacy of aqueous extracts of *Nauclea latifolia* and *Vernonia amygdalina* in the treatment of *Trypanosoma brucei brucei* infection in Wister albino rats was carried out. Parasite population after infecting 27 rats was determined using Herbert and Lumsden's rapid matching methods. Concentrations of 1000 and 2000mg/kg were administered orally to the respective groups. Both the T-test and two -way ANOVA carried out on the effect of the treatments and parasite and on the weights and blood parameters analyzed showed no significant difference (P> 0.05). The screening showed that the two different concentrations (2000 and 1000mg/kg) of *Vernonia* leaf extract and 2000mg/kg of the stem bark extract had the capacity to clear the parasites from the rats' blood for some days and also prolonged their life span after treatment. The *N. latifolia* leaf extract irrespective of the concentration had no effect on the number of parasites (parasitaemia) in the infected rats. The *Nauclea latifolia* stem bark, however, showed some trypanocidal activities by reducing the parasitaemia level from 8.4 million per ml to 5 thousand per ml on the 5th day; although the level of parasitaemia began to increase again from the 6th day until the day the animals died. Prepared sections of the liver and kidney tissues showed no major morphological abnormalities in those rats administered with the plant extracts when compared to the controls. This study suggested that the aqueous extracts of *V. amygdalina* plant and *N. latifolia* stem bark has antitrypanosomal activity and the capacity to prolong the life span of the experimental rats after treatment.
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Keywords: antitrypanosome, *Nauclea latifolia*, parasitaemia *Trypanosoma brucei brucei, Vernonia amygdalina*

1. Introduction

Trypanosomiasis is a worldwide disease caused by the species of the genus *Trypanosoma*, which affects humans, as well as domestic and wild animals. *Trypanosoma brucei* (Primmer and Bradford) is a parasitic protist species that causes Human African trypanosomiasis (or sleeping sickness) in humans and nagana in animals in Africa. Plants have been used thousands of years ago as the major source of drugs both in orthodox and traditional medicine. Plants have been used by man for prevention, treatment and management of diseases for a very long time without even the knowledge of the component and toxicity of the plant. Previous studies have reported antitrypanosomal potential of many medicinal plants (Biobaku *et al.,* 2009; Olukunle *et al.*, 2010; Mann *et al.,* 2011). *V. amygdalina* is traditionally used for analgesia and in the treatment of malaria infections (Anoka *et al*., 2008). It is used as anthelmintic, antiprotozoal and antibacterial agent (Huffman *et al*., 1996). The antitumour and antimicrobial properties of *V. amygdalina* have been traced to its bioactive principles (Koshimizu *et al*., 1994). It has hypoglycemic, antineoplastic and antibacterial properties (Izevbigie *et al*., 2004; Iwalewa *et al*., 2003). *V. amygdalina* has shown antiplasmodial properties, which has been attributed to the sequesterpene lactones isolated from the leaves (Masaba, 2000; Abosi and Raseroka, 2003; Tona *et al*., 2004). In Kano (Nigeria) *N. latifolia* is used as a chewing stick and as a remedy against stomach ache and tuberculosis (Deeni and Hussain, 1991). In Ivory Coast infusions and decoctions from stems and roots of *N. latifolia* are used against malaria by traditional healers (Benoit-Vical *et al*., 1998).The plant is also used in the treatment of ailments like malaria (Kokwaro, 1976; Akabue and Mittal, 1982; Boye, 1990), gastrointestinal tract disorders (Maduabunyi, 1995), sleeping sickness (Kerharo, 1974), prolong menstrual flow (Elujoba, 1995), hypertension (Akabue and Mittal, 1982) and as a chewing stick (Asubiojo *et al*., 1982). In Gabon, Congo and Nigeria infusions of leaves and bark are employed against fevers (DiGeorgio *et al*., 2006). In Kinshasa, DR Congo extracts and preparations together with other plants are applied against diarrhea (Tona *et al.*, 1999).

2. Methodology

2.1 Plant materials and preparation

Fresh leaves and stem barks of *Vernonia amygdalina* and *Nauclea latifolia* were harvested in

bushes around the University of Abuja, mini campus, Gwagwalada. Plant identification was carried out at the Biological garden of the Department of Biological Sciences of the University and at the National Institute for Pharmaceutical Research and Development (NIPRD), Idu, Abuja and a specimen of each of the plants was deposited there. Identification numbers of the plant specimens are as follows: *Vernonia amygdalina*- NIPRD/H/6555 and *Nauclea latifolia*- NIPRD/H/6559. Extraction and concentration of plant extracts was done according to Cuellar Cuellar and Okori (2010). The dried extracts were stored in small plastic containers (20ml capacity). The resulting extracts were reconstituted in distilled water to give the required doses of 1000 and 2000 mg/kg body weight respectively.

2.2 Animal grouping, infection and treatment

Twenty seven (27) healthy animals were used and grouped into nine groups of 3 rats each. An infected blood containing eleven (11) parasites per microscope field (X40 objective lens) after mixing with 5% dextrose saline was sub-inoculated into the experimental albino rats. Each rat received 0.3ml IP (intraperitoneal route) using 1ml syringe. Levels of parasitaemia were checked and extract administration started 3 days after.

The nine groups were labeled VL1, VL2, VS1, VS2, NL1, NL2, NS1, NS2 and C. Groups VL1 and VL2 were given 2000 and 1000mg/kg of *V. amygdalina* aqueous leaf extract respectively. Groups VS1 and VS2 were given 2000 and 1000mg/kg of *V. amygdalina* aqueous stem bark extract respectively. Groups NL1 and NL2 were given 2000 and 1000mg/kg *N. latifolia* aqueous leaf extracts respectively. Groups NS1 and NS2 were given 2000 and 1000mg/kg of *N. latifolia* aqueous stem bark extract respectively. Each of the extract was dissolved in 5ml of distilled water before administration. Each animal was given 1ml of extract as a daily dose for 5days. Group C served as the control group and was given 1ml of distilled water daily for five days. The extracts were administered orally using intragastric cannula attached to a 2ml syringe. After administration each animal was returned to its designated cage. Food and water were given *ad libitum*. The concentration for each group were arrived at using this formula

$$\frac{\text{Average weight of rats in each group x wt of extract}}{\text{1000g body weight (1kg)}}$$

2.3 Parasitological analysis

Blood samples obtained from the tail vein of infected rats were examined in wet mount under light microscope at ×40 magnification. Parasites were determined using the "Rapid matching method (Herbert and Lumsden, 1976). The method involves microscopic counting of parasites per field in pure blood or blood appropriately diluted with buffered phosphate saline (PBS, pH 7.2). Logarithm values of these counts obtained by matching with the table of Herbert and Lumsden (1976) is converted to antilog to provide absolute number of trypanosomes per ml of blood. Blood samples for parasite detection were taken 3days after infection and on daily basis thereafter. Drugs were administered on the third day post-infection and the blood analysis for parasite counts were done daily thereafter.

2.4 Collection of blood for haematological examination

Blood samples were collected through heart puncture of each chloroform anaesthetized rat into different EDTA bottles. The blood samples were analyzed for packed cell volume (PCV), haemoglobin concentration, mean corpuscular haemoglobin concentration (MCHC) and white blood cell (WBC) counts.

2.5 Post-mortem examination

Kidneys and livers of the animals in all the groups were collected and preserved in 10% buffered formal saline (10% buffered formalin in 0.85% saline solution was used in fixing the organs obtained from the rats) inside pre-labeled specimen bottles on the 14th day until they undergo histopathological analysis using the method of Carleton (1967).

2.6 Statistical analysis

The parasitaemia of the experimental animals before, during and after treatment was plotted on graphs to show the effects of the different plant extracts and concentrations on the number of parasites per ml of the experimental animals' blood. Two way analysis of variance without replication (two-way ANOVA) was used to analyze the relationship between haematological parameters and weight of the infected and treated animals and the different plants and concentrations of the plant parts.

3. Results

3.1 Body weights of animals infected and treated with the plant extracts

The weights of the infected and treated albino rats by their groups are shown on Table 1. The T-test analysis using Microsoft Excel 2007 showed that there is no significant difference in the weight of the experimental rats in relation to the different plant parts and concentrations ($p>0.05$).

3.2 Red blood cell indices and wbc in infected and treated rats

The result of the red blood cell (PCV) indices and white blood cell counts (WBC) of the infected and treated rats and the infected but not treated rats (control) are shown on Table 2. The PCVs and haemoglobin concentrations of the animals given *V.*

amygdalina stem bark extracts (groups VS1and VS2) were better (45.60%, 15.10g/dl and 40.00%, 13.10g/dl respectively) than the other groups while those given *N. latifolia* stem bark extracts (NS1 and NS2) had the lowest (27.55% , 9.75g/dl and 27.50%, 9.18g/dl respectively).

Table 1: Mean body weight of rats infected and treated with the plant extracts

Group	Initial weight ± S.E	Weight during treatment ± S.E	Weight after treatment ± S.E
VL1	195.00±5.00	223.57±2.69	190.00±4.23
VL2	172.50±7.50	173.00±4.10	183.91±2.43
VS1	197.50±2.50	185.63±3.95	169.29±3.01
VS2	197.50±2.50	192.78±3.24	170.00±0
NL1	177.50±17.50	173.13±6.19	0
NL2	210.00±10.00	201.00±2.77	186.25±8.51
NS1	165.00±5.00	172.00±3.82	171.33±3.47
NS2	167.50±2.50	164.00±4.00	156.67±4.13
CONTROL	200.00±0	211.67±4.78	0

Key- S.E = Standard error

Table 2: Red blood cell indices and WBC of the experimental rats.

Group	PCV (%)±S.E	Hb Conc (g/dl) ±S.E	WBC Count ($x10^3$/ml) ±S.E	MCHC (g/dl) ±S.E
VL1	34.10±0.10	11.60±0.20	7.70±0.10	32.80±0.70
VL2	35.50±0.50	12.50±0.50	5.60±0.30	33.25±0.25
VS1	45.60±040	15.10±0.10	9.60±0.20	32.05±0.45
VS2	40.00±0	13.10±0.10	6.10±0.30	33.10±0.80
NL1	33.20±0.20	10.50±0.50	6.00±0.20	33.20±0.10
NL2	31.85±0.15	10.60±0.20	5.35±0.15	32.45±0.15
NS1	27.55±0.45	9.75±0.45	6.15±0.15	32.45±0.75
NS2	27.50±0.50	9.18±0.18	5.40±0.30	33.15±0.15
Control	44.05±0.15	14.40±0.10	8.20±0.20	32.70±0.20
Normal range	37.60-50.60	11.50-16.10	6.600-12.60	31.50-36.00

Key: S.E- Standard Error
VL1= *Vernonia* leaf extract 2000mg/kg. VS1= *Vernonia* stem bark 2000mg/kg
VL2=*Vernonia* leaf extract 1000mg/kg. VS2= *Vernonia* stem bark 1000mg/kg
NL1= *Nauclea* leaf extract 2000mg/kg. NS1= *Nauclea* stem bark 2000mg/kg
NL2= *Nauclea* leaf extract 1000mg/kg. NS2= *Nauclea* stem bark 1000mg/kg

There was no significant difference in the effects of any of the plant extracts and the different concentrations on the different blood parameters ($p=0.1419$ and 0.3052) but there was significant difference among the blood parameters ($p< 0.05$).

3.3 Parasitological analysis

Figure 1 showed parasitaemia in relation to the days of treatment and after treatment using *V. amygdalina* leaf extract. The two concentrations used (2000 and 1000mg/kg) were compared with the control. There were total clearance of the parasite from the blood on the day 3 (4th day of treatment) and day 6 (2days after treatment) of treatment with 2000mg/kg and 1000mg/kg respectively. This lasted until day10 for group VL1 and day 8 for group VL2. Parasitaemia started increasing slowly again on day 9 in group VL2 until the last animal in the group died on day 15 while parasitaemia in group VL1 started increasing again on day 11 until day18 (14th day after treatment) when the rats were sacrificed.

Parasite number increased from day 0 (1st day of treatment) until day 4(8days after infection) when the last animal in the control group died. Parasites came up averagely in the blood of group VS1 rats on the 3rd day of treatment (day2) got cleared from the blood again on day 3(4th day of treatment) until day 8 (Figure 2). The parasites came up again on day 9 and increased until day 18 when the last 2 animals in the group were sacrificed. However, parasitaemia increased in group VS2 from day 0 to day 5. On day 6 a sharp decrease in the number of parasite was observed but there was no parasite clearance from the blood. The rats died on day 8 with high parasitaemia (Figure 2). Groups NL1and NL2 showed no decrease in the number of parasite and no parasite clearance was observed. The rats died on day 4 and 6

respectively (Figure 3). The groups given *N. latifolia* stem bark extract and the two concentrations (NS1 and NS2) showed no clearance of parasite from the blood but reductions in the number of parasites were observed on day 6 for NS1and on days 5and 6 in NS2. Increase in the number of parasites in groups NS1 and 2 were steady from day 7 respectively until day 10 when the increment became high. The rats in both groups died on day15 with high parasitaemia (Figure 4). The result of the trypanocidal screening of *V. amygdalina* and *N. latifolia* showed that the two different concentrations (2000 and 1000mg/kg) of *Vernonia* leaf extract have the capacity to clear the parasites from the rats' blood and also prolong their life span after infection and treatment. The plant's stem bark extract at a higher concentration of 2000mg/kg also showed similar activities. The *Nauclea* leaf extract irrespective of the concentrations seem to have little or no effect on the number of parasite (parasitaemia) in the infected rats. The *Nauclea* stem bark, however, showed some trypanocidal activities by reducing the parasitaemia on the 5th and 6th day after first day of treatment (day 0) before the parasitaemia started to increase again on the 7th day until the day the animals died.

3.4 Gross pathology

Control group: liver showed normal hepatocytes while kidney showed normal glomeruli and tubules (Plates 1&10).

Group VL1: the liver showed intense hepatocellular necrosis while kidney showed mild tubular necrosis [Plates 2&11].

Group VL2: the liver showed vascular congestion with moderate hepatocellular necrosis and sinusoidal congestion while the kidney showed intense glomerular and tubular necrosis [Plates 3&12].

Group VS1: the liver showed moderate hepatocellular necrosis while the kidney showed mild tubular necrosis (Plates 4&13).

Group VS2: the liver showed vascular congestion with kupfer cell hyperplasia while the kidney showed mild glomerular necrosis and lymphocyte hyperplasia (Plates 5&14).

Group NL1: liver showed kupfer cell hyperplasia, vascular congestion and lymphocyte hyperplasia with hepatocellular necrosis while the kidney showed mild glomerular necrosis (Plates 6&15).

Group NL2: liver showed lymphocyte hyperplasia and vascular congestion while the kidney showed mild tubular necrosis (Plates 7&16).

Group NS1: liver showed vascular congestion with mild hepatocellular necrosis and lymphocyte hyperplasia while the kidney showed normal features (Plates 8&17).

Group NS2: the liver showed vascular congestion and mild hepatocellar necrosis with sinusoidal congestion while the kidney showed normal features (Plates 9&18).

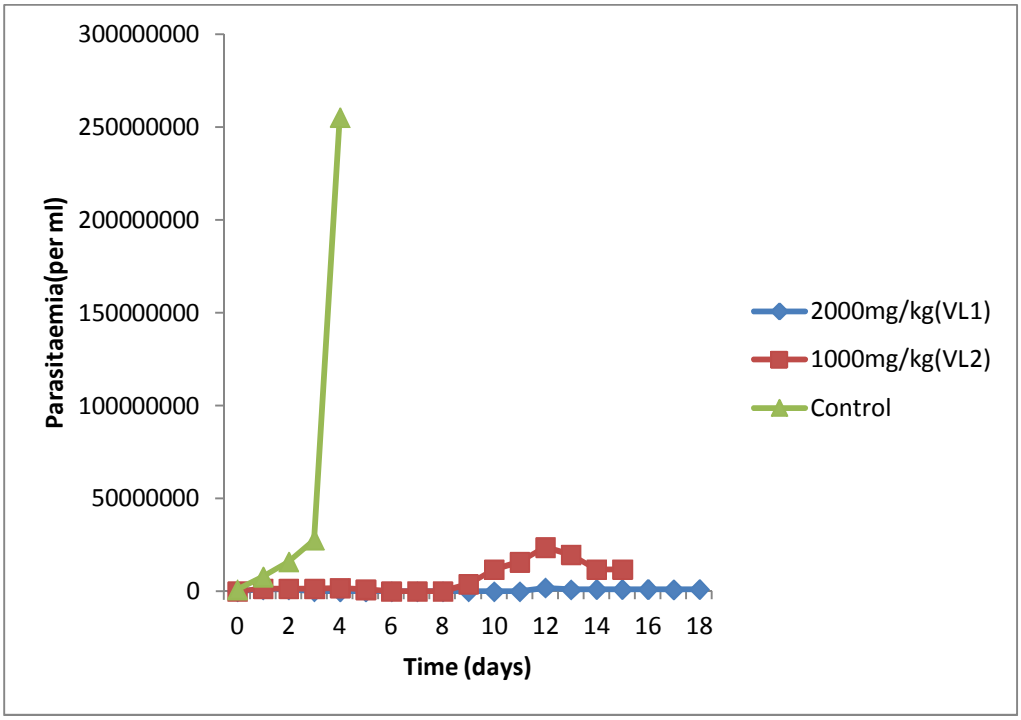


Figure 1: Antitrypanosomal activities of *V. amygdalina* aqueous leaf extract

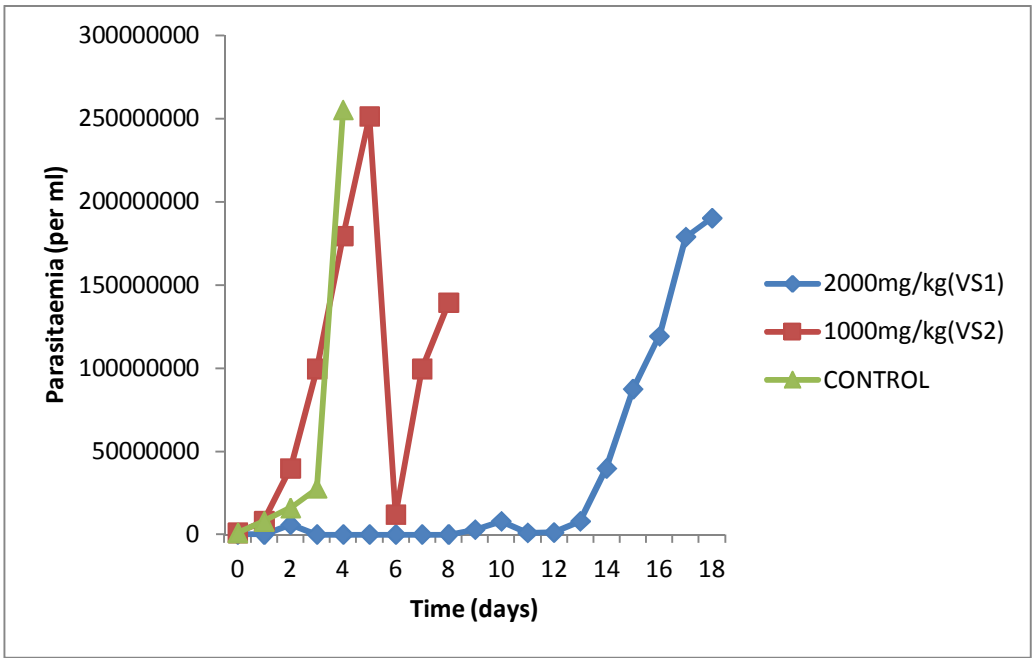


Figure 2: Antitrypanosomal activities of *V. amygdalina* aqueous stem bark extract

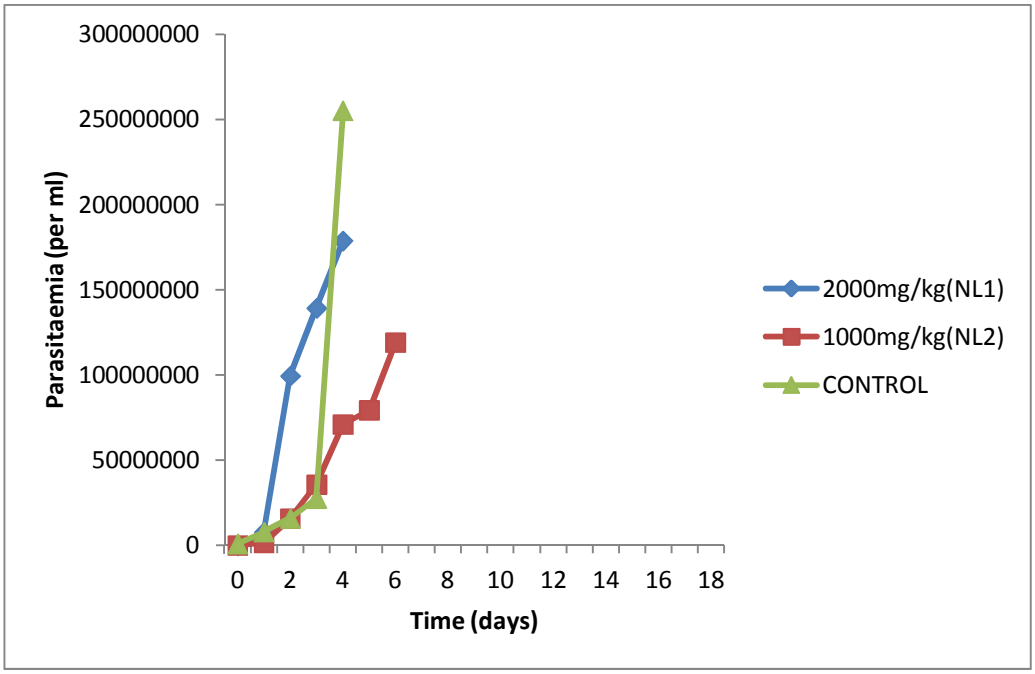


Figure 3: Antitrypanosomal activity of *N. latifolia* aqueous leaf extract

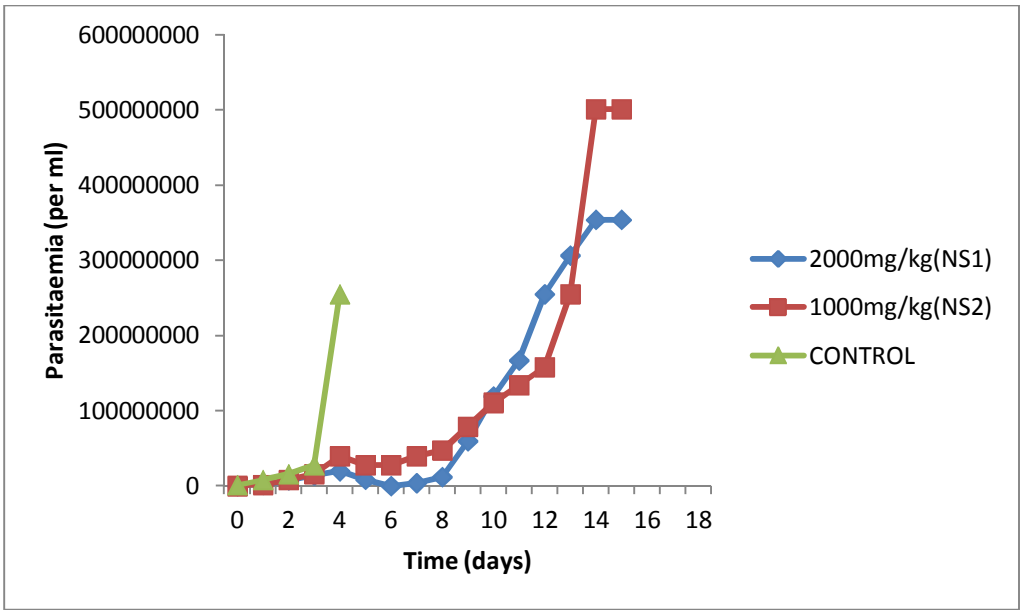
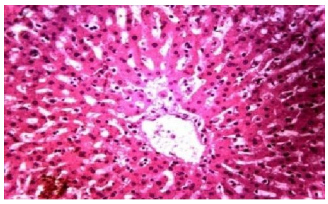


Figure 4: Antitrypanosomal activities of *N. latifolia* aqueous stem bark extract

The histopathology images of the liver and kidney of the infected and treated animals showed some vacuolations and necrosis. This has also been observed in the toxicity study which implies that the plant extracts had some histopathological effects on the internal organs although there may be physiological repair of the organs and eventually normalcy in the structure of the cells of the organs as it was observed in the animals in groups VL1 and NS1&2 (kidney). It is difficult to speculate the mechanism by which these extracts exhibit their trypanocidal action. However, accumulated evidences suggested that many natural products exhibit their trypanocidal activity by virtue of their interference with the redox balance of the parasites, acting either on the respiratory chain or on the cellular defenses against oxidative stress. This is because natural products possess structures capable of generating radicals that may cause peroxidative damage to trypanothione reductase that is very sensitive to alterations in redox balance. It is also known that some agents act by binding with the kinetoplast DNA of the parasite (Sepulveda-Boza and Cassels, 1996). Several findings have shown that *V. amygdalina* has strong antioxidant activity corresponding to mitigation of the generation of hydroxyl radicals. Yeh *et al.* (2003) and Battell *et al*. (1999) postulated that this antioxidant activity may provide possible rationale for the observed therapeutic effects of *V. amygdalina*.



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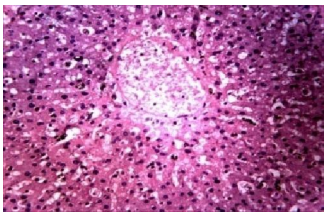

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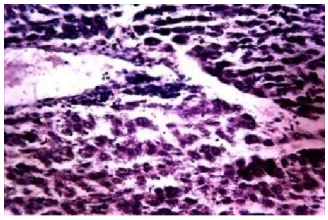

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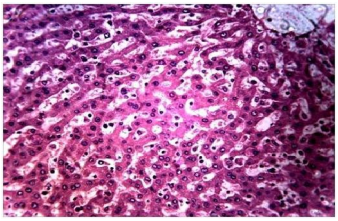

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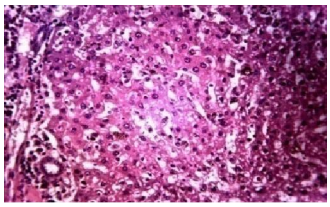

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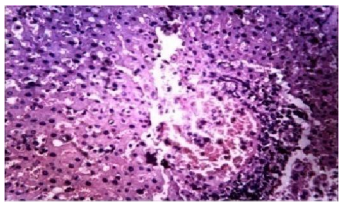

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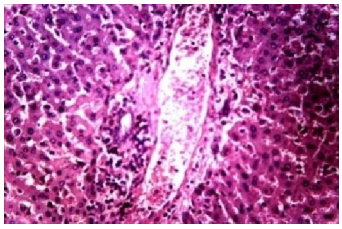

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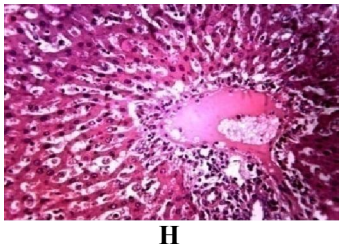

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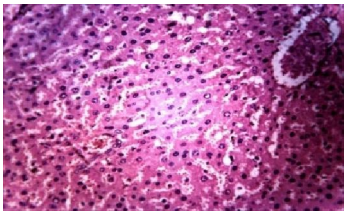

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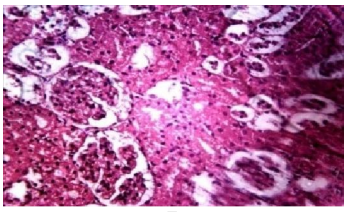

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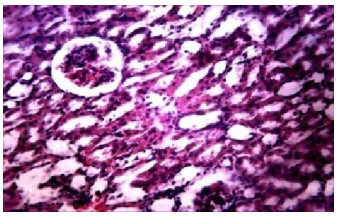

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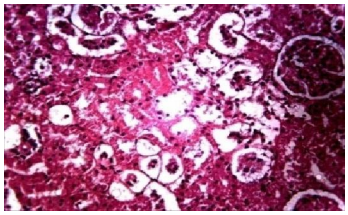

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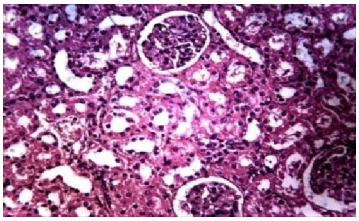

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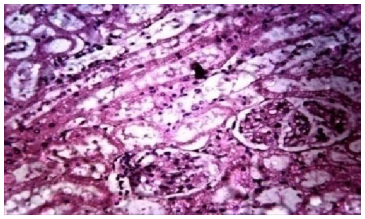

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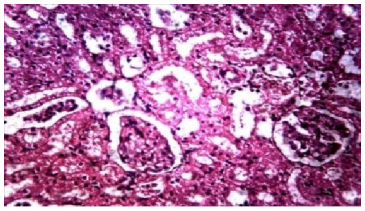

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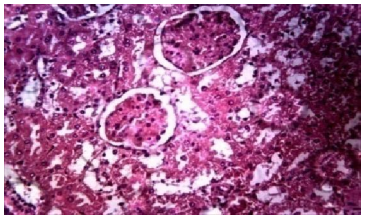

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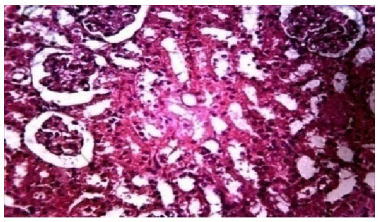

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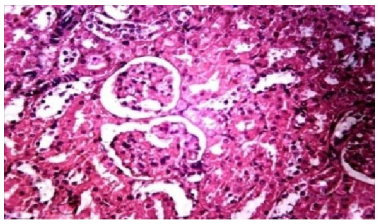

R

Plates a-r: Photomicrograph of liver and kidney tissues of the experimental animals. (a) Control group liver. (b) Group VL1 liver. (c) Group VL2 liver. **(d)** Group VS1 liver. (e) Group VS2 liver. (f) Group NL1 liver. (g) Group NL2 liver. (h) Group NS1liver. (i) Group NS2 liver. (j) Control group kidney. (k) Group VL1 kidney. (l) Group VL2 kidney. (m) Group VS1 kidney. (n) Group VS2 kidney. (o) Group NL1 kidney. (p) Group NL2 kidney. (q) Group NS1kidney. (r) Group NS2 kidney.

4.Conclusion and recommendation

The result on the whole showed that the aqueous leaf extract of *V. amygdalina* using 2000mg/kg and 1000mg/kg concentrations possess high trypanocidal activities and may clear off the parasite completely if administered for a longer period of time. Also the *Nauclea latifolia* stem bark at a higher concentration (2000mg/kg) has a good trypanocidal activity. The plant parts and concentrations with high trypanocidal activities also have the ability to prolong the life of the animals when compared to those with lower activity and the control group (infected but not treated).

An activity guided fractionation of these crude extracts should be carried out in an effort to locate and concentrate the fraction where the bioactivity against trypanosome lies.

An investigation should be carried out on the antirypanosomal activity of a combined therapy of the crude extracts of both plants.

An in vitro trypanocidal evaluation of the aqueous leaf and stem bark extracts of *N. latifolia* should be carried out.

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