Antidiarrhoeal Activity of the Ethanolic Extract of Unripe Fruit of *Limonia acidissima* L. (Thee-Thee)

Aye Hnin Thwe[1], Ei Ei Htway[2], Swe Swe[2], Sandar Linn[2], Myitzu Tin Oung[2], Zarni Htoon Lwin[3], Win Ko[4], Wah Wah Oo[5], Maung Maung Thet[1]

1= University of Traditional Medicine
2= Department of Medical Research (Pyin Oo Lwin Branch)
3= University of Medical Technology (Mandalay)
4= Member of Myanmar Traditional Medical Council
5= University of Medicine (Mandalay)

INTRODUCTION

- Diarrhoea results from an imbalance between the absorptive and secretory mechanisms in the intestinal tract, resulting in an excess loss of fluid in the faeces
- About 1.7 billion cases of diarrhoeal disease occur worldwide every year (WHO, 2013)
- Second leading cause of death in children under five years old (WHO, 2013)
- According to Health in Myanmar 2012, diarrhoea is the fourth leading cause of morbidity in Myanmar, 2010

- Several antidiarrhoeal drug in the market in Myanmar, including Modern antidiarrhoeal drugs, traditional medicine and herbal medicine.
- Myanmar Traditional Medicine for several years by using several herbal medicinal plants.
- Ashin Nagathein: leave of *Limonia acidissima* L
- Charak Sthanhitar encyclopedia: unripe fruit and seed of the *Limonia acidissima* L.

Limonia acidissima L.

- Known as Thee-Thee (Myanmar), Elephant apple and Wood apple (English) and Kapitha, Kapi (Sanskrit), Katbel (Hindi)
- Family - Rutaceae.
- Distribution: Asia tropical, Asia temperate, Southern and northern America,
  : Many parts of Myanmar, especially in Mandalay and Magway Division

Ayurvedic medicinal properties

- Rasa - Madhura, Kashaya
- Guna - Guru, Snigda
- Virya - Seeta (Vilarmaram, 2009)

Medicinal Uses of *Limonia acidissima* L. (Thee – Thee)

- In Ayurveda- The fruit is much used as a liver and cardiac tonic
  - Unripe fruit is used in treating diarrhoea, dysentery, hiccup, sore throat and diseases of the gums
- In Banglandesh- The unripe fruit is astringent and is used in diarrhoea and dysentery.
  - Seeds are used in heart diseases
  - Leaves are astringent and carminative, vomiting, indigestions, hiccup and dysentery.

- In traditional medicine principle, sweet and astringent tastes medicines are used to treat diarrhoea
- According to the literatures, *Limonia acidissima* L. also has antidiarrhoeal effect and it has sour, sweet and astringent taste
- But it has not been proved scientifically in Myanmar
- Therefore, the present study was carried out to explore the antidiarrhoeal effect of *Limonia acidissima* L. (Thee-Thee)

OBJECTIVES

- To determine the constituents of ethanolic extract of unripe fruit of *Limonia acidissima* L.
- To investigate the antidiarrhoeal effect of ethanolic extract of unripe fruit of *Limonia acidissima* L. in albino mice by using castor oil induced diarrhoea model including frequency of diarrhoea and percent of small intestinal transit

METHODOLOGY

Study Design

- Randomized controlled experimental animal study

Study Area

- (1) Department of Botany, University of Mandalay
- (2) Pharmacology Research Division, Department of Medical Research (Pyin-Oo-Lwin Branch)

Study Size

- ICR (Institute of Cancer Research) strain albino mice 60 in numbers
- were bred in Laboratory Animal Services Division, Department of Medical Research (Pyin-Oo-Lwin Branch)

Selection of Animal

ICR albino mice of both sexes weighing 25 g ± 3 g

Study Period

- From May 2012 to September 2013

Plant Collection and Identification

- were collected in the month of May, 2013 from garden of University of Traditional Medicine, Mandalay
- plant identification was carried out by the botanist, Department of Botany, University of Mandalay

Materials for this study (Chemicals)

- Ethanol
- Loperamide Hydrochloride
- Castor oil
- Charcoal
- Gum acacia
- Chloroform

Method for plant extraction

- Small dry pieces of unripe fruit of *Limonia acidissima* L.
- Reflux with 95% ethanol, at 60˚C, 6 hours for 2 times
- Filtered by using the filter paper
- Evaporated by using water-bath at 50˚ C until solid extract and kept in desiccator

Phytochemical screening

- Tested qualitatively phytochemical constituents, procedures as stated in Harborne, 1984 and Unani council, 1987

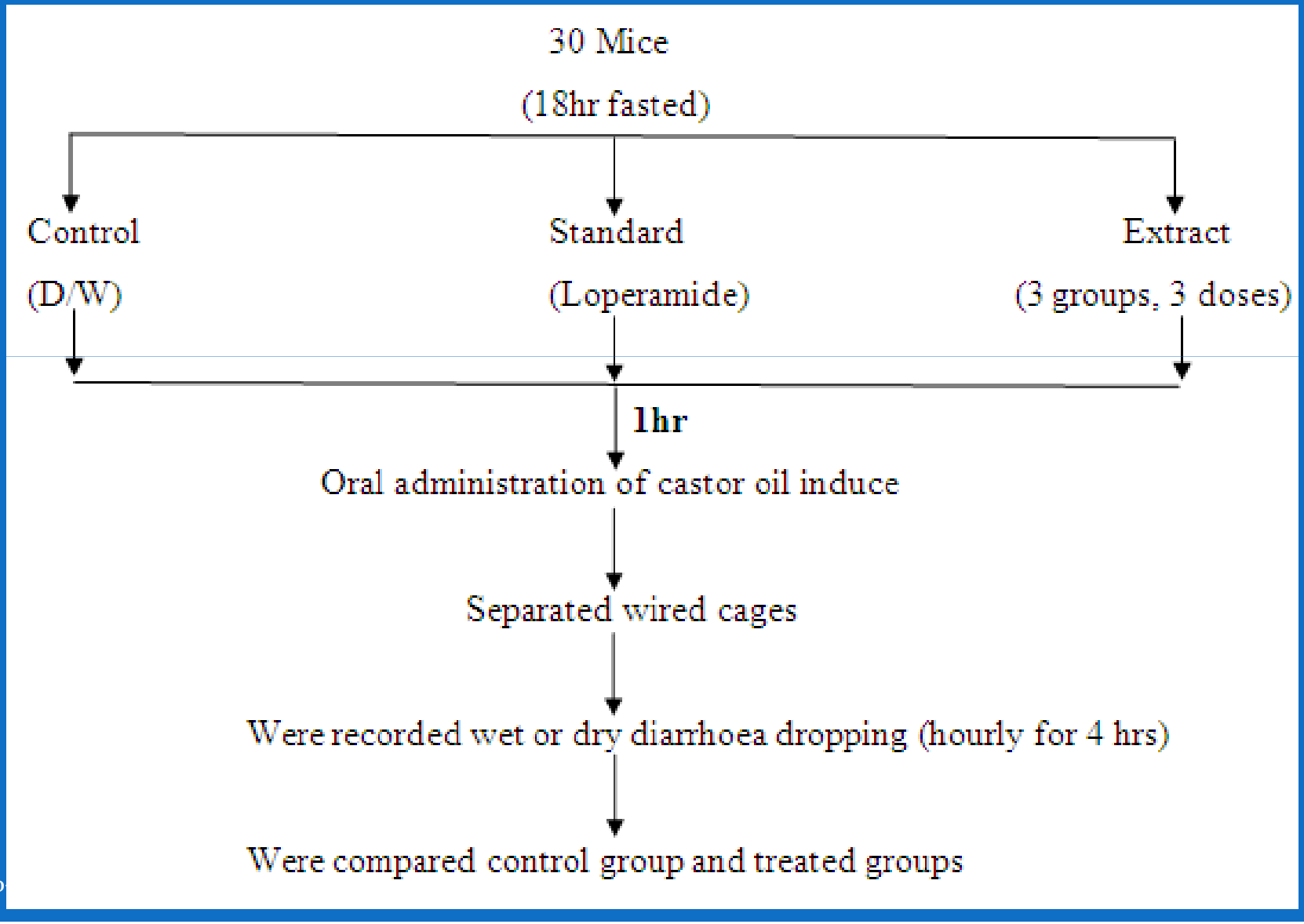
Antidiarrhoeal activity of ethanolic extract of *Limonia acidissima* L. in experimental animals

- Two methods

  (1) Castor oil induced diarrhoea

  (2) Castor oil induced small intestinal transit

(1) Castor oil induced diarrhoea

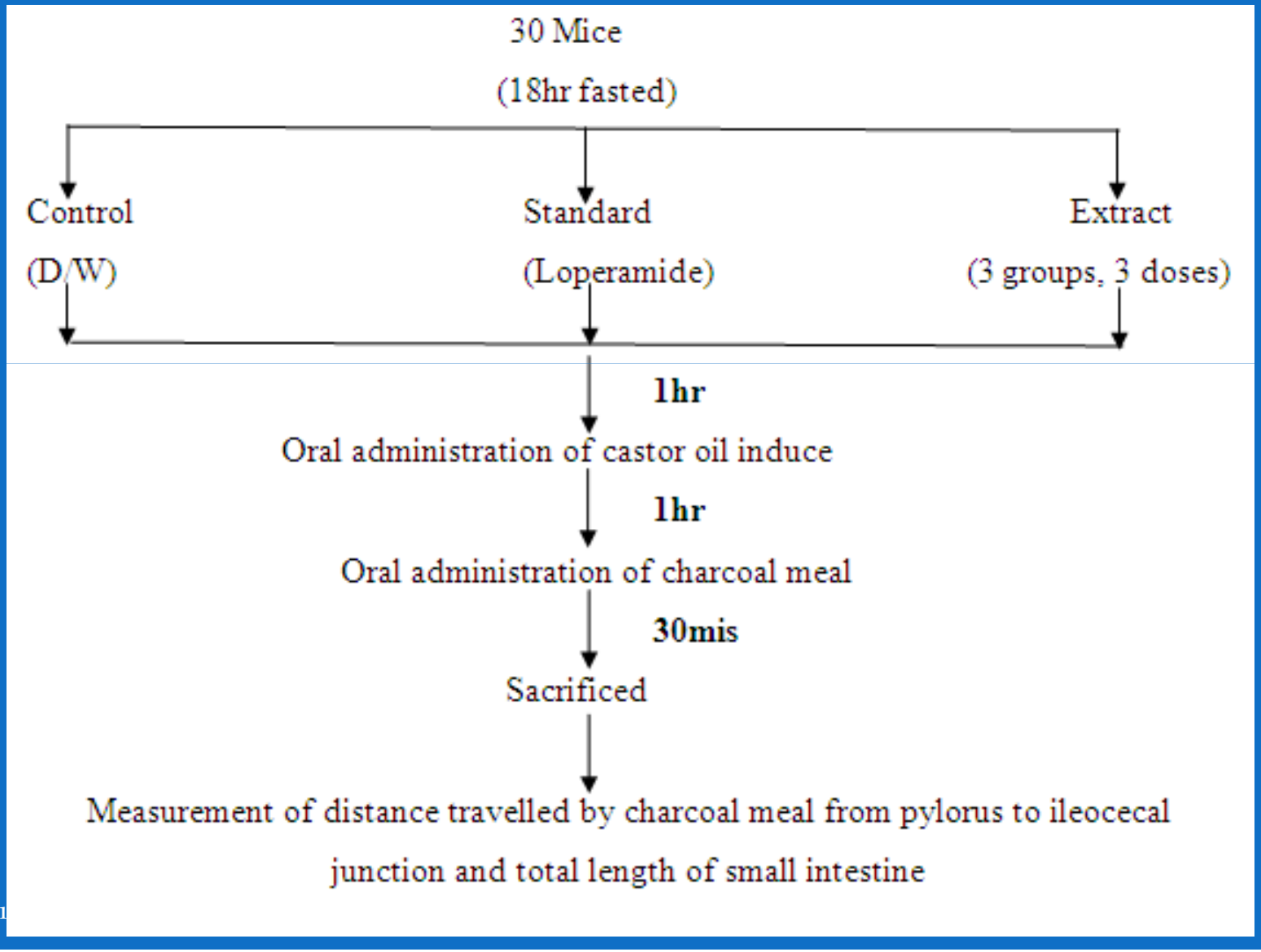



SHIMADZU
UNIT
28.83 g

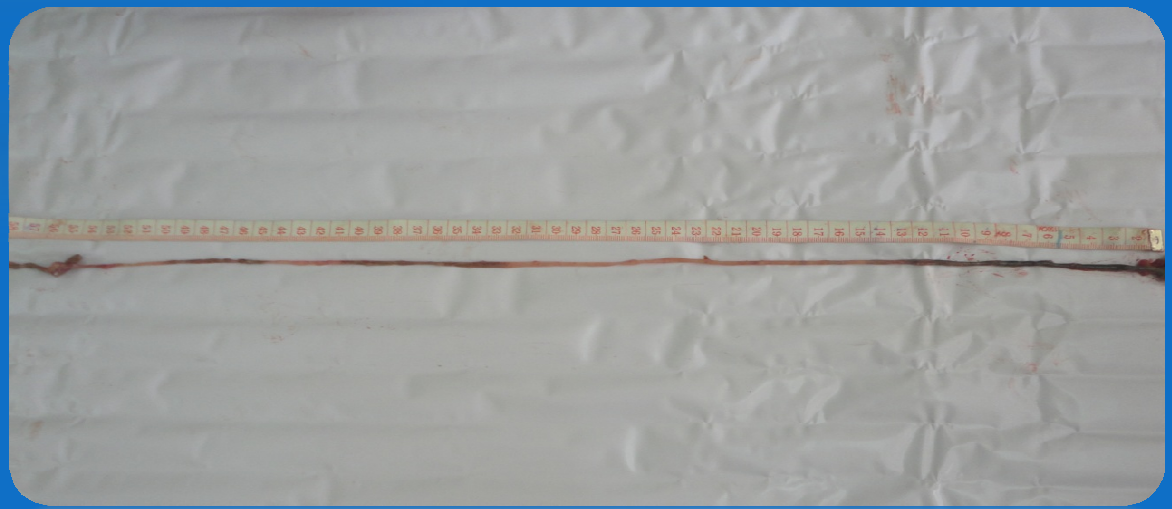
Observation of diarrhoea episodes of mice

$$\% \text{ inhibition} = \frac{(\text{Control} - \text{Test})}{\text{Control}} \times 100$$

(2) Castor oil induced small intestinal transit



Measuring the length of intestine of experimental animals



$$\% \text{ intestinal transit} = \frac{\text{Distance travelled by charcoal meal}}{\text{Total length of small intestine}} \times 100$$

Statistical Analysis

- SPSS software
- One way ANOVA test followed by Dunnett's was used
- $P < 0.05$ was considered significant

FINDINGS

➢ **Phytochemical analysis of *Limonia acidissima* L.**

- alkaloids, carbohydrates, glycosides, phenols, starch, steroids and tannins were present

➢ Results Castor oil induced diarrhoea

Table (1). Comparison of mean frequencies of diarrhoea of control with test groups at 1, 2, 3 and 4 hour after castor oil administration

Group	Treatment	Frequencies of diarrhea			
		1 hour	2 hour	3 hour	4 hour
		Mean ± SE	Mean ± SE	Mean ± SE	Mean ± SE
I	DW + CO	1.50 ± 0.72	1.67 ± 0.42	1.67 ± 0.21	0.83 ± 0.31
II	Lop (6 mg/kg) + CO	0.67 ± 0.49	0.50 ± 0.34	0.33 ± 0.33*	0.00 ± 0.00
III	Ext (240 mg/kg) + CO	1.67 ± 0.80	0.83 ± 0.31	0.50 ± 0.50	0.67 ± 0.33
IV	Ext (360 mg/kg) + CO	1.17 ± 0.48	1.17 ± 0.48	0.83 ± 0.31	0.33 ± 0.21
V	Ext (480 mg/kg) + CO	1.00 ± 0.37	0.33 ± 0.21	0.33 ± 0.21*	0.33 ± 0.21

DW = Distilled water (10 ml/kg) CO = Castor oil (10 ml/kg) Lop = Loperamide
Ext = Extract *$p < 0.05$ (p value versus control)

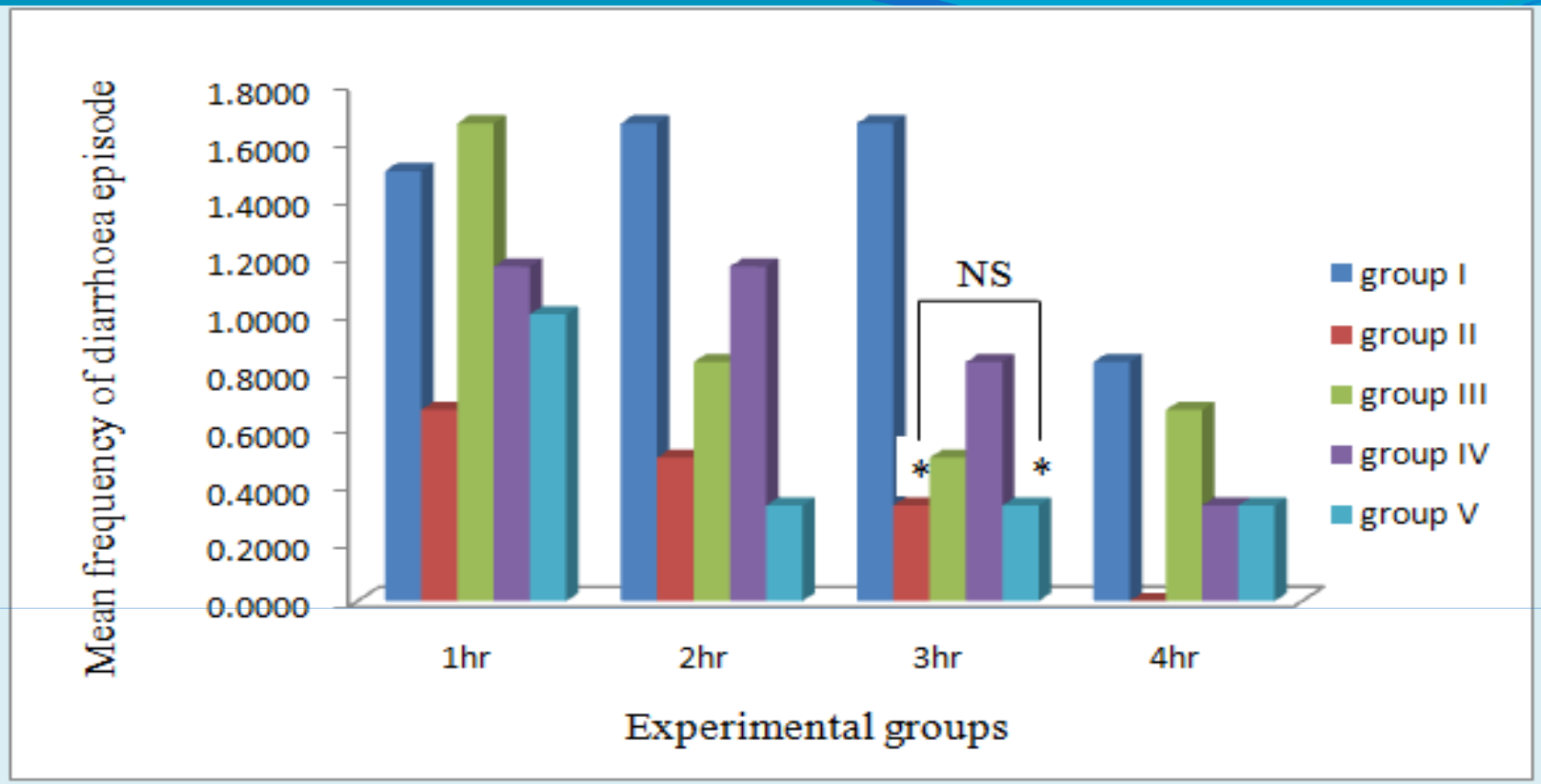


Figure (4). Effect of distilled water, loperamide and three different doses of the ethanolic extract of *Limonia acidissima* L. on frequencies of castor oil induced diarrhoea at 1, 2, 3 and 4 hour after castor oil administration

group I – Distilled water (10 ml/kg)

group II – Loperamide (6 mg/kg)

group III – Extract 240 mg/kg

group IV – Extract 360 mg/kg

group V – Extract 480 mg/kg

* $p < 0.05$ - versus control

NS = no significantly different – loperamide versus ethanolic extract 480 mg/kg

Table (2). Comparison of mean frequencies of diarrhoea of control with test groups within 4 hours after castor oil administration

Treatment	Frequencies of diarrhoea		
	Mean ± SE	P value versus distilled water	P value versus Loperamide
D/W+ CO	5.67 ± 0.67	p > 0.05	
Loperamide(6mg/kg)+CO	1.50 ± 0.72**	p < 0.01	
Extract (240mg/kg)+CO	3.67 ± 0.67	p > 0.05	
Extract (360 mg/kg)+CO	3.50 ± 0.99	p > 0.05	
Extract (480 mg/kg)+CO	2.00 ± 0.58**	p < 0.01	P > 0.05 P = 0.968

DW = Distilled water (10 ml/kg) CO = Castor oil (10 ml/kg)

**p < 0.01 (P value versus control)

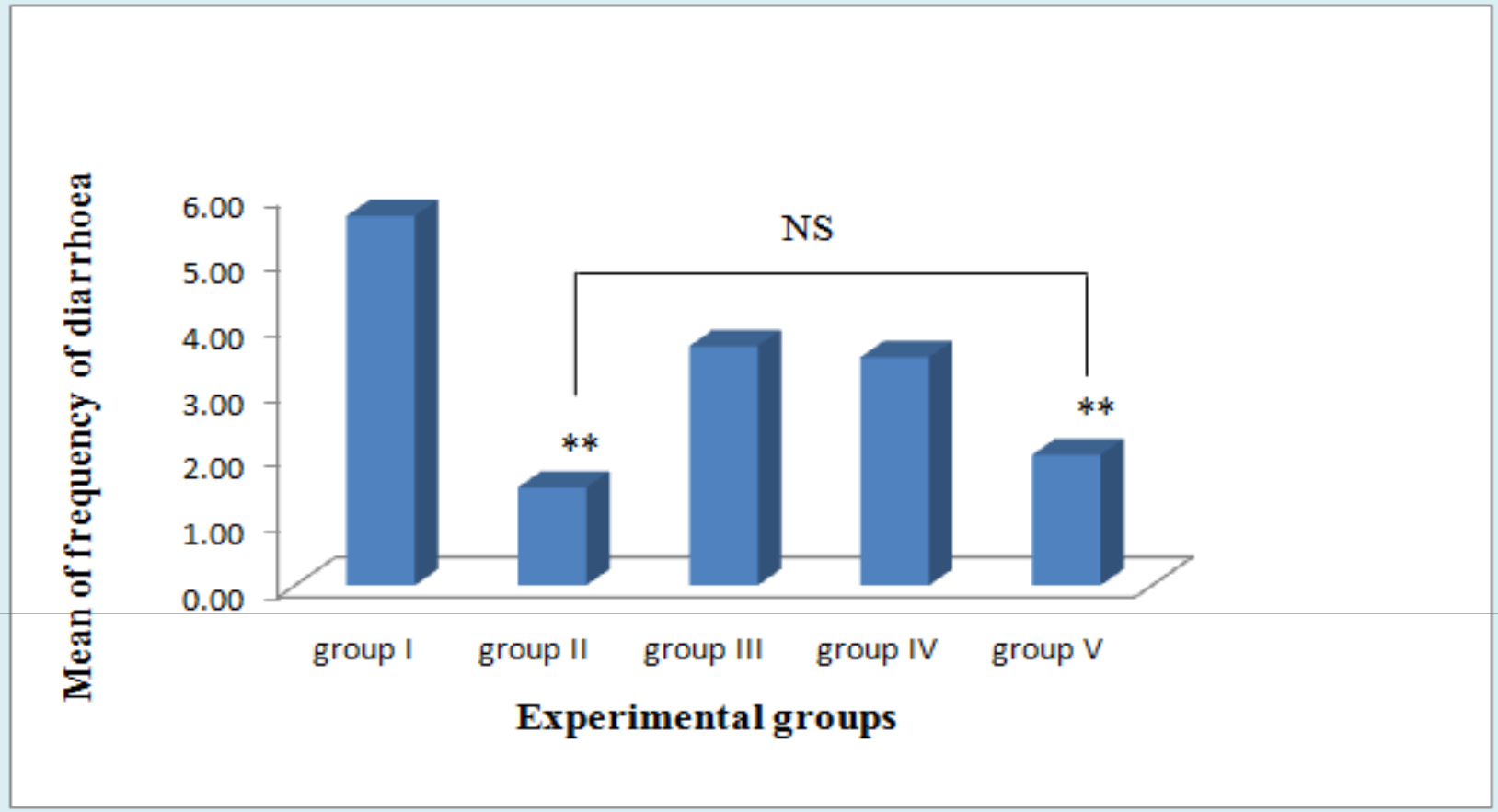


Figure (5). Effect of distilled water, loperamide and three different doses of the ethanolic extract of *Limonia acidissima* L. on frequencies of castor oil induced diarrhoea within 4 hours after castor oil administration

group I – Distilled water (10 ml/kg) group II – Loperamide (6 mg/kg)
group III – Extract 240 mg/kg group IV – Extract 360 mg/kg
group V – Extract 480 mg/kg $^{**}p < 0.01$ - versus control
NS = no significantly different – loperamide versus the ethanolic extract 480 mg/kg

Table (3). percent inhibition of castor oil induced diarrhoea at 1, 2, 3 and 4 hour after castor oil administration

Group	Treatment	% inhibition of diarrhea			
		1 hour	2 hour	3 hour	4 hour
I	DW + CO	-	-	-	-
II	Loperamide (6 mg/kg) + CO	56	70	80	100
III	Extract (240 mg/kg) + CO	-11	50	70	20
IV	Extract (360 mg/kg) + CO	22	50	50	60
V	Extract (480 mg/kg) + CO	33	80	80	60

CO = Castor oil (10 ml/kg) DW = Distilled water (10 ml/kg)

➢Result of Castor oil induced small intestinal transit

Table (4) comparison of mean percent intestinal transit of control with test groups

Treatment	Mean ± SE	% intestine transit	P value versus control	P value versus loperamide
Distilled Water(10 ml/kg po) + CO (10 ml/kg po)	59.39 ± 3.77	59.39		
Loperamide (6 mg/kg po) + CO (10 ml/kg po)	31.06 ± 4.83**	31.06	p < 0.01	
Extract (240 mg/kg po) + CO (10 ml/kg po)	45.46 ± 6.59	45.46	p > 0.05	
Extract (360 mg/kg po) + CO (10 ml/kg po)	44.99 ± 6.43	44.98	p > 0.05	
Extract (480 mg/kg po) + CO (10 ml/kg po)	35.50 ± 3.53*	35.53	p < 0.05 p = 0.011	p > 0.05 p = 0.931

CO = Castor oil po = per oral ** p < 0.01, * p < 0.05 - versus control

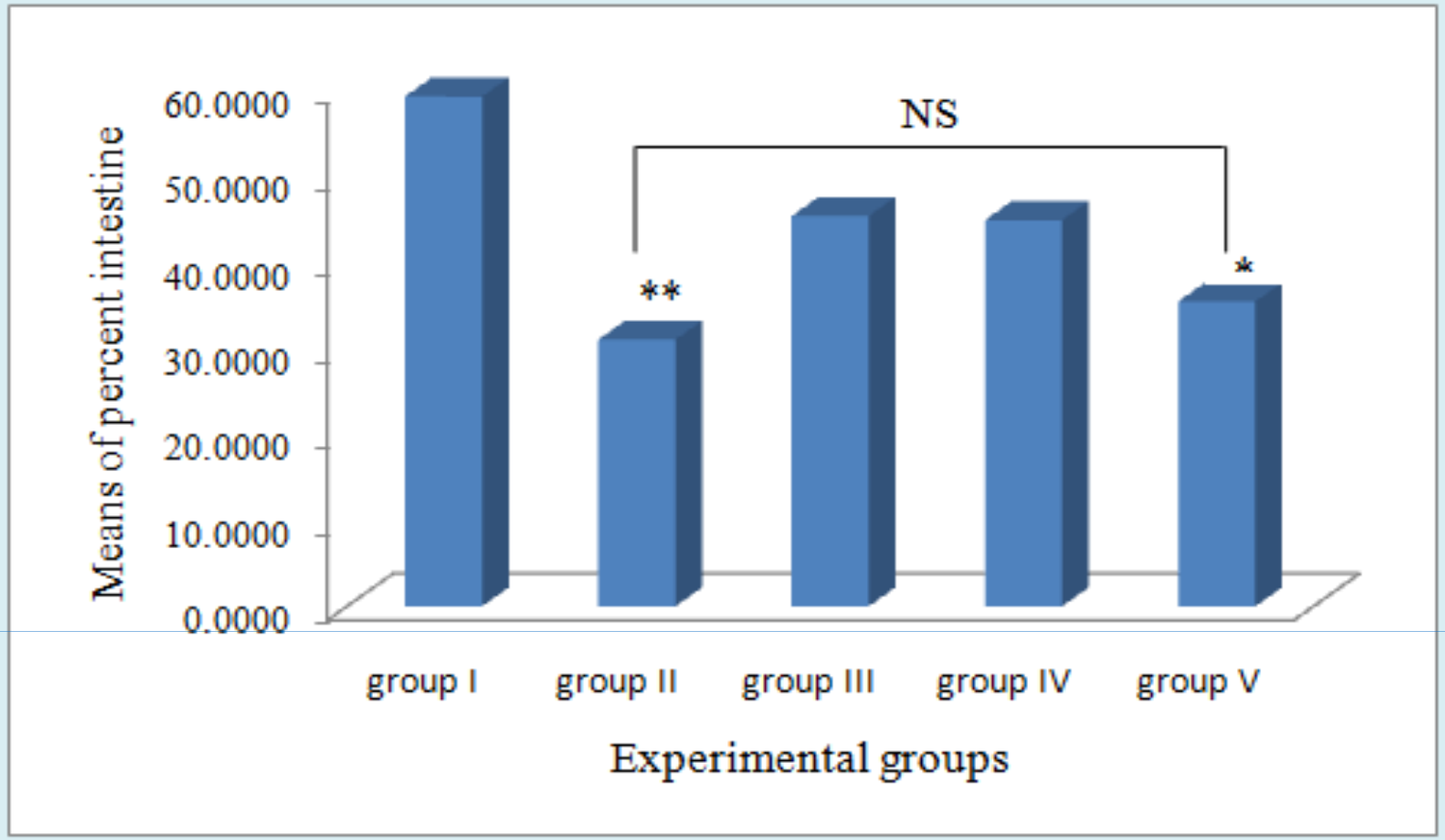


Figure (6). Effect of distilled water, loperamide and three different doses of the ethanolic extract of *Limonia acidissima* L. on castor oil induced small intestinal transit

group I – Distilled water
group II – Loperamide (6 mg/kg)
group III – Extract 240 mg/kg
group IV – Extract 360 mg/kg
group V – Extract 480 mg/kg
** $p < 0.01$, * $p < 0.05$ – Versus control
NS = no significantly different – loperamide versus the ethanolic extract 480 mg/kg

Discussion

- Alkaloid, tannins and flavonoid are responsible for antidiarrhoeal activity
- In this study, alkaloid and tannins were present
- Ethanolic extract 480 mg/kg is as nearly effective as loperamide
- This study showed that the unripe fruit of Thee-Thee has antidiarrhoeal activity
- Therefore, result of this study provided relevance background concept of Myanmar Traditional Medicine

CONCLUSION

- The presence of active antidiarrhoeal ingredients; alkaloids and tannins may be assumed to mediate antidiarrhoeal property
- Ethanolic extract of unripe fruit of *Limonia acidissima* L. (480 mg/kg) is potential antidiarrhoeal agent in ICR albino mice.
- It is possible to be used for symptomatic relief of acute diarrhoea

SUGGESTIONS

- Future detailed studies should be done to find out.
  - the toxic effect by the acute and subacute toxicity tests
  - the mechanism responsible for antidiarrhoeal activity
  - the pure active compound from the ethanolic extract of *Limonia acidissima* L.
  - the antidiarrhoeal activity of the other various extracts

ACKNOWLEDGEMENT

- The authors gratefully acknowledge Dr. Than Maung (Former Rector, University of Traditional Medicine, Mandalay),
- Dr. Yi Yi Myint (Director Deneral, Department of Traditional Medicine),
- Dr. Kyaw Zin Thant (Director General, Department of Medical Research),
- Daw Hnin Hnin Htun (Lecturer and Head, Department of Botany, University of Traditional Medicine, Mandalay),
- U Soe Myint Aye (Associated Professor, Department of Botany, University of Mandalay),
- U Maung Maung Oo (Managing Director, Great Wall Traditional Medicine Manufacturing Co, Ltd).
- And also we are thankful to all staffs of Pharmacology Research Division from Department of Medical Research (Pyin Oo Lwin Branch).

REFERENCES

- 1. နာဂသိန်၊ အရှင် (၁၉၇၆)၊ ပုံပြဆေးအဘိဓာန်၊ မင်္ဂလာပုံနှိပ်တိုက်၊ ရန်ကုန် ၃း ၄၁၂-၄၁၃။
- 2. နာဂသေနာဘိဝံသ၊ အရှင် (ဓမ္မာစရိယ) (၁၉၆၆)၊ စရကသံဟိတာကုထုံးကျမ်း၊ ဟံသာဝတီပုံနှိပ်တိုက်၊ ရန်ကုန် ၂း ၅၆၇။
- 3. Awouters, F., Niemegeers, C.J.E., Lenaerts F.M. & Janseen P.A.G (1978). Delay of castor oil diarrhoea in rats, a new way to evaluate inhibitors of Prostaglandin biosynthesis. *J.Pharm. Pharmacol.*, vol. 30, no. 1, pp. 41-45.
- 4. Aye-Than, Kulkarni, H.J., Wut-Hmone. & Tha, S.J (1989). 'Anti-diarrhoeal Efficacy of Some Burmese Indigenous Drug Formulations in Experimental Diarrhoeal Test Models', *Int. J. Crude. Drug. Res*, vol. 27, no. 4, pp. 195-200.
- 5. Bennet, P.N. & Brown, M.J (2008). Diarrhoea. In: *Clinical Pharmacology*. 10th Edition, Churchill Livingstone, Elsevier, London, pp. 574-575.
- 6. Central council for research in Unani medicines (1987). *Physiochemical standards of Unani Formulation*, New Delhi, India, vol. 2, pp. 202-204.
- 7. Chitme, H.R., Chandra, R. & Kaushik, S (2004). 'Studies on antidiarrhoeal activity of *Calotropis gigantea* R.BR. in experimental animals', *Journal of Pharmaceutical Science*, Bundelkhand University, India, vol. 7, no. 1, pp. 70-75.

- 8. Ching, F.P., Omogbai, E.K.I., Ozolua, R.I. & Okpo, S.O (2008). 'Antidiarrhoeal activities of aqueous extract of *Stereospermum kunthianum* (Cham, Sandrine Petit) stem bark in rodents', *African Journal of Biotechology*, Nigeria, vol. 7, no. 9, pp. 1220-1225.
- 9. Harborne, J.B (1984). Phytochemical methods. In: A guide to modern techniques of plant analysis. 2nd Edition, Chapman and Hall (USA), pp. 227-236.
- 10. Havagiray, R., Chandra, R. & Kaushik, S (2004). 'Studies on anti-diarrhoeal calotropis gigantean r.br. in experimental animals', *J Pharm Pharmaceut Sci* (www.ualberta.cal/-csps), vol. 7, no. 1, pp. 70-75.
- 11. Pradhan, D., Tripathy, G. & Patnaik, S (2012) 'Screening of antiproliferative effect of *Limonia acidissima* L. fruit extracts on Human breast cancer lines', *African Journal Pharmacy and Pharmacology*, vol. 6, no. 7, pp. 468-473.
- 12. Rani, S., Ahamed, N., Rajaram, S., Saluja, R., Thenmozhi, S. & Murugesan, T (1999). Antidiarrhoeal evaluation of *Clerodendrum phlomidis* Linn. leaf extract in rats. *Journal of Ethnopharmacology,* India, 6: pp. 315-319.
- 13. Ministry of Health (2012). *Health in Myanmar*. The Government of Union of Myanmar.
- 14. WHO (2013). Diarrhoeal diseases. Available at http://elbiruniblogspotcom.blogspot.com/2013/04/who-diarrhoeal-disease.html 18 November 2013.


Thank
You!!!