Diuretic Activity of *Oldenlandia corymbosa* L.

By

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Abstract

The plant Oldenlandia corymbosa L. locally known as su-la-napha, belongs to the family Rubiaceae. It is a naturally grown plant and collected from Yangon. The plant is used in the treatment of Jaundice and diuretic. The acute toxicity test was carried out with 70% ethanolic extract of su-la-na-pha by using albino mice. It was observed that the 70% ethanolic extract was free from acute toxicity or harmful effect during observation period of 2-weeks even with maximum permissible dose of 16g/kg. Diuretic activity of 70% ethanolic extract of O.corymbosa L. was tested by using albino rats at 6g/kg dose. The results showed that the 70% ethanolic extract of O.corymbosa L. significant for diuretic activity.

Introduction

- ➤The plant Oldenlandia corymbosa L. belongs to the family Rubiaceae.
- ➢It is an annual herb. It is found in hard or stony areas, graveled yards, sandy place and garden paths.
- ➤The family Rubiaceae consists of about 500 genera and about 6000 species (Trace & Evans 2002).
- ➤The plants occur in tropical and subtropical regions and widespread throughout the world (Cronquist, 1981).

➢In Myanmar, the plant is commonly known as su-la-na-pha and Mintwort (Collection of Commonly used Herbal plant, 2003) or Old diamond flower in English.

In Myanmar, the plant is used in traditional medicine especially in the treatment of cooling, febrifuge, Jaundice, diuretic and stomachic properties (Collection of Commonly used Herbal plant, 2003), pectoral , nervous depression caused by deranged bile (Henderson, 1949; Chopra, 1956; Wealth of India, 1959), diseases of liver and chronic malaria as a good febrifuge (Nadkarni, 1954)

Objectives

To investigate the acute toxicity and diuretic activity of ethanolic extract of *Oldenlandia corymbosa* L

Materials and Methods

Collection of Oldenlandia corymbosa L.

The plant *Oldenlandia corymbosa* L. were collected from South Okkalapa Township, Yangon Region.

The acute toxicity of ethanolic extract of *Oldenlandia corymbosa* L. on albino mice.

Preparation of 70% ethanolic extract

100 g of dried powder sample was extracted with 1 litre of 70% ethanol in a conical flask and heated in boiling water bath for 6 hours. After heating for 6 hours, it was cooled and filtered by using filter paper and filtrate was evaporated to dryness on a boiling water bath.

Materials

>Drug used

- Animals used 40 albino mice of both sexes, (body weight 24-30)g
 - different concentration of 70% ethanolic extract of *Oldenlandia corymbosa* L.
- >Apparatus used aluminium mouse cages
 - animal balance
 - "18" gauge intragastric dosing cannula
- Dose schedule 8g/kg in mice (on body weight basis)
 12g/kg in mice (on body weight basis)
 16g/kg in mice (on body weight basis)
 Period of two weeks

observation

Method

- The acute toxicity test on mice was carried out according to the method described by (Litchfield and Wilcoxon, 1949 and May Aye Than, 1994).
- ➤ 40 albino mice of the both sex, weighing (24-30) g were used in this study.
- The mice were separated into 4 groups and each group comprises of 10 mice. Each group was placed separately in 4-mouse cages.
- Food and water was withheld for the period of 19 hours before administration of drug.
- ➤ At first, the mice were individually marked with picrick acid staining on the part of body and the required dose were calculated.

- Group I was served as control group and administered 0.1ml/10g distilled water.
- Group II-IV were administered orally with different concentration of 70% ethanolic extract.
- The given doses of ethanolic extract were 8g/kg, 12g/kg and 16g/kg body weight respectively.
- After given the various concentration of extract orally, each group of mice was allowed access of food and water. Then, the observation of above treated mice were carried out.





Fig.1 Each group contains 10 mice Fig.2 Ethanolic extract was administered orally into albino mice

Diuretic effect of ethanolic extract of *Oldenlandia corymbosa* L. on albino rats.

Materials

- > Albino rat of female body weight (180-250)g
- Metabolism cages
- > "18" guage intragastric dosing cannula
- Normal saline solution (9% NaCl solution)
- > Test reagent 70% ethanolic extract of *Oldenlandia corymbosa* L.
 - diruton tablets.

"Total 8 rats" Fasting for food and water For 19 hours Normal saline (5ml/100g) body weight Urine collection (1 hourly up to 5hours) Rest for 1 week Same rats Fasting for food and water For 19 hours Standard drug diruton (100 mg/kg) body weight Urine collection (1 hourly up to 5hours) Rest for 1 week Same rats Fasting for food and water For 19 hours 70% ethanolic extract 6g/kg body weight Urine collection (1 hourly up to 5hours)

Flow diagram for testing diuretic activity on albino rats.



Fig.3 Administration of normal saline on albino rat.



Fig.4 An albino rat placed in the metabolism cage for urine collection.

Diuretic activity is calculated as follows:

Uringry excretion		volume of urine collected in 5 hours	\checkmark	100
Unitary excitation	_	volume of fluid administred		100
Divretic activity		Urinary excretion in test group		
Didictic activity	_	Urinary excretion in control group		
		% excretion in test group		
_		%excretion in control group		

Diuretic effect is presented if this ratio is more than 1.

Results

Diagnostic features of *Oldenlandia corymbosa* L.

Habit - Annual, slender herbs. 4-35 cm high.

Stem - quadrangular, ascending or spreading.



Fig.6 Habit

Leaves - simple, opposite and decussate, sessile, the margin recurved.



Fig.7 Leaves

Inflorescence – axillary, in corymbose cyme of generally 3-5 flowers.



Fig.8 Inflorescences

> Flower – small, white or pale purple.



Fig.9 L.S of flower



Fig.10 flowers

> Fruits – the capsule

with persistent sepals,

the top capsule

flattened.



Fig.11 Fruits

Acute toxicity of ethanolic extracts of Oldenlandia corymbosa L.

The acute toxicity test was done according to the method described by (Litchfiled and Wilcoxon, 1949 and May Aye Than, 1994)

➢ It showed that no lethality of the mice were observed daily up to 2 weeks. The results were shown in Table (1).

Table (1) Results of acute toxicity test of 70% ethanolic extract of *Oldenlandia corymbosa* L. on albino mice.

No	Types of drug	Dose (g/kg)	No.of animal test	% of lethality
1	Control	D/W(0.1ml/10g)	10	0%
2	70% ethanolic extract	8	10	0%
3	70% ethanolic extract	12	10	0%
4	70% ethanolic extract	16	10	0%

Therefore, it was observed that 70% ethanolic extract were free from acute toxic or harmful effectes.

Diuretic effect of *Oldenlandia corymbosa* L.on albino rats.

- Total '8' albino rats of female weighing (180-250)g are used in the present study.
- They were given by only 9% NaCl (5ml/ 100g) as control, reference drug chlorothiozide (diruton) (100mg/kg) as standard and 70% ethanolic extract (6g/kg) as test sample respectively.
- Afte administration of drug, the urine was collected hourly for 5 hours.
- \succ The results were shown in tables and figures.

Table 2. Mean cumulative urine excretion (in ml) of rats treated with
normalsaline, standard drug and ethanolic extract of
Oldenlandia corymbosa L. at various time interval

	Cumulative mean urine volume (in ml)				
groups	1hr	2hr	3hr	4hr	5hr
Control	0.344±0.11	1.02±0.32	1.601±0.39	2.056±0.51	2.355±0.49
Ethanolic extract of <i>O.corymbosa</i> L.					
6g/kg	1.037±0.19	2.981±0.37	3.479±0.46	3.664 ± 0.47	4.013±0.55
Diruton 100 mg/kg	0.788±0.31	3.139±0.48	4.348±0.74	5.266±0.80	6.434±0.63



Figure 12. Statistical comparison of cumulative urinary volumes (in ml)among control, diruton and ethanolic extract of *Oldenlandia corymbosa* L.

Each point represent the mean of observation and the vertical bars indicate standard errors of the means.

Table 3. Mean cumulative urine excretion of sodium (in g) of ratstreated with normal saline, standard drug and ethanolic extractof Oldenlandia corymbosa L. at various time interval

	Cumulative mean urinary sodium concentration (in g)					
groups	1hr 2hr 3hr 4h		4hr	5hr		
Control	0.005 ± 0.001	0.018±0.004	0.028±0.006	0.035±0.006	0.042 ± 0.007	
Ethanolic extract of <i>Oldenlandia</i> corymbosa L.						
6g/kg	0.036±0.007	0.091±0.017	0.145±0.009	0.177±0.013	0.203 ± 0.01	
diruton100mg/kg	0.011±0.005	0.052±0.006	0.084±0.016	0.096±0.018	0.113±0.018	



Figure.13. Statistical comparison of cumulative urinary sodium excretion(in g) among control, diruton and ethanolic extract of *Oldenlandia corymbosa* L.

Table 4. Mean cumulative urine excretion of potassium(in g) of rats treated with normal saline, standard drug and ethanolic extract of *Oldenlandia corymbosa* L. at various time interval

	Cummulative mean urinary potassium concentration (in g/L)					
groups	1hr	2hr	3hr	4hr	5hr	
Control	0.0009 ± 0.0006	0.029 ± 0.006	0.043 ± 0.01	0.051 ± 0.011	0.059±0.012	
Ethanolic extract of <i>O.corymbosa</i> L.						
6g/kg	0.107 ± 0.018	0.3 ± 0.036	0.367±0.046	0.378 ± 0.05	0.418±0.056	
diruton 100 mg/kg	0.015±0.007	0.071±0.005	0.086±0.008	0.096±0.008	0.128±0.015	



Figure.14. Statistical comparison of cumulative urinary potassium excretion (in g) among control, diruton and ethanolic extract of *Oldenlandia corymbosa* L.

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Table 5.Diuretic effects of individual rat treated with normal
saline (control)

Rat No.	Volume administered	Volume excreted within 5hr	Urinary excretion %	Diuretic activity
1	10	4.19	41.9	-
2	11.75	3.32	28.25532	-
3	10.75	2.48	23.06977	-
4	9.7	3.12	32.16495	-
5	10.5	0.6	5.714286	-
6	11.75	0.26	2.212766	-
7	11	3.21	29.18182	-
8	11	2.86	26	-
sum	86.45	20.04	188.4989	-
mean	10.80625	2.505	23.56236	-
se	0.261424	0.485033	4.713998	-

Table 6.Diuretic effects of individual rat treated with ethanolicextract of Oldenlandia corymbosa L.

Rat No.	Volume administered	Volume excreted within 5hr	Urinary excretion %	Diuretic activity
1	9.25	4.6	49.72973	1.186867
2	11.75	5.47	46.55319	1.64759
3	10.5	4.9	46.66667	2.022849
4	9.75	4.4	45.12821	1.403024
5	8.25	2.46	29.81818	5.218182
6	11.25	1.56	13.86667	6.266667
7	11.5	5.68	49.3913	1.692537
sum	72.25	29.07	281.1539	19.43772
mean	10.32143	4.152857	40.16485	2.776817
se	0.458469	0.549146	4.74996	0.729924

Table 7. Diuretic effects of individual rat treated with standarddrug diruton

Rat No.	Volume administered	Volume excreted within 5hr	Urinary excretion %	Diuretic activity
1	7.85	9.23	117.5796	2.806196
2	9.8	8.87	90.5102	3.203298
3	8.4	5.74	68.33333	2.96203
4	8.4	7.26	86.42857	2.687042
5	8.2	5.56	67.80488	11.86585
6	9.8	4.48	45.71429	20.65934
7	8.8	4.78	54.31818	1.861371
8	9.2	5.5	59.78261	2.299331
sum	70.45	51.42	590.4717	48.34446
mean	8.80625	6.4275	73.80896	6.043058
se	0.258505	0.642336	8.222386	2.384248

Discussion and Conclusion

- ➢ In this research, the morphological and diuretic activity of Oldenlandia corymbosa L. has been carried out.
- Su-la-na-pha, by the Myanmar name of this species which is an annual weedy herb. Leaves are opposite. Inflorescences are corymbose cymes. Fruits are capsules.

➤The acute toxicity of 70% ethanolic extract of the plant O. corymbos L. was free from acute toxic effect even with the maximum permissible dose of 16g/Kg body weight.

The medium lethal dose of 70% ethanolic extract of the plant *O.corymbosa* L. was supposed to be higher than 16g/Kg body weight.
Therefore, 70% ethanolic extract of the plant *O. corymbosa* L. was no acute toxic or harmful effect in this dosage. According to the toxicity

test, the plant may be used safely.

- In this research, after receiving 6g/kg body weight dose of the 70% ethanolic extract, the urinary sodium and potassium excretion were increased significantly in comparison with control (p < 0.05 0.0005).</p>
- The urinary sodium excretion of 70% ethanolic extract was significantly higher than control of 1, 2, 3, 4, 5 hours and potassium excretion of extract was significantly higher than control of 1, 2, 3, 4, 5 hours respectively.
- The urinary excretion of 70% ethanolic extract is higher than control. In this study, the mean of diuretic activity of standard drug diruton 100mg/kg was 6.043 and 70% ethanolic extract 6g/kg was 2.777.

If the ratio of percent excretion of urine in tested group and control group is more than 1, there is diuretic activity (Rosanthale, 1963).
 Ethanolic extract and standard drug diruton showed the mean of diuretic activity more than 1. Therefore, ethanolic extract and standard diruton have diuretic effect on albino rats.

According to this investigation, the ethanolic extract of O. corymbos L. has suitable for using as diuretic in edematous patients.

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