



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 \pm 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 <i>A. cepa</i>	49.00±0.58	45.8±4.30	46.23±0.67	38.6±1.67*
A2 200mg/kg <i>A. cepa</i>	47.75±5.26	46.80±1.92	46.80±1.92	42.40±1.95*
A3 400mg/kg <i>A. cepa</i>	48.50±0.65	45.60±3.84	45.60±3.84	40.80±3.85*
A4 800mg/kg <i>A. cepa</i>	45.00±1.73	45.40±3.05	45.40±3.05	43.33±0.33*
A5 1200mg/kg <i>A. cepa</i>	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 ($\times 10^6$ 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 <i>A. cepa</i>	10.03±0.64	9.22±0.79	9.18±1.06	7.52±0.76*
A2 200mg/kg <i>A. cepa</i>	9.70±1.11	9.19±0.88	8.97±0.95	8.30±0.63
A3 400mg/kg <i>A. cepa</i>	9.83±0.50	9.12±0.75	8.77±1.24	8.14±0.89
A4 800mg/kg <i>A. cepa</i>	9.35±0.79	8.75±0.60	8.72±0.54	8.54±0.79
A5 1200mg/kg <i>A. cepa</i>	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 $\times 10^6$ i.u) and 1200 mg/kg (7.41±0.62 $\times 10^6$ i.u) compare to the control (9.42±0.16 $\times 10^6$ 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 <i>A. cepa</i>	16.34±1.94	17.65±1.07	16.41±1.63	13.16±1.36*
A2 200mg/kg <i>A. cepa</i>	16.02±2.31	15.96±2.67	16.28±1.59	14.05±0.58*
A3 400mg/kg <i>A. cepa</i>	14.26±3.05	14.72±0.47	16.45±2.30	13.15±2.70*
A4 800mg/kg <i>A. cepa</i>	16.42±1.20	16.62±3.23	13.14±2.55*	13.92±0.57*
A5 1200mg/kg <i>A. cepa</i>	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 <i>A. cepa</i>	50.35±0.76	49.62±0.35	48.50±1.71	51.67±1.96
A2 200mg/kg <i>A. cepa</i>	51.38±1.37	51.17±1.54	51.47±2.22	51.21±0.76
A3 400mg/kg <i>A. cepa</i>	50.25±0.31	50.01±0.77	52.38±1.62	50.38±1.59
A4 800mg/kg <i>A. cepa</i>	50.82±0.16	51.94±1.17	52.09±0.86	53.37±2.40
A5 1200mg/kg <i>A. cepa</i>	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 <i>A. cepa</i>	16.28±0.62	19.22±0.80	21.07±2.71	17.61±0.99
A2 200mg/kg <i>A. cepa</i>	16.68±1.31	17.45±1.55	17.83±0.70	17.46±0.76
A3 400mg/kg <i>A. cepa</i>	14.56±1.50	14.80±1.02	19.24±2.21	15.66±1.68
A4 800mg/kg <i>A. cepa</i>	17.66±0.79	18.95±1.45	15.10±1.29	16.41±0.79
A5 1200mg/kg <i>A. cepa</i>	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 <i>A. cepa</i>	31.12±1.30	38.77±1.81	36.29±2.84	34.06±1.24
A2 200mg/kg <i>A. cepa</i>	34.11±1.93	33.95±2.33	34.76±1.23	33.18±0.84
A3 400mg/kg <i>A. cepa</i>	29.30±3.79	29.66±2.20	36.51±3.35	30.74±2.35*
A4 800mg/kg <i>A. cepa</i>	36.30±1.30	36.71±3.49	29.15±2.88	32.32±0.61
A5 1200mg/kg <i>A. cepa</i>	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

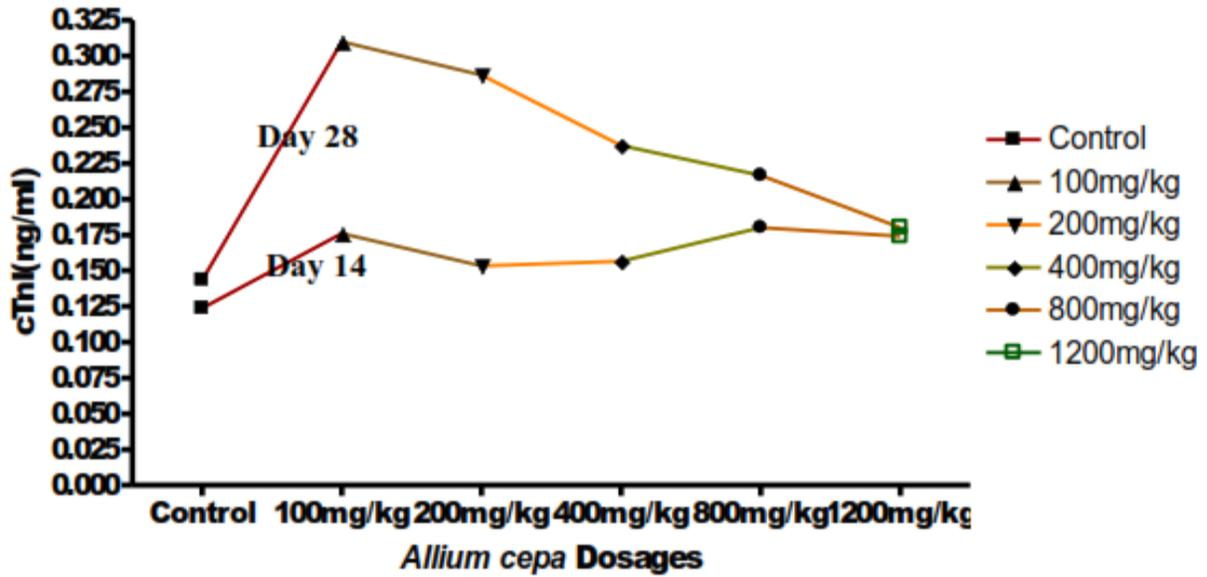


Figure 1: Effect of methanol extract of red cultivar *A. cepa* on cardiac troponin-I following 14 day and 28 day oral administration

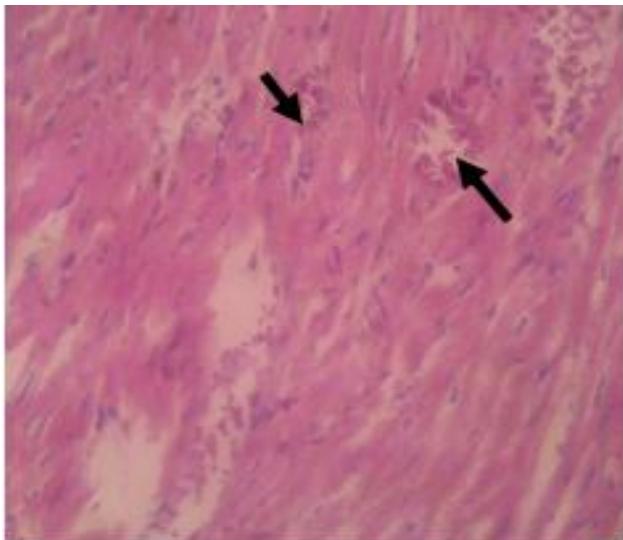


Plate I: Heart section of rat treated with 100 mg/kg methanol extract of red cultivar *Allium cepa* showing multiple foci of myofibres degeneration and necrosis (arrows) H & E X400

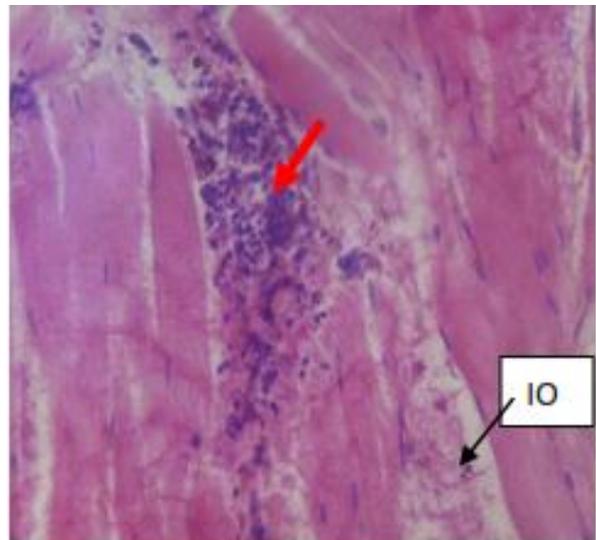


Plate II: Heart section of rat treated with 200 mg/kg methanol extract of red cultivar *Allium cepa* showing focal area of slight interstitial oedema (IO) and moderate myofibre degeneration and necrosis with mononuclear cells infiltration (mostly lymphocyte) (arrow) H & E X400

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 continuously. 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: haemorrhage, 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

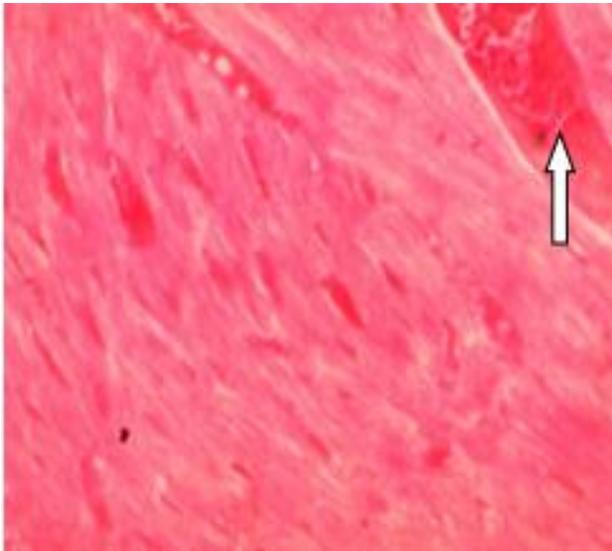


Plate III: Heart section of rat treated with 400 mg/kg methanol extract of red cultivar *Allium cepa* showing severe congestion of blood vessel (arrow). H & E X400

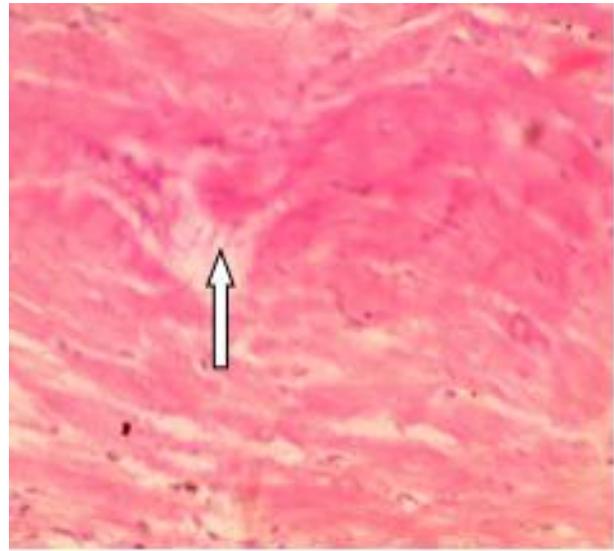


Plate IV: Heart section of rat treated with 1200 mg/kg methanol extract of red cultivar *Allium cepa* showing oedema in the interfiber spaces (arrow). H & E X400

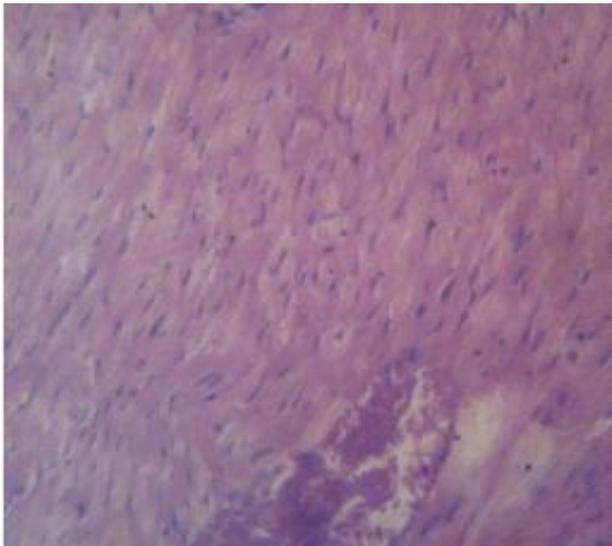


Plate V: Heart section of rat showing cross striated cardiomyocytes with centrally located nucleus. (H & E X400)

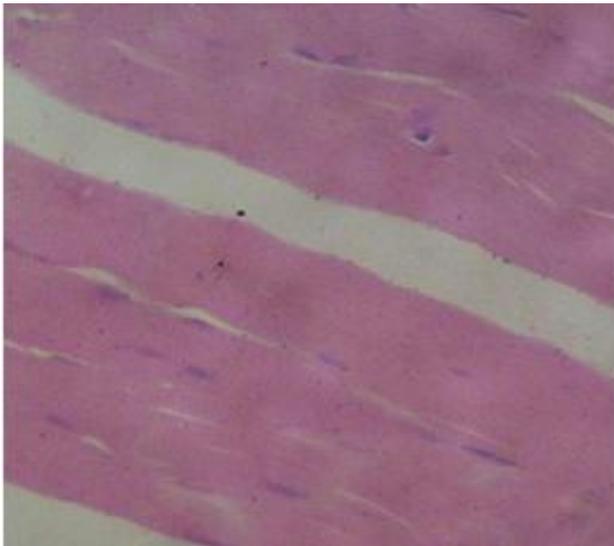


Plate VI: Heart section of rat showing cross striated cardiomyocytes with centrally located nucleus. (H & E X400)

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 the myocardial cells occur within 4–6 hours following onset of acute myocardial infarction with peak leakages at about 24 hours 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 ongoing 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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