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Genotoxicity Assessment and Toxic Metal Contamination of Traditionally used Anthelmintic Medicinal Plants in Assam, India

Amar Deep Soren[1*] and Arun K. Yadav[2]

[1]*PG and Research Department of Zoology, B. Borooah College, Guwahati, 781007, Assam, India; amar4deep@gmail.com*

[2]*Department of Zoology, North-Eastern Hill University, Shillong, 793022, Meghalaya, India*

Abstract

Medicinal plants possess several metals obtained naturally or through pollution. The study evaluates the genotoxic potentials and the presence of essential elements and toxic metals in *Sesbania sesban* var. *bicolor* (leaves), *Cyperus compressus* (roots), and *Asparagus racemosus* (roots), which are used as anthelmintics in the traditional medicine of the Santhals in India. A genotoxicity study was performed in rats as per Organization for Economic Co-operation and Development guidelines. Element contents were evaluated using inductively coupled plasma-optical emission spectrometry. Administration of *S. sesban* var. *bicolor* (400 mg/kg), *C. compressus* (700 mg/kg) and *A. racemosus* (500 mg/kg) did not cause any abnormalities in the chromosomes. Elemental analysis revealed the presence of lead (5.00±0.05, 4.98±0.01, 4.99±0.07 ppm), cadmium (4.99±0.04, 4.90±0.03, 5.00±0.08 ppm) and arsenic (0.98±0.03, 0.41±0.05, 0.92±0.03 ppm), respectively. Although their genotoxic potential was lacking, the presence of toxic metals in them is a cause for concern.

Keywords: *Asparagus racemosus*, *Cyperus compressus*, Genotoxicity, *Sesbania sesban* var. *bicolor*, Toxic Metals

1. Introduction

In recent years, a growing interest in herbal medicine has developed due to its long history of usage and the belief that it is natural and hence safe[1]. However, considering medicinal plants to be safe and efficacious because of their long history of traditional use is not only unreliable but also dangerous[2,3]. Several herbal medicines continue to be sold freely in markets and fairs, and by herbal vendors in the streets with little or no restriction[4]. Several countries like Bulgaria, and Nepal, including India, lack proper quality and control regulations for medicinal plants[5].

The toxicity associated with medicinal plants has been established by several workers. Medicinal plants as well as their constituents can cause genotoxicity in their users[6]. A study by Nath, *et al.*, revealed that *Acorus calamus*, used in folk medicine in India, caused genotoxic and hepatotoxic effects in its users[7]. Several medicinal plants from Ethiopia, Argentina, Serbia, and parts of Africa have also been shown to produce genotoxic effects[3,6,8,9]. In spite of their efficacy, medicinal plants can also be a source of toxic elements[10]. This has encouraged researchers to investigate medicinal plants for the presence of toxic elements in them[11]. Elements such as Al, As, Cd, Cu, Pb, Hg, Tl, Sn, and Zn are common

**Author for correspondence*

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adulterants found in medicinal plants[1]. Toxic potentials of herbal medicine are attributed to two major factors, intrinsic (direct) which include active chemical constituents present in the herbs and extrinsic (indirect) which include contamination, adulteration, and misidentification of the medicinal plants[12]. A vast majority of herbal medicines still await proper clinical trials and the assessment of their efficacy and safety has become a growing concern. Owing to reports of toxicity caused by usage of traditional herbal medicines, a thorough investigation on the safety profiles of medicinal plants seems necessary[7].

The leaves of *S. sesban* var. *bicolor* (Fabaceae) and the roots of *Cyperus compressus* (Cyperaceae) and *Asparagus racemosus* (Asparagaceae) are used in the traditional medicine system of the Santhals to treat helminth infections. *A. racemosus* has also been claimed to possess anti-mutagenic properties[13]. Since these medicinal plants are used frequently, this study was conducted to evaluate their potential genotoxic effects, if any, in rodent models and to evaluate the presence of both essential and toxic elements in them.

2. Materials and Methods

2.1 Chemicals

The following chemicals were procured from the respective companies to carry out this study. Colchicine (Sigma-Aldrich), disodium hydrogen phosphate (Na_2HPO_4) (Himedia), DPX (SDFCL), Giemsa stain (Sigma-Aldrich), glacial acetic acid (Himedia), methanol (Rankem), nitric acid (HNO_3) (Himedia), potassium chloride (KCl) (SDFCL), potassium dihydrogen phosphate (KH_2PO_4) (Rankem), sodium chloride (NaCl) (Sigma-Aldrich), and sulphuric acid (H_2SO_4) (Himedia).

2.2 Plant Material

Fresh leaves of *S. sesban* var. *bicolor* and roots of *C. compressus*, and *A. racemosus* were collected from natural habitats in Assam with the help of traditional healers. Plants were identified by a taxonomist from the Department of Botany, North-Eastern Hill University (NEHU) and voucher specimen numbers were assigned (NEHU – 12084: *Sesbania sesban* var. *bicolor*; NEHU – 12085: *Cyperus compressus;* and NEHU – 12098: *Asparagus racemosus*). A copy of each plant specimen was retained in the Parasitology and Ethnopharmacology Lab, Department of Zoology, NEHU.

Plant materials were washed, shade dried, and powdered in a kitchen mixer. The plant materials were then extracted in methanol using a Soxhlet apparatus and stored at 4°C in vials. The powdered plant material and plant methanolic extracts were used to carry out further analysis.

2.3 Experimental Animals

Laboratory inbred Wistar strain rats of both sexes procured from the animal room of the Department of Zoology, NEHU, weighing about 150 to 200 g, and aged 8-9 weeks, were used to perform genotoxicity studies. Animals were kept in separate cages with ad libitum access to water and food. Genotoxicity studies were performed after approval and written consent was obtained from the Department of Zoology and the Institutional Ethics Committee (IEC) (animal models), NEHU (vide, Member Secretary, IEC, NEHU, letter number 01, dated December 4, 2014). All the experiments on animals were performed strictly as per the guidelines laid out by the IEC, NEHU, Shillong and Animal Research: Reporting of *In Vivo* Experiments (ARRIVE) guidelines.

2.4 Genotoxicity Studies

Genotoxicity studies were conducted on Wistar rats following the protocol of Guo and Wu (2008) with minor modifications[14]. The animals were dosed according to the dosage administered by traditional healers to their clients. The recommended dose was taken as the median dose and two doses, one exponentially lower and the other higher than the median dose were selected for the study. Accordingly, animals were dosed 100, 200 and 400 mg/kg b.w. of *S. sesban* var. *bicolor* leaf extract, 175, 350 and 700 mg/kg b.w. of *C. compressus* root extract and 125, 250 and 500 mg/kg b.w. of *A. racemosus* root extract.

The animals were divided into three groups, with five animals in each. Group 1 served as the control and received only the vehicle. Groups 2 and 3 received the highest and lowest doses respectively. All the animals were dosed for 14 days. At the end of the dosing period, the animals were subjected to mitotic arrest by injecting colchicine intraperitonially (4mg/kg). The animals were then sacrificed after 2 h, according to the prescribed guidelines of the IEC (animal model) of NEHU.

The femurs were dissected out and flushed with pre-warmed (37±1 °C) 0.06 M KCl through a 40 µm cell strainer into a 50 ml centrifuge tube. The samples were kept in Carnoy's fixative, then centrifuged at 1500 rpm for 15 min (4 times) with KCl. After each centrifugation, the supernatant was discarded, and the pellet was used to carry out further

examinations. Finally, the cells were resuspended in 2 ml fixative and stored at -20° C for further use.

To make the slides, a few drops of the cell suspension were dropped onto grease free, methanol-cooled slides from a height of about 1 m. The slides were then warmed in a flame for 2 seconds to allow the cells to spread, following which they were allowed to dry and then stained in a 5% buffered Giemsa stain (pH 7) for 10 minutes. The slides were then washed in distilled water to remove excess stain and allowed to dry. The slides were mounted in DPX and observed in oil at 100 x for various chromosomal aberrations, which included Chromatid Breaks (CB), Isochromatid Breaks (ICB), Chromosomal Fragments (CF), Exchanges (E) and Sister Chromatid Unions (SCU). To score various chromosomal aberrations, about 100 well spread metaphase plates were examined per animal[7].

2.5 Elemental Analysis

To perform elemental analysis, the powdered plant material (1 g) was subjected to acid digestion using HCl and HNO_3 in the ratio 2:1. The acid mixture was made to evaporate on a hot plate, following which distilled water was added and then filtered. The filtrate was made to a final volume of 50 ml then subjected to elemental analysis by Inductively Coupled Plasma-Optical Emission Spectrometry (ICP-OES) using a Thermo Fischer iCAP 7600. Essential elements such as chromium (Cr), cobalt (Co), copper (Cu), iron (Fe), manganese (Mn) and nickel (Ni) were estimated. The toxic metals arsenic (As), cadmium (Cd), and lead (Pb) were also evaluated. The values were compared with the WHO/FAO permissible limits (ppm) and inferences were drawn[15-20].

2.6 Statistical Analysis

The results obtained in the study were analysed using OriginPro 8. All data are represented as mean ± Standard Error of Mean (SEM). $P \leq 0.05$ was considered significantly different.

3. Results

3.1 Genotoxicity Studies

Genotoxicity studies of the three test plants did not reveal any abnormalities or deviations from the control group. The photomicrographs of bone marrow metaphase chromosome spreads revealed intact and normal chromosomes (Figure 1). Abnormalities in the form of Sister Chromatid Unions (SCU), Chromosomal Fragments (CF), Exchanges (E), Chromatid Breaks (CB) and Isochromatid Breaks (ICB) were not observed in the study photomicrographs.

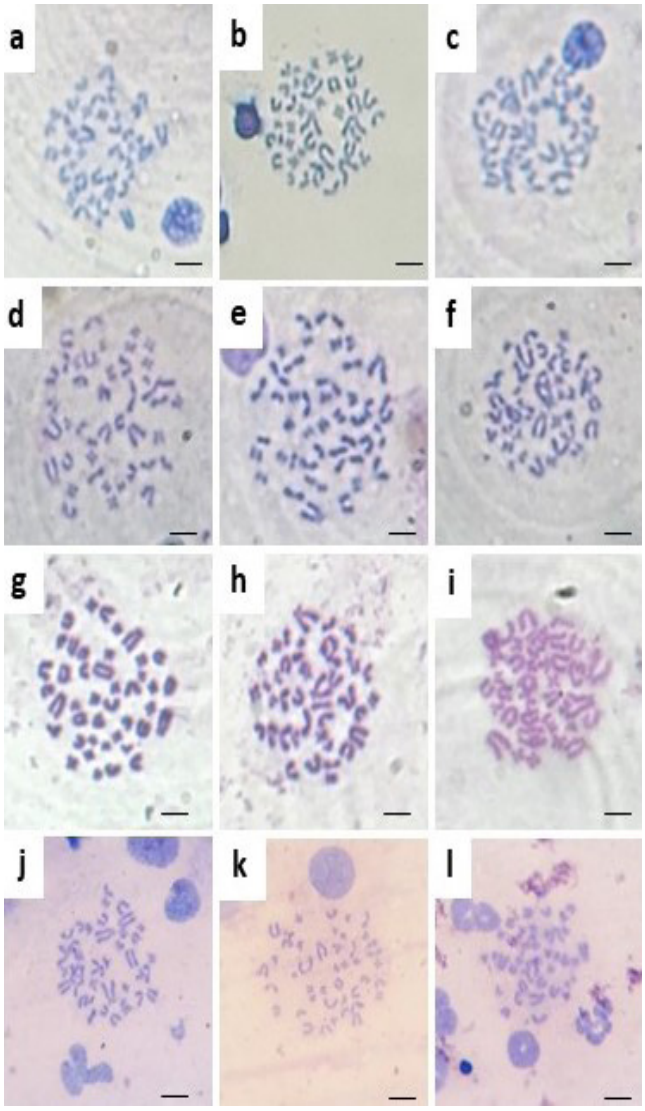


Figure 1. Photomicrographs of bone marrow metaphase chromosome spreads of rats. (**a, b, c**). control rat, showing normal set of chromosomes; (**d, e, f**). rat treated with *S. sesban* var. *bicolor* leaf extract; (**g, h, i**). rat treated with *C. compressus* root extract; (**j, k, l**). rat treated with *A. racemosus* root extract. Chromosome Breaks (CB), Chromosomal Fragments (CF) and Sister Chromatid Unions (SCU), Exchanges (E). Scale – 100x, (-) = 5µm.

3.2 Elemental Analysis

Elemental analysis of the studied plants revealed that the levels of essential elements, namely Co, Cr and Ni were found to be above the permissible limits as set by the WHO/FAO

Table 1. Elemental concentration in the test plants' samples

Plants	Essential elements							Potentially toxic elements		
	Cu	Zn	Fe	Co	Mn	Cr	Ni	Pb	Cd	As
S. sesban var. *bicolor*	9.95±0.03	4.82±0.03	5.91±0.01	5.00±0.03	8.15±0.04	9.88±0.05	4.93±0.05	5.00±0.05	4.99±0.04	0.98±0.03
C. compressus	9.06±0.02	4.86±0.02	ND	4.90±0.02	8.46±0.09	9.17±0.01	4.96±0.01	4.98±0.01	4.90±0.03	0.41±0.05
A. racemosus	9.97±0.05	5.00±0.05	7.94±0.07	5.02±0.05	9.97±0.02	9.92±0.07	4.99±0.07	4.99±0.07	5.00±0.08	0.92±0.03
FAO/WHO permissible limits	10	50	20	1.5	200	1.5	1.5	10	0.3	0.1

Data are expressed as mean ± SEM. Values are significantly different at $p < 0.05$. All concentrations in ppm (parts per million).

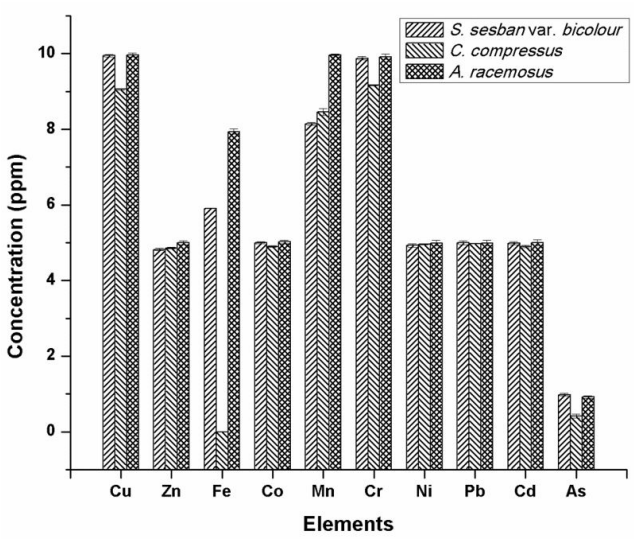


Figure 2. Concentration of various elements in the samples of *S. sesban* var. *bicolor*, *C. compressus* and *A. racemosus*.

in all three studied plant extracts. Cu, Zn, Fe, and Mn were found to be present in safe concentrations. Fe was however not detected in *C. compressus*. Two of the estimated toxic elements namely, Cd and As were found to be above the permissible limits of WHO/FAO in all three plant species. Although Pb was detected in all three plant extracts, it was found to be below the permissible limits (Table 1). The element contents are depicted in Figure 2.

4. Discussion

Efficacy and safety profiles of medicinal plants must be based on proper scientific validations[3]. This study has evaluated the genotoxic potentials of three medicinal plants as well as estimated the content of essential and toxic elements present in them. A genotoxicity study is conducted to observe for any damages caused by long-term administration of plant extract and has a significant role in the identification of toxic effects of any drugs[21]. A similar study by Nath, *et al.,* on the toxicity studies of *Acorus calamus*, revealed that the crude extract caused genotoxicity in the form of chromosome breaks, fragments, sister chromatid unions and exchanges[7]. Similar results were reported in a study by Seukep, *et al.,* on African medicinal plants where 33 plants were found to cause genotoxic effects in animal or bacterial systems[6]. However, Edziri, *et al.,* performed a similar study on *Cleome amblyocarpa* a medicinal plant used in Tunisia and observed that it did not cause any genotoxic effects[22]. Similarly, the findings of this study also revealed that the administration of the plant extracts did not cause abnormalities at the chromosomal level in animals, indicating that they lack genotoxic potentials.

Metals are essential nutrients required for various biochemical and physiological functions. Essential elements are important for human health and the prevention and control of diseases. Several authors have estimated the elemental contents of medicinal plants[23,24]. In the present study, all the studied plants revealed high levels of essential elements such as Co, Cr and Ni. All the plants also showed the presence of high amounts of the toxic elements Cd and As. Pb was also detected within safe limits in all three studied plants. Since plants absorb minerals from the soil, this accumulation of toxic elements in them can be attributed to this phenomenon[25].

Exposure to Pb results in bone marrow and kidney damage, neurotoxicity, and gastrointestinal and neurodevelopmental defects[26] whereas the main cause of Cd exposure occurs through ingestion of foods containing Cd[27]. It is a pulmonary and gastrointestinal irritant, and its ingestion or inhalation could cause death[28]. It is also carcinogenic, and its chronic exposure could result in renal damage and pulmonary fibrosis[26]. Exposure to As occurs through ingestion, inhalation, dermal contact and parenteral route[29] and is known to cause vitamin A deficiency resulting in night

blindness[30]. The exposure to these metals may affect different organ systems, including the cardiovascular, dermatologic, nervous, hepatobiliary, renal, gastro-intestinal, and respiratory systems[29].

Cr exposure may cause oxidative stress in the cell, resulting in damage to DNA and proteins[31]. It possesses mutagenic properties and is categorized as a Group 1 human carcinogen[32]. On the other hand, Ni induced toxicity occurs by pulmonary absorption, and it is a known hematotoxic, immunotoxic, neurotoxic, genotoxic, reproductive toxic, pulmonary toxic, nephrotoxic, hepatotoxic, and carcinogenic agent[33]. Excess Co intake is characterized by neurological, cardiovascular, and endocrine deficits[34]. A study by Tschinkel, *et al.,* revealed similar results where several medicinal plants used in Brazil showed high amounts of Pb and Cd in them[35]. Also, Silva, *et al.,* reported high concentrations of Ba, Cr, Fe, Hg, Se and Ni in *Achillea millefolium, Senna alexandriana* and *Malva sylvestris* used in traditional medicine in Brazil[36]. However, some authors have reported normal levels of elements in medicinal plants in their studies[11,37].

5. Conclusions

This is the first study in the region that has detected the presence of toxic elements in medicinal plants. Although the studied plants did not reveal genotoxic effects, the presence of toxic metals in them is a cause for concern since these medicinal plants are used widely. The accumulation of these toxic elements can be attributed to their presence in the soil of the studied area. Their uncontrolled consumption can result in adverse toxic effects in their users and hence their usage in the area should be done with caution.

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