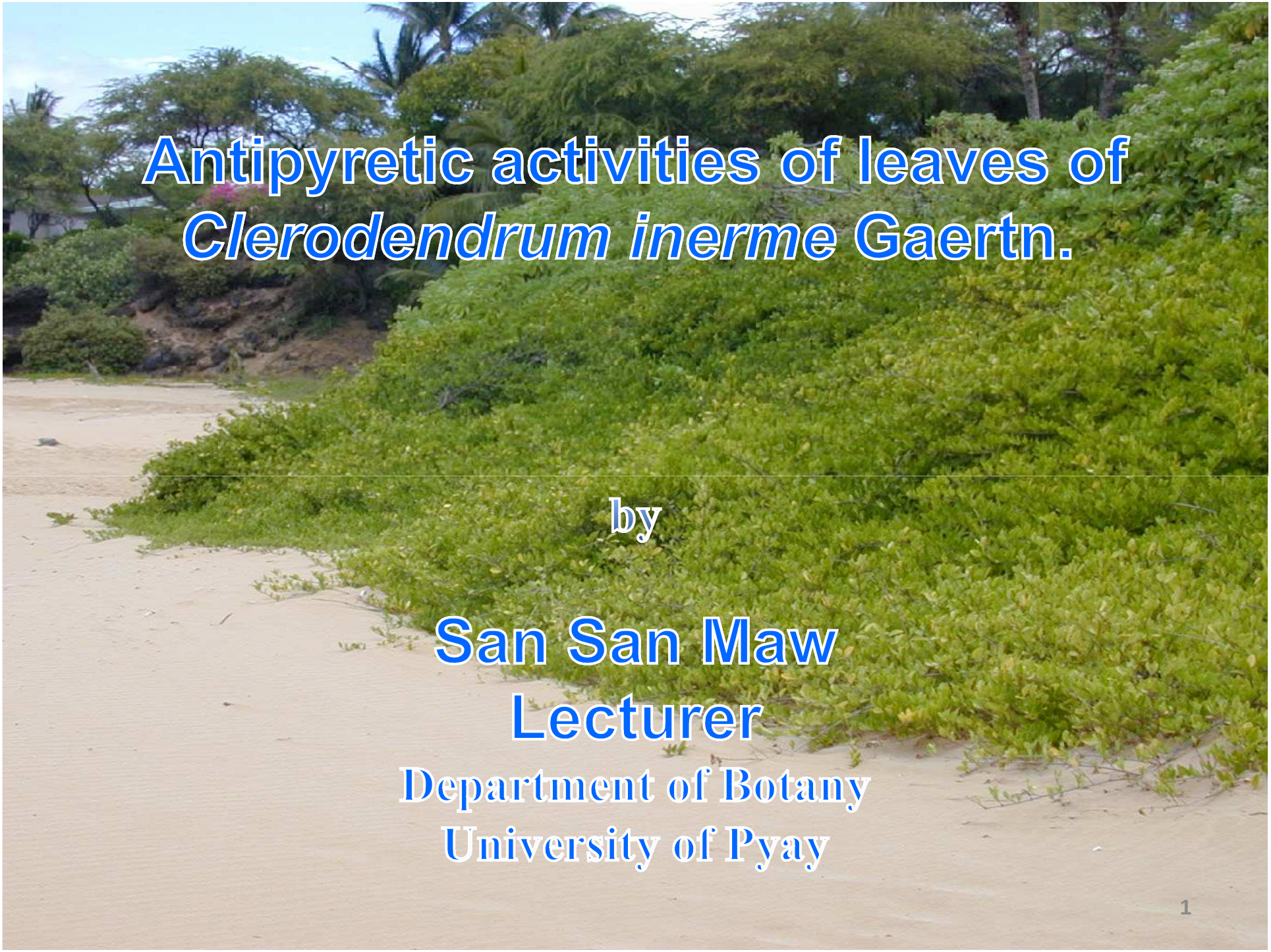

Antipyretic activities of leaves of *Clerodendrum inerme* Gaertn.

by

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Abstract

The evergreen mangrove plant *C. inerme* Gaertn. is belonging to the family Verbenaceae, locally known as Pinle-kyauk-pan. The specimens of this plant were collected from Ka-Mar-Aung creek in Dalla Township. It is used in herbal medicine especially in the treatment of fever, rheumatism, skin diseases and urinary infection.In this research, the acute toxicity test of 70% ethanolic extract of the leaves of *C. inerme* Gaertn was tested on albino mice. It was showed that there was no toxicity effect with maximum permissible dose as high as 16 g/kg body weight. In addition, the antipyretic activity of 70% ethanolic extract of the leaves was conducted on albino rats.

In the antipyretic activity, the test was evaluated against brewer's yeast induced-pyrexia and the tested drugs were run with the standard paracetamol (50 mg/ kg) and control distilled water (10 ml/kg). Rectal temperatures were recorded before and after inducing pyrexia at interval of one hour to five hours. It was showed this extract can be used to reduce fever on albino rats when compared to that of a standard paracetamol.

Introduction

- Medicinal plants are important for pharmacological research and drug development. It is namely Garden quinine by (Ashin Narga-thein, 1972) and Glory bower in English. In Myanmar, *Clerodendrum inerme* Gaertn. is known as Pinle-kyauk-pan or Kywe-yan-nge (Hundley, 1987 and Kress, 2003).

- The plant *C. inerme* Gaertn. is an evergreen straggling shrub, found growing in marshy places at the edge of mangrove swamps and throughout the year, belongs to the family Verbenaceae. The family consists of about 98 genera and 2600 species (Datta, 1965).

- The plant is used as traditional medicine for the treatment of various diseases. The leaves contain bitter principle and is considered as substitute for quinine and used in traditional medicine for tonic, antipyretic and efficient febrifuge (Datta, 1965).

- In Malay, the leaves smeared with oil are heated over the fire and applied to recent wounds. In Bombay, half an ounce of juice of the leaves is great reputation as a febrifuge.

- The dried leaves are used in decotion with aromatic and a poultice used to resolve buboes (Wealth of India,1948 and Kirtikar & Basu, 1975).

- Antipyretic activity of the leaves of *C.inerme* Gaertn. has not been tested scientifically in Myanmar. In the present work, acute toxicity test and antipyretic activity on animal models had been conducted by using 70% ethanolic extract of leaves.

Objectives

- To evaluate the acute toxicity test and antipyretic activity of 70% ethanolic extract of the leaves of *Clerodendrum inerme* Gaertn.

Materials and Methods

Preparation of 70% ethanolic extract

Hundred grams of dried powdered leaves was mixed with 400 ml of 70 % ethanol in a conical flask, occasionally shaken and kept for two weeks. After two weeks, it was filtered by using filter papers and filtrate was evaporated to dryness on boiling water bath at 70 °C. When completely dried the residue was weight and kept in a dessicator.

Acute Toxicity Tests

Materials

Animals used	-	40 albino mice of both sexes, (body weight 24-30) g
Drug used	-	70 % ethanolic extract of leaves of *C. inerme* Gaertn.
Apparatus used	-	aluminium mouse cages, animal balance, 18 guage intragastric dosing cannula, rubber gloves and mask
Dose schedule	-	8g/kg body weight, 12g/kg body weight, 16g/kg body weight
Period of observation	-	two weeks.

Methods

The acute toxicity test of ethanolic extract on mice was evaluated by the method of (Litchfield and Wilcoxon 1949 and May Aye Than 1994). In this test, forty albino mice of both sexes were randomly divided into 4 groups and each group contains 10 mice Fig.(2). They were kept in mouse cages. Food was withheld for period of 18 hours before administration of the extract dose.

At first, the mice were individually marked with picric acid staining on the parts of body and weighed and required doses were calculated which basis on body weight.

Group I was used for control by administration 0.1 ml/10g distilled water. Group II to IV were treated orally with various concentration of 70 % ethanolic extract of the leaves.

The require doses were used by intragastric dosing cannula to every mouse as shown in Fig. (3). After administration, the mice were allowed access to standard food and water. Treated mice were observed carefully for their lethality and motility for two weeks.

Acute toxicity test

Fig. (1) Weighing mouse in balance

Fig. (2) Each group contains 10 mice

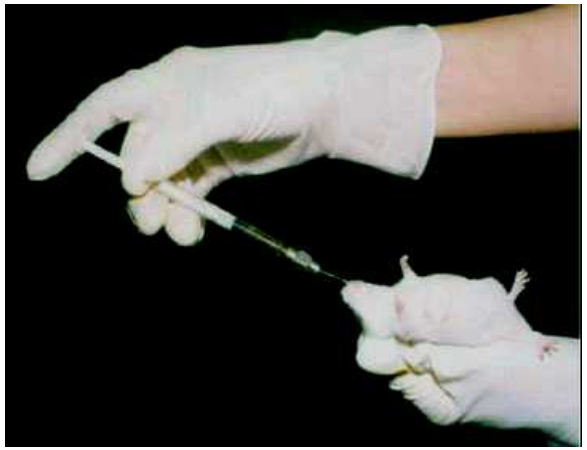

Fig. (3) Ethanolic extract was administered orally into albino mice

Antipyretic activity of 70 % ethanolic extract of the leaves of *Clerodendrum inerme* Gaertn.

Materials

Animal used	-	18 adult albino rats (body weight 200-250) g
Apparatus used	-	aluminium rat cages, animal balance, intragastric dosing cannula, disposable syringe, rubber gloves and mask.
Drug used	-	70 % ethanolic extract of *C. inerme* Gaertn. Brewer's yeast (*Saccharomyces cerevisae*) and paracetamol (MPF).
Doses schedule	-	3 g/kg body weight

Methods

The Brewer's yeast, paracetamol and both sexes of 18 adult albino rats (body weight 200-250) g were used for antipyretic activity. Before the experiment, the rats were marked, the weight of the rats and required dose were calculated. For the antipyretic activity, the normal temperature of each rat was measure rectally by using YSI TELE thermometer and YSI 400 series probe.

Introduction of fever by yeast induced pyrexia

In this experiment, the albino rats were separated into three groups of six animals in each, placed in the aluminum rat cages Fig. (4). And then, the body temperature of each rat was recorded by measuring rectal temperature of predetermined time intervals. 15 % suspension of Brewer's yeast was injected into rat subcutaneously to induce fever according to a standard method of (Murugesan *et. al*., 2000 and Chattopathyay, 2005). A thermister probe was inserted 4 cm deep into the rectum, after fastened the tail, the basal rectal temperature was recorded.

The animals were then given subcutaneous injection of 15 % Brewer's yeast suspended in 0.5% methylcellulose solution. And then, food were withdraw for 19 hours. After 19 hours of yeast injection, the rats were again restrained in individual cage to record their rectal temperature, immediately after recorded rectal temperature, the distilled water, paracetamol and 70 % ethanolic extract were administered orally with dose calculated according to their respective body weight. The measurement was repeated after one hour. Group I of animals received distilled water as control group (dose 10 ml/kg body weight). Group II of animals received paracetamol of standard drug (dose 50 mg/kg body weight). Group III of animals received 70 % ethanolic extract (dose 3g/kg body weight).

Rectal temperature of all rats were recorded at 19^{th} hours immediately before administration of distilled water, paracetamol and 70 % ethanolic extract hourly up to 24^{th} hours as shown in Fig. (8).

Statistical analysis

The data are expressed as Mean ± SEM and p value were analyzed statistically by student's t-test significant levels were at $p < 0.05$.

Antipyretic activity test

Fig. (4) Grouping of rats

Fig.(5)Weighing mouse in balance

Fig. (6) Administration of ethanolic extract to the rat

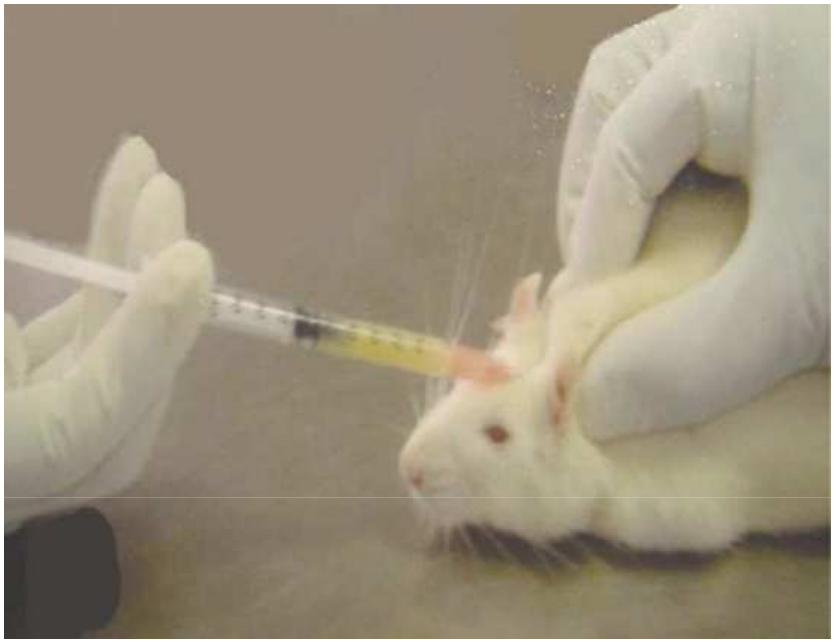

Fig. (7) Yeast induced-pyrexia in rat

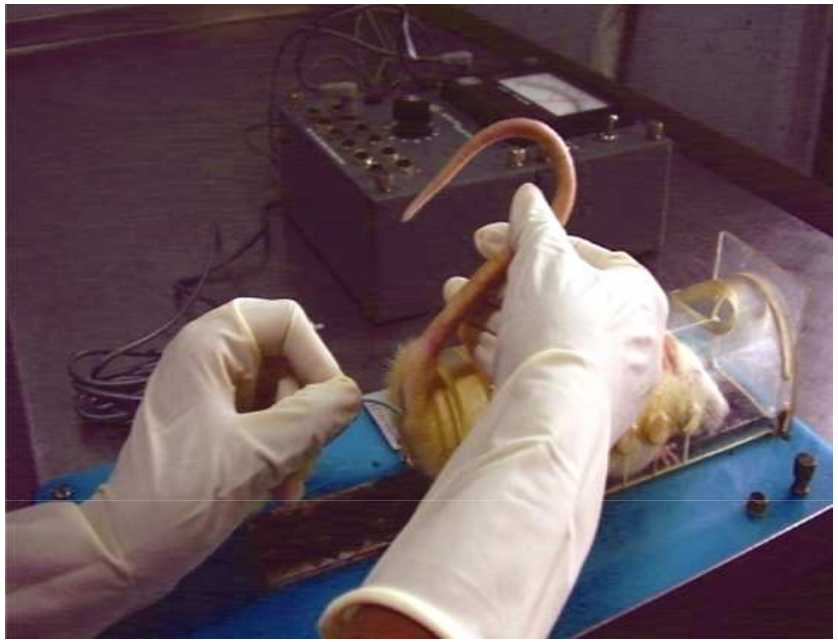

Fig.(8) Measuring rectal temperature

RESULTS

Acute toxicity study of the 70 % ethanolic extract of leaves of *Clerodendrum inerme* Gaertn.

The acute toxicity test was determined by the methods of (Litchfield and Wilcoxon, 1949 and May Aye Than, 1994). The lethality of the albino mice were observed within two weeks. The results were shown in Table (1).

According to this result, the mice were found to be alive and healthy within two weeks. Therefore, it was observed that the extract was free from acute toxic or any harmful effects up to 16g/kg body weight.

Table 1. Acute toxicity test of 70 % ethanolic extract of the leaves of *C. inerme* Gaertn. on albino mice

Group No.	Types of drug	Dose (g/kg)	No. of animal test	Ratio of dead and tested animal
1	Distilled water	0.1 ml/10g	10	0/10
2	70 % ethanolic extract	8g/kg	10	0/10
3	70 % ethanolic extract	12g/kg	10	0/10
4	70 % ethanolic extract	16g/kg	10	0/10

Antipyretic activity of 70 % ethanolic extracts of leaves of *Clerodendrum inerme* Gaertn.

Effect of body temperature of individual rats after administration of distilled water and paracetamol on yeast induced fever in albino rat was shown in Table (2 and 3). Mean body temperature (in ºC) of control and paracetamol on yeast induced fever in albino rat was shown in Table (5). Mean body temperature (in ºC) of control rats at 0, 19^{th}, 20^{th}, 21^{st}, 22^{nd}, 23^{rd}, and 24^{th} hours were 37.17 ± 0.19, 37.50 ± 0.12, 38.05 ± 0.09, 38.22 ± 0.08, 37.60 ± 0.13, 37.40 ± 0.09 and 37.42 ± 0.18 respectively. Mean body temperature (in ºC) of paracetamol treated rats at 0, 19^{th}, 20^{th}, 21^{st}, 22^{nd}, 23^{rd}, and 24^{th} hours were 37.10 ± 0.15, 37.60 ± 0.08, 36.58 ± 0.18, 36.53 ± 0.10, 36.67 ± 0.21, 36.82 ± 0.08 and 36.95 ± 0.22 respectively.

Significant reduction in body temperature at 20^{th} to 23^{rd} hours after administration of paracetamol was showed when compared with that of control ($P<0.01$- $P < 0.00005$).

Effect of body temperature of individual rat after administration of 70 % ethanolic extract of leaves of *C. inerme* Gaertn. on yeast-induced fever in albino rats was shown in Table (4). Mean body temperature (in ºC) of 70 % ethanolic extract on yeast-induced fever in albino rats was shown in Table (6). The mean body temperature (Mean ± SEM) of 70 % ethanolic extract of leaves of *C. inerme* Gaertn. at 0, 19^{th} , 20^{th} , 21^{st} , 22^{nd} , 23^{rd} , and 24^{th} hours were 36.17 ± 0.08, 36.58 ± 0.08, 36.72 ± 0.32, 36.41 ± 0.11, 36.41 ± 0.11, 36.25 ± 0.08 and 36.00 ± 0.08 respectively. The fever was significantly reduced ($P<0.005$ -$P< 0.00005$) in albino rat at 21 to 24 hours when compared with that of control.

Comparison of the effect on body temperature between tested drug (70% ethanolic extract of *C. inerme* Gaertn. and paracetamol) with control on yeast induced pyrexia in albino rat at various time interval was shown in Fig. (9 and 10). In this figure, after the injection of yeast, the body temperature had significantly increased at 20^{th}, 21^{st} hours and reduced at 22^{nd}, 23^{rd}, 24^{th} hours. After administration of paracetamol, the body temperature was significantly reduced at 20^{th} to 23^{th} hours. After administration of 70% ethaolic extract, it was reduced at 21^{st} to 24^{th} hours when compared with that of control. The results showed that paracetamol as well as 70 % ethanolic extract reduced fever in yeast-induced pyrexia in albino rat. This extract was as effective as that of paracetamol. Therefore, it showed the leaves of *C. inerme* Gaertn. can be used to reduce fever.

Table (2) Effect of body temperature (in ºC) of individual rat after administration of distilled water on yeast-induced fever on albino rat

No.	0 hr	19 hr	20 hr	21 hr	22 hr	23 hr	24 hr
1	37.0	37.5	38.0	38.3	37.8	37.5	38.0
2	37.0	37.5	38.2	38.4	37.5	37.4	37.5
3	37.5	37.5	38.0	37.8	37.8	37.6	37.5
4	37.0	37.5	38.0	37.0	37.0	37.1	36.5
5	36.5	37.0	37.7	37.5	37.5	37.1	37.5
6	38.0	38.0	38.4	38.0	38.0	37.7	37.5
Sum	223.0	225.0	228.3	225.6	225.6	224.4	224.5
Mean	37.16667	37.5	38.05	38.21667	37.6	37.4	37.41667
SE	0.19245	0.117851	0.087401	0.083055	0.131233	0.094281	0.183207

Table (3) Effect of body temperature (in °C) of individual rat after administration of paracetamol (50 mg/kg) on yeast-induced fever on albino rat

No.	0hr	19hr	20hr	21hr	22hr	23hr	24hr
1	37.0	37.7	36.5	36.3	36.0	36.5	36.5
2	37.5	37.5	36.5	36.5	36.9	37.0	37.2
3	37.0	37.5	36.0	36.2	36.2	36.6	37.0
4	36.5	37.4	36.5	36.6	36.4	37.0	38.0
5	37.5	38.0	36.5	37.0	37.5	37.0	36.5
6	37.5	37.5	37.5	36.6	37.0	36.8	36.5
Sum	223.0	225.6	219.5	219.2	220	220.9	221.7
Mean	37.1	37.6	36.58333	36.53333	36.66667	36.81667	36.95
SE	0.152753	0.08165	0.183207	0.104527	0.210379	0.083055	0.222049
pvalue	NS	NS	<0.005	<0.00005	<0.01	<0.005	NS

Table (4) Effect of body temperature (in ºC) of individual rat after administration of ethanolic extract of *C. inerme* Gaertn. (3 g/kg) on yeast-induced fever on albino rat

No.	0hr	19hr	20hr	21hr	22hr	23hr	24hr
1	36.0	36.2	36.5	37.0	37.5	36.5	36.0
2	36.0	36.5	36.0	36.0	36.0	36.5	36.0
3	36.5	36.8	38.6	36.5	36.5	36.0	36.0
4	36.0	36.5	36.5	36.5	36.0	36.0	36.0
5	36.0	36.5	36.0	36.0	36.0	36.0	36.0
6	36.5	37.0	37.0	36.5	36.5	36.5	36.0
Sum	217.0	219.5	220.0	218.5	218.5	217.5	216.0
Mean	36.16667	36.58333	36.72	36.41667	36.41667	36.25	36.0
SE	0.078567	0.084802	0.322077	0.114531	0.114531	0.083333	0.081011
pvalue	0.005	0.0005	0.05	0.00005	0.00005	0.0005	0.005

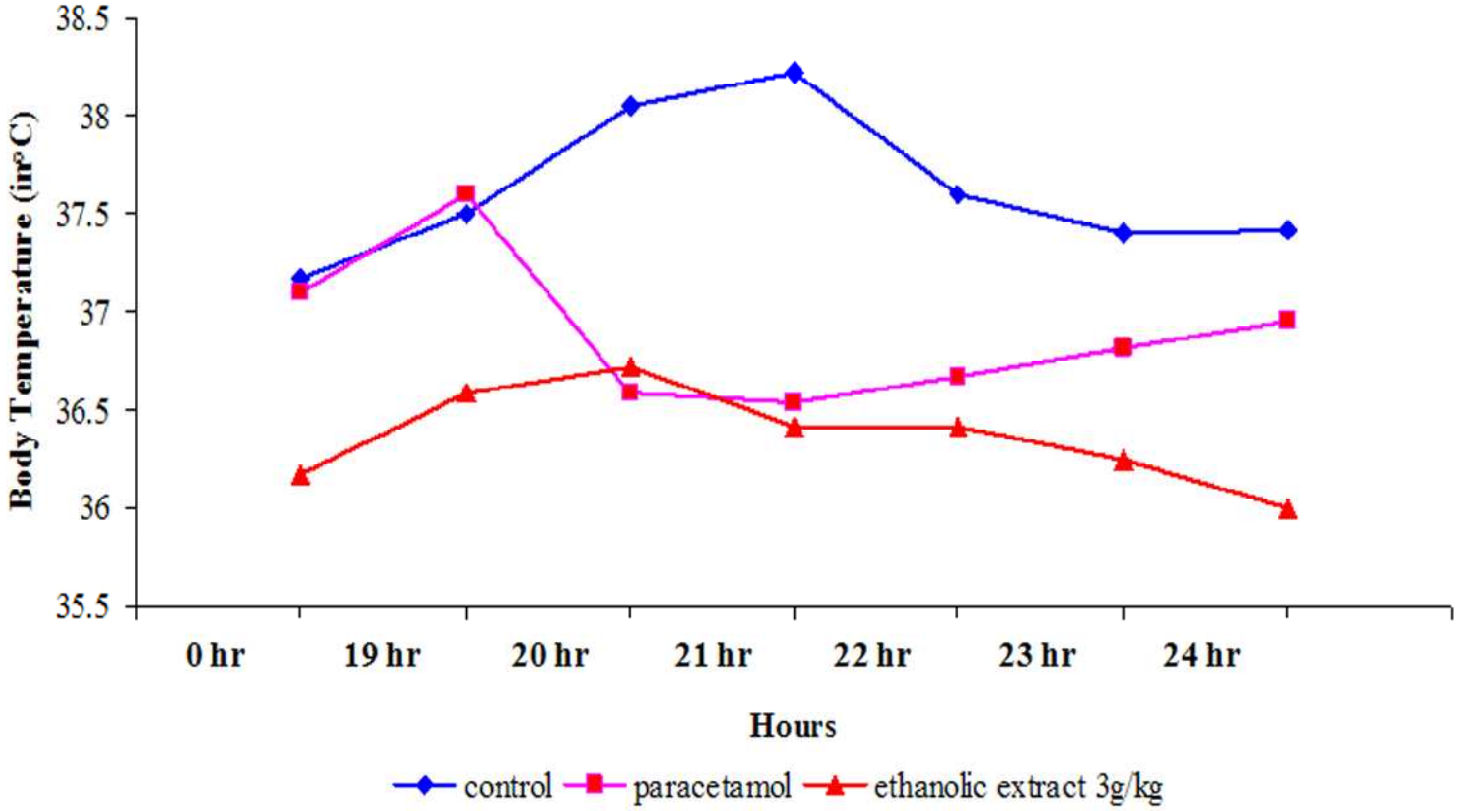

Fig.(9) Comparison the effects on body temparature between test drugs (ethanolic extract of *Clerodendrum inerme* Gaertn. and paracetamol) with control on yeast induced fever on albino rats.
Each point represents the mean of observations.

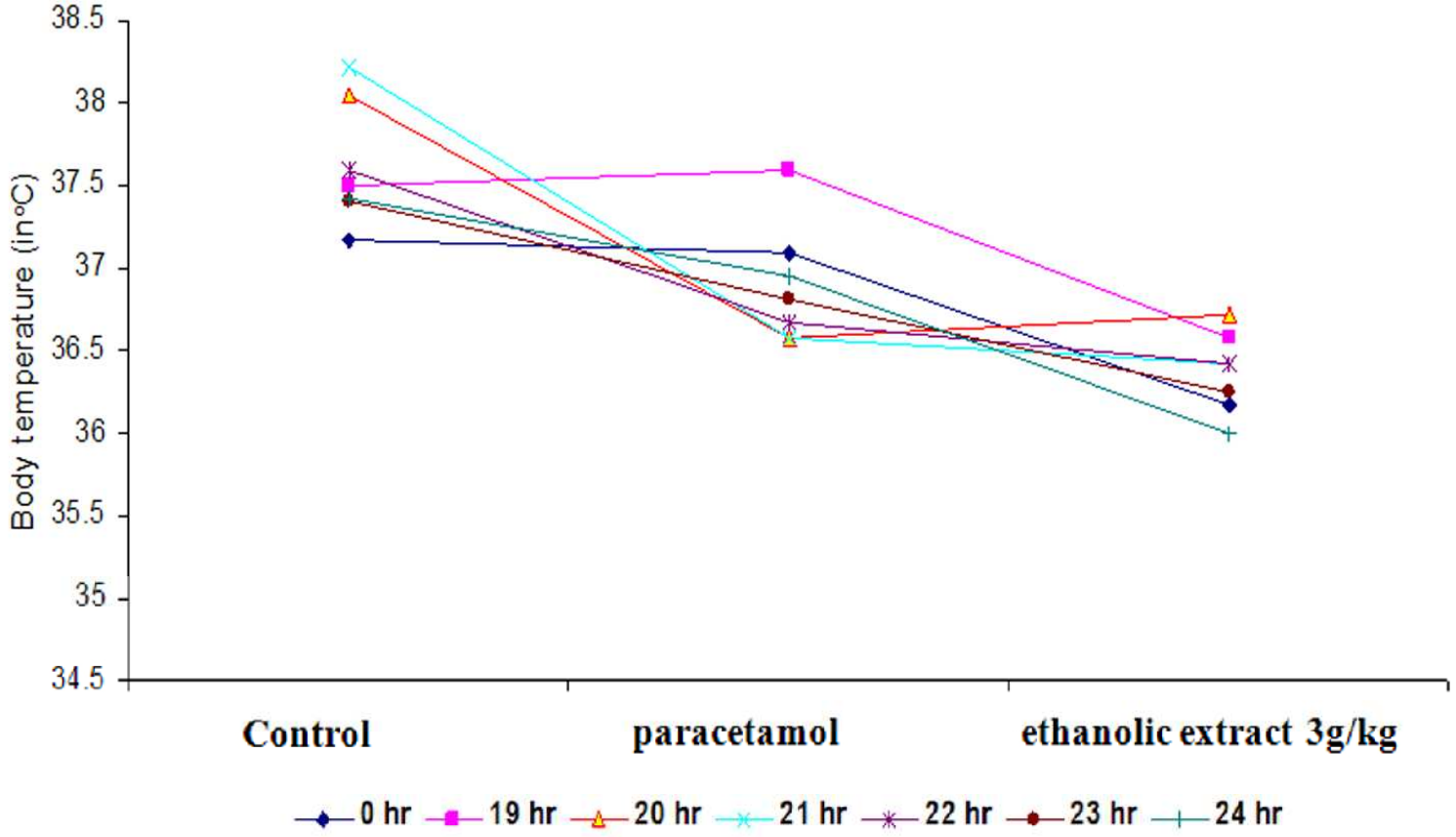

Fig.(10) Time course effects on body temperature between test drugs (ethanolic extract of *Clerodendrum inerme* Gaertn. and paracetamol) with control on yeast induced fever on albino rats in various time interval.

Table (5) Mean body temperature (in ºC) of control and paracetamol on yeast-induced fever on albino rat

Groups	Body Temperature (mean ± SEM in ºC)						
	0hr	19hr	20hr	21hr	22hr	23hr	24hr
Control (n=6)	37.17 ± 0.19	37.50 ± 0.12	38.05 ± 0.09	38.22 ± 0.08	37.60 ± 0.13	37.40 ± 0.09	37.42 ± 0.18
Paracetamol (n=6)	37.10 ± 0.15	37.60 ± 0.08	36.58 ± 0.18**	36.53 ± 0.10***	36.70 ± 0.21*	36.80 ± 0.08**	36.95 ± 0.22

denote p<0.01 ** denote p<0.005 denote p<0.00005**

Table (6) Mean body temperature (in ºC) of control and ethanolic extract of *Clerodendrum inerme* Gaertn. on yeast-induced fever on albino rat

Groups	Body Temperature (mean ± SEM in ºC)						
	0hr	19hr	20hr	21hr	22hr	23hr	24hr
Control (n=6)	37.17 ± 0.19	37.50 ± 0.12	38.05 ± 0.09	38.22 ± 0.08	37.60 ± 0.13	37.40 ± 0.09	37.42 ± 0.18
ethanolic extract (n=6)	36.17 ± 0.08**	36.58 ± 0.08***	36.72 ± 0.32*	36.41 ± 0.11*****	36.41 ± 0.11*****	36.25 ± 0.08***	36.00 ± 0.08***

*** denote p<0.05** denote p<0.005*** denote p<0.0005**** denote p<0.00005**

Discussion and Conclusion

The acute toxicity test, this extracts was administered on albino mice models of both sexes, the maximum permissible dose of 16g/Kg body weight of ethanolic extract for two weeks. The medium lethal dose LD_{50} study of this extracts were safe at dose of 16g/kg body weight. The mice were alive and healthy during observation period. So, 70% ethanolic extract of the leaves of *C. inerme* Gaertn. was free from acute toxicity or any harmful effect and no evidence of toxicity on mice.

According to the antipyretic activity study, 70% ethanolic extract of leaves inhibited significant ($p < 0.05$) of fever on rats. They have shown significant fall in body temperature up to 5 hours after its administration. In the antipyretic activity, highly significant reduction in body temperature at 20 to 23 hours after administration of paracetamol when compared with that of control. The fever was significantly reduced at 21 to 24 hours in ethanolic extract treated albino rats ($p < 0.05$) when compared with that of control.

These results showed that paracetamol as well as 70% ethanolic extract of the leaves reduced fever in albino rats. So, this extract can be used to reduce fever. In future, it should be studied clinically on human being for the applications as antipyretic drugs form of traditional medicine. The purpose of this study is to ascertain the local medicinal plant resources to be effectively used in health for Myanmar people.

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THANK YOU