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Toxicological studies of the water extract of green leafy vegetable Sessile joy weed (*Alternanthera sessilis*)

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***Alternanthera sessilis* (L.) DC. (Sessile joy weed; Amaranthaceae) is a popular leafy vegetable in Sri Lanka and also used as traditional medicine in China, Taiwan, India and Sri Lanka. Histopathological test revealed degenerative and necrotic changes in the liver and kidney in Swiss mice, caused by oral administration of water extract of *A. sessilis* in high doses. The major reason for these changes could be due to the effects of cytotoxic substance/s in *A. sessilis*.**

Keywords: *Alternanthera sessilis*, cytotoxicity, histopathology, leafy vegetables.

GREEN leafy vegetables (greens) play a major role in the Sri Lankan diet, probably due to the influence of traditional herbal medicine, easy accessibility and low cost[1]. Further, green leaves are considered as a main source of vitamins, minerals and fibre for the local consumers. Due to their dietary importance, many scientific studies have been carried out on the nutritive values of green leaves[1–3]. However, there is lack of scientific literature on the toxic effects of green leafy vegetables consumed in Sri Lanka. Due to various reasons, including scientific and other information, certain herbs, e.g. *Sauropus androgynus* (L.) Merr. (Mella) have recently been removed from the diet and the people have been encouraged to consume some other greens such as leaves of *Passiflora edulis* Sims (passion fruit). Information as well as misinformation regarding the true nutritional and/or undesirable effects plays a major role in this selection process. Hence, correct information would be needed to educate the general public on their choice of greens for consumption. This requires a systematic scientific evaluation of the nutritional properties as well as the potential toxic effects of locally available greens for human consumption. Removal of a nutritional herb from the diet based on inadequate or erroneous information will deny the consumers of a readily available cheap source of nutrients. On the other hand, inclusion or potential addition of toxic herbs, when little is known about them can be endangered to human health. There are reports published worldwide about the risk of misidenti-

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Table 1. Clinical, post-mortem and histopathological findings in Swiss mice fed with water extract of *A. sessilis* for 14 consecutive days

Dose	Mice	Clinical findings	Post-mortem findings	Histopathological findings	
				Liver	Kidney
(Treatment I)	M 1	NAD	NAD	NAD	NAD
16.9 mg/mice	M 2	NAD	TI	++	NAD
	M 3	NAD	TI	+	NAD
	F 1	NAD	NAD	NAD	NAD
	F 2	NAD	NAD	NAD	NAD
(Treatment II)	M 1	NAD	NAD	+	+
33.8 mg/mice	M 2	NAD	NAD	+	+
	M 3	NAD	TI	++	+
	F 1	NAD	NAD	NAD	+
	F 2	AD on day 3	TI	++	+
(Treatment III)	M 1	DR on day 5	NAD	+	+
67.6 mg/mice	M 2	WN, DR days 10–14	NAD	+++, N	++, N
	M 3	WN days 8–14	NAD	+++, N	++
	F 1	D-day 7	AP	++	++, N
	F 2	NAD	NAD	+++, N	++, N
Control	M 1	NAD	NAD	NAD	NAD
0 mg/mice	M 2	D-day 3	AP	NAD	NAD
	M 3	NAD	NAD	NAD	NAD
	F 1	NAD	NAD	NAD	NAD
	F 2	NAD	NAD	NAD	NAD

M, Male; F, Female; D, Dead; AD, Activity decreased; DR, Diarrhoea; WN, Wounded; TI, Tympanic intestine; NAD, No abnormality detected; N, Necrosis; AP, Asphyxia; +++, Severe degenerative changes, ++, Moderate degenerative changes; +, Mild degenerative changes.

fication of culinary herbs as well as the consumption of toxic herbs, which can cause irreparable damage to vital organs and body functions[4], including death. In our previous bioassay study[5], potential lethal effects of green vegetables and culinary herbs consumed in Sri Lanka, was tested using brine shrimp (*Artemia salina*) lethality bioassay[6] to check their cytotoxicity. Even though cytotoxicity of most of the tested plant extracts was found to be not significant, *A. sessilis* showed significant level of cytotoxicity on brine shrimp. Reports showed that *A. sessilis* is used for the treatment of biliousness, dyspepsia associated with sluggish liver, chronic congestion of liver, acute and chronic pyelitis, cystitis, gonorrhoea, strangury and snake-bite in Sri Lanka. Further, it increases the production of milk in nursing mothers[7]. In Taiwan, *A. sessilis* is used in the treatment of renal diseases and is claimed to have hepatoprotective effects[8]. Alkaloidal extract of *A. sessilis* is reported to have altered the liver and kidney functions and metabolism of mice[9]. Moreover, this plant which is claimed to have antibacterial activity[10] is used to treat gastrointestinal disorders in India and Sri Lanka.

A. sessilis is a popular leafy vegetable among the Sri Lankans due to its taste and comparatively low cost and availability. Therefore, large quantities of the cooked plant parts (stem, leaves, flowers) are often consumed in a single meal. As a result, more investigations on toxicological aspects of the plant to evaluate its potential health risks are needed. This study was, therefore, aimed to investigate the histopathological changes caused by oral administration of water extract of *A. sessilis* in Swiss mice.

Six-week-old Swiss mice weighing 35 ± 10 g were used as experimental animals. Before starting the feeding trials, 20 mice were kept in the animal house at the Veterinary Research Institute, Gannoruwa, Sri Lanka, for 5 days for acclimatization to laboratory conditions. The animals were separated into four experimental groups, each containing three males and two females. All the animals were given a regular diet of commercial feed (consisting maize; rice polish; rice bran; wheat bran; fish meal; soya meal; milk powder; molasses; soya oil; bone meal; mineral mix including Ca, P, Mg, Na; Vitamin mix, including vitamins A, D, E, K; methionine and vitamin B complex to give a metabolic energy of 12.6 MJ/kg and crude protein 230 MJ/kg) and water *ad libitum* and kept under observation throughout the experiment.

Aerial parts (leaves, stems, flowers) of fresh *A. sessilis* plants were collected from a known locality where the plants were grown without using any chemicals such as pesticides and herbicides. The collected plants were washed thoroughly with water, macerated with distilled water and filtered using a muslin cloth. Subsequently, the filtrate was freeze-dried to obtain a green colour dry

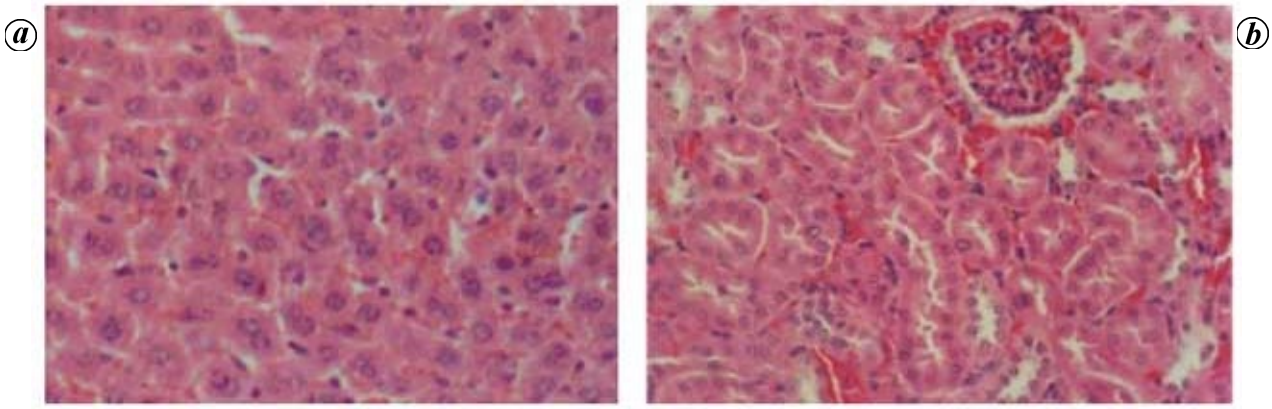

Figure 1. ***a***, Normal hepatic tissue; ***b***, Normal renal tissue of control (H&E stain), X 40.

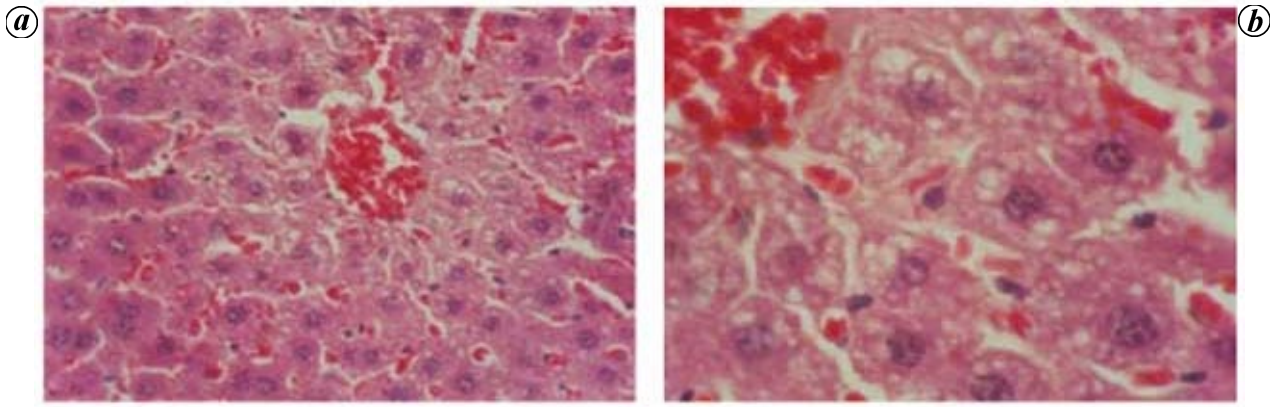

Figure 2. Hepatic tissue of a mouse from treatment III fed with a high dose of *Alternanthera sessilis* water extract showing moderate hepatocyte degeneration and necrosis in the centrilobular area, accompanied by widespread sinusoidal congestion (H&E stain). ***a***, X 40; ***b***, X 100.

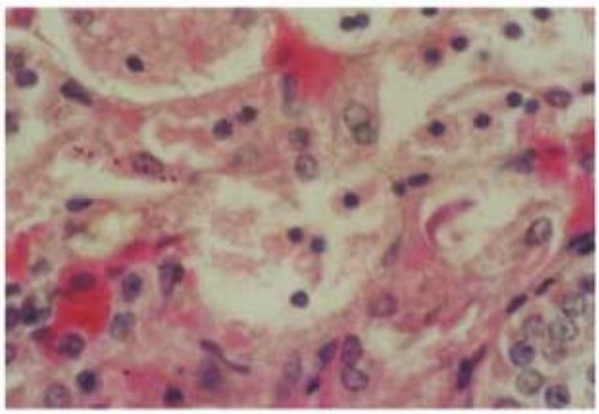

Figure 3. Renal tissue of a mouse from treatment III fed with a high dose of *A. sessilis* water extract showing degeneration and necrosis of renal tubular cells accompanied by hyperaemia and congestion (H&E stain, X 100).

powder. One hundred grams of fresh *A. sessilis* yielded 4.8 g of the dry powder. This dry powder was used as the test substance.

Experimental dose was calculated assuming a daily intake of 50 g of fresh *A. sessilis* consumed by a human of an average weight of 50 kg, which is equivalent to 2.4 g of freeze-dried extract. Dose for unit weight of an average human was calculated ($2.4\ g/50 \times 10^3\ g$) and converted to the average weight (35 g) of mice, which amounted to 1.69 mg per mouse per day. Each animal of the first group (treatment I) received ten times the calculated dose (16.9 mg), the second group (treatment II) received twenty times (33.8 mg), and the third group (treatment III) received forty times the calculated dose (67.6 mg) per day. The calculated dose of the test substance was dissolved in 0.2 ml of water and fed using an oral cannula to each animal daily for 14 consecutive days. An equal volume of water was fed to the fourth group of mice, kept as the control. Throughout the experiment, all the animals were provided with their regular diet and water *ad libitum*.

The mice were observed daily for changes in general behaviour and clinical signs suggesting toxicity, such as decreased or increased activity, loss of hair, anorexia, constriction or dilatation of pupils, salivation, nasal discharge, diarrhoea and death during the experimental period.

On the 15th day of study, the animals were euthanized by cervical dislocation and post-mortem examinations were carried out soon after death. The visceral organs, namely liver, kidney, spleen, stomach, small intestine, large intestine, caecum, pancreas, urinary bladder, lungs, heart, brain, skeletal muscles and reproductive organs were carefully dissected out and fixed in 10% formal saline for histopathological examinations. Micro sections of 5 μm cut from the tissues embedded in paraffin wax were stained with haematoxylin and eosin (H&E) and examined under light microscope for histopathological changes[11].

As indicated in Table 1, the experimental animals did not show severe clinical signs suggestive of toxicity during the experiment. However, one animal (treatment III, male-2) fed with the highest dose of test substance developed diarrhoea during the latter part of the experiment. Apart from mild to moderate dilatation of the intestine due to gas accumulation (tympanic changes) in four animals, the gross post-mortem findings did not show any significant damage. These observations indicate that oral administration of water extract of *A. sessilis* does not cause acute clinical disease in Swiss mice. However, histopathological changes were found in liver and kidney specimens of the mice fed with the plant extract (Table 1). In contrast, mice in the control group were found with no histopathological changes or damage in their body (Figure 1 *a* and *b*). Moreover, the histological lesions were relatively severe in treatment-III (highest dose) when compared with the other groups. These lesions were characterized by moderate to severe hepatocyte degeneration in centrilobular area associated with widespread sinusoidal congestion and focal hepatocellular necrosis (Figure 2 *a* and *b*). Four of the five mice in treatment-II had mild to moderate hepatocyte degeneration and necrosis. The degenerative changes of hepatocytes found in treatment-I was not consistent and did not appear in all the animals. As shown in Table 1, kidney lesions were found only in animals of treatments II and III. Kidney specimens of treatment III showed moderate degeneration of renal tubular cells and necrosis (Figure 3), whereas treatment II showed only mild degenerative changes of the renal tubular cells.

These findings indicate that oral administration of water extract of *A. sessilis* in high doses leads to histopathological changes in the liver and kidney tissues of Swiss mice. An earlier study carried out using an alkaloidal extract of *A. sessilis* administered intra-peritoneally resulted in alteration of liver and kidney functions in Swiss mice[9]. Based on all these findings, it can be concluded that changes in the liver and kidney functions are due to lesions in the liver and kidney caused by cytotoxic substance/s in *A. sessilis*. However, further investigations are necessary in order to understand the long-term effect of the consumption of cooked *A. sessilis* in smaller quantities.

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Preliminary analysis of cuprome of *Anabaena doliolum* using two-dimensional gel electrophoresis

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This study provides first-hand information on the initial characterization of copper-induced changes in the global proteome (hereafter called cuprome) of *Anabaena doliolum* subjected to short- and long-term treatments. PD Quest analysis revealed that out of 215 protein spots in the control, 79 showed alterations (26 up- and 36 down-regulation, and 5 up- and 12 down-regulation res-

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