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TOXICOLOGY OF SOME ETHNOVETERINARY PLANTS USED FOR WOUND HEALING

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ABSTRACT

The long-term use of an ethnomedicinal plant or parts thereof does not equate to safety as not often are these plants subjected to rigorous pre- and post-market evaluations demonstrating safety. A study was therefore conducted to determine possible toxic effects and/or properties of extracts from *Cissus quadrangularis* (whole aerial plant parts), *Adenium multiflorum* (whole aerial plant parts) and *Erythrinaabys sinica* (leaves and bark) using BALB/c female mice based on observations, histological and haematological evaluations. Acute and sub-acute toxicity results showed that *C. quadrangularis* extracts did not lead to any observable organ (liver and kidney) damage. *Adenium multiflorum* and *Erythrinaabys sinica* (leaves and bark) extracts exhibited some possible extract associated liver and kidney damage in the sub-acute experiments. Extracts of *A. multiflorum* at 300mg/kg body weight doses were 100% lethal in 48hours and its LD_{50} was estimated to be 250mg/kg body weight in mice. There were no notable variations of haematological or biochemical markers induced by the all extracts relative to the control. *Cissus quadrangularis* is probably safe for both short and long term oral use, while caution is need for long term use of *E. abyssinica*. *Adenium multiflorum*is toxic in both short/acute and long term uses. Further evaluations maybe necessary to ascertain damage to other organs and possible dose-related toxic effects.

Key words: Ethnomedicinal, Histological, Haematological, *Cissus quadrangularis*, *Adenium multiflorum*, *Erythrinaabys sinica*, Toxicity.

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INTRODUCTION

The ever-growing markets for herbal medicines, hence their wider usage world-over, is increasing concerns on their safety as only about 10% are properly characterised and are being produced based on strict

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quality standards (Ifeoma *et al*., 2013). Usage of herbal medicines from

pre-historical times does not guarantee safety. Phytoconstituents in herbals vary with geographic regions, growth stages and seasons. Possible contamination points are abundant throughout herbal medicine production stages. Thus, the World Health Organization (WHO) recommends Good Manufacturing Practises (GMP) for ensuring quality as well as efficacy and safety (WHO, 2011). Several plants have toxic effects, including some that are useful in traditional medicine. In addition, some plants contain phytoconstituents that have high toxicity potentials to vital organs and other living tissues e.g. cardiac glycosides and some alkaloids. Therefore, evaluation of potential toxic effects of plants and their products is essential in assuring public health (Jothy *et al*., 2011). Adulterations and falsification are also rampant as many countries do not have standards to monitor, regulate and control herbal medicine trade and use. WHO thus recommends standardization and use of approved fingerprinting tools to assure efficacy, safety and quality (Ukwuani AN *et al*., 2012). Unlike with modern medicines, which often contain one active chemical, evaluation of potential toxicity on plant extracts is complicated by the fact that they often contain hundreds or thousands of different molecules in a complex chemical matrix (George P, 2011). Herbals or extracts thereof may contain cytotoxic, carcinogenic and/or heavy metals among other potentially toxic phytoconstituents.

Cissus quadrangularis L. (Vitaceae) is one of about 350 species in the genus and a perennial climber of grape family (Vitaceae) widely distributed in sub-tropical and tropical regions of the world including sub-Saharan Africa (Vijayalakshmi G *et al*., 2015; Ghouse and Mohammed S, 2015). It has many applications in traditional/herbal medicine and nutritional supplementation which speaks well to its potential safety. The *Adenium* is a small genus with about five species belonging to the family Apocynaceae found naturally in sub-tropical and tropical regions of Africa and Arabia (Neuwinger and Hans D, 1996). All species of the *Adenium* genus (e.g. *Adenium multiflorum* Klotzsch) have applications in folklore ethnoveterinary medicine, witchcraft, homicide, magic, landscaping/decorations and as arrow poisons (hunting game and fishing) (Gueye EF, 1999; Zorloni and Alberto, 2007; Adedeji OS *et al*., 2013; Kumar and Dharmendra, 2014; Shukla and Susmita, 2015; Marandure T, 2016). The applications suggest possible toxicity of the adeniums. *Erythrina* is a genus belonging to the Fabaceae family with about 290 species of trees, shrubs and herbicaceous plants that are widely distributed throughout the warm parts of the sub-tropics and tropical regions of the world including sub-Saharan Africa (Irungu and Stanely, 2012). *Erythrina abyssinica* Lam. Ex. DC. produces many phytochemicals such as flavonoids, isoflavonoids, pterocarpans, terpenoids, saponins and alkaloids withwide ranging biological effects (pharmacological or toxic effects).

A study was carried out to determine toxic effects and/or properties of extracts from *C. quadrangularis* (whole aerial plant parts), *A. multiflorum* (whole aerial plant parts) and *E. abyssinica* (leaves and bark) using BALB/c female mice based on observations, histological and haematological evaluations. These plants are often used in the management of animal wounds in Zimbabwe based on a preliminary survey (Araújo J *et al.*, 2012).

MATERIALS AND METHODS

Plant collection and identification

The plant materials were collected from Mberengwa, Midlands Province (*Cissus quadrangularis* L. (Vitaceae) – 20°28′09.0″S 29°55′23.3″E.), Karoi, Mashonaland West Province (*Erythrina abyssinica* Lam. Ex DC. (Fabaceae) – 16°49′44.1″S 29°41′19.8″E) and Buhera, Manicaland Province (*Adenium multiflorum* Klotzsch (Apocynaceae) – 19°17′10.7″S 31°25′20.2″E) of Zimbabwe during the months of October – December 2016. Species identification was done by qualified botanists from the National Herbarium and Botanic Garden, Harare and University of Zimbabwe.

Preparation and extraction

Fresh whole aerial plant parts samples from *C. quadrangularis*, whole aerial plant parts of *A. multiflorum*, leaf and bark samples of *E. abyssinica* were separately oven dried at 50 °C for 48 h. Dried plant materials were ground into powders and extracted (1:20 w/v) with 50% aqueous methanol in an ultrasonic bath for one hour. The extracts were filtered under vacuum through Whatman's No. 1 filter paper. The extracts were then concentrated under pressure using a rotary evaporator at 30 °C and completely dried with a freeze drier overnight. The dried crude extracts were kept in airtight glass containers away from light in a cool dry place for subsequent use.

Animal Husbandry

A total of 52 albino female mice (BALB/c) aged 8 – 12 weeks were obtained from the Department of Livestock and Veterinary Services - Ministry of Agriculture, Mechanisation and Irrigation Development,

Zimbabwe for the *in vivo* toxicity assays. The weights of the adult female mice used ranged between 20 and 30 grams with an average weight of 25.07 grams (standard deviation of 2.45). The animals were acclimatized for five days in the experimental laboratory with room temperature of 22±5°C, relative humidity of 80±10% and approximately 12hour cycles of night and day. The animals were fed on standard mouse feed with water always available through standard water bottles. Protocols and procedures were approved by the Ethics and Animal welfare sub-committee, Division of Veterinary Services, Department of Livestock and Veterinary Services - Ministry of Agriculture, Mechanisation and Irrigation Development, Zimbabwe, as well as the Faculty Higher Degrees by Research (HDR) Committee, Faculty of Veterinary Science, University of Zimbabwe. The animals were handled and treated following the principles outlined in the "Guide for the Care and Use of Laboratory Animals" prepared by the National Academy of Sciences and published by the National Institutes of Health.

Ethical considerations

Ethical clearance for protocols and procedures were sought and granted by the Departmental Ethics and Animal welfare sub-committee, Division of Veterinary Services, Department of Livestock and Veterinary Services - Ministry of Agriculture, Mechanisation and Irrigation Development, Zimbabwe as well as the Faculty Higher Degrees Committee, Faculty of Veterinary Science, University of Zimbabwe. The mice were not subjected to unnecessary stresses and those to be put-down we done so in humane way. Anaesthesia was used during incisions to minimize pain. The animals were handled and treated following the principles outlined in the "Guide for the Care and Use of Laboratory Animals" prepared by the National Academy of Sciences and published by the National Institutes of Health.

Acute and sub-acute toxicity

Toxicity studies were conducted as per internationally accepted protocol drawn under OECD (Organization for Economic Cooperation and Development) guidelines as cited by Ukwuani et al (2012). Animals were grouped into groups of five (5) animals depending on doses to be given, with one group serving as the control group. Each of the four (4) plant extracts had three groups for the acute toxicity (i.e. 100mg/kg, 200mg/kg and 300mg/kg). They were fasted overnight and dosed with the respective plant extracts as humanely as possible by gavaging and were observed for 48 hours. For sub-acute each plant extract had one group that was maintained at 200mg/kg body weight for 16 days. Observations were made throughout the course of the experiments. The animals that died at any stage of both experiments and those put-down at the end had post-mortem carried out on them. Their livers and kidneys were preserved in formalin for histopathological examinations. The histological evaluations were done by a veterinary pathologist and a histologist. All evaluations were done relative to the negative control group.

Histological examinations

Organ collection (livers and kidneys) was done immediately after euthanasia and placed in 10% formalin for preservation before histopathological examinations. The histological evaluations were done by a veterinary pathologist and a histologist within hours of being put down. All evaluations were done relative to the negative control group.

Haematological studies and markers of toxicity

At the end of the sub-acute assay, blood samples were collected from each animal and the following parameters were analysed: aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP), total and conjugated bilirubin, albumin, total protein, urea, uric acid, electrolytes, creatinine, haematocrit (HCT), haemoglobin (Hb), red blood cell (RBC), mean cell volume (MCV), mean corpuscular haemoglobin (MCH), mean corpuscular haemoglobin concentration (MCHC), white blood cell (WBC)lymphocytes, neutrophils, monocytes, basophils and platelets.

Statistical analysis

Data on the measured parameters were analysed for analysis of variance (ANOVA) in a completely randomized design using general linear model of SPSS version 21.0. Differences among treatment means were separated using multiple comparisons LSD test at 5% level of significance.

RESULTS

Acute toxicity

All the animals in the acute toxicity assay for *A. multiflorum* 300mg/kg body weight died or had to be put down to limit suffering. Two died and two had to be put done. The deaths in the acute assay were attributed to the gastrointestinal disturbances (excessive gassing) leading to distended intestines (Figure 1).

Animals on all the doses of C. *quadrangularis* and *E. abyssinica* leaf and bark extracts 100mg/kg & 200mg/kg body weight were active and exhibiting baseline characteristics and behaviours as before extract administration and/or just as the control group at 24hours. Those on *E. abyssinica* bark extracts 300mg/kg body weight and *A. multiflorum* 100 & 200mg/kg body weight were active and exhibiting baseline characteristics and behaviours as before extract administration and/or just as the control group at 48hours. The lethal dose in 50% of the population (LD_{50}) of *C. quadrangularis*, *E. abyssinica* leaves and *E. abyssinica* bark could not be precisely

estimated based on the doses used as all doses used were not lethal (thus it is considered to be above 300 mg/kg body weight for all the said extracts). The LD_{50} for *A. multiflorum* extracts was estimated to be between 250 to 300 mg/kg body weight.

Sub-acute toxicity

Regular observations of behaviours, physical and general activity parameters such as body weight, food consumption and water intake of all the sub-acute groups did not differ relative to the control group throughout the 16 day period of the experiments.

Cissus quadrangularis extracts did not lead to any observable changes to the structure or function of the organs assayed. Based on histological observations (Table 1.) the liver and kidney were normal after 16 days of daily 200mg/kg body weight for the *C. quadrangularis* extracts. All the other extracts exhibited some drug(s)/extract(s) related damages (Table 1.) to the livers and kidneys (Table 2. and Table 3.). Possible damage on heart muscles/cells could not be ascertained.

Variations of the full blood count (FBC) parameters in all extracts from the control in Table 4. could suggest some effects on cellular composition of the blood. The assays did not however show conclusively haemolysis or other blood related toxicity of the extracts. This may be however be different if high does are used or if the period is increased. Variations in the neutrophils with *E. abyssinica* barks and *A. multiflorum* aerial parts with rather very low values relative to the control and also lymphocytes with *E. abyssinica* barks and *A. multiflorum* having very high relative to the control (Table 5.), these may suggest some kind of inflammatory activity. The variations of biochemical parameters with *E. abyssinica* leaves and *A. multiflorum* (Table 6.) particularly urea rather higher levels of urea, lower levels of potassium and sodium as well as those of creatinine in all extracts may point to some impairment of renal or heart functions. Cardiac enzymes for the test groups did not vary significantly much from that of the control as shown in Table 8. Liver function parameter variations from the control shown in Table 7., does not point to significant liver damage with an extract but that maybe because of the period of the tests, a longer period might exhibit significant liver damage. The rather high total and/or conjugated bilirubin associated with *E. abyssinica* leaves (Table 7.) might suggest possible liver insufficiency or interference with bile flow, this however is not conclusive on its own as other sources not related to the liver may be contributing as well.

Table 1. Histological observations in the sub-chronic groups of mice fed with extracts of selected plants used in animal wound management

	Organ	
	Liver	**Kidney**
Treatment		
CQ	Normal structure of the hepatocytes	Normal structure (normal glomerulus, proximal tubules - cuboidal cells)
EAb	Congested blood vessels, perivascular inflammatory infiltration Inflammatory cell infiltration around the central vein	Loss of tubular structure, tubular cell death/tubular necrosis
EAl	Dilated and congested blood vessels Necrotic foci, inflammatory cell infiltration Several hepatocytes with double nuclei in view	Necrotic foci in papillary region Loss of tubules in necrotic foci, vacuolated cells in place of original cells
AM	Hepatocyte vacuolation, necrotic foci Necrotic focus, inflammatory cell infiltration, large nuclei in hepatocytes, hepatocyte enlargement	High number of small dark nuclei in cells lining tubulesLow tubular cells with small dark nuclei, necrotic foci

CQ – *Cissus quadrangularis*, EAb – *Erythrina abyssinica* barks, EAl – *Erythrina abyssinica* leaves and AM – *Adenium multiflorum*

Table 4. Full blood count (FBC) of the groups on various extracts and the control group of mice fed with with extracts of selected plants used in animal wound management

Treatment	**WBC x10^9/L**	**RBC x10^12/L**	**HB g/dL**	**HCT %**	**MCV fl**	**MCH pg**	**MCHC g/dL**	**PLT x10^9/L**	**RDW**
CQ	5.38^{a}±0.060	8.86^{d}±0.006	14.7^{b}±0.058	42.67^{c}±0.437	48.6^{b}±0.035	16.5^{b}±0.058	34.1^{c}±0.017	1505.0^{b}±2.88	15.4^{d}±0.017
EAb	2.69^{d}±0.038	9.39^{b}±0.006	15.8^{a}±0.058	46.1^{a}±0.058	49.1^{a}±0.02	17.2^{a}±0.384	34.4^{b}±0.011	1373.0^{c}±1.73	16.2^{b}±0.006
EAl	3.76^{c}±0.011	9.75^{a}±0.029	16^{a}±0.058	45.2^{b}±0.058	46.3^{d}±0.023	16.4^{b}±0.06	35.4^{a}±0.035	1547.0^{a}±1.73	15.5^{c}±0.023
AM	4.36^{b}±0.012	9.13^{c}±0.017	14.7^{b}±0.058	42.8^{c}±0.058	46.8^{c}±0.029	16.1^{b}±0.058	34.4^{b}±0.006	1166.7^{d}±4.41	16.5^{a}±0.029

Control	2.25^{e}±.0173	8.84^{d}±0.023	15.7^{a}±0.116	47.4^{a}±0.116	45.4^{e}±0.057	14.8^{c}±0.057	32.6^{d}±0.023	1147.0^{d}±1.155	16.5^{a}±0.011

$^{a, b, c}$ *Means in the same column with different superscripts are significantly different (P < 0.05)* **CQ –** *Cissus quadrangularis*, EAb – *Erythrina abyssinica* barks, EAl – *Erythrina abyssinica* leaves and AM – *Adenium multiflorum*
WBC – white blood cell count, RBC – red blood cell count, HB – haemoglobin, HCT haematocrit, MCV – mean capsular/cell volume, MCH mean cell haemoglobin, MCHC – mean cell haemoglobin concentration, PLT – platelets, RDW – red blood cell distribution width.

Table 5. Differential count of the groups on various extracts and the control group

Treatment	Neutrophils X10^9/L	Lymphocytes X10^9/L	Monocytes X10^9/L	Eosinophils X10^9/L	Basophils X10^9/L
CQ	25.9^{b}±0.057	74.0^{d}±0.577	0^{c}	0^{b}	0.37^{a}±0.265
EAb	6.8^{d}±0.115	93.2^{b}±0.231	0^{c}	0^{b}	0^{b}
EAl	19.7^{c}±0.116	78.7^{c}±0.173	1.5^{a}±0.06	0.37^{a}±0.262	0^{b}
AM	1.7^{e}±0.23	98.3^{a}±0.04	0^{c}	0^{b}	0^{b}
Control	40.3^{a}±0.058	59.6^{e}±0.058	0.34^{b}±0.230	0^{b}	0^{b}

$^{a, b, c}$ *Means in the same column with different superscripts are significantly different (P < 0.05)*CQ – *Cissusquadrangularis*, EAb – *Erythrinaabyssinica* barks, EAl – *Erythrinaabyssinica* leaves and AM – *Adeniummultiflorum*

Table 6. Biochemical parameters of the groups on various extracts and the control group

Treatment	Urea Mmol/L	Sodium Mmol/L	Potassium Mmol/L	Chloride Mmol/L	Creatinine Mmol/L
CQ	6.9^{d}±0.115	92.1^{b}±0.00	10.94^{b}±0.023	24.0^{d}±0.058	89.4^{d}±0.23
EAb	6.9^{d}±0.173	86.4^{c}±0.00	8.86^{c}±0.023	24.7^{c}±0.115	27.0^{e}±0.462
EAl	11.28^{a}±.0202	82.7^{d}±0.00	7.12^{e}±0.011	29.7^{a}±0.115	158.4^{b}±1.154
AM	10.0^{b}±0.006	72.0^{e}±0.00	7.63^{d}±0.0115	23.9^{d}±0.058	97.8^{c}±0.608
Control	8.21^{c}±0.023	131.8^{a}±0.00	13.69^{a}±0.0346	25.3^{b}±0.029	436.2^{a}±0.643

$^{a, b, c}$ *Means in the same column with different superscripts are significantly different (P < 0.05)*CQ – *Cissusquadrangularis*, EAb – *Erythrinaabyssinica* barks, EAl – *Erythrinaabyssinica* leaves and AM – *Adeniummultiflorum.*

Table 7. Liver function tests on the various groups of mice fed with with extracts of selected plants used in animal wound management

Treatment	ALT IU/L	AST IU/L	ALP IU/L	GGT IU/L	Albumin G/L	Total Protein G/L	Globulin G/L	Total Bilirubin µmol/L	Conjugated Bilirubin µmol/L
CQ	39.0^{b}±0.058	206.0^{d}±1.155	0	0	20.67^{c}±0.882	50.67^{c}±0.881	30.0^{a}±0.116	28.0^{e}±0.577	21.0^{d}±0.57
EAb	39.0^{b}±0.057	242.0^{c}±1.14	0	0	27.0^{b}±0.057	55.0^{b}±0.231	28.0^{ab}±0.058	50.0^{c}±1.155	35.0^{c}±0.057
EAl	19.0^{c}±0.461	261.0^{b}±1.155	0	0	33.0^{a}±0.577	59.0^{a}±0.173	26.0^{ab}±0.173	121.0^{a}±1.732	85.0^{a}±01.155
AM	52.0^{a}±0.577	157.0^{e}±1.155	0	0	26.0^{b}±0.116	56.0^{ab}±0.058	30.0^{a}±0.116	42.0^{d}±0.577	33.0^{c}±0.173
Control	40.0^{b}±0.057	351.3^{a}±1.76	0	0	28.0^{b}±0.058	55.0^{b}±0.231	27^{b}.0±0.057	80.0^{b}±1.155	55.0^{b}±0.577

$^{a, b, c}$ *Means in the same column with different superscripts are significantly different (P < 0.05)*
CQ – *Cissus quadrangularis*, EAb – *Erythrina abyssinica* barks, EAl – *Erythrina abyssinica* leaves and AM – *Adeniummultiflorum*, ALT – Alanine transaminase, AST – Aspartate transaminase, ALP – Alkaline phosphatase and GGT - γ-Glutamyl transpeptidase

Table 8. Cardiac enzymes of the various groups of mice fed with with extracts of selected plants used in animal wound management

Treatment	CKiU/L	LDHU/L
CQ	38.0^{c}±0.115	3149.0^{d}±5.77
EAb	36.5^{cd}±0.115	3231.0^{c}±4.04
EAl	60.0^{b}±0.289	4471.5^{a}±2.94
AM	36.0^{d}±0.058	1903.5^{e}±1.10
Control	87.0^{a}±1.155	3591.5^{b}±2.37

$^{a, b, c}$ *Means in the same column with different superscripts are significantly different (P < 0.05)*
CQ – *Cissus quadrangularis*, EAb – *Erythrina abyssinica* barks, EAl – *Erythrina abyssinica* leaves and AM – *Adenium multiflorum*. CK – Creatine Kinase and LDH – Lactate dehydrogenase (lactic acid dehydrogenase)

Fig 1. Mice very sick and intestines distended to the point of bursting of those that died on *A. multiflorum* acute toxicity group

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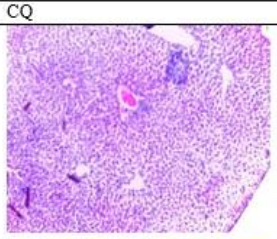
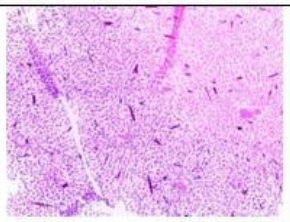
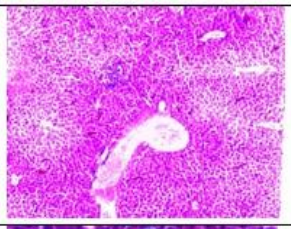
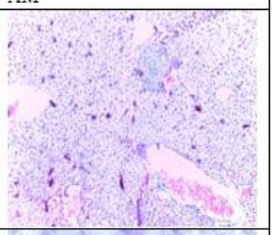
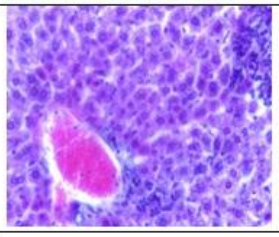
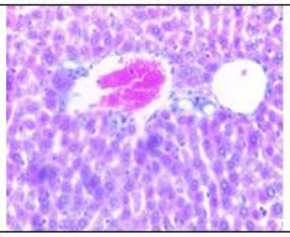
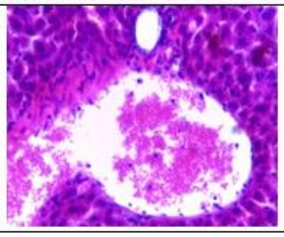
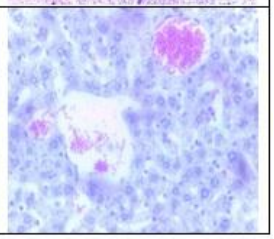
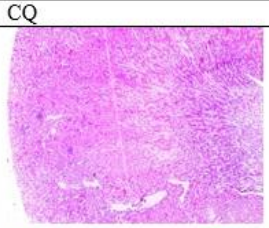
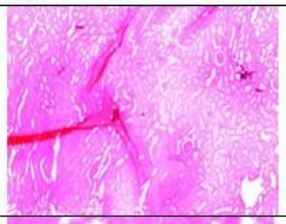
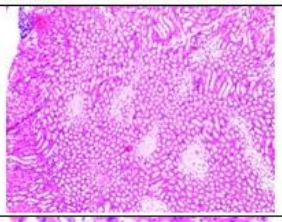
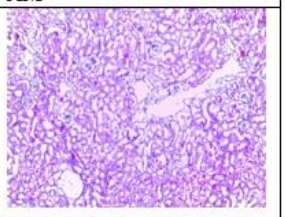
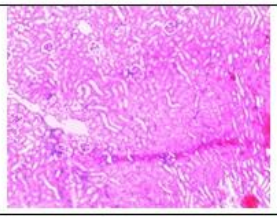
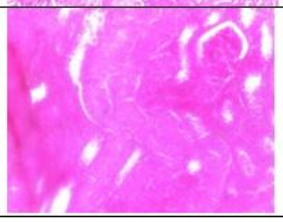
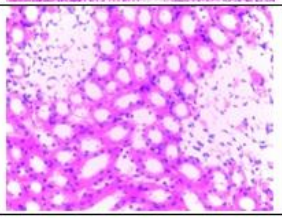
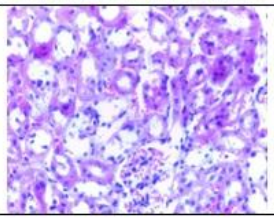
Table 2. Images of the livers from the various groups of mice fed with with extracts of selected plants used in animal wound management

Magnification	CQ	EAb	EAl	AM
Low				
High				

CQ – *Cissus quadrangularis*, EAb – *Erythrina abyssinica* barks, EAl – *Erythrina abyssinica* leaves and AM – *Adenium multiflorum*

Table 3. Images of the kidneys from the various extract groups

Magnification	CQ	EAb	EAl	AM
Low				
High				

CQ – *Cissus quadrangularis*, EAb – *Erythrina abyssinica* barks, EAl – *Erythrina abyssinica* leaves and AM – *Adenium multiflorum*

DISCUSSION

The death and gastrointestinal observations in acute toxicity assays in the *A. multiflorum* 300mg/kg body weight group is consistent with other findings as extracts of the adeniums have been shown to cause digestive disturbances, cardiac insufficiency and ventricular fibrillation (Burrows, 2013). Safety studies on *C. quadrangularis* in rats and mice have also shown no toxic effects at dosages as high as 2000 mg/kg of body weight (Raj *et al*., 2011). In addition, the fact that *C.*

quadrangularis is part of many diets in many parts of the world and is used internally for a variety of reasons (oral rehydration, nutritional supplementation, constipations, eye diseases, piles, anaemia, asthma, irregular menstruation, burns and wounds) demonstrate safety of the plant and or its extracts (Sen *et al*., 2012). *Erythrinaabyssinica* and other species in its genus have been in use in ethnomedicine both topically and internally for various human and animal ailments including bacterial and fungal infections, liver disorders, skin diseases, brucellosis, oedema, hygroma, dropsy, sterility, eye diseases, fever and inflammation (Kone *et al*., 2011). Wide use however does not guarantee safety instead may highlight the need for more toxicological assays of the plants and/or their extracts. Thus there might be need for more evaluations of safety of *Erythrina* species as not much literature is available about their safety or toxic potentials. The safety associated with long-term use of the plant(s) or parts thereof in ethnomedicine may be as a result of lower doses being used or very short duration of exposure.

Damages to the liver and kidney are usually expected from herbal extracts. This is because the liver is the primary site of detoxification and kidneys are key in xenobiotic and/or its metabolite elimination. Biochemical/haematological marker analyses are affected by many factors that occur before sampling such as fasting or other environmental and the blood itself may be the source of variations (e.g. haemolysis, collection procedures, anticoagulants used, etc.) (Fernandez *et al*., 2010).

The results for extracts are consistent with other findings particularly that of *A. multiflorum* which also did not show significant haematological changes in many other toxicity assays (Sharma *et al*., 2015). The predominant toxic effect of adeniums is thought to be cardiotoxicity attributed to compounds like cardenolide glycosides: e.g. echujine (Bradfield *et al.*, 2015), thus the need for more cardiac evaluations. Adenium genus poisoning after intravenous administration in cats has been shown to lead to the following signs and symptoms: incontinence, restlessness, rapid breathing, and convulsions (Bradfield *et al.*, 2015), but none of the mice showed these signs.

The γ-Glutamyl transpeptidase (GGT) is found in many cells in the body e.g. hepatocytes, biliary epithelial, renal tubules, pancreas and intestine thus other diseases like renal failure may elevate GGT (Giannini *et al*., 2005). Alkaline phosphatase (ALP) elevation could result from liver and bone diseases. ALP is a more specific test for hepatic injury, its magnitude of increase is often higher relative to other enzyme markers (Boone L *et al*., 2005). Bilirubin is a product of red blood cell destruction/metabolism elevation of unconjugated may suggest high production or impaired liver uptake will elevation of conjugated may point to obstruction of the bile duct or some liver disease like cirrhosis or hepatitis. Thus these haematological markers need to be correlated with other signs or patient characteristics as well as histological evaluations to be conclusive, also the liver may appear normal based on these parameters alone in the presence of other liver diseases or damage (Boone *et al*., 2008; Thapa BR and Anuj W, 2007). Resources permitting, a longer period of similar studies will reveal more on toxic potentials of these and other plant extracts. As part of further analysis there is need for inclusion of the heart, spleen, lungs, intestines and testis/ovary in histological evaluations. Also evaluations of possible dose-related damages to internal organs maybe essential. Given complex nature of haematological and/or biochemical analysis; to get conclusive results there is need for larger sample sizes and longer periods of study.

CONCLUSION

In the dosage ranges used, the study has shown the safety of *Cissus quadrangularis* extract for oral use in both short and relatively long term use. Caution however should be applied to possible long term oral use of *Erythrina abyssinica* and/or extracts thereof as it has shown possible liver and kidney damage. *Adenium multiflorum* being toxic in the acute and sub-acute settings (possible gastrointestinal disturbance, and liver and kidney damage) should not be used orally. If oral use is to be done very low doses are recommended.

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Nil

CONFLICT OF INTEREST

No interest

REFERENCES

Araújo J, Joao X, Mariana SG, et al. A Phytochemical and Ethnopharmacological Review of the Genus Erythrina. In *Phytochemicals - A Global Perspective of Their Role in Nutrition and Health*, 1, 2012, 327–52.

Adedeji OS, Ogunsina TK et al. Ethnoveterinary Medicine in African Organic Poultry Production. *International Food*

Research Journal, 20 (2), 2013, 527–32.

Boone L, Meyer M, et al. Selection and Interpretation of Clinical Pathology Indicators of Hepatic Injury in Preclinical Studies Position Paper. *Veterinary Clinical Pathology*, 34(3), 2005, 182–88.

Burrows, George E and Ronald J. *Toxic Plants of North America*. Second. Iowa: Wiley-Blackwell, 2013.

Bradfield, et al. Southern African Arrow Poison Recipes , Their Ingredients and Implications for Stone Age Archaeology. *Southern African Humanities* 27 (11), 2015, 29–64.

EFSA. 2009. EFSA Compendium of Botanicals That Have Been Reported to Contain Toxic , Addictive , Psychotropic or Other Substances of Concern. *EFSA Journal*, 7, 2009, 281.

Fernandez, et al. Clinical Biochemistry Parameters in C57BL/6J Mice after Blood Collection from the Submandibular Vein and Retroorbital Plexus." *Journal of the American Association for Laboratory Animal Science*, 49(2), 2010, 202–6.

Giannini, Edoardo G, Roberto T and Vincenzo S. Liver Enzyme Alteration: A Guide for Clinicians. *Canadian Medical Association Journal*, 172(3), 2005, 367–79.

George P. Concerns Regarding the Safety and Toxicity of Medicinal Plants - An Overview. *Journal of Applied Pharmaceutical Science*, 1(6), 2011, 40–44.

Ghouse and Mohammed S. A Pharmacognostical Review on Cissus Quadrangularis Linn. *International Journal of Research in Pharmacy and Biosciences*, 2(7), 2015, 28–35.

Gueye EF. Ethnoveterinary Medicine against Poultry Diseases in African Villages." *World's Poultry Science Journal*, 55(6), 1999, 1–9.

Ifeoma, Obidike, and Salawu O. Screening of Herbal Medicines for Potential Toxicities. In *New Insights into Toxicity and Drug Testing*, edited by Sivakumar Gowder, 2013, 63–88.

Irungu and Stanely. Isolation and Characterization of Antimicrobial Compounds From the Plants, Erythrina Abyssinica Dc . and Chasmanthera Dependens Hochst. Kenyatta University, 2012.

Jothy, Subramanion L, Zuraini Z, et al. Acute Oral Toxicity of Methanolic Seed Extract of Cassia Fistula in Mice. *Molecules*, 16 (6), 2011, 5268–82.

Kone, Witabouna M, Kakou NE, et al. Assessing Sub-Saharian Erythrina for Efficacy: Traditional Uses, Biological Activities and Phytochemistry. *Pakistan Journal of Biological Scieneces*, 14(10), 2011, 560–71.

Kumar and Dharmendra. Phytochemical Investigation and Pharmacological Screening of Some Medicinal Plants, Shobhit University, 2014.

Marume A, et al. Antioxidant Properties, Protein Binding Capacity and Mineral Contents of Some Plants Traditionally Used in the Management of Animal Wounds. *South African Journal of Botany*, 111, 2017, 23–28.Shukla and Susmita. Callus Induction of Adenium Obesum through Leaf Explant - An Ornamental Tree of Medicinal Value. *International Journal of Tropical Agriculture*, 33(2), 2015, 1369–72.

Marandure T. Concepts and Key Issues of Ethnoveterinary Medicine in Africa: A Review of Its Application in Zimbabwe. *African Journal of Agricultural Research*, 11(20), 2016, 1836–41.

Neuwinger and Hans D. *African Ethnobotany: Poisons and Drugs : Chemistry, Pharmacology, Toxicology*, 1996.

Raj, Justin S and Baby J. Pharmacognostic and Traditional Properties of Cissus Quadrancularis Linn -an Overview. *International Journal of Pharma and Bio Sciences*, 2 (1),2011, 131–39.

Sen, Monokesh K and Biplab K. A Review on Phytochemical and Pharmacological Aspects of Cissus Quadrangularis L. *International Journal of Green Pharmacy*, 6(3), 2012, 169–73.

Sharma, Yash, Anshita N and Susmita S. Antimicrobial Activity and Pytochemical Screening of Adenium Obesum (Desert Rose) Leaf. *International Journal of Pharma and Bio Sciences*, 6(3), 2015, 85–92.

Thapa BR and Anuj W. Liver Function Tests and Their Interpretation. *Indian Journal of Pediatrics*, 74, 2007, 67–75.

Ukwuani AN, et al. Toxicological Studies of Hydromethanolic Leaves Extract of Grewia Crenata. *International Journal of Pharmaceutical Sciences and Drug Research* 4(4), 2012, 245–49.

Vijayalakshmi G, et al. Antibacterial and Phytochemical Analysis of Cissus Quadrangularis on Selected UTI Pathogens and Molecular Characterization for Phylogenetic Analysis of Klebsiella Pneumoniae. *World Journal of Pharmacy and Pharmaceutical Sciences*, 4(11), 2015, 1702–13.

WHO. Who Expert Committee On Specifications For Pharmaceutical Preparations." Geneva, 2006.

Zorloni and Alberto. Evaluation of Plants Used for the Control of Animal Ectoparasites in Southern Ethiopia. University of Pretoria, Pretoria, 2007.