

ACUTE TOXICITY TEST AND UTERINE RELAXANT ACTIVITY OF *ZINGIBER CASSUMUNAR* ROXB.

Ni Ni Htun¹

Abstract

This paper is a study to examine the medicinal properties of *Zingiber cassumunar* Roxb. (Meik-tha-lin), a medicinally important aromatic herb in South East Asia including Myanmar. *Z. cassumunar* Roxb. was collected in flowering time from Pyin-oo-lwin and surrounding areas of Yangon. According to the morphological characters present in the vegetative and reproductive parts of the plants, these plants have been identified by using available literature. Furthermore, acute toxicity test on animal models has been conducted by using aqueous extract of Meik-tha-lin rhizome. As a result, the lethality of mice was found to be 10% in 12g/kg dose, 60% in 16g/kg dose and 90% in 20g/kg dose. The uterine relaxant activity of Meik-tha-lin rhizome was tested on the isolated rat uterus. It was found that the aqueous extract of Meik-tha-lin rhizome caused the relaxation of the oxytocin-induced contraction in dose response manner.

Keywords; *Zingiber cassumunar*, acute toxicity, uterine relaxant activity

Introduction

Z. cassumunar Roxb. belongs to the family Zingiberaceae which is a tropical group of monocotyledons. This family usually consists of aromatic herbs that are useful in medicine. Aromatic herbs have been utilized by man since very ancient times in food, cosmetics and medicinal preparations. Due to their characteristics of aroma and flavour, they should naturally be among the first plants that attracted man's attention.

All the plant parts of *Zingiber spp.* possess numerous secretory cells which produce oleoresins and essential oils. The essential oils occurring in aromatic plants play a very significant role in the economy of man.

Many species of *Zingiber* were used as herbal medicine for treatment of stomachache, carminative, loss of appetite, flatulence, inflammation, muscle problem, acute cold and diarrhoea in Myanmar. *Zingiber cassumunar* Roxb. was found to be

¹ Dr. Associate Professor, Department of Botany, Banmaw University

used in most of the Myanmar traditional medicines. Also according to the literature, it was frequently mentioned that it is very useful as the traditional folk medicine.

In most of rural area, the rhizomes of this plant is widely used during puerperium in order to promote uterine involution. So, it was chosen to investigate the medicinal value and effect on the uterus of rhizome of *Z. cassumunar* Roxb. (Meiktha-lin) in this research.

Materials and Methods

Collection, Identification and Preparation

In this investigation, *Z. cassumunar* Roxb. was collected during flowering period (July to November) from different locations. After the collection, all vegetative and reproductive parts of the fresh specimens were studied, measured in detail and recorded. The relevant data for taxonomic description of the species were also recorded. Based on the resulting data, the plants were identified with the help of literature (Backer; 1963, Burkill; 1966, Hooker; 1875, Kirtikar&Basu; 1935).

Acute Toxicity Test

Materials

Animals used - 50 albino mice of both sexes, weighing between 24-36 g,

Drugs used - different concentrations of aqueous extracts of *Z. cassumunar* Roxb.

Dose schedule - 10 g/, 12 g/kg, 16 g/kg, 20g/kg in mice (on body weight basis)

Apparatus used - aluminium mouse cages, animal balance, intragastric dosing cannula, disposable syringes

Period of observation - two weeks

Method

The acute toxicity test on mice was carried out according to the method described by Litchfield and Wilcoxon (1949). 50 albino mice of both sexes, weighing 24 – 36 g, were used in this study. Mice were separated into 5 groups and each group comprises of 10 mice. Each group was placed separately in the 5 mouse cages. Food was withheld for 12 hours before administration of drug. At first, the mice were individually marked with picric acid staining on the parts of body and weighed and the required dose were calculated. Group I was served as control group and administered 0.1 ml/ 10 g distilled water. Group II –V were administered orally with different concentrations of aqueous extract. The given dose of aqueous extract was 10

g/kg, 12 g/kg, 16 g/kg and 20 g/kg body weight receptively. After giving the various concentration of extract orally, each group of mice was allowed access to food and water. Then, they were carefully observed for 24 hours. Any mortality of mice within 24 hours was recorded and the survivors were subsequently observed up to 14 days.



Administration of extracts on albino mice Aluminium mouse cages

Uterine Relaxant Activity

Materials

Uterus of a freshly killed female rat which has been primed by estrogen for 1 day Isolated organ bath with water jacket, Physiograph (two channel recorder), Thermometer for controlling temperature, The aqueous extract of Meik-tha-lin rhizome, Drugs (Oxytocin, Salbutamol, Aminophylline, Propranolol), De Jalon's solution (Sodium chloride 9.0 g, Potassium chloride 0.42 g, Calcium chloride 0.06 g, Sodium bicarbonate 0.5 g, Glucose 0.5 g), Distilled water 1 liter, Oxygen cylinder with gas pipe.

Method

To study the effects of aqueous extract of Meik-tha-lin rhizome on smooth muscle, isolated rat uterus was used. The set-up was performed according to the method described by Perry, 1970. Female rats weighing between 250 – 300 g, treated with stibesterol(2 mg/ml) subcutaneously 24 hrs prior to the experiment, were used. Animals were anaesthetized with chloroform and the abdomen was opened. The two horns of the uterus are dissected out and transferred to a petridish containing De Jalon's solution.

The two horns were cut into longitudinal strips approximately 1 cm long and 1 mm wide. Then, a thread was tied at antimesentric border of each end, making sure

that the threads did not close the lumen. The preparation was mounted in a 50ml isolated organ bath containing De Jalon's solution which was aerated with oxygen and maintained at 32°C. One thread is attached to a fixed pin and the other to a transducer which recorded on the paper of two channel recorder. The preparation usually takes 1 (or) 1 hrs to settle down before regular responses are obtained. The contraction and relaxation of uterine muscle after drug administration was recorded isometrically using force displacement transducer (UGO BASILE) connected to a physiograph (two channel recorder "GEMINI" 7070).

After tissue stabilization, firstly, the effect of oxytocin (0.002 IU/ml b.c) on the isolated rat uterus was examined. When an adequate response had been obtained, the bath was rinsed three times with De Jalon's solution. Then the aqueous extract of Meik-tha-lin rhizome (0.5 mg/ml b.c) was introduced into organ bath, left for 1 min and followed by the same amount of oxytocin (0.002 IU/ml b.c) without washing. The inhibition of the extract on the effect of oxytocin was recorded and the bath was rinsed three times. As the same manner, the experiment was carried on with the different doses of aqueous extract (1.0 mg/ml, 1.5 mg/ml, 2.0 mg/ml, 2.5 mg/ml and 3.0 mg/ml b.c) on the contraction induced by oxytocin (0.002 IU/ml b.c) was studied. The height and width of contraction were measured and compiled and analysed statistically.



2 – horns of uterus in female rat Isolated rat uterus



Set up of apparatus for isolated rat uterus preparing

Results and Discussion

Morphological characters of *Zingiber cassumunar* Roxb.

Perennial rhizomatous herbs; the rhizomes thick, ovoid with fleshy roots, bright yellow inside, with pale brown scale leaves at each node and a strong camphoraceous odour. Aerial stems herbaceous, 4 – 6 ft high. Leaves alternate, simple; the leaf-blades oblong-lanceolate, the margins entire; the petioles sessile; the leaf sheaths oblong, reddish green, the margins pubescent; the ligules small, triangular, 2-clefted, membranous, glabrous. Inflorescences terminal cone-like spike, the spikes oblong-ellipsoid, red, the peduncles terete, enveloped by bladeless leaf-sheaths, pinkish, glabrous; bracts broadly ovate, the tips subacute, brightly red, pubescent, persistent; bracteoles lanceolate, whitish, glabrous, persistent. Flowers infundibuliform, creamy color, complete, bisexual, zygomorphic, 3-merous, sessile, epigynous; calyx 3-dentate, fused, tubular, valvate, white, glabrous, deciduous; corolla 3-lobed, fused, corolla tubes cylindrical, glabrous, deciduous, the lobes lanceolate, posterior lobes concave, lateral lobes deflexed, yellowish white, glabrous; fertile stamens inserted, epipetalous, the filaments very short, the anthers oblongoid, ditheous, basifixed, introrse, longitudinal dehiscence, yellowish white, the connective appendix or beaks or crests curved, yellowish white; lips or labellum shorter than the corolla segments, 3-lobed, the middle lobes orbicular, yellowish white, unspotted, the tips obtuse, the margins wavy, the two lateral lobes or basal auricles obovate, yellowish white with red streak below, the tips obtuse, the margins entire; ovaries inferior, oblongoid, 3-carpelled, syncarpous, 3-loculed, the placentation axile, the styles simple, white, the stigmas infundibuliform, white, with a whorl of cilia on mouth.

**Habit****Rhizome****Inflorescence****Acute Toxicity Test**

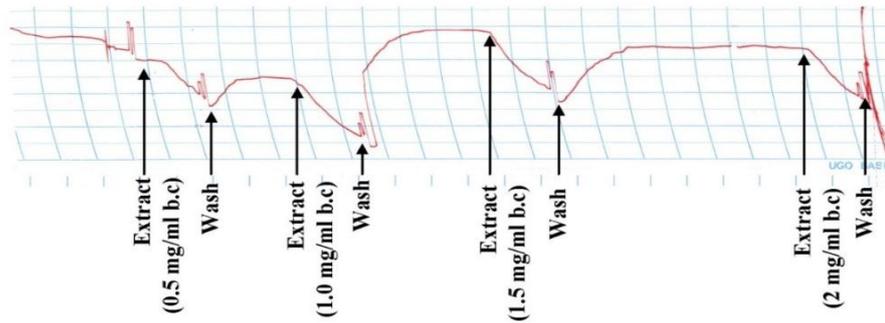
The lethality of the mice was observed up to 14 days with the maximum dose 20g/kg of aqueous extract of Meik-tha-lin rhizome. The results of acute toxicity test were shown in Table 1. According to the results, it was observed that the aqueous extract of rhizome of *Z. cassumunar* Roxb. was free from acute toxic or harmful effects up to 10g/kg dose.

Table 1. Results of Acute Toxicity Tests of Aqueous Extract of Meik- tha-lin Rhizome on Albino Mice.

Group No.	Group	Mice number	Dosage	Ratio of dead & tested	Percentage of death
I	Control (distilled water)	10	0.1 ml/10 g	0/10	0%
II	Aqueous extract	10	10 g/kg	0/10	0%
III	Aqueous extract	10	12 g/kg	1/10	10%
IV	Aqueous extract	10	16 g/kg	6/10	60%
V	Aqueous extract	10	20 g/kg	9/10	90%

Uterine Relaxant Activity

The aqueous extract of *Zingiber cassumunar* Roxb. caused the relaxation of uterus as seen by decrease in resting tone in tracing recorded by 2-channel recorder.



The standard drug oxytocin 0.002 iu/ml b.c induced significant contraction on the isolated rat uterus. Preadministration of aqueous extract (0.5, 1.0, 1.5, 2.0, 2.5 and 3.0 mg/ml b.c) was found to cause a decrease in uterine contraction induced by oxytocin.

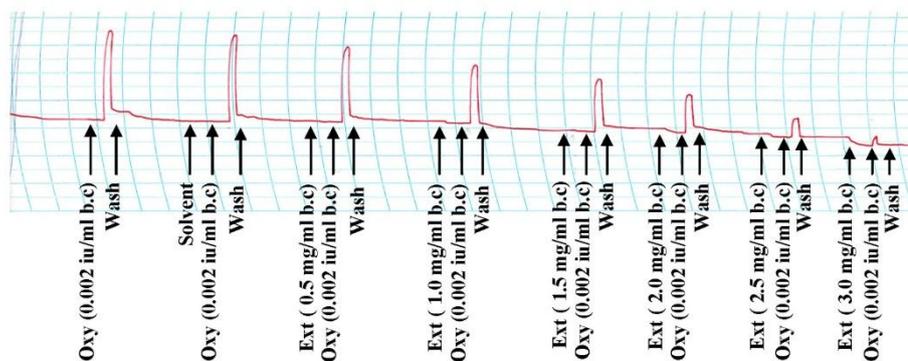


Table 2. Effect of Aqueous Extract of *Zingiber cassumunar* Roxb. on Uterine Contraction (height) Induced by Oxytocin Tested with Individual Isolated Rat Uterus

Experiment No.	Height of contraction (in mm)						
	(Oxy) only	Oxy + Ext. 0.5mg	Oxy + Ext. 1mg	Oxy + Ext. 1.5mg	Oxy + Ext. 2mg	Oxy + Ext. 2.5mg	Oxy + Ext. 3mg
1	19	17	14	12	8	6	4
2	42	40	35	29	23	20	9
3	33	23	19	15	10	6	4
4	30	26	22	18	15	12	7
5	31	29	27	24	20	15	3
6	32	27	21	19	14	7	3
SUM	187	162	138	117	90	66	30
MEAN	31.17	27	23	19.5	15	11	5
SE	3.00	3.11	2.96	2.52	2.34	2.34	1

The effect of aqueous extract of *Z. cassumunar* Roxb. on isolated rat uterus showed the uterine relaxant activity. Addition of extract and oxytocin produced decrease in uterine contraction in a dose related manner. The contraction effect of oxytocin decreases with the increase of different dose of extract. Finally, aqueous extract of 3 mg/ml b.c completely inhibits the uterine contraction induced by oxytocin. Considering it, we can know that the aqueous extract of *Z. cassumunar* Roxb. have uterine relaxant activity and antioxytotic activity. It was also reported that the plant produced relaxation of the uterine muscle during the premature labour or abortion.

Conclusion

Zingiber cassumunas Roxb. (Meik-tha-lin) was chosen to pin-point this research on as it is known to be widely used in Myanmar folklore medicines. Acute toxicity test was made on albino mice by using aqueous extract of rhizome to know the lethal dose of the drug. This test revealed no evidence of toxicity in mice when given 10g/kg body weight. But when the dose was increased above 10g/kg, it was found to have lethal effect on mice.

To ascertain its uterine muscle relaxation activity, the aqueous extract of rhizome was tested on isolated rat uterus. It was found that this aqueous extract was effective in relaxing the uterus with dose response manner.

This uterus relaxation properties can be clinically used in two ways, that is (1) to delay or prevent premature parturition in selected individuals and (2) to slow or arrest delivery for brief periods in order to undertake other therapeutic measure.

It is hope that further and more detailed study of these aspects will make a great contribution to the improvement in Myanmar indigenous medicines. Thus, it is believed that all the new findings containing in this research will make a considerable contribution to the improvement of Myanmar traditional medicine.

References

- Andrew Wilson & Schild, H.O (1968) Applied pharmacology. J. & A. Churchill Ltd., London.
- Backer, C.A. (1968) Flora of Java. Vol. III, Noordhoof-Groninen Co., Netherland.
- Bhattacharyya, B. & B.M. Johri (1998) Flowering plants (Taxonomy and Phylogeny). Narosa Publishing House, New Delhi, Madras, Bombay, Calcutta, London.
- British Pharmacopoeia (1973) Published on the recommendation of the Medicines Commission, London.
- Burkill, I.H. (1935) A Dictionary of the Economic Products of the Malay Peninsular. Vol. II, Crown Agents for the Colonies, London.
- Datta, S.C. (1931) A Hand Book of Systematic Botany. Asia publishing house, Bombay.
- Henderson, M.R. (1954) Malayan Wild Flowers, Monocotyledons. The Malayan Nature Society, Kuala Lumpur.
- Hooker, J.D. (1881) Flora of British India. Vol. VI, Reeve & Co., Ltd., London.
- Hundley, H.G & Chit KoKo (1987) Trees, Shrubs, Herbs and Principal Climbers, etc. Government Printing Press, Yangon, Myanmar.
- John Kress, W., Robert A. Defilipps, Ellen Farr and Daw Yin YinKy (2003) A Checklist of the Trees, Shrubs, Herbs and Climbers of Myanmar.

- Kanjanapothi D.et.al (1986) A Uterine Relaxant Compound from *Zingiber cassumunar*. *Planta Medica*, 53:4:329 – 32.
- Kirtikar, K.R. & Basu B.D. (1935) *Indian Medicinal Plants*. Vol IV. 2nd edition, Booksellers & Publishers, Dehra Dun, India.
- Litchfield, J.T. & Wilcoxon, F.A (1949) *A simplified method of evaluating dose-effect experiments*, Stamford Research Laboratories, American Cyanamid Co., Stamford, Connecticut.
- Loomis Ted. (1968) *Essential of Toxicology*, Lea & Febiger Philadelphia.
- Mya Mya Win (1987) *Morphology and Anatomy of some of the Burmese species of the genus Zingiber*. Department of Botany, University of Yangon, Myanmar.
- Perry, W.L.M. (1970) *Pharmacological Experiments on Isolated Preparations*, E.&S. Livingstone, Edinburgh and London.
- Pharmacopoeia of India* (1966) *The Indian Pharmacopoeia*. 2nd Ed.,
- Rendle, A.B. (1953) *The classification of flowering plants*. Vol. 1, 2nd Ed., Cambridge University Press.
- Sinei, K.A.& Mwangi, J.W. (1995) Effect of the tuber of *Adeniaglobosa* on Isolated Rat Uterus Preparation. *International Journal of Pharmacognosy*, Vol. 33:4:346 – 347.
- Specification of Thai Medicinal Plants* (1986) *A Guide to the Identification and Authentication of Some Thai Medicinal Plants*, Thailand.
- Trease and Evans (1978, 2002) *Pharmacognosy*. 11th edition, 15th edition, Bailliere Tindall, London.
- World Health Organization (1998) *Quality control methods for medicinal plant material*. Geneva.