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Heavy Metals Evaluation and Acute Toxicity Study of *Artemisia annua* L. (Asteraceae)

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Abstract: *The present work was aimed to evaluate the level of heavy metals and medial lethal dose (LD_{50}) of aqueous and hexane leaf extracts of Artemisia annua L. (Asteraceae) obtained from Kano State, Nigeria. The heavy metals analysis revealed that chromium, arsenic lead, cobalt and nickel were not detected, while only traces of copper (0.0322 ± 0.01 mg/kg), cadmium (0.0005 ± 0.00 mg/kg) and manganese (0.1208 ± 0.04 mg/kg) were detected. The LD_{50} of hexane and aqueous leaf extracts were estimated to be 2154.07 and 3807.89 mg/kg body weight respectively. The findings in this study showed that aqueous and hexane leaf extracts of A. annua were slightly toxic, while the levels of copper, cadmium and manganese detected did not exceed the World Health Organization (WHO) permissible limit for medicinal plant species.*

Keywords: *Artemisia annua, heavy metals, medial lethal dose, permissible limit.*

INTRODUCTION

Medicinal plants are used in many aspects of health care, particularly in developing countries; this is because of their natural origins and lower costs when compared to synthetic or modern drugs. Medicinal plants contain elements that are essential for humans, which may become toxic when present at high concentrations (Rajurkar and Damame, 1997; Obianjunwa *et al.,* 2004; Elekes *et al.,* 2010).

Heavy metals are simply defined as the group of elements whose densities are greater than 5 gcm^{-3}. They are also referred to as non-biologically degradable metals or metalloids that cause an environmental problem or pollution, examples of such elements include lead, chromium, arsenic, mercury, copper, nickel, cadmium, cobalt etc (Herrera-Estrella and Guevara-Garcia 2009). Because of the widespread presence of heavy metals in the environment, their residues also reach and assimilated into medicinal plants (Meena *et al.,* 2008).

Evaluation of heavy metals and acute toxicity tests are some of the essential steps in improving the overall safety and quality of medicinal plants and herbal products (Street *et al.,* 2008). Toxicity studies are usually the first tests conducted in order to predict the potential hazards of medicinal plants, herbal products and other chemicals (Maheshwar and Shaikh, 2016).

The common health problems associated with higher levels of heavy metals are cancer, asthma, cardiovascular disorders, allergic reactions, overproduction of red blood cells, anxiety, depression, premenstrual syndrome, respiratory problems, weakened immune systems, kidney, liver damage, inactivation

of enzymes involved in DNA synthesis and repair, nausea abdominal pain, severe diarrhea etc (Henok and Ariaya, 2013).

Due to the above mentioned health problems, the World Health Organization (WHO) has emphasized that medicinal plants and herbal products should not be used without qualitative and quantitative analysis of heavy metals content (WHO, 2005).

Although, the permissible levels of heavy metal contaminants in herbal medicine are not yet standardized because many of them are considered as micronutrients; the European Pharmacopoeia has drafted limits for cadmium, lead and mercury. Also, the European Commission established limits in March 2001 for lead, cadmium, and mercury. Similarly, WHO and Food and Agriculture Organization (FAO) have proposed the acceptable levels of toxic substances that can be ingested on a weekly basis, i.e the provisional tolerable weekly intake (PTWI) for arsenic, lead, cadmium, and mercury (IPCS, 2009). However, there are few established safe limits for the content of heavy metals in medicinal plants (Wang *et al.,* 1985).

Artemisia annua commonly known as sweet worm wood or sweet annie; belongs to the family Asteraceae. It has been identified as the main source of artemisinin for the production of artemisinin-based combination therapy (ACT), an effective antimalarial drug recommended by WHO. The phytochemical and *in vitro* antiplasmodium activity of *A. annua* cultivated in Kano State, Nigeria was previously reported by Abubakar et al (2018). The present study was aimed to evaluate the level of heavy metals and acute toxicity index of the aqueous and hexane leaf extracts of this important medicinal plant.

Materials and Methods

Animals

Adult Swiss albino mice of both sexes, weighing 20-35 g were obtained from the animal house of the Department of Pharmacology and Therapeutics, Bayero University Kano, Nigeria and maintained under normal laboratory conditions of humidity, temperature and light for 7 days before the experiment and allowed free access to food and water. All experiments performed on the laboratory animals in this study were approved by the Local Ethical Committee for animal experimentation in the Department of Pharmacology, Faculty of Pharmaceutical Sciences, Bayero University, Kano, Nigeria.

Collection and Identification of *A. annua*

The plant was collected, identified and authenticated at the Ethnobotany Unit of Bioresources Development Centre, Kano, a reference sample number; BDCKN/EB/1896 has been deposited in the Herbarium. The leaves were then dried and powdered using pestle and mortar.

Extraction of the Powdered Plant Material

The powdered plant material (100 g) was successively macerated with hexane and distilled water (500 ml each) for 48 hours, the mixture was shaken occasionally. The filtrate obtained was evaporated to dryness at 40^{o}C using a rotary evaporator and a water bath.

Acute Toxicity Test (Median Lethal Dose (LD_{50}) Determination)

This was conducted in two phases using the method described by Lorke (1983). In the first phase, mice were divided into 3 groups of three mice each. The first group received the extract (i.p) at a dose of 10 mg/kg body weight, followed by 100 mg/kg and 1000 mg/kg to the second and third group respectively. The animals were then observed for 24 hours for signs and symptoms of toxicity and death.

In the second phase, mice were divided into 3 groups of one mouse each, the extract was then administered to group 1, 2 and 3 at the doses of 1, 600, 2, 900 and 5, 000 mg/kg body weight respectively. The LD_{50} was calculated from the results of the final phase as the square root of the product of the lowest lethal dose and the highest non-lethal dose.

Evaluation of Heavy Metals

The methods described by Henok and Ariaya (2013) and Samali et al (2017) were employed for the determination of heavy metals in *A. annua*. Approximately 1gram sample was weighed in glass beaker and 25ml of an acid mixture of nitric acid (65%) and perchloric acid (70%) at molar ratio 4:1was added. The mixture was wet digested at 130 ^{0}C in fuming hood near to dryness. The procedure was repeated until the sample digestion process was completed as indicated by appearance of white fumes and residue almost gets to dryness. The solutions were left to cool to room temperature, and each sample was filtered into a 50 ml volumetric flask and was diluted up to the mark with distilled water. The samples solutions were analyzed for chromium (Cr), arsenic (As) lead (Pb), copper (Cu), nickel (Ni), cadmium (Cd), cobalt (Co) and manganese (Mn) using Atomic Absorption Spectrophotometer available in the Department of Soil Science, Faculty of Agriculture, Bayero University Kano, Nigeria.

Statistical Analysis

Heavy metals determinations were carried out in triplicates, and data was expressed as mean ± standard deviation (SD).

Results

The acute toxicity test of the hexane leaf extract of *A. annua* resulted in mortality and other behavioural changes such as loss of appetite and general weakness (Table 1). The LD_{50} was estimated to be 2154.07 mg/kg body weight.

Table 1: Acute Toxicity Test of Hexane Extract

First Phase		Second Phase	
Dose (mg/kg)	Mortality	Dose (mg/kg)	Mortality
10	0/3	1600	0/1
100	0/3	2900	1/1
1000	0/3	5000	1/1

Also, deaths were recorded in animals administered with the aqueous leaf extract of *A. annua* (Table 2), while behavioural changes observed include loss of appetite and general weakness. The LD_{50} of the aqueous extract was estimated to be 3807.89 mg/kg body weight.

Table 2: Acute Toxicity Test of Aqueous Extract

First Phase		Second Phase	
Dose (mg/kg)	Mortality	Dose (mg/kg)	Mortality
10	0/3	1600	0/1
100	0/3	2900	0/1
1000	0/3	5000	1/1

Heavy Metals Analysis

Chromium, arsenic lead, cobalt and nickel were not detected, while only traces of copper (0.0322 ± 0.01 mg/kg), cadmium (0.0005 ± 0.00 mg/kg) and manganese (0.1208 ± 0.04 mg/kg) were detected in the leaves of *A. annua* (Table 3);

Table 3: Levels of Heavy Metals in the Leaves of *A. annua*

S/N	Elements	Concentration (mg/kg)
1	Pb	ND
2	Ni	ND
3	Mn	0.1208 ± 0.04
4	Cu	0.0322 ± 0.01

5	Co	ND
6	Cr	ND
7	As	ND
8	Cd	0.0005 ± 0.00

All values were mean ± standard deviation of triplicate determinations.
ND = Not detected

Discussion

Acute systemic toxicity determines the adverse effects that occur following exposure of organisms to a single or multiple doses of a plant extract or chemical within 24 or 48 hours by a known route for example oral, subcutaneous, dermal etc (Saganuwa, 2016).

The present study indicates that the LD_{50} of the hexane extract was estimated to be 2154.07 mg/kg body weight, while that of aqueous extract was estimated to be 3807.89 mg/kg body weight. These findings agreed with the work of Emmanuel et al (2014) who also reported an LD_{50} of 2750 mg/kg body weight for the hexane extract of *A. annua* obtained from Langtang, Plateau State, Nigeria.

Plant extracts with LD_{50} of 500-5000 mg/kg body weight are considered to be slightly toxic, thus, both hexane and aqueous extracts of *A. annua* could be considered as slightly toxic (Lorke, 1983; Loomis and Hayes, 1996).

Results obtained from the acute toxicity study could serve as a guide in dosage selection for long term toxicity studies as well as other pharmacological studies that involve the use of animals (Maheshwari and Shaikh, 2016).

The result of the heavy metals evaluation showed that chromium, arsenic lead, cobalt and nickel were not detected. However, the absence of lead and nickel did not agree with the previous study (Negi et al., 2012). The lower quantities of copper, cadmium and manganese detected in this study did not exceed WHO permissible limit for medicinal plant species (WHO, 2007). Also, the level of cadmium reported in this work was lower than the value reported in the previous study (Negi et al., 2012).

Some heavy metals like copper, manganese and nickel are essential nutrients at trace levels, however, at high concentrations could be toxic and harmful, while heavy metals like lead, cadmium and chromium were reported to be non-essential to humans (Serafim et al., 2012). In view of that, WHO recommended the qualitative and quantitative determination of heavy metals in medicinal plant species (WHO, 2005).

Bioaccumulation and uptake of heavy metals in plant materials are usually influenced by climate, atmospheric deposition, concentration and nature of the soil on which the plants are grown and the degree of maturity of the plant at the time of harvest (Lake et al., 1984).

Conclusion

The present study has shown that the aqueous and hexane leaf extracts of *A. annua* were slightly toxic, while the levels of copper, cadmium and manganese detected did not exceed WHO permissible limit for medicinal plant species.

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