

Preliminary Toxicology Evaluation and Heavy Metal Determination of Selected Malaysian Medicinal Plants

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ABSTRACT: The safety and toxicity information of herbal medicines or products are required prior to expanded clinical studies and to support the registration of the herbal products with the Drug Control Agency. The presence of toxic heavy metals such as arsenic (As), lead (Pb), cadmium (Cd) and mercury (Hg) in herbal products at levels that could be toxic pose serious risk to public health. These heavy metals when consumed in considerable amount can result in damaged or reduced mental and central nervous function, damage to blood composition, lungs, kidneys, liver, and other vital organs. Long-term exposure may result in muscular and neurological disorders that mimic Alzheimer's disease, Parkinson's disease, and muscular dystrophy. Several medicinal plants used in Malaysian traditional medicines were collected from different location in Malaysia and evaluated for their heavy metal contents and toxicity. The heavy metal contents of their aqueous extracts were evaluated using the Inductively Coupled Plasma-Mass Spectrometer (ICP-MS), toxicities were determined using the brine shrimp lethality assays and acute *in vivo* toxicity determination in Sprague-Dawley rats. All the aqueous extracts were found to be non-toxic against the brine shrimp and rats (dosage up to 0.3% of body weight). The aqueous extracts were also found to contain heavy metals much less than the permissible limit.

Keywords: heavy metals, herbal medicine, toxicity

Introduction

The safety and toxicity information of herbal medicines or products are required prior to expanded clinical studies and to support the registration of the herbal products with the Drug Control Agency. The presence of toxic heavy metals such as arsenic (As), lead (Pb), cadmium (Cd) and mercury (Hg) in herbal products at high levels pose serious risk to public health (Smith, 1973; Mahafey, 1985; Chen, 1992; Caldas and Machado, 2004; Ernst, 2004; Phillips, 2004). These heavy metals when consumed in considerable amount can result in damaged or reduced mental and central nervous function, damage to blood composition, lungs, kidneys, liver, and other vital organs. Long-term exposure may result in muscular and neurological disorders that mimic Alzheimer's disease, Parkinson's disease, and muscular dystrophy.

Several medicinal plants used in Malaysian traditional medicine were collected from various locations throughout Malaysia and their aqueous extracts were evaluated for their heavy metals

content and toxicity. The heavy metal content of their aqueous extracts were evaluated using the Inductively Coupled Plasma-Mass Spectrometer (ICP-MS) with toxicities being determined using the brine shrimp lethality assays and the acute *in vivo* toxicity determination was carried out in Sprague-Dawley rats.

Materials and Methods

Toxicity evaluation of extracts

In order to obtain a preliminary indication of the extracts potential cytotoxic activities, biological evaluation was conducted using the brine shrimp (*Artemia salina* Leach) lethality assay and the acute toxicity test in rats. The aqueous extracts were also evaluated for heavy metal content.

Brine shrimp lethality assay

The brine shrimp lethality test was carried out using the 96-well microplate method as described by Solis et al. (1993). Extracts were first diluted to 10 mg/ml with sea-salt (Sigma) solution. An aliquot (100 µl) of the 10 mg/ml sample solution was dispensed (in triplicates) into the first and second well of the microplate row. Two fold serial dilutions with 100 µl sea salt solution were made across the plates starting from well number 2 to 7 (inclusive) to give a final concentration of 0.156 µg/ml. The serial dilution was performed in triplicate. A suspension containing 7-

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15 mature *Artemia salina* nauplii was then added to each well and the covered plates were left at room temperature for 24 hours.

The plates were then examined under a binocular microscope (x12.5) and the numbers of dead nauplii were counted. Methanol (100 µl) was then added to each well to kill the remaining nauplii. The total number of nauplii was counted and the average percentage of deaths and survival at each dose were recorded. Emetine hydrochloride (1 mg/ml) and sea-salt solution (containing < 1% DMSO) were used as the negative and positive controls, respectively.

Acute toxicity study in rats

A total of 15 adult Sprague Dawley female rats weighing between 180 - 220 g were used in the toxicity study. The animals were purchased from the Animal House of Institute of Medical Research, Kuala Lumpur and were divided equally into 3 groups. Each group (A, B and C) contained 5 rats. The rats in group A and B were force-fed using a feeding needle (gavage) with aqueous extracts of the plants in a single oral dose (expressed as percent of the extract per body weight). The rats in group A and B received 0.2% and 0.3% of extracts per kg of their body weight, respectively. The rats in control group C received only an equal volume of distilled water. The animals were kept in a room where they were maintained under a controlled temperature on a 12 h light/12 h dark cycle, and were provided with food and water. All the animals were put under

observation for up to 3 weeks for any signs of toxicity.

Heavy metal evaluation

Plant extracts were sent to RAS Laboratory for heavy metal evaluation. The heavy metal content of the aqueous extracts was determined using Inductively Coupled Plasma-Mass Spectrometer (ICP-MS).

Results and Discussion

Toxicity evaluation of extracts

The results indicated that there was no indication of toxicity in both the brine shrimp and the laboratory animals. The LC₅₀ of all the aqueous extracts tested against the brine shrimp were found to be > 5 mg/ml (**TABLE 1**). The LC₅₀ for the positive control, emetine hydrochloride, was found to be 0.03 mg/ml. The LC₅₀ < 1 mg/ml against the brine shrimp is considered toxic. The acute toxicity study performed in rats also indicated that there is no evidence of toxicity at oral dose equivalent in amount of up to 0.3% of the body weight (**TABLE 2**).

Heavy metal evaluation

The results obtained indicated that the heavy metal contents of the aqueous extracts were found to be below the permissible limits of heavy metals content in herbal product (**TABLE 3**).

TABLE 1- Toxicity evaluation of the aqueous extracts using brine shrimp lethality assays

Plant species	Local name	Plant parts	LC ₅₀ (mg/ml)
<i>Ficus deltoidea</i>	Serapat angin	leaves	> 5.0
<i>Ficus deltoidea</i>	Delima sudip	leaves	> 5.0
<i>Cephalala leucena</i>	Petai belalang	Fruit	> 5.0
<i>Androgaphis paniculata</i>	Hempedu bumi	Aerial part	> 5.0
<i>Muntingia calabura</i>	Ceri	leaves	> 5.0
<i>Lagerstroemia speciosa</i>	Bangur	leaves	> 5.0
<i>Orthosiphon stamineus</i>	Misai kucing	Aerial part	> 5.0
<i>Cibotium</i>	Ayam emas	rhizome	>5.0
Emetine HCl (+ve control)	-	-	0.030
Sea-salt solution (-ve control)	-	-	-

TABLE 2-Acute in-vivo toxicity evaluation of the aqueous extracts in rats

Plant species	Local name	Observable toxic sign or death (up to 3 weeks)	
		Dosage (0.2% body weight)	Dosage (0.3% body weight)
<i>Ficus deltoidea</i>	Serapat angin	None	None
<i>Ficus deltoidea</i>	Delima sudip	None	None
<i>Cephalaleucena</i>	Petai belalang	None	None
<i>Androgaphis paniculata</i>	Hempedu bumi	None	None
<i>Muntingia calabura</i>	Ceri	None	ND
<i>Lagerstroemia speciosa</i>	Bangor	None	None
<i>Orthosiphon stamineus</i>	Misai kucing	None	ND
<i>Cibotium</i>	Ayam emas	None	None

ND – not determined

TABLE 3- Heavy metal contents of the aqueous extracts

Plant species	Local name	Location	Heavy metals content(ppb)					
			Cd	As	Pb	Hg	Mn	Zn
<i>Ficus deltoidea</i>	Cotek emas (Serapat angin)	Kerteh (oil palm plantation)	0.04	0.05	1.14	0.27	432	106
<i>Ficus deltoidea</i>	Cotek emas (Delima sudip)	Jenjarum (oil palm plantation)	0.13	0.42	15.2	0.38	387	114
<i>Cephalaleucena</i>	Petai belalang	Selayang (Roadside)	0.36	0.31	7.14	1.2	227	61.5
<i>Androgaphis paniculata</i>	Hempedu bumi	Medan (Indonesia)	0.44	0.11	4.12	1.12	65.7	50
<i>Muntingia calabura</i>	Ceri	Bagan Serai (Roadside)	0.2	0.53	7.14	ND	32.6	71.2
<i>Lagerstroemia speciosa</i>	Bangor	Kota Belud (Sabah)	0.03	0.2	1.05	2.1	69.5	208
<i>Orthosiphon stamineus</i>	Misai kucing	Dengkil, Selangor	0.2	0.33	2.82	1.1	134	63.3
<i>Cibotium</i>	Ayam emas	Sarawak	0.06	0.7	6.4	0.98	301	77.3

Cd – cadmium (3.0); As – arsenic (5.0); Pb – lead (10.0); Hg – mercury (5.0); Mn- Manganese; Zn- Zinc (Permissible limits of heavy metals content in herbal product, ppm)

Conclusion

All the aqueous extracts were found to be non-toxic against the brine shrimp and rats (dosage up to 0.3% of body weight). The aqueous extracts were also found to contain heavy metals much less than the permissible limit.

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