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Anti-Inflammatory Effect of Aqueous Extract of TMF-27 (*PJI-LOUN:-CHAN:-THA-HSEI:*) on Albino Mice

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Abstract:- This investigation was performed to find out whether the freeze dried powder of aqueous extract of TMF-27 possess anti-inflammatory activity. The dried powder ofTMF-27 was extracted with distilled water. In order to study the anti-inflammatory effect of freeze dried powder of aqueous extract of TMF-27, the experiment was carried out on 30 albino mice of both sexes. Digital Plethysmometer was used to measure the volume changes of the paw edema. Inflammation was induced by subplantarinjection of 0.1 ml of 1% λ-carrageen an in right hind paw of albino mice. Anti-inflammatory effect of freeze dried powder of aqueous extract of TMF-27 was investigated by using 3 doses levels, i.e, 150mg/kg, 300mg/kg, 600mg/kgrespectively. Significant anti-inflammatory effect was started to show with the median dose of the aqueous extract 300 mg/kg at 6 hour ($p < 0.05$) and the high dose of 600mg/kg started at 2 hour up to 6 hour ($p < 0.05$ to $p < 0.01$) after λ-carrageenaninjection. The positive control group (aspirin, 300 mg/kg) was started to show at 2hour and 3 hour ($p < 0.01$), 4 hour and 5 hour ($p < 0.001$), and 6 hour ($p < 0.01$).The result of this study supported that the freeze dried powder of aqueous extract of TMF-27 has potential anti-inflammatory effect and therefore it is possible to be used as anti-inflammatory drug in Myanmar Traditional Medicine.

Keywords:- *Anti-Inflammatory, TMF-27, Λ-Carrageenan, Aspirin.*

I. INTRODUCTION

Inflammation has been defined as the reaction of vascular and supporting elements of tissue to injury. There are two types of inflammation according to the onset as acute and chronic inflammation. Acute inflammation is rapid in onset and of short duration, lasting from a few minutes to as long as a few days, and is characterized by fluid and plasma protein exudation and a predominantly neutrophilicleukocyte accumulation. Chronic inflammation may follow acute inflammation but may be more insidious, as a low grade, smoldering and often asymptomatic reaction. It is longer duration (days to years) and is typified by influx of lymphocytes and macrophages with associated vascular proliferation and fibrosis (scarring) (Kumar *etal.*, 2013).

In this study, the mice were inflamed by injecting the carrageenan. Necas & Bartosikova (2013) reported that it is a widely used test to determine the anti-inflammatory activity. Carrageenan induced paw edema in albino mice of footpad has all the cardinal features of acute inflammation such as redness (rubor), heat (color),swelling (tumor), pain (dolar) and loss of function.

Acetyl Salicylic acid (aspirin) was used as a standard drug. It is a no steroidal anti-inflammatory agent which pharmacologic activity includes anti-inflammatory,analgesic and antipyretic effects.(Vane & Botting, 2003).

Herbal drugs are being used for the treatment of pain and inflammation. TMF-27 is composed of 16 kinds of medicinal plants and all of its ingredients are easily available and cost-effective. Some of plants ingredient in TMF-27 have been proved scientifically as anti-inflammatory effect in literatures. The stem of *Tinosporacordifolia* Miers. includes mostly in TMF-27. Among the 57 numbers of Myanmar Traditional Medicine Formulation, TMF-27 is commonly used by many Traditional Medicine Practitioners. TMF-27 is a drug that has been used by ancient Traditional Medicine Practitioners by generation to generation.

According to the scientific study of TMF-27, (Pharmacological and Toxicological Evaluation of Myanmar Traditional Medicine Formulations, DMR, 1989), this drug has no toxicity in animal models. In pharmacological study, this drug has antihypertensive effect especially in mild and moderate hypertensive patients (Moe-Kyaw-Myint*et al.*, 2012).

In this study, TMF-27 was extracted with water by freeze drying process because water was a universal and good solvent to extract plant products because more substances dissolve in water than any other chemicals (Tiwari *et al.*, 2011). Freeze drying is a process of drying in which water is moved from the product after it is frozen and placed under a vacuum, allowing the ice to change directly from solid to vapor without passing through a liquid phase. It can remove water from the product without excessive heating, enhance product stability in dry state and minimize chemical decomposition (Nireesha*et al.*, 2013).

TMF-27 is one of the conventional drugs and also it has been used in Traditional Medicine Hospitals and Clinics. This drug is extensively used for pain, inflammation, tingling and numbness by Traditional Medicine Practitioners. The results of this experiment may provide one of the useful information in the development of Myanmar Traditional Medicine from Traditional Medicine formulations. Regarding the above mentions, the present study was carried out to evaluate the anti-inflammatory effect of freeze dried powder of aqueous extract of TMF-27 in animal models.

II. OBJECTIVES

➢ *General Objective*

To study the anti-inflammatory effect of aqueous extract TMF-27 (*PJI-LOUN:-CHAN:-THA-HSEI:*) on albino mice

➢ *Specific Objectives*

1. To assess the organoleptic characteristics of TMF-27
2. To evaluate the anti-inflammatory effect of freeze dried powder of aqueous extract of TMF-27 on albino mice

III. MATERIAL AND METHODS

This study was done by laboratory based experimental animal study design from 1st August 2016 to 31st July 2017 and approved by Protocol Board of University of Traditional Medicine. Total 30 albino mice were tested according to inclusion criteria as apparently healthy Albino mice of both sexes (body weight: 27 ± 3 g) and excluded in pregnant albino mice, lactating mice and body weight > 35 g of mice at Department of Medical Research (PyinOoLwin Branch).

Random sampling procedure was done by lottery method. Thirty albino mice were numbered and identified by individually specified markings. For treatment groups, a letter marked by ‘a’ were represent group I (negative control), ‘b’ were represent group II (positive control), ‘c’ were represent group III (150 mg/kg of freeze driedpowder of aqueous extract, TMF-27),‘d’ were represent group IV (300 mg/kg of freeze dried powder of aqueous extract, TMF-27), ‘e’ were represent group V (600mg/kg of freeze dried powder of aqueous extract, TMF-27).

TMF-27 was prepared by the method of DTM (2016). The raw materials of TMF-27 were collected from local market. These were carefully washed with tap water to remove dust and any foreign materials and they were air dried under shade. The air-dried parts of the plant materials were chopped into small pieces and the ingredients were made into fine powder in Traditional Medicine Pharmaceutical Factory, Mandalay. This drug was stored in the air tight bottle.

The aqueous extract was done by 100 g powder of TMF-27 with 1000 ml of DW by using reflux extractor, 3 hours for three times at 60˚C. The extract was filtered using filter paper and filtrate was concentrated by using vacuum rotary evaporator at 50˚C. The residues were made powder by freeze dryer (FD-1, Japan).

➢ *Detail procedure of animal testing*

In this experiment, the mice were taken from Laboratory Animal Service Division, DMR (POLB). The non-fasted mice of both sexes in the weight range of 27 ± 3 g were used. Food and water were withheld during the experimental period. The screening was done on 30 albino mice (ICR strain). They were divided into five groups (negative control group, positive control group, three tested groups) and each group contains six albino mice. The solution of the freeze dried powder of aqueous extract of TMF-27 was suspended in vehicle (DW). Negative control group was given vehicle (10 ml/kg) orally, positive control group was given Acetyl Salicylic Acid (300 mg/kg) and three tested groups were given by different dose levels of freeze dried powder of aqueous extract of TMF-27 as low dose (150 mg/kg), median dose (300 mg/kg) and high dose (600 mg/kg) respectively. Animals were marked with marker pen at the lateral malleolus. Then the basal paw volumes were measured by volume displacement methods using digital Plethysmometer (Model LE 7500).

Freshly prepared suspension of λ-carrageenan 50 μL (1.0% in 0.9% NaCl) was injected under the plantar aponeurosis of the right hind paws of the albino mice with a hypodermic needle (No-29) gauge by the method of Winter *et al.*, 1962. The measurement of the paw volume was carried out by taking baseline volume (0 hour) and then 1 hour, 2 hour, 3 hour, 4 hour, 5 hour and 6 hours after λ-carrageenan injection. The volume of the paw was expressed in terms of milliliter (ml). The average volume of right hind paw at each animal was calculated from 3-reading which does not deviate more than 4%. The paw volume changes were recorded in paw edema volume and the mean paw volumes of test groups and positive control groups were compared statistically with those of negative control groups for each time interval and express as the percent edema inhibition. The percentage of edema inhibition in paw edema was calculated as follows.

Percent inhibition of paw edema volume = [1- (Vt / Vc)] × 100

(Vt and Vc are paw volume in the drug treated and control group)

➢ *Data Management and Data Analysis*

The results were expressed as mean ± SE. Statistical analysis were performed with SPSS software, version 21. Statistical analyses were carried out using one-way analysis of variance(ANOVA) followed by Dunnett test. The values of drug treated group were compared with negative control group. p value < 0.05 was considered as statistically significant.

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IV. FINDINGS AND DISCUSSION

- *Organoleptic Characteristics of Freeze Dried Powder of Aqueous Extract of TMF-27*

The organoleptic characteristics of raw powder and freeze dried powder of aqueous extract of TMF-27 via the senses including appearance, taste, odor, color and touch by senses of human with ten people.

No	Parameters	Character
1.	Appearance	Powder
2.	Taste	Bitter
3.	Odor	*Tinosporacordifolia*
4.	Color	Beigy brown
5.	Touch	Fine

- *The Effect of Freeze Dried Powder of Aqueous Extract of TMF-27 on λ-Carrageenan Induced Paw Edema of Albino Mice*

In acute inflammation models, the groups were orally administered with the freezedried powder of aqueous extract of TMF-27 in the dose of 150 mg/kg, 300 mg/kg and600 mg/kg. Paw edema volumes were measured before (0 hour) and hourly up to 6hour after injection of λ-carrageenan by digital Plethysmometer. The paw volumes oftreated group were compared with negative control group (vehicle, 10 ml/kg).In this study, aspirin 300 mg/kg was used as a positive control group. The results of mean increase in paw edema volume in ml after treatment with positive control (aspirin, 300 mg/kg) were 0.06 ± 0.024 at 1hour, 0.04 ± 0.013 at 2 hour, 0.06± 0.013 at 3 hour, 0.07 ± 0.016 at 4 hour, 0.06 ± 0.016 at 5 hour and 0.05 ± 0.018 at 6hour respectively. The paw edema volume of mice reduced start at 2 hour and 3 hour($p < 0.01$), 4 hour and 5 hour ($p < 0.001$) and 6 hour ($p < 0.01$).

There was no significant increase in paw edema volume at low dose (aqueousextract of TMF-27, 150 mg/kg) up to 6 hour. The results of mean increase in pawedema volume in ml after treatment with aqueous extract of TMF-27, 150 mg/kg were
0.09 ± 0.024 at 1hour, 0.06 ± 0.013 at 2 hour, 0.08 ± 0.013 at 3 hour, 0.09 ± 0.016 at 4
hour, 0.08 ± 0.016 at 5 hour and 0.08 ± 0.018 at 6 hour respectively.

The results of mean increase in paw edema volume in ml after treatment with aqueous extract of TMF-27, 300 mg/kg were 0.06 ± 0.024 at 1hour, 0.05 ± 0.013 at 2hour, 0.06 ± 0.013 at 3 hour, 0.08 ± 0.016 at 4 hour, 0.07 ± 0.016 at 5 hour and 0.05 ±0.018 at 6 hour respectively. The paw edema volume of mice was significantly reduced in the median dose (aqueous extract of TMF-27, 300 mg/kg) at 6 hour ($p <0.05$).

The results of mean increase in paw edema volume in ml after treatment with aqueous extract of TMF-27 (600 mg/kg) were 0.06 ± 0.024 at 1hour, 0.04 ± 0.013 at 2 hour, 0.06 ± 0.013 at 3 hour, 0.07 ± 0.016 at 4 hour, 0.05 ± 0.016 at 5 hour and 0.05 ±0.018 at 6 hour respectively. The paw edema volume of mice was significantly reduced the highest dose (aqueous extract of TMF-27, 600 mg/kg) at 2 hour and 3hour ($p < 0.05$); 4 hour, 5 hour and 6 hour ($p < 0.01$).

Table 1.Comparison between the effect of positive control group (aspirin, 300 mg/kg) and tested group (freeze dried powder of aqueous extract of TMF-27, 600 mg/kg) on λ-carrageenan induced paw volume increase in (ml)

Treatment (Dose)	1 hr	2 hr	3 hr	4 hr	5hr	6 hr
Positive control group (Aspirin, 300 mg/kg)	0.06 ± 0.024 -	0.04 ± 0.013 **	0.06 ± 0.013 **	0.07 ± 0.016 ***	0.06 ± 0.016 ***	0.015 ± 0.018 **
Tested group (aqueous extract, 600 mg/kg)	0.06 ± 0.024 -	0.04 ± 0.013 *	0.06 ± 0.013 *	0.07 ± 0.016 **	0.05 ± 0.016 **	0.05 ± 0.018 **

The results are in mean ± S.E
n = 6
* $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$

Table 2. Percent inhibition of λ-carrageenan induced edema after treatment with aspirin and freeze dried powder of aqueous extract of TMF-27

Treatment (Dose)	1 hr	2 hr	3 hr	4 hr	5 hr	6 hr
Aspirin (300 mg/kg)	40%	50%	45%	46%	50%	58%
Aqueous extract of TMF- 27 (150 mg/kg)	10%	25%	27%	31%	33%	33%
Aqueous extract of TMF-27 (300 mg/kg)	40%	37%	45%	38%	42%	58%
Aqueous extract of TMF-27 (600 mg/kg)	40%	50%	45%	46%	58%	58%

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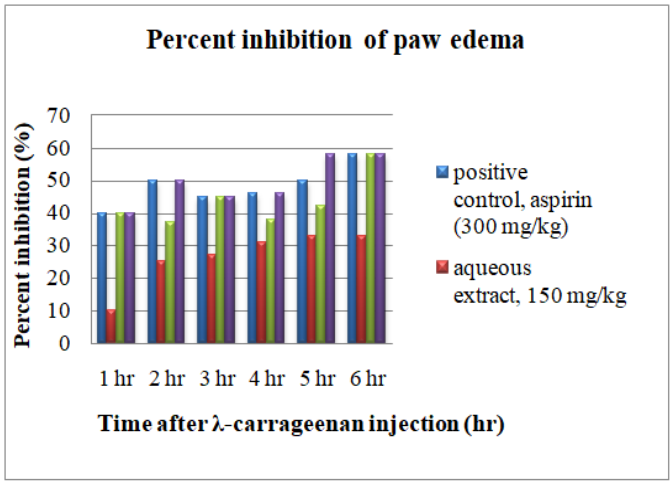

Figure 1:- Percent inhibition of λ-carrageenan induced edema after treatment with aspirin and freeze dried powder of aqueous extract of TMF-27

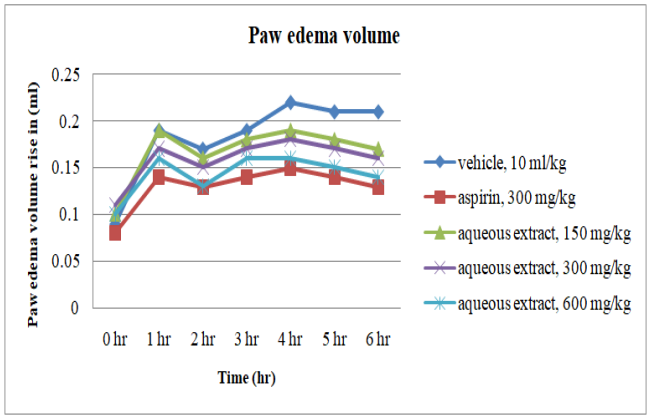

Figure 2 :- Effect of negative control (vehicle), positive control (aspirin) and test drug (freeze dried powder of aqueous extract of TMF-27) on λ-carrageenan induced edema volume rise in (ml). (n = 6)

V. DISCUSSION AND CONCLUSION

In the present study, it can be concluded that the freeze dried powder of aqueous extract of TMF-27 has significant anti-inflammatory effect on λ-carrageenan induced edema. This study was to find out the organoleptic characters and the anti-inflammatory effects of freeze dried powder of aqueous extract of TMF-27 in albino mice. The sample size was small and the study period was limited. Therefore, it is recommended that the study should be done repeatedly with larger sample and longer duration. The anti-inflammatory effect of freeze dried powder of aqueous extract of TMF-27 should also be investigated in other pharmacological effects. Finally, the follow up study should also be continued for clinical trial in chronic inflammation should be performed.

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