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Annals of Biological Research, 2014, 5 (6):15-19
(http://scholarsresearchlibrary.com/archive.html)

Annals of Biological Research
Scholars Research Library
ISSN 0976-1233
CODEN (USA): ABRNBW

Investigation on Toxicity, hypoglycemic effect of the root bark of *Securidaca longepedunculata* Fresen (Polygalaceae) and determination of heavy metals in it

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ABSTRACT

The ethanol extract of the root bark of the plant Securidaca longepedunculata Fresen (Polygalaceae) was found to be toxic against the albino mice at medium (600 mg/kg) and higher doses (1000 mg/kg) when administered orally through gavages. The LD_{50} calculated was found to be 547.72 mg/kg. The root bark did not show any significant hypoglycemic effect on Streptozotocin induced albino mice. Six heavy metals were analyzed but only three metals (Zn, Cu, Mn) could be detected in the root and root bark of this plant after microwave assisted digestion at optimized condition using Flame Atomic Absorption Spectroscopy (FAAS) technique. Out of these six, three metals (Cr, Pb and Cd) were found below the method detection limit and the rest (Zn, Mn and Cu) were within the permissible range according to specification made by the World Health Organization (WHO).

Keywords: Toxicity, hypoglycemia, *Securidaca longepedunculata***,** Polygalaceae, albino mice, heavy metals.

INTRODUCTION

Securidaca longipedunculata Fresen (family: Polygalaceae) is a semi-deciduous shrub or small tree that grows up to 12 meter (36 feet) tall. It is a widely used medicinal plant in most of the African countries such as Angola, Benin, Botswana, Burundi, Cameroon, Chad, Cote d'lvoire, Democratic Republic of Congo, Eritrea, Ethiopia, Gambia, Ghana, Guinea, Kenya, Malawi, Mali, Mozambique, Namibia, Niger, Nigeria, Rwanda, Senegal, Sierra Leone, South Africa, Sudan, Tanzania, Uganda, Zambia and Zimbabwe. The tree also grows in other countries like Cuba, Malaysia and many Asian countries. In Ethiopia it is known as 'Es a manahi' (Amharic name), 'shotora' (Tigrigna name). The common English names are: violet tree, fiber tree and Rhodesian violet.

The flowers are in abundance at the beginning of rainy season, sweetly scented, bright purple or violet racemes and the fruit is winged. Fruit is more or less a round nut, somewhat heavily veined occasionally smooth, bearing a single, oblong, rather curved, membranous wing up to 4 cm long; purplish-green when young, becoming pale, straw-colored when mature. It is a beautiful flowering tree with potential as an ornamental in parks, gardens and along the road sides. Fruits often hang on the trees for many months and those that stay the longest are said to germinate best. The young leaves are eaten as a vegetable and in sauces. The flowers are suitable for honey production. In Eritrea, this tree is one of the most valuable low land honey sources. The timber is resistant to termites and decays and used for poles and hut construction. The flowers yield oil and used for some purposes. Bark, roots and seeds are used in arrow poison and root as a snake repellent. Roots are 100% effective as a molluscicide. Due to the presence of

saponins, the bark, root bark and crushed seeds give a soapy solution in water and are used as soap for washing clothes.

In Northern Nigeria, it is called "Uwar Magunguna" in Hausa language, literally translated "the mother of all drugs", a tribute to its very numerous medicinal uses[1]. In Tanzania, the plant is known as 'Mlyangabako' among the Hehe people in Iringa and Masukemengi" and Zigua people of Tanga. In Iringa, it is used for the management of some manifestation of non-insulin dependent diabetes. A decoction of the dried bark is used to treat bacterial infection and inflammation[2,3], insanity and epilepsy[4]. The leaves are used for treating wounds, sores, cough, venereal diseases, snake bite and as a purgative[5,6], to treat tuberculosis[7,8], bilharzias[9], skin diseases[10], convulsion in children[11]. The decoction of the root is used to hasten (accelerate) labour[12,13], to treat malaria[5], rheumatism[14], gonorrhea, palpitations, pneumonia, syphilis[15] and asthma[16].

In many parts of Africa, the plant is employed in traditional medicine principally for its psychotropic properties; the aqueous extract of the root is used as psychopharmacological agents[17]. They also reported the presence of ergot alkaloid in the extract. The plant has been employed for various rheumatic and inflammatory diseases and as anti-helmintic or purgative agents[18]. The use of the plant against snakebites, fish poisoning and in different diseases have been documented[10,18,19,20]. Its use in bacterial and malarial chemotherapy has also been investigated[21,22]. The anti-inflammatory activity of the methanol extract of this plant was reported[23].

Heavy metals may cause serious health hazards such as renal failure, skin diseases, and liver damage when they are above permissible limit[24]. Accumulation of heavy metals, namely Pb, Cd, Cu, Ni, Cr, Zn and Mo were found in different herbal drugs[25,26]. Therefore, we became interested to investigate the general toxicity against albino mice and the presence of heavy metals in root and root bark of the plant. Furthermore, some investigation was carried out to establish the scientific validity regarding the anti-diabetic activity of the plant as claimed and used by the common people in Tanzania. Despite the widespread use of this plant in all parts of Africa, there is no report in the literature about presence of heavy metals and hypoglycemic activity in the plant. The results of our investigation are described here.

MATERIALS AND METHODS

The instruments used were: Microwave (BMS1 BUCK Scientific Germany-210), FAAS (BUCK-210 Scientific USA), PH Meter HK-3C and Glucometer (PRODIGY, USA). The drugs Streptozotocin and Glibenclamide used were obtained from Sigma-Aldrich and Bon Marche, En France respectively.

Plant material

The sample was collected from a place close to Ajora falls (SNNPR), Kambeta Tembaro Zone, Hadero Tunto Zuria Woreda, Ethiopia. The falls is located at altitude of 1447 meter (approximately 4340 feet) and 300 km away from the capital city Addis Ababa in Ethiopia. The plant was authenticated by the taxonomist in Addis Ababa University National Herbarium, Addis Ababa. After collection the root was thoroughly washed with tap water to remove any dust particles, then by distilled water and finally by de-ionized water. Afterwards the bark was separated from the root by peeling and both were then air dried under shade. The air dried materials were then ground separately with local metal coffee bean grinder. The materials after grinding were kept in separate glass containers before extraction and metal analysis.

Extraction of root bark with ethanol

The powdered sample (root bark, 200g) was soaked in 96% ethanol (800 ml) contained in 1-litre Erlenmeyer flask using maceration procedure and kept at room temperature for 72 hours with a constant shaking in a shaker. After extraction the solvent was filtered through Whatmann filter paper No.1 and the filtrate concentrated in a rotary evaporator, a light yellow coloured semi-solid obtained (22.15 g, yield 11.1%).

Toxicity of ethanol extract of the root bark

A total number of 33 mice were used for the experiment. The extract (soluble in water) was dissolved in distilled water and different concentrations (dosages) namely 75, 125, 250, 500, 1000 and 2000 mg/kg body weight were prepared. All the mice (age 5 weeks, weighing 24-30 g) were acclimatized for 7 days before the experiment started. The animals were fasted for three hours prior to oral administration of the extract. The 33 mice were divided into two batches namely 1st batch (consisting of total 18) and 2nd batch (consisting of total 15). The 1st batch was then

arranged in 6 groups having 3 mice in each group. Similarly the 2nd batch was arranged in 3 groups having 5 mice in each group **(Table1)**. The 1st group in each batch (1st and 2nd) received only distilled water (as control) and was provided with normal food after 30 minutes the experiment started. The acute toxicity of the extract of the root bark was carried out by oral administration of the above dosages by gavages (only extract) and the mice were closely observed for 24 hours. The food was provided to all mice after 30 minutes of oral administration of the extract. The observation was continued for next seven days for any delayed effect according to the literature[27,28].

Hypoglycemic effect of the root bark

Preparation of reagent

A buffer solution was prepared from a weak acid (citric acid) and its sodium salt for dissolving streptozotocin. Sodium citrate (1.47 g) was dissolved in distilled water (50 ml) and the pH adjusted to 4.5 by gradual addition of citric acid. The hyperglycemia in mice was induced by intraperitoneal injection of the above solution. The 30 male mice were selected (age 5 weeks, weighing 24-30 g), acclimatized for seven days and fasted for 4 hours before inducing streptozotocin. The mice were distributed into 5 cages containing 6 mice in each cage as shown in **Table 2**. The cage 5 was used as control.The procedure was followed according to literature [29].

Streptozotocin (STZ) is a drug that increases the blood glucose level in mice by distracting the β-cells of the animals. After induction of STZ, 10% sucrose solution (in water) was provided to the mice along with normal food and then left for 15 hours. Afterwards the 10% sucrose solution was replaced by normal water, normal food provided and the animals left for another 33 hours. When the glucose level reached above 250 mg/dl, the blood was taken from the tails of the mice and the blood glucose level measured by using Glucometer. Standard drug, ethanol extract and distilled water were administered through gavages (**Table 4**).

Determination of heavy metals

All parameters like temperature, time, power, reagent volume and mass of the sample were optimized for microwave digestion. Thoroughly homogenized plant material (0.2 g) was digested in polytetraflouro ethylene vessel with HNO_3 (6 ml, 65%) and H_2O_2 (3 ml, 30%) and the process repeated three times. The blank reagent containing HNO_3 (6 ml, 65%) and H_2O_2 (3 ml, 30%) was digested in microwave and the process repeated six times. Each digestion of the plant material and blank was quantitatively transferred to a 25 ml volumetric flask and the volume made up to the mark with distilled de-ionized water.

Different standard solutions containing 10 mg/l for each metal was prepared from standard stock solutions containing 1000 mg/l of each metal. These standards solutions were diluted with de-ionized water to obtain four working standards for each metal of interest. Calibration curves were drawn from 4 working standard concentrations for each metal. The efficiency of the method was assessed by spiking root sample with known amounts of metals and gave a good recovery, 86-108%. Three replicate determinations were carried out for each sample. Mn, Cd, Cr, Zn, Pb and Cu were analyzed with FAAS. The results obtained were subjected to statistical analysis using excel 2007 and origin pro 8 software.

RESULTS AND DISCUSSION

Regarding Toxicity

The results obtained in **Table 1** showed that the plant was toxic at medium and higher dosages but it has less toxic effect at lower doses. The LD_{50} calculated was found to be 547.72 mg/kg. The procedure was followed according to literature[28] who worked on another plant *Lycium shawii* (Solonaceae). Their work did not show any toxicity of the plant below the dosage of 3000 mg/kg when monitored for 14 days. Similarly, the plant *Nypa fruticans* Wurmb (Arecaceae) also did not show any toxicity below the dosage of 3000 mg/kg[30]. But in our investigation we observed that *Securidaca longepedunculata* started showing toxicity even at dosage of 500 mg/kg and above.

Regarding hypoglycemic effect

Based on the results obtained in **Table 4**, the statistical analysis t-test followed by one way ANOVA showed that there was no significant difference between the diabetic control and the experimental value (diabetic induced + extract 200 mg/kg) at probability of 0.05 at all measured time but there was a significant difference between diabetic control and diabetic induced + glibenclamide (5 mg/kg) at 2 hours. This showed that the ethanol extract of the plant had no significant hypoglycemic effect on mice.

Regarding Determination of heavy metals

Table 5 showed the presence of much higher amount of Zn and Mn but lower amount of Cu in the root than that of the root bark. The amount of Cu present is very close in both the cases when compared. The other metals such as Cr, Cd and Pb were not detected in the root as well as root bark since they were below the method detection limit. All the metal concentrations detected in *Securidaca longepedunculata* were found to fall within the range recommended by WHO. According to the specification made by WHO, the plant under investigation is safe for oral administration as medicine from the toxicity point of view concerning the presence of heavy metals determined.

Table 1: Toxicity of ethanol extract of the root bark

Batch	Group	Dose in mg/kg	Observation from 0 hour to 7 days	LD_{50} mg/kg
1	1 (3 mice)	0 (H_2O, Control)	No mortality and normal behavior	547.72 mg/kg
	2 (3 mice)	75	No mortality and normal behavior	
	3 (3 mice)	125	No mortality and normal behavior	
	4 (3 mice)	250	They were inactive during first 1 hour and showed normal behavior afterwards	
	5 (3 mice)	500	They were inactive during first 4 hours and showed normal behavior afterwards	
	6 (3 mice)	600	Mortality of 2 mice after 20 hours and rest 1 mice survived	
2	1 (5 mice)	0 (H_2O, Control)	No mortality and normal behavior.	
	2 (5 mice)	1000	All were unable to feed, 3 died after 15 hours and the rest 2 died after 22 hours	
	3 (5 mice)	2000	All were unable to feed, 3 died after 14 hours and the rest 2 died after 20 hours	

Table 2: Distribution of male mice and induction of Streptozotocin into the animal

Cage (6 mice in each cage)	Dosage of STZ in mg/kg
1 (24 g each)	200
2 (25 g each)	200
3 (26 g each)	200
4 (28 g each)	200
5 (30 g each)	Control (buffer)

Table 3: Test for hypoglycemia using glibenclamide and ethanol extract

Cage (6 mice in each cage)	Material administered
1	Diabetic induced + glibenclamide (5 mg/kg) *
2	Diabetic induced + ethanol extract (200 mg/kg)●
3	Non diabetic (normal) + distilled water (0.2 ml)
4	Diabetic induced (control) + distilled water (0.2 ml)

** Glibenclamide is a standard drug (hypoglycemic agent, positive control) that brings the glucose level down in the blood.*

● Administration of ethanol extracts (200 mg/kg) of the root bark.

Table 4: Hypoglycemic effect of the ethanol extract of root bark after administration of Streptozotocin.

Cage (6 mice in each)	Mice under observation	Level of blood glucose in mg/dl at different interval of time			
		0 hr	1 hr	2 hr	4 hr
1	Diabetic induced + glibenclamide (5 mg/kg)	464.6 ± 96.23	389.6 ± 157.2	304.2 ± 94.2	352.6 ± 93.8
2	Diabetic induced + ethanol extract (200 mg/kg)	446.4 ± 54.58	403.2 ± 83.73	368.2± 105.2	340.8 ± 83.43
3	Normal control	104.6 ± 10.21	103.8 ± 9.73	104 ± 16.45	105 ±10.1
4	Diabetic induced (control)	401.7 ± 82.58	442 ± 18.48	432.8 ± 52.4	392.8 ± 12.93

Table 5: Detection of heavy metals in the root and root bark

Name of element	Root (in mg/kg)	Root bark (in mg/Kg)	Upper limit recommended by WHO (1984) and literature value for edible plants*
Zn	121.92 ± 0.99	19.38 ± 0.89	Not given
Cu	3.95 ± 0.37	5.8 ± 0.29	20 (mg/Kg)
Mn	62.85 ± 0.45	43.29 ± 0.32	300 (mg/Kg)
Cr	ND	ND	----------
Cd	ND	ND	----------
Pb	ND	ND	----------

*ND = Not Detected, *Ref [32]*

CONCLUSION

The root bark of the plant was found to be toxic and showed more toxicity at higher dosage and less toxicity at medium dosage against the albino mice. But it did not show any significant hypoglycemic effect on Streptozotocin induced albino mice. Therefore, there are no strong scientific facts that were found in our experiment which could support the use of this plant in traditional medicine against diabetes. The root possesses higher concentration of Zn and Mn as compared to root bark. The concentration of copper was found higher in root bark than that of root. The toxic heavy metals such as Pb and Cd were not detected because their concentrations were below the method detection limit. The optimum concentration of Zn and Mn are very important since they possess very essential enzymatic action in their permissible limit on human physiological system [31]. Hence, the toxicity of the plant could be due to the presence of toxic organic compounds since there was no heavy metal found above the permissible limit that could contribute towards toxicity. Further work is in progress to isolate the active compound (s).

REFERENCES

[1] Dapar LPM, Aguiyi CJ, Wannang NN, Gyang SS and Tanko MN. *African J Biotechnology*, **2007**, **6**(5), 591-595.
[2] Arnold HJ and Gulumian M. *J.Ethnopharmacology*, **1984**, **12**, 35-74.
[3] Tommasi NDe, Placente S, Simone FDe and Pizza C, *J Natural Products*, **1993**, **56**,134-137.
[4] Mathias ME, *Taxon*, **1982**, **31**, 488-494.
[5] Chhabra SC, Mahunnah RLA and Mshiu EN, *J Ethnopharmacology*, **1991**, **33**,143-157.
[6] Hedberg I, Hedberg JO, Madati PJ, Mshigeni KE Mshiu EN and Samuelsson G, *J Ethnopharmacology*, **1983**, **9**, 237-260.
[7] Akah PA and Nwambie AI, *J Ethnopharmacology*,**1994**, **42**1, 79-82.
[8] Asres K, Bucar F, Kartnig T, Witvrouw M, Pannecoupue C and Clercq Ede, *Phytother Res*, **2001**, **15**, 62-69.
[9] Kamwendo WY, Chiotha SS and Msonthi JD, *Fitoterapia*, **1985**, **56**, 29-32.
[10] Odebiyi OD, *Nigerian J Pharmacy*, **1978**, **9**, 29-30.
[11] Sofowora A, *J Ethnopharmacol*, **1980**, **2**, 109-118.
[12] Kokwaro JO, Medicinal plants of East Africa 1st Edition, East African Literature Bureau, Nairobi, **1976**, p.57.
[13] Yu GD, *Chung Yao Tung Pao*, **1982**, **7**, 6-7.
[14] Kloos H, Tekle A, Yohannes LW, Yosef A and Lemma A, *Ethiopian Medical J*, **1978**, **16**, 33-43.
[15] Desta B, *J Ethnopharmacol*, **1993**, **39**,129-139.
[16] Akah PA, Gamaniel KS, Samson A and Wambebe CO, *J Ethnopharmacol*, **1997**, **55**, 87-92.
[17] Winkleman M and Dobkin de Rios M, *J Psycho act* Drugs, **1989**, **21**, 51-59.
[18] Neuwinger HD, African Ethnobotany: Poisons and Drugs: Chemistry, Pharmacology, Toxicology, Chapman and Hall, London, England, **1996**, p.743-753.
[19] Burkill HMP, The Useful Plants of West Africa, Royal Botanic Gardens, Kew 4, **1997**, p. 447- 458.
[20] Oladije OA, Awe SO and Makinde JM, *Fitoterapia*, **1998**, **69**(3), 245-248.
[21] Akinniyi JA, Manawadu D and Sultanbawa M, Ethnobotany and ethnopharmacology of Nigerian medicinal plants research and traditional medicine in malaria: In: state of medicinal plants research in Nigeria; **1996**, p.35-49.
[22] Msonthi JD, *J Agricultural Sci*,**1986**, **2**, 335- 349.
[23] Okoli CO, Akah PA and Ezugworie U. *African J Traditional, Complementary and Alternative Medicines*, **2005**, **2**(3), 54 – 63.
[24] Kirmani MZ, Mohiuddin S, Naz F, Naqvi II and Zahir E, *J Basic and Appl Sci*, **2011**, **7**(2), 89-95.
[25] Baranowska K, Srogi A, Wlochowicz K and Szczepanik, *Polish J Env Studies*, **2002**, **11**, 467-471.
[26] Rai V, Kakkar P, Khatoon S, Rawat AKS and Mehrotra S, *Pharmaceutical Biol*, **2001**, **39**, 384-387.
[27] Okolo CE, Akah PA and Uzodinma SU, *Phytopharmacol* , **2012**, **3**(1), 12-18.
[28] Sher H and Alyemeni MN, *J Medicinal Plant Res*, **2011**, **5**(15), 3387-3395.
[29] Mujeeb M, Khan SA, Ali M, Abhishek Mall A and Ahmad A, *J Pharmacy Res*, **2009**, **2**:132-139.
[30] Reza H, Haq WM, Das AK, Rahman S, Jahan R and Rrahmatullah M, *Pak J Pharm Sci*, **2011**, **24**(4), 485-488.
[31] Whitney E, Whitney EN and Rolfes SR, Editors. Understanding Nutrition. 12th edn: Thomas Learning, Inc; **2009**.
[32] Bhowmik S, Datta BK and Saha AK, *J Agricultural Technol*, **2012**, **8**, 1467-1476.