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Assessment of cardiotoxic potential of methanol extract of red cultivar *Allium cepa*

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Abstract

The effects of oral administration of crude methanol extract of red cultivar *Allium cepa* (Onion) on serum cardiac troponin (cTnI) in cardiac muscle and some haematological parameters were investigated in this study. Fifty five (55) male albino rats were housed and fed with standard growers ration and water *ad libitum*. There were three major groups; A, B and C containing twenty five (25), twenty five (25) and five (5) rats respectively. Group C was the control group while groups A and B were sub-divided into 5 groups of 5 rats each. Group A was administered with red cultivar *A. cepa* extract at doses of 100 mg/kg, 200 mg/kg, 400 mg/kg, 800 mg/kg and 1200 mg/kg for 14 days while group B rats were administered with the doses of red cultivar *A. cepa* for 28 days. Blood samples were collected from the retro-orbital sinus for haematology and cardiac troponin-I assay, histopathological examination of the heart was also done. Haematology showed significant ($p<0.05$) progressive decrease in packed cell volume (PCV), red blood cell (RBC) and haemoglobin (Hb) concentration and there was progressive elevation of mean corpuscular volume (MCV). Dose-independent elevation of serum cardiac troponin-I (cTnI) with varying degrees of myocardial injuries was observed. This study further postulates a correlation between the *A. cepa*-induced anaemia and increased cTnI which may be caused by myocardial ischaemia. In conclusion, this study reported the capability of red cultivar *A. cepa* to induce anaemia and cause myocardial injury as expressed with statistical significant ($p<0.01$) increase in serum cTnI. Medicinal use of red cultivar *A. cepa* is therefore recommended to be limited to lower doses and for short duration to prevent the haemotoxic and cardiotoxic potentials.

Keywords: *Allium cepa*, Cardiac troponin-I, Cardiotoxicity, Haemotoxicity, Medicinal, Red cultivar

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Introduction

Allium cepa, commonly called onion, is known for its nutritional and medicinal uses (Nwaoguikpe, 2009). It is also used traditionally as anti-cancer, anti-asthmatic, anti-hyperglycaemic and anti-hyperlipidaemic (Stajner & Varga, 2003). Notwithstanding these beneficial uses, Oyewusi *et al*. (2015) reported some behavioural abnormalities such as anorexia, depression and unsteady gait in rats following oral administration of high doses of methanol extract of *A. cepa*. Some physiological derangements such as respiratory distress, muscular rigidity and partial paralysis of the limbs were observed following intraperitoneal administration of *A. cepa* (Oyewusi *et al*., 2015).

It was reported by Jain (1993) and Tang *et al*. (2008) that oral administration of *A. cepa* caused Heinz body haemolytic anaemia in horses, cattle, sheep, and dogs. *A. cepa* has been shown to contain n-propyl disulfide, an oxidative chemical that depletes the enzyme glucose 6-phosphate dehydrogenase which is found within the erythrocytes. Depleting action of this oxidative chemical prevents the reduction of oxidized glutathione, hence diminishing the protective ability of this antioxidant (Cope, 2005). Arena *et al*. (2000) reported the occurrence of

tachycardia in a patient following ingestion of raw or lightly cooked onions.

Other clinical signs of *Allium* species toxicosis observed in dogs and cats include depression, haemoglobinuria, presence of haemosiderin, urinary casts, icterus, tachycardia, tachypnea, weakness and exercise intolerance. Following ingestion of 5 g/kg (cats) and 15-30 g/kg (dogs) or 0.5% of body weight, *Allium* toxicosis may appear several days post ingestion resulting into clinically significant haematological changes (Cope, 2005).

There is a dearth of information on the cardiotoxic potential of *A. cepa*, which is an important aspect before further investigations on the pharmacological effects are pursued. Cardiac injury is the disruption of normal cardiac cellular membrane integrity which leads to a loss of essential intracellular constituents like troponin, creatinine kinase, lactate dehydrogenase, and myoglobin (Jaffe & Morrow, 2015). These are essential constituents that serve as biomarkers for cardiac injury. Previously, creatinine kinase, lactate dehydrogenase, myoglobin and fatty acid binding proteins were depended upon for the diagnosis and prognosis of myocardial injury (Jaffe & Morrow, 2015). However, troponin has been found to be the biomarker of choice for the detection of cardiac injury (Luciano & Jaffe, 2005) and this was also supported by U.S. Food and Drug Administration; Biomarker Qualification Review Team (BQRT) based on results of several medical and veterinary studies (O'Brien *et al*., 2011). One major sequel of cardiac damage is myocardial infarction (MI) or acute myocardial infarction (AMI), which occurs as a result of stoppage of blood flow to a part of the heart leading to further damage of the heart muscle.

This work was therefore designed to investigate the cardiotoxic potential of the methanol extract of *A. cepa* using changes in serum cardiac Troponin-I (cTnI), haematological parameters and cardiac cellular integrity as biomarkers of cardiac injury.

Materials and Methods

Experimental design

Fifty five male Wistar rats fed on standard growers ration (Vital Feed Nig. Ltd, Jos, Nigeria) and provided with clean water *ad libitum* were used for this study. The fifty five rats were divided into 3 groups A, B and C with 25, 25 and 5 rats respectively. Groups A and B were further sub-divided into 5 groups of 5 rats each. Groups A1, A2, A3, A4 and A5 were orally administered with methanol extract of *A. cepa* red cultivar at doses of 100 mg/kg, 200 mg/kg, 400 mg/kg, 800 mg/kg and 1200 mg/kg body weight respectively for 14 consecutive days, while groups B1, B2, B3, B4 and B5 were treated with the same graded doses for 28 consecutive days. The rats were treated with the extract daily with the aid of oral cannula. Group C rats served as negative control for the study and were administered with distilled water (10 ml/kg).

Blood sample collection

Following proper restraint technique (one hand scruff restrain), blood samples were collected from the retro-orbital venous sinus using the lateral canthus approach. The blood samples were collected on days 14 and 28 into plain bottles for serum troponin I analysis, while it was collected weekly into lithium heparinized bottles for haematological studies as applicable for groups A and B. Rats in each of the sub-groups in group A were bled on days 7 and 14 while those in sub-groups in group B were bled on days 21 and 28. Group A rats were sacrificed on day 14, while Group B rats were sacrificed on day 28.

Blood and serum analysis

The method described by Jain (1986) was used to determine haematological parameters; packed cell volume (PCV), haemoglobin concentration (Hb) and red blood cell count (RBC). Cardiac troponin-I (cTnI) was analyzed using ELISA machine within four hours of collection as recommended by the manufacturer of the troponin-I reagent (Life Diagnostics, Inc., West Chester, U.K.). Serum cTnI values were derived from a standard curve which was constructed by plotting the absorbance values of each reference standard serum against its concentration in ng/ml as described by the manufacturer. The derived cTnI concentrations were multiplied by the dilution factor (x4) to obtain the actual serum cTnI concentration.

Statistical analysis

Data generated from this study was presented as mean ±SD. The differences between the means in the treated and in the untreated groups were compared by two way analysis of variance (ANOVA) using the Prism GraphPad Statistical software (Prism 5).

Results

Packed Cell Volume

On days 7, 14 and 21 post oral administration of red cultivar *A. cepa* extract, there was a progressive non-significant ($p>0.05$), dose dependent decrease in

Table 1: Percentage (%) Packed Cell Volume of rats administered with the crude methanol extract of red cultivar *A. cepa*

Trt grps	7 days post-treatment	14 days post-treatment	21 days post-treatment	28 days post-treatment
Negative Control	49.00±4.34	48.66±1.76	48.67±0.67	49.00±4.34
A1 100mg/kg *A. cepa*	49.00±0.58	45.8±4.30	46.23±0.67	38.6±1.67*
A2 200mg/kg *A. cepa*	47.75±5.26	46.80±1.92	46.80±1.92	42.40±1.95*
A3 400mg/kg *A. cepa*	48.50±0.65	45.60±3.84	45.60±3.84	40.80±3.85*
A4 800mg/kg *A. cepa*	45.00±1.73	45.40±3.05	45.40±3.05	43.33±0.33*
A5 1200mg/kg *A. cepa*	46.40±0.68	44.00±1.41	44.0±1.41	41.00±2.31*

*Indicates significant ($p<0.05$) difference compared to the control values for the day of observation

Table 2: Red Blood Cell count ($x10^6$ i.u) of rats administered with the crude methanol extract of red cultivar *A. cepa*

Trt grps	7 days post-treatment	14 days post-treatment	21 days post-treatment	28 days post-treatment
Negative Control	10.22±1.93	9.80±0.1	9.69±0.57	9.42±0.16
A1 100mg/kg *A. cepa*	10.03±0.64	9.22±0.79	9.18±1.06	7.52±0.76*
A2 200mg/kg *A. cepa*	9.70±1.11	9.19±0.88	8.97±0.95	8.30±0.63
A3 400mg/kg *A. cepa*	9.83±0.50	9.12±0.75	8.77±1.24	8.14±0.89
A4 800mg/kg *A. cepa*	9.35±0.79	8.75±0.60	8.72±0.54	8.54±0.79
A5 1200mg/kg *A. cepa*	9.21±0.21	8.86±1.00	8.45±0.59	7.41±0.62*

*Indicates significant ($p<0.05$) difference compared to the control values for the day of observation

mean packed cell volume (PCV) in all the groups when compared to the control. However on day 28, a significant decrease ($p<0.05$) in the mean PCV was observed in all the groups (38.60±1.67 %, 42.40±1.95 %, 40.80±3.85 %, 43.33±0.33 % and 41.00±2.31 %) compare to that of the control group (49.00±4.34 %) (Tables 1).

Red blood cell count

There was no significant ($p>0.05$) decrease in RBC count in all the groups on days 7, 14, 21 and 28, except on day 28 in rats treated with A. cepa red cultivar at doses of 100 mg/kg (7.52±0.16 $x10^6$i.u) and 1200 mg/kg (7.41±0.62 $x10^6$i.u) compare to the control (9.42±0.16 $x10^6$i.u) (Tables 2).

Haemoglobin concentration

There was no significant difference in the haemoglobin concentration values obtained on day 7 and day 14 compared to the control values. There was significant decrease ($p<0.05$) in the haemoglobin concentration values on day 21 at 800mg/kg (13.14±2.55 g/dl). On day 28, all the test animals (13.16±1.36 g/dl, 14.05±0.58 g/dl, 13.15±2.70 g/dl, 13.92±0.57 g/dl and 13.82±0.38 g/dl) had significantly ($p<0.05$) lower Hb concentrations compared to the control group (18.50±1.17 g/dl) (Tables 3).

Table 3: Haemoglobin concentration (g/dl) of rats administered with the crude methanol extract of red cultivar *A. cepa*

Trt grps	7 days post-treatment	14 days post-treatment	21 days post-treatment	28 days post-treatment
Negative Control	18.50±4.07	18.50±4.07	18.50±1.17	18.50±1.17
A1 100mg/kg *A. cepa*	16.34±1.94	17.65±1.07	16.41±1.63	13.16±1.36*
A2 200mg/kg *A. cepa*	16.02±2.31	15.96±2.67	16.28±1.59	14.05±.0.58*
A3 400mg/kg *A. cepa*	14.26±3.05	14.72±0.47	16.45±2.30	13.15±2.70*
A4 800mg/kg *A. cepa*	16.42±1.20	16.62±3.23	13.14±2.55*	13.92±0.57*
A5 1200mg/kg *A. cepa*	15.78±1.45	18.88±0.94	15.64±1.61	13.82±0.38*

*Indicates significant ($p<0.05$) difference compared to the control values for the day of observation

Table 4: Mean Corpuscular Volume (fL) of rats administered with the crude methanol extract of red cultivar *A. cepa*

Trt grps	7 days post-treatment	14 days post-treatment	21 days post-treatment	28 days post-treatment
Negative Control	50.29±0.66	49.77±1.40	50.48±2.15	50.61±0.62
A1 100mg/kg *A. cepa*	50.35±0.76	49.62±0.35	48.50±1.71	51.67±1.96
A2 200mg/kg *A. cepa*	51.38±1.37	51.17±1.54	51.47±2.22	51.21±0.76
A3 400mg/kg *A. cepa*	50.25±0.31	50.01±0.77	52.38±1.62	50.38±1.59
A4 800mg/kg *A. cepa*	50.82±0.16	51.94±1.17	52.09±0.86	53.37±2.40
A5 1200mg/kg *A. cepa*	50.35±0.30	52.17±2.52	52.16±1.17	55.41±0.94

*Indicates significant ($p<0.05$) difference compared to the control values for the day of observation

MCV (pg), MCH (fl) and MCHC (%)

There was no significant effect on the MCV, MCH and MCHC on day 7. There was statistically insignificant increase in the MCV and significant ($p<0.05$) increase in both MCH and MCHC at 1200mg/kg on day 14 post-treatment. On day 21 (48.50±1.71 fL, 51.47±2.22 fL, 52.38±1.62 fL, 52.09±0.86 fL and 52.16±1.17 fL) and 28 (51.67±1.96 fL, 51.21±1.96 fL, 50.38±1.59 fL, 53.37±2.40 fL and 55.41±0.94 fL) post-treatment, there was a further statistically insignificant increase in MCV and there was no significant effect in MCH and MCHC (Tables 4-6).

Serum cardiac troponin-I (cTnI)

The results of cardiac troponin I ELISA assay at day 14 showed a non-significant ($p>0.05$) increases in cTnI concentration in all rats administered methanol extract of *A. cepa* (0.18±0.00 ng/ml, 0.15±0.01 ng/ml, 0.16±0.00 ng/ml, 0.18±0.02 ng/ml and 0.17±0.02 ng/ml) compared to the control group (0.12±0.03ng/ml). Twenty eight days post-treatment, cTnI values of rats administered doses of 100 mg/kg, 200 mg/kg, 400 mg/kg and 800 mg/kg increased significantly ($p<0.01$) to 0.31±0.03 ng/ml, 0.29±0.02 ng/ml, 0.24±0.04 ng/ml and 0.22±0.05 ng/ml compared to control values (0.14±0.00 ng/ml). The value of the cTnI for rats administered doses of 100 mg/kg, 200 mg/kg, 400 mg/kg were significantly

Table 5: Mean Corpuscular Haemoglobin (pg) of rats administered with the crude methanol extract of red cultivar *A. cepa*

Trt grps	7 days post-treatment	14 days post-treatment	21 days post-treatment	28 days post-treatment
Negative Control	18.10±1.34	17.99±0.20	19.09±1.29	19.40±0.28
A1 100mg/kg *A. cepa*	16.28±0.62	19.22±0.80	21.07±2.71	17.61±0.99
A2 200mg/kg *A. cepa*	16.68±1.31	17.45±1.55	17.83±0.70	17.46±0.76
A3 400mg/kg *A. cepa*	14.56±1.50	14.80±1.02	19.24±2.21	15.66±1.68
A4 800mg/kg *A. cepa*	17.66±0.79	18.95±1.45	15.10±1.29	16.41±0.79
A5 1200mg/kg *A. cepa*	17.13±0.68	23.40±1.28*	18.65±1.56	18.72±0.74

*Indicates significant ($p<0.05$) difference compared to the control values for the day of observation

Table 6: Mean Corpuscular Haemoglobin Concentration (%)of rats administered with the crude methanol extract of red cultivar *A. cepa*

Trt grps	7 days post-treatment	14 days post-treatment	21 days post-treatment	28 days post-treatment
Negative Control	32.03±1.96	34.13±1.65	34.79±1.32	37.75±1.01
A1 100mg/kg *A. cepa*	31.12±1.30	38.77±1.81	36.29±2.84	34.06±1.24
A2 200mg/kg *A. cepa*	34.11±1.93	33.95±2.33	34.76±1.23	33.18±0.84
A3 400mg/kg *A. cepa*	29.30±3.79	29.66±2.20	36.51±3.35	30.74±2.35*
A4 800mg/kg *A. cepa*	36.30±1.30	36.71±3.49	29.15±2.88	32.32±0.61
A5 1200mg/kg *A. cepa*	34.01±1.35	43.05±1.40*	35.61±2.20	33.75±0.85

*Indicates significant ($p<0.05$) difference compared to the control values for the day of observation

higher ($p<0.05$) at day 28 compare to those of day 14 for the same doses (Figure 1).

Histopathology

Histopathology of the cardiac muscles following administration of the extract at various concentrations revealed varied degrees of cardiac injury as shown in plates I - IV. The pathological lesions observed include mild necrosis (100 mg/kg/ at day 14), multifoci of myofibre degeneration, interstitial oedema (400 mg/kg/ at day 28), congestion of the blood vessels and necrosis with mononuclear cells infiltration (1200 mg/kg/ at day 28). Normal heart section from the control revealed no pathological lesions (Plates V and VI).

Discussion

In this study, red cultivar *A. cepa* caused responsive anaemia indicated by significant reduction of packed cell volume (PCV), red blood cell count (RBC) and haemoglobin concentration (Hb) by day 28 post administration with progressive increase in the MCV. Continued administration till day 28 showed further significant decline in these red cell parameters and further increase in MCV. This clinically implies that there was reticulocytosis which may translate to reduction in the oxygen and nutrient carrying capacity of RBC. Clinically, blood and blood forming elements are investigated to confirm animal exposure to toxic substances and other conditions

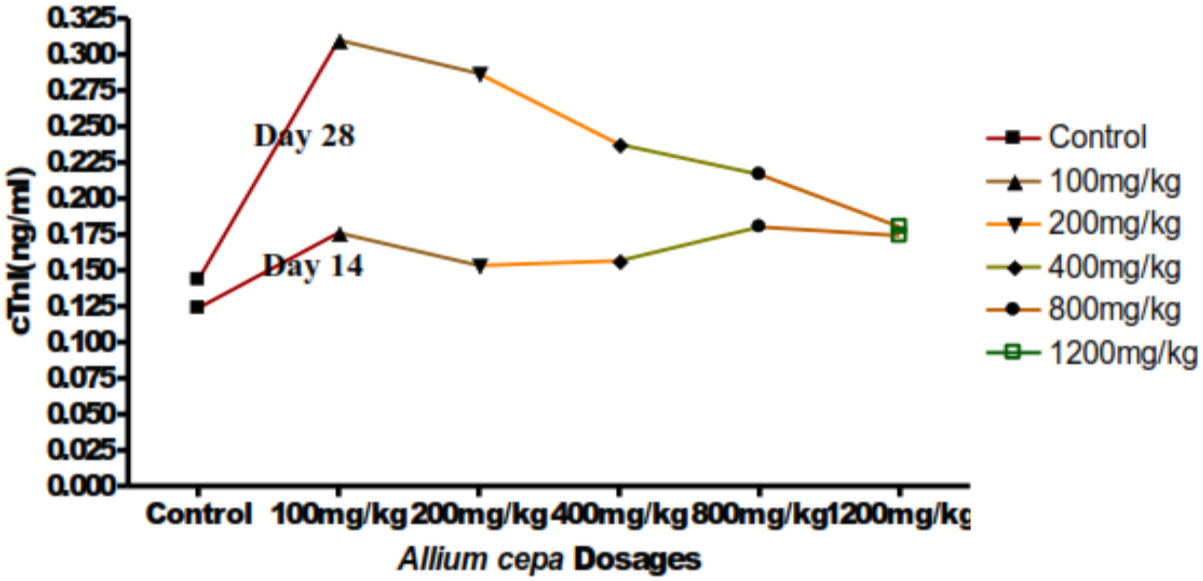

Figure 1: Effect of methanol extract of red cultivar *A. cepa* on cardiac troponin-I following 14 day and 28 day oral administration

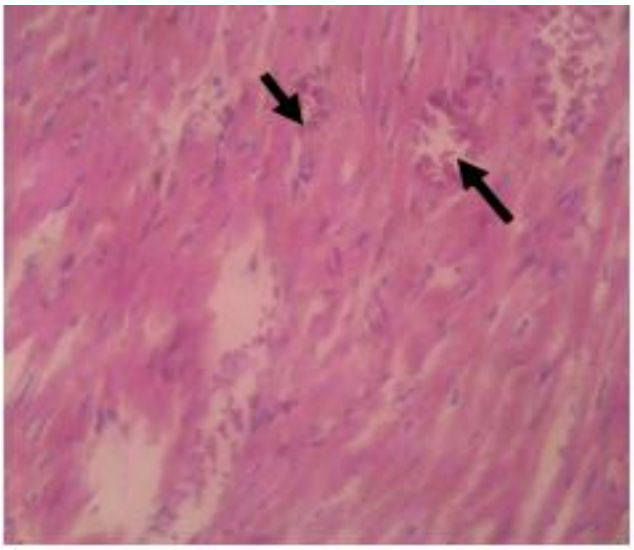

Plate **I**: Heart section of rat treated with 100 mg/kg methanol extract of red cultivar Allium cepa showing multiple foci of myofibres degeneration and necrosis (arrows) H & E X400

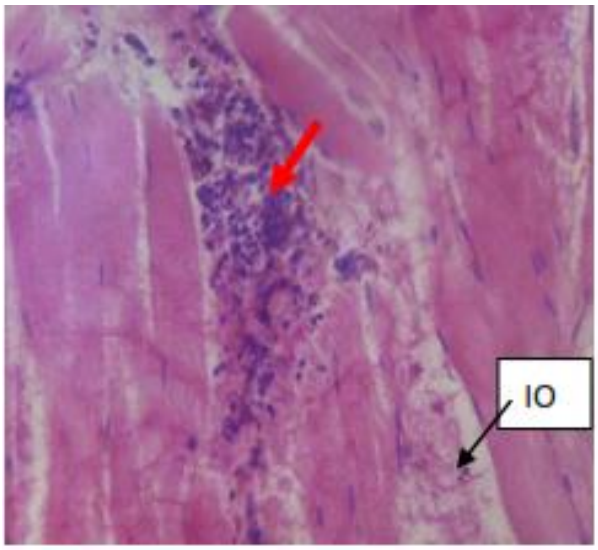

Plate II: Heart section of rat treated with 200 mg/kg methanol extract of red cultivar Allium cepa showing focal area of slight interstitial oedema (IO) and moderate myofibre degeneration and necrosis with mononuclear cells infiltration (mostly lymphocyte) (arrow) H & E X400

relating to destruction of blood cells (Bamishaiye *et al*., 2009). Results of this study suggest that red cultivar *A. cepa* is capable of inducing macrocytic hypochromic anaemia if consumed at a dose equal to or more than 100 mg/kg and if used for more than 7 days continuosly. Anaemia is defined as decrease in the number of red blood cells (RBCs) and or the amount of haemoglobin in the circulatory system (Boden, 2005) as observed in this study. Anaemia can be caused by any of the following sources: haemorhage, decreased or faulty red blood production and destruction of red blood cells (Peter, 2015). RBC loss may be due to haemorrhage (whole blood loss) or haemolysis (premature destruction of RBC) (Chineke *et al*., 2006). The anaemia may be mild if the bone marrow compensates for the loss by

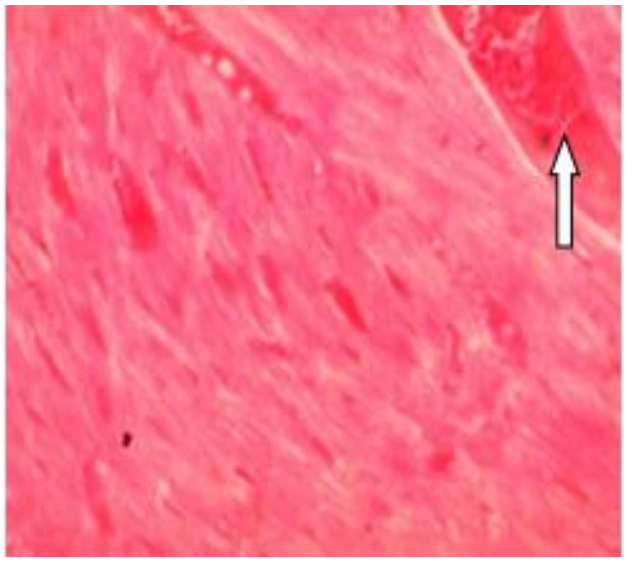

Plate III: Heart section of rat treated with 400 mg/kg methanol extract of red cultivar Allium cepa showing severe congestion of blood vessel (arrow). H & E X400

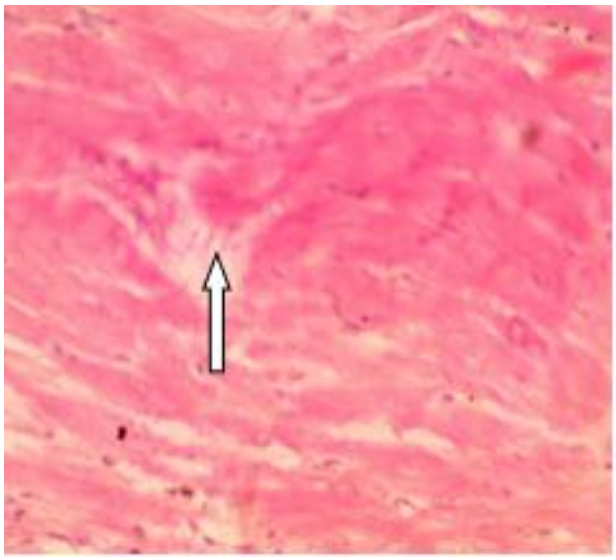

Plate IV: Heart section of rat treated with 1200 mg/kg methanol extract of red cultivar Allium cepa showing oedema in the interfiber spaces (arrow). H & E X400

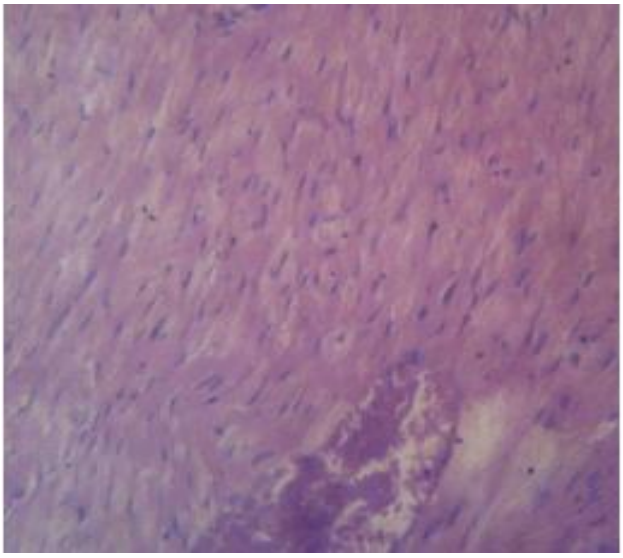

Plate V: Heart section of rat showing cross striated cardiomyocytes with centrally located nucleus. (H & E X400)

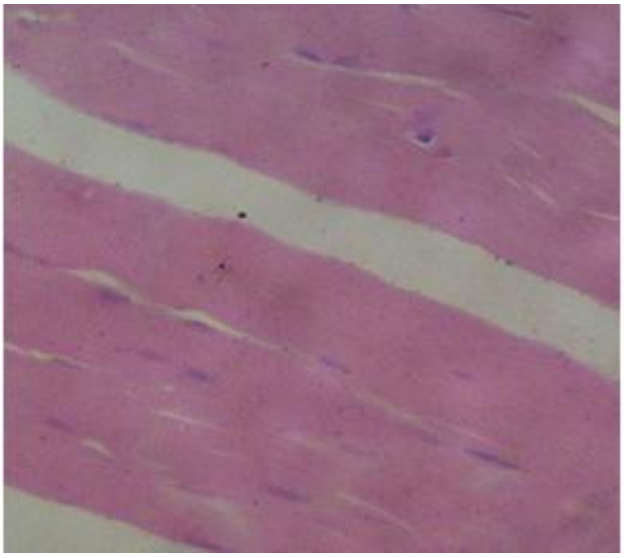

Plate VI: Heart section of rat showing cross striated cardiomyocytes with centrally located nucleus. (H & E X400)

increasing RBC production. In this study, the anaemia was haemolytic and, responsive or compensatory. Chronic therapeutic use of red cultivar *A. cepa* may degenerate the anaemia to aplastic anaemia if administered at high doses and for more than 28 consecutive days. However, that is if there is no compensatory response by the bone marrow as observed with certain drugs (e.g. chloramphenicol), infection, cancer and toxic substances (e.g. lead) (Peter, 2015).

The results of the present study are in agreement with previous researchers who reported the haemolytic potential of *A. cepa* (Ebubekir *et al.,* 2009) in dogs fed with a single dose of *A. cepa* juice. Ugwu and Omale (2011) observed a significant decrease in haematological indices of rats following sub-chronic administration with *A. cepa* extract. The study by Ostrowska *et al*. (2004) reported decrease in erythrocytes count and haemoglobin concentration in pigs orally administered with brown onions. An earlier study in human subjects involving administration of encapsulated *A. cepa* also reported decreases in haematocrit (Mayer *et al.,* 2001).

This study further investigated specific cardiac damage using cardiac troponin I as biomarker for cardiac injury, particularly to the myocardium.

Cardiac troponins I and T are cardiac regulatory proteins that control the calcium mediated interaction between actin and myosin (Sharma *et al.*, 2004). The cardiac forms of these regulatory proteins are coded by specific genes and theoretically have the potential of being unique to the myocardium. Elevated cardiac troponin concentrations are now accepted as the standard biochemical marker for the diagnosis of myocardial infarctions (Bertrand *et al.*, 2000). Cardiac troponin-I level above 0.16 ng/ml may be a risk factor for congestive heart failure (CHF) (Williams *et al*., 2002). Cardiac troponins (I and T) leakage out of themyocardial cells occur within 4–6 hours following onset of acute myocardial infarction with peak leakages at about 24hours after the attack (Eisenman, 2006) due to a gradual degeneration of myofibrils with release of the troponin complex (Bertinchant *et al.*, 1996).

Serum cardiac troponin levels remain elevated for up to about 2 weeks unlike creatinine kinase which is metabolized more rapidly. Persistent presence of troponins makes it a better diagnostic marker of myocardial injury compared to creatinine kinase. Troponins also have almost complete tissue specificity, hence the preferred markers for evaluating myocardial injury (Eisenman, 2006) and cardiac troponin I (cTnI) has not been identified in other tissues outside the myocardium (Bodor *et al.*, 1995).

Clinically significant ($p<0.05$) increases were observed in the treated rats, indicating marked increased risk of development of myocardial infarction and subsequently congestive heart failure. The cardiotoxic potential of *A.cepa* was both dose- and time-dependent with further increases with higher doses and at day 28 observation. The on-going myocardial damage in the rats was confirmed by the histopathology of the heart.

Histology sections from the heart of rats administered graded doses of *A. cepa* showed various degrees of injuries ranging from mild necrosis, multifoci of myofibres degeneration and interstitial oedema to necrosis with mononuclear cells infiltration.

Several mechanisms leading to elevated cardiac troponins have been postulated with the most favoured being myocardial ischaemia in the setting of acute coronary syndrome or myocardial infarction (Hamm, 1994). This may be explained by the reduced oxygen pressure sequel to anaemia, which *A. cepa* clearly caused in this study. Previous study by Ebubekir *et al.* (2009) also reported occurrence of first degree heart block and bradycardia 12 hours- and tachycardia 24 hours-post administration of onion juice but cardiac troponin was not assayed. To the best of our knowledge, this is the first report on the effect of *A. cepa* on serum Cardiac Troponin-I (cTnI) as evidence of possible *Allium species* cardiotoxicity. This study therefore postulates a relationship or link between the *A. cepa*-induced anaemia and increased cTnI which may be caused by myocardial ischaemia. Further studies need to be conducted to evaluate the effect of other *Allium* species on serum cTnI levels and the mechanism of toxicity.

In conclusion, this study has shown that red cultivar *A. cepa* is capable of inducing anaemia and has cardiotoxic potentials which may result in congestive heart failure or worsen cardiac condition if consumed at dose up to 100 mg/kg and for a protracted period of time.

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In vivo antitrypanosomal effects of stem-bark extracts of *Securidaca longipedunculata* in rats experimentally infected with *Trypanosoma brucei brucei*

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Abstract

The efficacy of stem-bark extracts of *Securidaca longipedunculata* against *Trypanosoma brucei brucei* infected rats was investigated. For curative study, forty adult Wistar rats of both sexes were randomly divided into 8 groups of 5 rats each. Each rat was infected with 10^6 cells of trypanosomes per ml of blood intraperitoneally (*ip*). Rats in groups 1 and 2 received the crude methanol extract (CME) at 0.7 and 0.35 mg/kg, respectively. Similarly, rats in groups 3 and 4 received ethyl acetate fraction (EAF) at 0.7 and 0.35 mg/kg, respectively; while 5 and 6 were treated with 0.9 and 0.45 mg/kg of aqueous methanol fraction (AMF), respectively. Rats in groups 7 and 8 were treated with diminazene aceturate (3.5 mg/kg) and phosphate buffered saline, PBS (2 ml/kg), respectively. Four rats (group 9) were neither infected nor treated and served as neutral control. In the prophylactic studies, 25 rats of both sexes were randomly divided into V groups of 5 rats each. Rats in groups I, II, and III were pre-treated with CME at 0.7 mg/kg *i.p*. for 3, 5 and 7 days, respectively; while group IV received PBS for 7 days and served as negative control. The rats were then individually infected with 10^6 parasites per ml of blood on days 3, 5 and 7 for groups I, II and III, respectively. Rats in group V were neither treated nor infected and served as neutral control. CME of *S. longipedunculata* suppressed level of parasitaemia and prolonged the survival period of rats when compared to other groups ($P<0.05$). Pre-treatment of animals with CME before challenge with the parasite could not prevent infection. Thus, stem-bark extract of *S. longipedunculata* exhibited some levels of curative antitrypanosomal effect against *T. brucei brucei* infection in rats despite its low margin of safety.

Keywords: Curative effect, High toxicity, *In vivo*, Phytochemical screening, Prophylactic effect
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Introduction

Tsetse-transmitted trypanosomosis is an important constraint to livestock development in sub-Saharan Africa with estimated direct annual losses to producers and consumers exceeding US$1 billion (Kristjanson *et al*., 1999; Simukoko *et al*., 2007). The disease is ranked among the top 10 global cattle diseases affecting livestock production in sub-Saharan Africa. (Perry *et al*., 2002). The scarcity of modern effective drugs for the treatment and management of trypanosomosis, combined with their high cost has created a growing public interest in the pursuit of alternative natural drugs from botanicals (Etet & Mahomoodally, 2012). Phytotherapy is the oldest form of therapeutic treatment world-wide with the use of over 21, 000 plant species as herbal medicine (Efferth, 2010).

Natural products are important sources of lead compounds in the development of new drugs (Kayser *et al*., 2003). However, the great potential of plants as lead to the discovery of newer antitrypanocidal drug is still at its lowest ebb (Adams *et al*., 2013). Many existing drugs were derived from natural compounds (Newman & Cragg, 2012). There are only three available trypanocidal drugs for the management of trypanosomosis in ruminants. Diminazene aceturate with only therapeutic activity, homidium as well as isometamidium both with therapeutic and prophylactic activities (Tauheed *et al*., 2016a) Therefore, screening natural products may provide a link to the discovery of a new compounds with unique structure of high activity and selectivity.

Securidaca longipedunculata (Polygalaceae) also known as violet tree, fibre tree or Rhodesian violet in English, and popularly known as *uwar magunguna* (mother of all medicines) in Hausa speaking communities of northern Nigeria (Tauheed *et al*., 2016b). It is a small tree of up to 6-9 m high with a pale grey, smooth bark and oblong, more or less hairless alternate leaves of varying size and shape and crowded towards the stem tips (Van Wyk *et al*., 2009). Whole plant, root, stem-bark and leaves of the plant are used for medicinal purposes in folkloric medicine. The plant is widely used in African traditional medicine as a general remedy for cough, malaria, backache, venereal disease, snakebite, erectile dysfunction and tuberculosis (Mongalo *et al*., 2015). The aim of this study was to determine the anti-trypanosomal effect of stem-bark extracts of *Securidaca longipedunculata* against *T. brucei brucei* experimental infection in rats.

Materials and Methods

Plant collection and identification

Fresh stem-bark of *Securidaca longipedunculata* was collected from Zaria, Nigeria. The plant was identified in the Herbarium, Department of Biological Sciences, Ahmadu Bello University (A.B.U.), Zaria, Nigeria where a voucher number specimen of 900213 was assigned. The identified stem-bark was dried in an open air in the Laboratory and the dried sample was kept in polythene bags until required for preparation of the extract.

Plant extraction, concentration and fractionation

Seven hundred and ninety grams (790) g of the pulverised stem-bark of *S. longipedunculata* was extracted with absolute methanol in a soxhlet extractor. The liquid extract was concentrated to dryness over a water-bath at 60^0C. About 73 g of the crude methanol extract was dissolved in 300 ml of distilled water. The solution was transferred to 1 L separating funnel and partitioned with 600 ml of ethyl acetate for 8 hours. The lower denser aqueous fraction was collected into a separate conical flask and upper portion (ethyl acetate fraction) was dispensed into a clean conical flask. The process was repeated two more times and similar fractions were pooled together. The fractions were concentrated to dryness over a water-bath at 50^0C and 70^0C for ethyl acetate and aqueous methanol fractions, respectively.

Phytochemical screening

S. longipedunculata extract and fractions were evaluated for the presence of carbohydrates, anthraquinones, flavonoids, tannins, alkaloid, saponins, cardiac glycosides, steroids and triterpenes using standard procedures (Trease & Evans, 1983).

Experimental animals

Adult male and female Wistar rats weighing between 170 to 190 g were obtained from the animal house, Department of Physiology, Faculty of Medicine, Ahmadu Bello University (A.B.U.), Zaria, Nigeria. They were allowed to acclimatize for 2 weeks in the Laboratory at the Department of Veterinary Pharmacology and Toxicology, A.B.U., Zaria. They were housed in clean plastic cages with wood shavings as bedding, which was changed twice a week. The rats were fed standard rat feed and given access to clean water *ad libitum*. The approval for the use of animal was obtained from the Ethical Committee on Animal Use and Care, A.B.U., Zaria, Nigeria.

Test organism

Trypanosoma brucei brucei was obtained from the Department of Veterinary Parasitology and Entomology, A.B.U., Zaria. The parasite was maintained in rats by continuous passage. Each cycle of passage was done when parasitaemia was in the range of 35 – 40 parasites per field, which corresponded to an interval of 6 days post-infection. For several passages, about 3 ml of blood was obtained from an infected rat by cardiac puncture after light chloroform anaesthesia into 5 ml syringe and emptied into a vial containing 9 ml of phosphate buffered saline (PBS). About 1 x 10^6 cells trypanosomes contained in 0.2 ml was used to infect a trypanosome-free rat by *i.p.* route.