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Acute Toxicity and Some Elemental Assessment of Senna Tora Linn Thylakoids in Natural Population of Northeastern Nigeria

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Abstract

Water extract of thylakoids of Senna tora linn, a legume in the subfamily of caesalpiniodeae which grow wild in the tropic and considered as weed in many places but with great medical value worldwide was screened using standard laboratory procedures for acute toxicology and some elements. The LD50 screening was found to be higher than 5000mg/kg body weight, while elemental screening demonstrated the presence within permissible levels of iron (8.44mg/kg), copper (0.09mg/kg), zinc (0.44mg/kg), magnesium (3.38mg/kg) and calcium (1.72) in varying concentrations. Lead and Cadmium were below the detection levels of the methodology used.

Key words:-herbs; thylakoids; chlorophyll; acute toxicity; elements

Introduction.

Medicinal herbs used for therapeutic purposes are known to have many phytochemical and are rich sources of bioavailable forms of elements for human consumption, however, environmental conditions such as soil type, rainfall, agricultural activity and vicinity and type of industry influence the level of bioavailability in plants (Cui et al. 2004, Choudhury and Garg 2007, Delbari and Kulkarni 2013). The role of metals in animal and plant metabolism has long being established (Hunt and Nielsen 1981), but the effect and influence of these elements on administration of herbal medicine have received relatively little attention. Diets has long been considered as the major source of human exposure to trace element and consequently the levels in basic

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foodstuff (Levander 1986), however, the indiscriminate use of herbal preparations by 70 – 80% of world's population (Vijayan et al 2007) with regards to the proportion of various active constituents, dosage of a particular formulation and the possibility of having some traces of toxic metal in plant material with the possibility of side effects has been a major challenge facing traditional herbalists, toxicologists and nutritionists (Osadebe et al 2014).

The use of herbs is as old as man's history, many plants have efficacy in treating various disease states. Senna Tora Linn (originally described by Linn as cassia tora) a legume in the subfamily caesalpiniodeae. It grows wild in most of the tropics and is considered a weed in many places. Its native origin is not well known but probably South Asia (Ogunkunle and Ladejobi 2006; Okigbo et al 2009), and often confused with Chinese senna or sickle pods obtusifolia. It is known in Nigeria by different names, Rere Ako in Yoruba community, Tapasa in Hausa and Ochigichi in Igbo. Personal interaction with herbalists and community elders in Northeastern Nigeria showed their relevance in food and local herbal formulation.

Senna tora linn either whole or parts are used by herbalists as liver stimulant, mild laxative, heart tonic, feverish condition, laxative, antiperodic, leprosy, ringworm, bronchitis, and ophthalmic, skin diseases, cough, hepatic disorder, and haemorrhoids (Elujoba et al 1999; Manjsha et al 2011; Santosh et al 2013). It has been reported that leaves of Cassia tora has antioxidant activity and contain many active substances including chrysophenol, emodin, rhein (Park et al 2004; Ingle et al 2012). The seeds, when roasted and grinded are used as beverage drink by some tribes in Nigeria.

Inside plant cells and other eukaryotic cells with photosynthetic ability, are tiny, bacteria-sized organelles, called chloroplasts with membranous Thylakoids (Nathan and Charles 2006). Thylakoids constitute the site of the light dependent reactions of photosynthesis with favourable nutritional composition with 70% membrane proteins and 30% galactolipids (Albertsson 2001; Kubmarawa et al 2011).

Thylakoids of various green leaves are of great import in herbal formulation of Nigerian Nation, aqueous extract of Neem leaves is well accepted as treatment for malaria parasite, Gossypium herbaceum leave extract is taken as blood tonic, aqueous extract of Trichodesma africanum leaves is known for the treatment of diarrhea, dysentery, diuretic and insect sting. Thylakoids extract from spinach have been extensively studied (Kohnke 2009), the suppression of appetite, induction of satiety, reduction in body weight in obese animals and human (Kohnke 2009). The aim of this study is to investigate and document acute toxicological effects and some elements content of water extract of senna tora linn thylakoids naturally found in Maiduguri, Northeastern Nigeria which to our knowledge has not been documented before.

Materials and Methods.

Plant Material.

Senna Tora linn was collected from the natural population growing at Shagari Low-Cost Housing Estate along Gubio Road in Maiduguri, Borno State, Nigeria in August 2014. Maiduguri (latitude 11.86811^0 24'N, longitude 13.19781^0 8'E) is in Sahel belt with raining season spanning the period between June/July and September/October. *Senna Tora linn* was authenticated by experts form Forestry Department of University of Maiduguri, Maiduguri, Borno State, Nigeria

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Thylakoids Extraction

The fresh leaves of *Senna Tora Linn* were washed thoroughly in tap water, followed by distilled water and then deionized water. The extraction was carried out according to Rickard Kohnke el al 2009. Briefly 1000g of the leaves were homogenised with 1500ml of deionized water using stainless domestic blender (Master Chef® China). The homogenate was filtered using filter cloth, followed by Number 1 filter paper. The pH of the filtrate was adjusted to pH 4.7 and kept at 4^0C for 4 hours to settle, after which the green thylakoids sediment and the yellow supernatant were harvested into separate container. The sedimentation process was repeated three times. The harvested green thylakoids was rewashed with deionized water and the sedimentation repeated at the same pH 4.7) which was adjusted to Ph 7.0 and the yellow supernatant were dried using lyopolizer (FreeZone® 4.5 Liter Freeze Dry System: Labconco, U.S.). The dried extracts were kept in air tight containers and store at 4^0C for further use.

Acute Toxicity Study

Oral Medial lethal dose (LD_{50}) was determined by modified method of Lorke 1983. The method was divided into two phases, in the first phase; twelve mice were randomly divided into three groups of four mice and were given 10, 100 and 1000mg/kg body weight per oral respectively. The mice were observed for sign of toxicity and death within 24 hours. The second phase was based on the result of the first phase (0/4 for all the groups) where 1250, 2500 and 5000mg/kg were administered per oral for three group of a mouse each respectively. The outcome was still 0/1 for each group. Thus, the LD_{50} per oral was above 5000mg/kg body weight.

Screening for some elements (Lead, Copper, Zinc, Cadmium, Iron, Calcium and Magnesium)

Sample preparation: The extract was blend for homogeneity after which 2.5g of the extract was weighed and pre-ashed on a heating mantle on hot plate at 100 – 150^{o}C to remove all volatile substances. The extract was ashed in a carbonite furnace at 500 – 550^{o}C for hours until the extract is completely ashed. Few drops of concentrated HCl was added to the ashed extract after it has cool, the essence of HCl is to enhance the dissolution of the ash.

The ash was dissolved in 1% HNO_3 and transferred into 100ml volumetric flask and make up to the mark. The solution was filtered, and the filtrate transfer into sample vials for analysis using Atomic Absorption Spectrophotometer (Shimadzu AA-6800 Japan).

Results

Oral acute toxicological assessment in our study showed the LD_{50} of the extract to be higher than 5000mg/kg body weight.

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Table 1: Elemental screening of Thylakoids extract of Senna Tora Linn

Elements	Thylakoids extract (mg/kg)
Iron	8.44 mg/kg
Copper	0.09 mg/kg
Zinc	0.44 mg/kg
Magnesium	3.38 mg/kg
Lead	BDL
Cadmium	BDL
Calcium	1.72mg/kg

BDL = Below Detection Level.

Table I show the result of elemental screening of the extract which revealed the presence of Iron, magnesium and calcium in high concentrations while copper and zinc were present in traces. Lead and cadmium

Discussion.

The use of metals in different anthropogenic activities is an age old practice. Increase industrial and mining activities during the late 19th and early 20th century have augmented the environmental pollution due to metal (Pati 2014). Some metals are relatively uncommon in the earth crust, therefore not readily available for absorption by plants, however, unintentional process such as volcanic eruption, erosions and intentional activities such as metal manufacturing and fossil fuel burning liberate them into earth crust. (Gochfeld 2003, Selinus et al 2005).

Elemental screening of plant extract furnishes us the preliminary information about the elemental composition, probable toxic metal, the biological role and medicinal import of the plant (Omoruyi et al 1995). Variation in elemental concentration of any plant is attributed to the differences in botanical structure, and mineral composition of the soil in which the plants grow (Shaw 1995). Other factors responsible for variation in elemental content are preferential absorbability of the plant, use of fertilizers, irrigation water and climatological conditions (Zhang 2007).

Elemental screening of the extract in this study (Table I) showed the presence of varying concentrations of iron, copper, zinc, magnesium and calcium, with known pharmacological impacts (Arsenian 2007), the concentration of elements detected were within the safety limit recommended by WHO (1996).

Lead and cadmium were not detected with the methodology employed in this study. Some trace essential elements have significant useful functions in the human body. Iron and calcium play important role in human health and disease. Elements like zinc, magnesium and copper have been postulated to have hypoglycaemic effects and reduces the incidence of lower respiratory infection in infants and preschool children (Jaryum et al 2010), but the antidiabetic roles of some of them are not well understood.

Zinc is an important part of insulin, it is known to assists in the regulation of insulin levels in the blood and improvement of insulin sensitivity in the management of diabetes (Prasad 1991). Similarly, some years ago there was only limited public health interest in zinc, now it is widely recognized that zinc deficiency is associated with suboptimal growth and reduced immunocompetence in children (Black, 2001). Zinc plays an important role in the structure and inactivation some of enzymes

(Black, 2001), has a potentiating role on some hormones and vitamins and has a major role in neuromuscular transmission, blood pressure, vasomotor tone and cardiac excitability (Seelig 1980).

The main functions of iron are related its participation in blood oxygen transport and tissue respiration. Iron is a component of hemoglobin and red blood cells. It is found in muscles as myoglobin, as transferrin in serum, as uteroferină in placenta, as lactoferrin in milk and in liver as ferritin and hemosiderin (Chanarin 1999) . It also plays an important role in the body as a component of several metabolic enzymes. Physiological role of copper component of ceruloplasmin, consists in the participation in hematopoesis, therefore iron absorption and his utilization in the synthesis of porphyrin III and heme iron enzymes is conditional. In the absence of copper in the body, anemia that does not succumb to iron administration, but only in association with copper occurrence (Jaryum et al. 2010). Acute toxicological screening of the extract implied its non toxicity as the LD_{50} is greater than 5000mg/kg.

The thylakoids extract may be postulated to have effect on rebuilding weak skeletal bone, hypoglycaemic activities, and hematonic potentials as a result of its elemental compositions. Further studies are ongoing in our laboratory to elucidate the beneficial effects of non toxic Senna Tora linn thylakoids

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