Acute Toxicity of Senna Didymobotrya Fresen Irwin Roots Used as a Traditional Medicinal Plant in Kenya

Lydia Bonareri Nyamwamu

Moses Ngeiywa

Margaret Mulaa

University of Eldoret
Department of Biological Sciences
Eldoret, Kenya

Agola Eric Lelo

Johnstone Ingonga

Kenya Medical Research Institute
Centre for Biotechnology Research and Development
Nairobi, Kenya

Albert Kimutai
University of Kabianga
Department of Biological Sciences
Kericho, Kenya

Abstract

Medicinal plants play an important role in the treatment of various illnesses in Kenya and the whole world. Senna didymobotya is one of such plants used traditionally in Kenya to treat illnesses such as diarrhea, malaria, ringworm, jaundice and intestinal worm. The main aim was to determine the acute toxicity of Senna didymobotya roots. The methanol total and dichloromethane crude root extracts of S. didymobotrya were the most toxic and they killed 80% of mice at a dose of 5000mg/kg body weight with an LD_{50} of 1927mg/kg after a period of 14days.

Keywords: *Senna didymobotrya,* toxicity, roots

1.0 Introduction

The use of medicinal plants is as old as man (Anthoney *et al*., 2013). In the past few decades medicinal plants have been tested extensively and found to have several pharmacological uses such as, antibacterial activity, antifungal activity, anti-diabetic activity, anticancer activity, antioxidant activity, hepatoprotective activity, haemolytic activity, anti-inflammatory activity, larvicidal activity, anthelmintic activity, central nervous system activity and pain relief activity (Sukirtha *et al*., 2012; Mir *et al*., 2013). Many side effects associated with allopathic medicines and dependencies are common reasons why many people are hospitalized today. In order to counteract the effects, many people are now turning to nature in pure form to prevent and cure diseases using natural medicinal herbs or natural health alternatives (Deshpande, 2010). Many species of the plants belonging to the genus *Cassia* possess potential larvicidial, ovicidal, repellant activities against wide species of immature and adult vector mosquitoes (Govindarajan *et al*., 2011b).

Senna didymobotrya is a potential medicinal plant and the medicinal values are explored well in many parts of the world by traditional practitioners (Nagappan, 2012). In Kenya, traditionally the Kipsigis community has been using these plants to control malaria as well as diarrhea (Korir *et al*., 2012). The pastoralists of West Pokot peel the bark, dry the stem and burn it into charcoal that they use to preserve milk (Tabuti, 2007). In addition, it has been used to treat skin conditions of humans and livestock infections as well (Njoroge and Bussmann, 2007). It is also used in the treatment of animal diseases such as removal of ticks (Njoroge and Bussmann, 2006).

In Congo, Rwanda, Burundi, Kenya, Uganda, Tanzania, root decoction of this plant has been used for the treatment of malaria, other fevers, ringworm, jaundice and intestinal worm (Nagappan, 2012). The root or leaf mixed with water or decoction of fresh parts has been used to treat abscess of the skeletal muscle and venereal diseases (Kamatenesi-Mugisha, 2004). The plant is also useful for the treatment of fungal, bacterial infections, hypertension, haemorrhoides, sickle cell anemia, a range of women's diseases such as inflammation of fallopian tubes, fibroids and backache, to stimulate lactation and to induce uterine contraction and abortion (Tabuti, 2007). The antibacterial activities of hexane extract against *Microsporum gypsum*, has been reported (Korir *et al*., 2012). According to Reddy *et al*., (2010), presence of phenolic compounds, flavonoids and carotenoids in the ethyl acetate extract of leaves are responsible for pronounced antibacterial activities. A decoction or infusion from the leaves, stems and roots of *S. didymobotrya* is drunk as a laxative and purgative for the treatment of abdominal pains, while in large quantities it is taken as an emetic (Singh *et al*., 2003). The leaf sap in water is given as a drink to treat diarrhoea, dysentery, and taken as a diuretic and emetic (Sunarno, 1997). A decoction made from the roots is used as an antidote for poisoning, to expel a retained placenta, and to treat East Coast fever and blackleg (Njoroge and Bussmann, 2007). Much research has not been done to test the toxicity of *S. didymobotrya*. This study was carried out to investigate the toxicity of the roots of *S. didymobotrya*.

2.0 Materials and Methods

2.1 Experimental Animals

Eight week old BALB/c mice were obtained from Kenya Medical Research Institute's (KEMRI), Nairobi animal house facility and were infected in accordance with Institutional Animal Care and Use Committee approved protocols (Asgharpour *et al*., 2005). The animals were maintained under specific pathogen free conditions. The experiments using mice were done in compliance with Animal Care and Use Committee guidelines of KEMRI.

2.2 Collection of *Senna* Didymobotrya Roots

The roots of *S. didymobotrya* were collected randomly from Bomet County during the month of October-November, 2012 and were authenticated. The plant materials were taxonomically identified by a taxonomist and the voucher specimens were preserved at the Centre for Biotechnology Research and Development of the Kenya Medical Research Institute (KEMRI), Nairobi for future reference.

2.3 Preparation of Plant Material for Solvent Extractions

The sample preparation and extraction procedure were carried out as described by Harbone, (1994).The roots were washed, cut into small pieces and air-dried for three weeks under a shed. The dried specimens were shred using an electrical mill in readiness for extraction. Sequential extraction was carried out on the plant material with distilled water, dichloromethane, ethyl acetate, hexane and methanol as the solvent system.

2.4 Organic Solvent Extractions

300g of dried powder were taken in 600ml of hexane in a conical flask, plugged with cotton wool and then kept on a rotary shaker at 190-220rpm for 24hrs. After 24hrs the supernatant was collected and the solvent evaporated. The residue obtained was collected and stored at 4°C in airtight bottles. The process was repeated sequentially for ethyl acetate, dichloromethane and methanol.

2.5 Aqueous Extraction

300g of dried powder were added to 600ml of distilled water in a conical flask and boiled on slow heat for 2hrs. It was then filtered using No. 1 Whitman filter paper and centrifuged at 5000 rounds per minute (rpm) for 10min. After 6hrs, the supernatant was collected at an interval of every 2 hrs pooled together and concentrated using a rotary evaporator. The residue obtained was collected and stored at 4°C in airtight bottles.

2.6 Determination of acute toxicity

BALB/c mice were used in the study. Healthy mice weighing 20-22g were divided into two groups (control and treatment) each cage with five mice. The mice were allowed to have access to water and food except for a short fasting period of 12hrs before oral administration of a single dose of the test between 1250-5000mg kg^{-1} body weight. In this study, three dose levels of 1250, 2500 and 5000mg kg^{-1} body weight were used. The general behavior of mice was observed continuously for 1hr after the treatment and then intermittently for 4hrs and thereafter over a period of 24hrs (Twaij *et al*., 1983). The mice were observed further for up to 14 days following treatment for any sign of restlessness and the latency of death (LD_{50}).

The LD_{50} value was determined according to a method described by Miller and Tainter (1944). During the experiment all the dead mice were disposed according to KEMRI biosafety guidelines. After the experiment all the mice were sacrificed using chloroform and the carcasses were safely incinerated.

3.0 Results

During the 24hour period dichloromethane crude root extract of *S. didymobotrya* killed 60% of mice at a dose of 5000mg/kg and 20% at a dose of 2500mg/kg (Table 1), ethyl acetate crude root extract of *S. didymobotrya* killed 60% of mice at a dose of 5000mg/kg and 40% at a dose of 2500mg/kg, methanol total crude root extract of *S. didymobotrya* killed 80% of mice at a dose of 5000mg/kg, 60% at a dose of 2500mg/kg and 20% at a dose of 1250mg/kg. Methanol successive crude root extract of *S. didymobotrya* killed 60% of mice at a dose of 5000mg/kg, 40% at a dose of 2500mg/kg and 20% at a dose of 1250mg/kg, hexane and water crude root extracts of *S. didymobotrya* killed 40% of mice at a dose of 5000mg/ml and 20% at a dose of 2500mg/kg. During the 14days period dichloromethane and methanol total crude root extracts of *S. didymobotrya* killed 80% of mice at a dose of 5000mg/kg body weight, 60% at a dose of 2500mg/kg and 40% at a dose of 1250mg/kg with an LD_{50} of 1927mg/kg (Table 2). Ethyl acetate crude root extract killed 60% of mice at a dose of 5000mg/kg body weight, 40% at a dose of 2500mg/kg, 20% at a dose of 1250mg/kg and its LD_{50} was 3892mg/kg. Methanol successive and hexane crude root extracts of *S. didymobotrya* killed 60% of mice at a dose of 5000mg/kg body weight, 40% at a dose of 2500mg/kg and their LD_{50} was 4092mg/kg, while water crude root extract of *S. didymobotrya* killed 60% of mice at a dose of 5000mg/kg, 40% at a dose of 2500mg/kg and its LD_{50} was 4433mg/kg.

Discussion

This study investigated the acute toxicity of crude root extracts of *S. didymobotrya*. The crude root extracts were considered safe when the death of mice was less than 50% which was observed at a lower dose of less than 5000mg/kg body weight except methanol total whose safety is below 2500mg/kg. It was found out that the water crude root extract of *S. didymobotrya* (LD_{50} of 4433mg/kg) was less toxic and this is in agreement with a study carried out by Muthaura *et al*. (2007), who found out that the water extract was less toxic compared to dichloromethane crude root extract. *Senna didymobotrya* is widely used among the Kipsigis community in treating and managing skin and diarrhea infections. However the method of preparation such as burning into ashes and then mixing with margarine for skin conditions and mixing with hot water and milk for stomach problems may be a way of reducing toxicity otherwise the population may be continuously exposed to the toxic plant (Korir *et al*., 2012).

Conclusion

Dichloromethane and methanol total crude root extracts of *Senna didymobotrya* were more toxic with an LD_{50} of 1927mg/kg therefore, for safety measures they should be taken at lower doses. *Senna didymobotrya* is generally mixed with milk or margarine for oral use; this is basically to reduce toxicity.

Acknowledgements

The authors are very grateful to Ms Emily Wanjiku and Milkah Mwangi for their assistance during data collection.

Table 1: Acute Toxicity of Senna Didymobotrya Crude Root Extracts in BALB/c Mice

	Mortality after 24Hour							
	5000mg/kg		2500mg/kg		1250mg/kg			
Test extracts	Deaths	% Deaths	Deaths	% Deaths	Deaths	%	Deaths	LD50
Dichloromethane	3/5	60	1/5	20	5/0	0		4432
Ethyl acetate	3/5	60	2/5	40	5/0	0		4092
Methanol total	4/5	80	3/5	60	1/5	20		2342
Methanol successive	3/5	60	2/5	40	1/5	20		3892
Hexane	2/5	40	1/5	20	0/5	0		5329
Water	2/5	40	1/5	20	o/5	0		5329

Table 2: Acute Toxicity of Senna Didymobotrya Crude Root Extracts in BALB/c Mice

	Mortality after 14days						
	5000mg/kg		2500mg/kg		1250mg/kg		
Test extracts	Deaths	% Deaths	Deaths	% Deaths	Deaths	% Deaths	LD50
Dichloromethane	4/5	80	3/5	60	2/5	40	1927
Ethyl acetate	3/5	60	2/5	40	1/5	20	3892
Methanol total	4/5	80	3/5	60	2/5	40	1927
Methanol successive	3/5	60	2/5	40	0/5	0	4092
Hexane	3/5	60	2/5	40	0/5	0	4092
Water	3/5	60	1/5	20	0/5	0	4433

References

Mir, A.M., Sawhney, S.S. and Jassal, M.M.S. (2013). Qualitative and quantitative analysis of phytochemicals of Taraxacum officinale,Wudpecker; J Pharm Pharmacol; 2: 01-05.

Sukirtha, K. and Growther, L. (2012). Antibacterial, analysis of selected medicinal plants. J Nat Prod and Plant Res; 2: 644-648.

Deshpande, D.J. (2010). A handbook of medicinal herbs. Agrobios, Joghpur, India.

Govindarajan, M., Mathivanan, T., Elumalai, K., Krishnappa, K. and Anandan, A. (2011b). Ovicidal and repellent activities of botanical extracts against Culex quinquefasciatus, Aedes aegypti and Anopheles stephensi (Diptera: Culicidae). Asian Pac J Trop Biomed; 43-48.

Nagappan, R. (2012). Evaluation of aqueous and ethanol extract of bioactive medicinal plant,Cassia didymobotrya (Fresenius) Irwin & Barneby against immature stages of filarial vector, Culex quinquefasciatus Say (Diptera: Culicidae). Asian Pacific J Trop Biomed; 707-711.

Korir, R.K., Mutai, C., Kiiyukia, C. and C. Bii, C. (2012). Antimicrobial Activity and Safety of two Medicinal Plants Traditionally used in Bomet District of Kenya. Res J Med Plant; 6: 370-382.

Tabuti, J.R.S. (2007). Senna didymobotrya (Fresen.) H.S. Irwin & Barneby. In: Schmelzer, G.H. and Gurib-Fakim, A. (Editors). Prota 11(1): Medicinal plants/Plantes médicinales 1. PROTA,Wageningen, Netherlands.

Njoroge, G. N. and Bussmann, R. W. (2006). Herbal usage and informant consensus in ethnoveterinary management of cattle diseases among the Kikuyus (Central Kenya). J Ethnopharmacol; 108 : 332-339.

Kamatenesi-Mugisha, M. (2004). Medicinal plants used in reproductive health care in Western Uganda: documentation, phytochemical and bioactivity evaluation. Ph.D. thesis. Makerere University, Kampala, Uganda; 228.

Singh, H.B., Singh, R.S. and Sandhu, J.S. (2003). Herbal Medicine of Manipur: A colour Encyclopaedia, 11.

Sunarno B. (1997). Senna didymobotrya (Fresenius) Irwin & Barneby[Internet] Record from Proseabase. Faridah Hanum, I & van der Maesen, L.J.G. (Editors). PROSEA (Plant Resources of South-East Asia) Foundation, Bogor, Indonesia. http://www.proseanet.org.

Asgharpour A., Gilchrist C., Baba D., Hamano S. and Houpt E. (2005). Resistance to intestinal Entamoeba histolytica infection is conferred by innate immunity and Gr-1+ cells. Infection and Immunity; 73: 4522 – 4529.

Twaij H.A.A., Kery A. and Al-Khazraji N.K. (1983). Some pharmacological, toxicological and phytochemical investigations on Centaurea phyllocephala. Journal of Ethnopharmacology; 9: 299-314.

Miller L.C.and Tainter M.L. (1944). Estimation of LD50 and its error by means of log-probit graph paper. Proceedings of the Society for Experimental Biology and Medicine; 57:261.

Muthaura C.N., Rukunga G.M., Chhabra S.C., Omar S.A., Guantai A.N., Gathirwa J.W., Tolo F.M., Mwitari P.G., Keter L.K., Kirira P.G., Kimani C.W., Mungai G.M. and Njagi E.N. (2007). Antimalarial activity of some plants traditionally used in Meru District of Kenya. Phytother Res; 21: 860-867.