Prime Scholars Library

Full Length Research Paper

Available online at
https://primescholarslibrary.org/

Medical Advances and Case Report

Vol. 6 (2), pp. 13 – 17, April, 2018

Prognosticate toxicity profile associated with repeat-dose administration of root extracts of *Zanthoxylum chalybeum* using laboratory rat models

A.J Shittu, Olonire Olufemi, Tayo Adetokunbo and Mariam Odunaiye

Natural Chemotherapeutics Research Laboratory, Ministry of Health, P.O. Box 4864, Kampala, Uganda.

Abstract

Zanthoxylum chalybeum **is a traditional medicinal plant used in the treatment of various ailments in the African region. In sickle cell disease a decoction of the root bark extract is administered for life. The safety of long term use of this plant is not documented. This study investigated the systemic effects of daily administration of low and high oral doses of the root bark extract of this plant in rodents. Three groups of six young Albino wistar rats each were used. The first and second groups received a daily dose of 100 and 4000 mg/kg of the extract respectively orally for 4 weeks. Animal weight, renal, liver function tests, heamatological indices, plasma electrolytes and tissue pathology were used to assess safety. No serious adverse event was observed with both study doses in the experimental animals. Histology revealed presence of squamous cell growth in the small and large intestines of the rats that received the dose of 4000 mg/kg. This group also showed significant elevations in plasma creatinine, sodium and potassium levels ($p< 0.05$). Long term administration of low doses of the root bark extract of *Z. chalybeum* is safe in experimental animals. High doses however may be associated with impaired renal function and intestinal neoplasms. We recommend cautious dosing in traditional use of the root bark extracts of *Z. chalybeum* as there is a possibility of dose- dependant toxicity. There is need for further studies to document the effectiveness of these extracts in sickle cell disease**

Keywords: *Zanthoxylum chalybeum,* safety, repeat dose

INTRODUCTION

Zanthoxyleum chalybeum (Rutaceae) is a traditional medicinal plant of Eastern Africa. Decoctions of this plant are traditionally used in treatment of malaria, sickle cell disease, measles, skin infections and coughs (Olila et al., 2002). Studies carried out on the Zanthoxylum species in Nigeria have shown that root extracts have antisickling effect on red blood cells and hence the basis for use in sickle cell patients (Durosimi et al., 1989). In sickle cell disease lifelong treatment is administered. The duration of such treatment tends to raise safety concerns. The acute toxicity documented in mice with the methanolic root extract (Ogwal-Okeng et al., 2003) and the ethanol-acetic root extract (Ogwang et al., 2006) is not sufficient to allay the safety concerns associated with long term use of the root extracts from this plant. This study was carried out to predict the toxicity profile associated with repeat-dose administration of root extracts of *Z. chalybeum* using laboratory rat models.

STUDY METHODS

Plant material

Z. chalybeum fresh root bark and aerials parts were collected from Katakwi district, about 400km North-East of Kampala, Uganda. Plant materials were harvested during rainy season with the help of a traditional healer and brought to the Natural Chemotherapeutics Research Laboratory, Ministry of Health, Kampala, Uganda. The identity of the plant was confirmed by a taxonomist at the laboratory

.

and a voucher specimen; number NCJ 256 was deposited at the Natural Chemotherapeutics Research Laboratory herbarium.

Preparation of the test extract of *Z. chalybeum*

Fresh roots were dried under shade, debarked and pulverized. A portion of the powder (100g) was extracted by cold maceration in ethanol-acetic acid 9:1 solvent system for three days with occasional shaking and then filtered. The filtrate was concentrated using a Rotary evaporator, BIBBY STERLIN LTD Model RE 100 to obtain a viscous solid. The viscous solid was oven dried between 40 - 50°C for 8 h and then air dried for 24 h to obtain a dry extract giving 12% yield. The crude extract was suspended in distilled water for administration to the test models. The suspension was found to be very stable and was stored between 2 - 8°C in fridge till end of study.

Experimental Animals

Male Albino wistar rats, 8 weeks old, average weight 74 ± 14 g were provided by the animal house of the Faculty of Veterinary Medicine, Makerere University Kampala. The animals were maintained in a standard environmental condition, in clean cages and provided with water and food ad lib. All precautions were taken and the animals handled according to the international biosafety guidelines (Laboratory Biosafety Guidelines, 2004) . The experimental procedures were approved by the Institutional Research and Ethics Committee of the Natural Chemotherapeutics Research Laboratory.

Experimental design

Eighteen male Albino wistar rats aged 28 days were acclimatized in the laboratory for 1 week. The test animals were fed on standard diet provided by Ugachick (U) Ltd, local manufacturers of animal feeds and maintained at room temperatures with 12 hour day light and 12 h of no light. At 35 days (5weeks) the rats were weighed and randomized into three groups and each rat numbered on the tail. Rats in group one were given 100mg/kg body weight daily by oral route while those in group two received incremental doses of the extract daily by the same route starting at 100 mg/kg body weight up to 4,000 mg/kg, a dose level that had caused 50% death in mice (Ogwang et al., 2006) . The third group received distilled water (solvent used as diluent of the extract) by same route. The extract and the water were administered to the test animals daily for 28 days. Blood (1 ml) was drawn from the tail of each test animal for baseline haemoglobin determination and thereafter weekly. The animal weights were taken every third day and animals were given sufficient food and water each day.

Measurements

The measurements included; survival rate, weight changes, hemoglobin, renal and liver function tests. At 28 days the rats were anaesthetized with ether and blood was drawn from the venacava for haematological and biochemical analysis. The kidney, liver, brain and gastrointestinal tract were harvested and sent for gross pathology and histopathology at the Faculty of Veterinary Medicine, Makerere University Kampala.

Data analysis

Data was analyzed using prism graph pad Version 3.02 and the excel spreadsheet 2000. A difference was considered significant a t $p< 0.05$. The gross changes, post-mortems and histology were analyzed qualitatively; photographs of the histology picture under microscope could not be taken due to equipment failure.

Ethical issues

The study was approved by the Research and Ethics Committee of Makerere University Medical School, Kampala, Uganda and the Ethics Committee of Natural Chemotherapeutics Research Laboratory-Ministry of Health. The study registration number is NCRL/06-2. Study animals were handled in conformity with guidelines for the care and handling of laboratory animals provided by the two Institutions.

RESULTS

General observations

No death was observed for any of the rats in the study groups and there were no remarkable changes in general appearance or animal behavior.

Changes in animal weight

The mean weight gain in the low dose group was 78.30 ± 3.3, in high dose group was 80.40 ± 6.7 g and 74.50 ± 5.6 g in the control group. The changes in weight were however not significant compared to the controls ($p = 0.05$). The changes in weight are shown in Figure.1.

Effect of extract on hematological indices, kidney and liver functions

The haemoglobin mean gains were 7.4 ± 0.5, 7.5 g ± 0.7 and 7.3 ± 0.5 g/dl for 100 mg/kg, 4000 mg/kg and water groups respectively. The gains in both study groups were not significantly different from control group $P = 0.8$ and 0.79 respectively at 95% confidence interval (Figure 2). The study groups had a significantly higher total white blood cell count with a means of 5, 726 ± 69 /l and 5, 826 ± 39 /l for 100 and 4000 mg/kg groups respectively compared to 4,733 ± 143 /l for control ($P<0.0001$ at 95% confidence interval). The differentials white blood cell count showed reduction in neutrophils and increase in lymphocytes levels. Creatinine levels were significantly elevated in the 4000 mg/kg group $p = 0.001$ at 95% confidence interval (Table1).

Gross pathology and histopathology changes in experimental animals

Gross pathology showed no apparent toxicity on all the organs. The histology picture showed Squamous cell growths in the large and small intestines in 100% of 4000 mg/kg group and in 33.3% of the 100 mg/kg group and 33% of the control group. There was however no signs of fast dividing cells in the squamous cell neoplasm

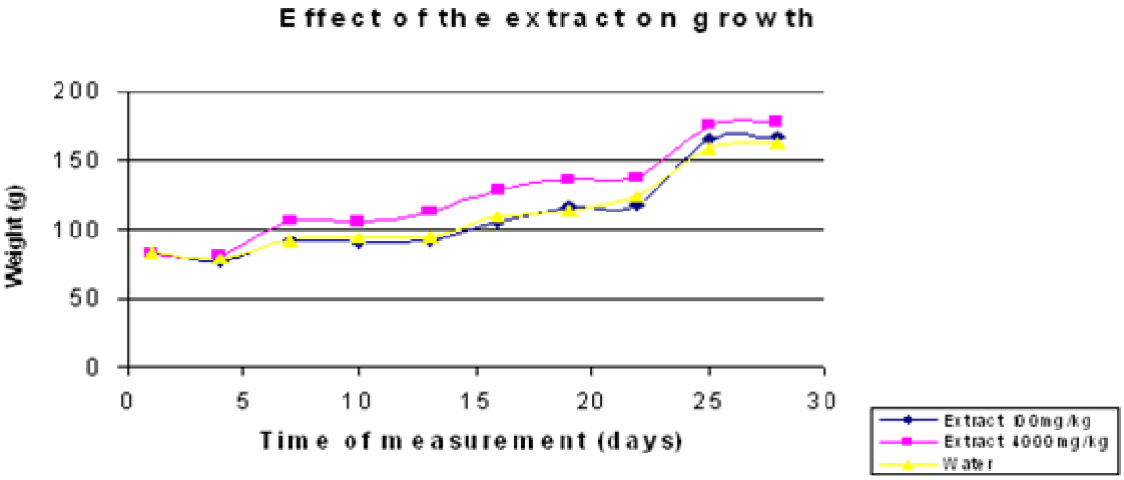

Figure 1. The effect of repeat-dose administration of the root bark extracts of *Z. chalybeum* on weight in male albino wistar rats.

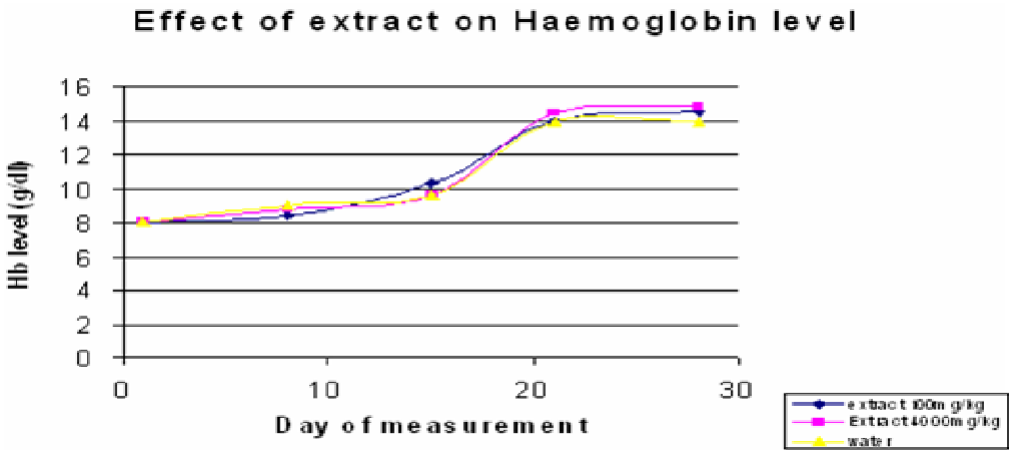

Figure 2. The effect of repeat-dose administration of the root bark extracts of *Z. chalybeum* on haemoglobin (Hb) levels in young growing Albino Wistar Rats.

Table 1. Effect of *Z. chalybeum* extract on selected body systems in male wistar albino Rats

Group	Weight change (g)	Haemoglobin Change (g/dl)	Leucocytes /L	Differentials (%)	Creatinine	ALT	AST
Extract group 1 (n = 6)	78.30±3.3	7.4± 0.5	5,726± 143	N(59.0±1.4) L(41.0±1.4)[c]	52.30±3.4[a]	7.36±0.07[b]	5.0±0.18[c]
Extract group2 (n = 6)	80.40±6.7[a]	7.5 g ± 0.7[a]	5,826± 39[b]	N(59.0±1.4) L(41.0±1.4)[c]	69.67±4.2[b]	7.16±0.07[c]	5.2±0.18[c]
Control (n = 6)	74.50±5.6	7.3± 0.5	4,733± 143	N(64.7±5.0) L(35.3±5.0)	51.67±4.2	7.75±0.05	6.35±0.4

[a]p > 0.05 considered not significant
[b]p< 0.05 considered significant.
[c]p< 0.005 Considered extremely significant.
ALT- Alaninine Amino Transferase, AST- Aspartate Amino Transferase, N- Neutrophils, L-Lymphcytes.

DISCUSSIONS

This study showed that root extracts of *Z. chalybeum* have no significant systemic effects in experimental animals at the lower dose of 100 mg/kg. The extract also has no effect on weight gain, an important measure of growth. The absence of growth retardation in the study animals even at doses as high as 4000 mg/kg could suggest safety of the extract in children. Chloroquine is the drug widely used in sickle cell children to prevent malaria; however chloroquine is known to retard growth (Kinney et al., 1999). A Zanthoxylum extract is reported to inhibit liver cycle of *P. falciparum* at IC50 of 4.9 ug/ml (Olakuule et al., 2005) . This property of Z. *chalybeum* together with the level of safety observed in this study makes it a potential alternative to chloroquine use in sickle cell patients in Uganda. This study finding also justifies the indigenous knowledge of the Masai tribe in Kenya, who add the root decoction to the milk of their babies to boost appetite (Megan *E* et al., 2007). Although the gains in haemoglobin in the extract groups were not significantly different from control group, most drugs when administered at such high dose would cause severe anemia. The Z. *Chalybeum* extract in this study appears not to affect heamoglobin formation or hasten erythrocyte degradation.

The study of Osoba et al. (1989) showed the usefulness *Z. Chalybeum* root extract as antisickling agent. This property was attributed to the presence of membrane stabilizing benzoic acid derivatives in the roots of *Zanthoxylum* species. The ability to stabilize red blood cell would be an added value to sickle cell patients who usually suffer from sickle anaemia (Kaine et al., 1983). The significant rise in lymphocytes may point to the potential usefulness of the plant as immune system stimulant (Kinney et al., 1999) a factor that may justify the use of the plant decoction in treatment of measles in Uganda. The high creatinine level in the 4000 mg/kg group predicts the potential of the extract to compromise renal output if used in very large doses. However, in usual traditional medicine low doses of plant extracts are used and such high doses are only likely to occur in cases of acute poisoning or over dose.

This study used a rodent model to study the safety of the extracts, rodents are however known to metabolize drugs more rapidly than higher mammals hence the need to interpret results with care. The doses of the extracts used in traditional use are not documented; however, important biological activity of *Z. chalybeum* has been attributed to the rich alkaloidal content which include; fagaronine, fagaramide, chelythrine and berberine (Trease and Evans, 2002). On this basis we used alkaloids as the biomarker in this study and a method that concentrates alkaloids described by Gosh et al. (2005) was used. It is possible that actual safety profile of the extract is much better than what is reported in this study. It should also be noted that the study lasted only 28 days and hence the results should be applied to life long treat ment with caution.

Conclusion

Long term administration of low doses of the root bark extract of *Z. chalybeum* is safe in experimental animals. High doses however may be associated with impaired renal function and intestinal neoplasms. We recommend cautious dosing in traditional use of the root bark extracts of *Z. chalybeum* as there is a possibility of dose-dependant toxicity. There is need for further studies to document the effectiveness of these extracts in sickle cell disease

ACKNOWLEDGEMENTS

The authors are grateful to the management and staff of the Natural Chemotherapeutics Research Laboratory, Ministry of Health, Uganda, for the financial support to this work.

REFERENCES

Amit A, Saxena VS, Pratibha N, Bagchi M, Bagchi D, Stohs SJ (2003). Safety of a Novel Botanical Extract Formula for Ameliorating Allergic Rhinitis; Toxicology Mechanisms and Methods 13: 4253 261.

Ghosh R, Sharatchandra K, Rita S, Thokchom IS (2004). Hypoglycemic activity of Ficus hispida (bark) in normal and diabetic albino rats. Indian J. Pharmacol. [cited 2005 May 19]; 36: 222-225.

Ghosh, MN (1984). Fundamentals of Experimental Pharmacol. 2nd Edition. Scientific Book Agency, Culcutta. pp. 154-157.

Ichiro Shimizu, Yue-Rong Ma, Yoko Mizobuchi, Fei Liu, Tetsuo Miura, Yoichiro Nakai, Mitugi Yasuda, Masako Shiba, Takahiro Horie, Sakae Amagaya, Norifumi Kawada, Hitoshi Hori , Susumu Ito (1999). Effects of sho-saiko-to, a japanese herbal medicine, on hepatic fibrosis in rats . Hepatology. 29(1): 149-160

John O Grady ,Pieter H Joubert (1999). Reproductive and Developmental Toxicity Testing. Handbook of Phase I/II Clinical Drug Trial, page 41.

Little JA, McGowan VR, Kato GJ, Partovi KS, Feld JJ, Maric I, Martyr S, Taylor JG, Machado RF, Heller T, Castro O, Gladwin MT, (2006). Combination erythropoietin-hydroxyurea therapy in sickle cell disease: experience from the national Institutes of Health and a literature review. Haematologica ,91,8, 1076-83.

Megan E Parker, Sophie Chabot, Brian J. Ward, Timothy Johns,200. Traditional dietary additives of the Maasai are antiviral against the measles virus. J. Ethnopharmacol. Vol. 114, Issue 1, Pages 146-152

Ogwal-Okeng JW, Obua C, Anokbonggo WW (2003). Acute Toxicity Effects of Methanolic Extract of Fagara chalybeum Root Bark. African Health Sciences, 3, 3, 124-126.

Ogwang PE, Tumusiime HR, Mugisha Gerosome, Nalika Nusula , Agwaya Moses,2006.Ethanol-Acetic acid extract of Zanthoxyleum chalybeum root bark shows greater potency than the methanol extract. The Makerere Pharmaceutical Journal, 1(,4) .23.

Olila D, Olwa-Odyek , Opuda Asibo J.,2002. Screening of extracts of *Zanthoxylum chalybeum* and *Warburgia ugandensis* for activity against measles virus (Swartz and Edmonston strains) in vitro African Health Sciences, 2,1, 2-10 .

Osoba OA, Adesanya SA, Durosimi MA (1989). Effect of Zanthoxylum xanthoxyloides and some substituted benzoic acids on glucose-6-phosphate and 6-phosphogluconate dehydrogenases in Hbss red

blood cells. J. Pharmacol, 27(1-2):177-83
Tagoe CN, Ofori -Adjei D. (1995). Effects of chloroquine and its enantiomers on the development of rat embryos *in vitro*. Teratology. 52(3): 137-142.
Thomas R. Kinney, Ronald W. Helms, Evin E. O'Branski, Kwaku Ohene-Frempong ,1999. Safety of Hydroxy Urea in children with sickle cell anaemia: Results of the HUG-KIDS study, a phase I/II trial. Blood, 5, 1550-1554.
Trease and Evans, 2002. Pharmacognosy, SAUNDERS imprint of Elsevier Science, 15th Edition, page 490-492.