

**Research Article****Safety profile of an antihypertensive traditional herb: *Oldenlandia auricularia*****D. Inoka Uluwaduge<sup>1\*</sup>, M. Ira Thabrew<sup>2</sup>**<sup>1</sup>Department of Allied Health Sciences, Faculty of Medical Sciences, University of Sri Jayewardenepura, Gangodawila, Nugegoda, 10250, Sri Lanka<sup>2</sup>Institute of Biochemistry, Molecular Biology and Biotechnology, University of Colombo, Colombo 03, 00700, Sri Lanka

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**Abstract**

**Objective:** The decoction of *Oldenlandia auricularia* (synonyms; *Hedyotis auricularia*, *Exallage auricularia*) is recommended as a treatment of hypertension by traditional Ayurvedic and folk medical practitioners in Sri Lanka. Since the therapeutic benefits of any pharmaceutical agent depends not only on its efficacy but also its lack of toxicity, scientifically controlled experiments were carried out to evaluate any possible toxicological effects mediated by the short term or long term administration of a decoction of this plant. **Materials and Methods:** Experiments were conducted with ICR mice to determine the approximate lethal dose and to investigate short term toxicological effects (on liver function, kidney function and haematological parameters) or long term toxicological effects (on liver function, kidney function, haematological parameters and histopathology of major body organs) of administration of the plant extract. **Results:** Investigations showed that the decoction of this plant had no short term or long term adverse effects on liver function, kidney function or on the haematological parameters as well as the histology of body organs such as liver, kidney, heart, lung and intestine of mice. **Conclusion:** *Oldenlandia auricularia* therefore appears to be free from any major toxic or unacceptable effects when administered for a period of one month and indicates safety of the plant material as an anti-hypertensive agent.

**Keywords:** *Oldenlandia auricularia*, *Hedyotis auricularia*, *Exallage auricularia*, anti-hypertensive, toxicity

**Introduction**

Traditional plants play an important role in the healing of many diseases. In modern medicine, most of the active ingredients are purified and isolated from traditional herbs. According to anecdotal evidences, *Oldenlandia auricularia* is extensively used in Sri Lankan complementary and alternative medicine (CAM) as a treatment for lowering blood pressure despite the lack of strong scientific evidence to validate such claims.

*Hedyotis* or *Oldenlandia* is a genus of flowering plants in the family Rubiaceae and includes approximately 200 species (John et al., 2016). *Oldenlandia auricularia* (synonyms; *Hedyotis auricularia*, *Exallage auricularia*) is a herb distributed from India to southern China and through Malaya to Australia

(Ahamed et al., 2005; John et al., 2016). This plant (Figure 1) has not been studied by many investigators and therefore scanty literature is available on the scientific or medicinal merit of the plant. The plant is widely distributed in wetlands of Sri Lanka and it is reported as a common weed found in rice fields by Chandrasena (1987). It is a sub erect or diffused herb with branches of 15 to 45 cm long. Small leaves are ovate-lanceolate, 2 to 7.5 cm long, and 0.8 to 1.5 cm wide, smooth on the upper surface, and often hairy beneath. The flowers are white with very short stalks and fruits are rather tiny, crowded and ovoid in shape (John et al., 2016).

Traditionally the plant is used as an emollient and prescribed for dysentery and cholera in India (John et al., 2016). The decoction of *O. auricularia* is used as a remedy in Sri Lanka for the treatment of dysentery and diarrhea (Jayaweera, 1982).

Phytochemical analysis of the *O. auricularia* has demonstrated the presence of three glycosides (hydroxy-1-methoxy ethyl glucopyranoside, 1'-Oethyl--D-galactopyranoside, 2-formyl-5-hydroxymethylfuran,

**\*Address for Corresponding Author:**

D. Inoka Uluwaduge,  
Department of Allied Health Sciences,  
Faculty of Medical Sciences, University of Sri Jayewardenepura,  
Gangodawila, Nugegoda, 10250, Sri Lanka  
Email: [deepthi@sjp.ac.lk](mailto:deepthi@sjp.ac.lk), [deepthiuluwaduge@yahoo.com](mailto:deepthiuluwaduge@yahoo.com)

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stigmasta-5 and 22-diene3-O--D-glucopyranoside) together with ursolic acid and oleanolic acid (Tuen et al., 2007). A water soluble alkaloid, auricularine isolated from the plant showed both hypotensive and broncho dilating properties (Mukerjee et al., 1967).



**Figure 1.** Morphology of *Oldenlandia auricularia* flowering aerial part

Decoction prepared by the aerial parts of the fresh plant of *O. auricularia* is a well-established anti-hypertensive Ayurvedic treatment among traditional healers of Sri Lanka. Even though sometimes there is no scientific rationale, the heritage of passing the knowledge on traditional treatments continues from generation to generation amongst traditional healers in Sri Lanka. It is of utmost important to safeguard such practices for future generations and adding scientific rationale to those will intensify the value and confidence in those treatments.

Value of any therapeutic agent depends not only on its potency and efficacy but also its lack of toxicity. According to the information provided by the traditional healers, the decoction of the plant has to be administered to the hypertensive patients over a relatively long period. It must therefore be free of acute as well as chronic toxic effects. However no scientifically controlled studies have been carried out to date, to verify the therapeutic efficacy or to investigate the toxic potential of *O. auricularia*. Investigations were therefore conducted to evaluate any possible toxicological effects mediated by the short term and long term administration of aqueous extract of *O. auricularia*.

The effects of this plant extract on (a) the histopathology of various organs (liver, kidney, heart, lung and intestine) in the body, (b) some haematological parameters (RBC count, WBC count, PCV and Hb concentration) (c) liver function as assessed by the effects on alanine aminotransferase (ALT), aspartate aminotransferase (AST) and alkaline phosphatase (ALP) levels in serum and (d) kidney function as assessed by serum creatinine, were investigated by using male ICR mice as the animal model.

## Materials and methods

## Plant material

Whole plants of *O. auricularia*, were collected freshly from paddy fields of Pannipitiya, Colombo district, Sri Lanka. The botanical identity of the plant was determined by using the descriptions in books and confirmed by well-recognized traditional medical practitioners and the botanist of the Bandaranayake Memorial Ayurvedic Research Institute, Nawinna, Sri Lanka (Jayaweera, 1982). A voucher specimen (M/12) has been deposited at the Department of Allied Health Sciences, Faculty of Medical Sciences, University of Sri Jayewardenepura, Sri Lanka.

The plant extract was prepared according to the method used by Ayurvedic and other traditional medical practitioners for administration to hypertensive patients. The method was confirmed by discussions with several traditional medical practitioners in Sri Lanka. Aerial parts of the fresh plants (60g) were cut into small pieces and boiled in distilled water (1500ml/8 cups) over a gentle heat and the final volume was reduced to 150ml. The filtered extract was collected for the toxicological studies.

## Experimental animals

Healthy, adult male, ICR mice weighing 100-150 g were used in this study. The animals were kept in plastic cages in the animal house of the Faculty of Medicine, University of Kelaniya, Ragama, Sri Lanka, under standard conditions. The animals were fed with pelleted food and tap water. All the experiments were conducted in accordance with the rules of the internationally accepted laboratory animal use and care (based on Helsinki convention), and the guidelines and the rules of Ethics Review Committee of the Faculty of Medicine, University of Kelaniya, Ragama, Sri Lanka for animal experimentation (protocol approval No: 08/10).

## Determination of the approximate lethal dose (LD<sub>50</sub>)

Thirty (30) mice were randomly assigned into five groups (n=6 per group). Group 1 served as the control and was administered distilled water. Mice in groups 2, 3, 4 and 5 was orally administered the extract of *O. auricularia* at doses of 4, 8, 10, 15 g/kg body weight respectively once daily in the morning between 7-8 a.m. for a period of one week via a gastric gavage. The 8 g/kg body weight was comparable to that given to humans on a weight for weight basis. Three other doses, one lower dose (4 g/kg) and two higher doses (10, 15 g/kg) were considered in evaluation of lethal dose. The dose was calculated according to data published by Dhawan and Srimal (2010). The mice were observed throughout the period for any mortality, loss of consciousness, hyperesthesia, salivation, muscle tremors, urinary frequency, defaecation, piloerection, changes in

locomotor activity, changes in posture, ataxia and loss of reflexes.

### Short term toxicity studies

Experimental mice were divided into 2 groups, a control and test (n=6 per group). Plant extract (at a dose of 8g/kg body weight) equal to the human therapeutic dose was administered to the test group once daily for a period of one week. On the 8<sup>th</sup> day the experimental animals were sacrificed by decapitation and blood was collected by cardiac puncture into clean dry centrifuge tubes for assessment of liver enzymes and serum creatinine. For assessment of haematological parameters, blood was collected into clean, dry sample bottles containing dry anticoagulant (EDTA). The serum levels of alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP) and creatinine were estimated by previously reported methods by using commercially available diagnostic kits purchased from Analytical Instruments Pvt. Ltd., Sri Lanka (Bruits et al., 2008).

Blood collected into anticoagulant containing bottles was used for assessment of the RBC count, WBC count, PCV and Hb concentration by methods described by the International Committee for Standardization in Haematology and compared with corresponding values in control animals (Lewis et al., 2006).

### Long term toxicity studies

The animals (n=6 in each group) of the test group were orally administered the *O. auricularia* water extract at a dose of 8g/kg body weight (test group) once daily for 30 days. The control group was similarly treated with distilled water equal to the volume of extract received by the test group. On the 31<sup>st</sup> day of the experiment, all animals were sacrificed by decapitation and blood was collected by cardiac puncture for assessment of liver function (AST, ALT and ALP), serum creatinine and haematological parameters as described in the short term toxicity studies.

### Histopathological studies

From the animals sacrificed for obtaining blood for liver function tests, creatinine and haematological indices, the body organs (liver, kidney, heart, lung and intestine) were excised and fixed in 10% phosphate-buffered formalin for histological assessment of tissue damage after haematoxylin-eosin staining. The histological examinations were carried out by the pathologist in the Department of Pathology, Faculty of Medicine, University of Kelaniya, Ragama, Sri Lanka.

### Statistical analysis

The results were expressed as Mean  $\pm$  Standard Error of Mean (SEM). The significance of difference in the parameters tested between test and control groups were analyzed by Student's test.

Difference were considered significant if  $p < 0.05$ .

## Results

### Ld<sub>50</sub> of *O. auricularia*

All the animals used in the study were active during the entire period of one week regardless of the dose of the plant extract (4, 8, 10 and 15 g/kg body weight) they received. No abnormalities in external appearance or loss in body weight were noted. Food intake was also normal during the entire period of investigation.

No deaths or any other apparent adverse effects such as loss of consciousness, hyperesthesia, salivation, muscle tremors, urinary frequency, defaecation, piloerection, changes in locomotor activity, and changes in posture, ataxia and loss of reflexes were observed during the experimental period. Therefore these results did not allow the calculation of the LD<sub>50</sub>, but they indirectly indicate the absence of acute toxic effects of *O. auricularia*.

### Short term toxicity studies

The effects of administration of *O. auricularia* extract for one week on serum levels of ALT, AST, alkaline phosphatase, creatinine and haematological parameters are depicted in table 1.

It is evident that administration of the extract for one week did not produce any acute toxicological effect on liver function, kidney function or on the haemoglobin concentration and blood cell counts.

### Long term toxicity studies

The effects of administration of *O. auricularia* extract for thirty days on serum levels of ALT, AST, alkaline phosphatase, creatinine and haematological parameters are summarized in table 2.

As shown, administration of the plant extract (compatible to

**Table 1.** Effect of the aqueous extract of *O. auricularia* (dose: 8g/kg body weight) on serum levels of liver enzymes, creatinine and haematological parameters, after 1 week

Parameters	Test group	Control group
<b>Key hepatic enzymes</b>		
ALT (U/L)	96.6 $\pm$ 6.5*	88.3 $\pm$ 7.0
ALP (U/L)	23.8 $\pm$ 2.0*	20.8 $\pm$ 1.2
AST (U/L)	106.0 $\pm$ 6.3*	113.0 $\pm$ 8.2
Creatinine (mg/dL)	0.66 $\pm$ 0.05*	0.79 $\pm$ 0.1
<b>Haematological indices</b>		
PCV (%)	37.4 $\pm$ 3.2*	32.6 $\pm$ 2.0
Hb (mg/dL)	15.1 $\pm$ 1.0*	12.8 $\pm$ 1.8
WBC / mm <sup>3</sup>	4.6 $\pm$ 0.26*	4.28 $\pm$ 0.32
RBC / mm <sup>3</sup>	9.4 $\pm$ 0.23*	9.9 $\pm$ 0.26

Data shown are the mean  $\pm$  SEM of 6 determinants. \*Not significantly different from the corresponding control values by Student's *t* test ( $p > 0.05$ )

**Table 2.** Effect of the aqueous extract of *O. auricularia* (dose: 8g/kg body weight) on serum levels of liver enzymes, creatinine and haematological parameters, after 30 days

Parameters	Test group	Control group
<b>Key hepatic enzymes</b>		
ALT (U/L)	68.6 ± 4.6*	71.0 ± 3.8
ALP (U/L)	18.3 ± 2.0*	23.1 ± 1.6
AST (U/L)	109 ± 3.6*	113.0 ± 8.2
Creatinine (mg/dL)	1.07 ± 0.05*	1.08 ± 0.2
<b>Haematological indices</b>		
PCV (%)	34.8 ± 2.6*	30.0 ± 3.0
Hb (mg/dL)	14.2 ± 2.6*	13.8 ± 1.6
WBC / mm <sup>3</sup>	3.34 ± 0.2*	3.32 ± 0.4
RBC / mm <sup>3</sup>	6.52 ± 0.2*	7.0 ± 0.3

Data shown are the mean ± SEM of 6 determinants. \*Not significantly different from the corresponding control values by Student's *t* test ( $p > 0.05$ )

the human therapeutic dose) to mice for a period of one month also did not produce any toxicological effect on liver function, kidney function or on the haemoglobin concentration and blood cell counts.

#### Effects on organ histology

No morphological differences were observed on light microscopic observation of haematoxyline and eosin stained histological sections of the organs (liver, kidney, heart, lung and intestines) of control animals and those of animals treated with the plant extract for 30 days. These findings indicate that the test plant extract does not produce any toxic effects on the major body organs even after 30 days of treatment.

#### Discussion

The therapeutic value of any plant extract depends not only on its pharmacological potency but also on its lack of acute and chronic toxicity. A perusal of published literature has shown that there are some medicinal plants that contain toxic compounds which have the potential to produce serious adverse reactions (Tamilselven et al., 2014). Assessment of toxicity is especially important in the case of the treatment for hypertension which requires the therapeutic agent to be administered over a relatively long period of time. According to anecdotal evidences a few plants that have been reputed to be used, by Sri Lankan traditional medical practitioners, for the treatment of hypertension are *O. auricularia*, *Biophytum reinwardtii* (Family: Oxalidaceae, Local name: Gas nidikumba / Heen nidikumba, English name: Sikerpud, Tamil name: Nilaccurunki, Tintanali) and *Rauwolfia serpentina* (Family: Apocynaceae, Local name: Sarpghandha, Ekaweriya, English name: Black snake root, Tamil name: Chevanamalpodu). Of these, toxicity has evaluated only in

*Rauwolfia serpentina* and it has been reported to be a safe and effective agent against hypertension (Lobay, 2015).

As evident from results obtained in the present study, administration of *O. auricularia* extract to mice for one month did not produce any toxic effects of importance. Thus, the extract used had no significant effects on liver function, kidney function, haematological parameters or on the histopathology on the body organs of the experimental animals. The general condition of the animals also did not change and they remained in good health throughout the experimental period. *O. auricularia* therefore appears to be free from any major toxic effects when administered even up to a period of one month.

Since it has been reported that *O. auricularia* contains auricularine, an anti-hypertensive compound, it may be possible to develop a safe and effective anti-hypertensive drug from this plant in the future. However for a more definitive conclusion with regard to the clinical use of this plant, scientifically controlled studies are needed.

#### Competing Interests

The authors declare that they have no competing interests regarding publication of this paper.

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