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Acute Toxicity and Antipyretic Activity of Traditional Medicine Formulation (TMF-76) *Apu-Kja-Hsei:* on Animal Models

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Traditional Medicine Formulation-76 (TMF-76) (*Apu-Kja-Hsei:*) is a special antipyretic drug with herbomineral preparation of 4 kinds of ingredients which is useful in the treatment of pyrexia. This study was performed to evaluate the acute toxicity and antipyretic activity of TMF-76 on animal models. The study was carried out from October 2019 to September 2020. Physicochemical properties of TMF-76 were analyzed for quality control purpose. Acute toxicity test of TMF-76 was performed by using main test according to OECD 425 (Organization for Economic Co-operation and Development) guideline (2008). Antipyretic activity of TMF-76 was carried out on Brewer's yeast induced pyrexia albino rats with oral administration of three different doses (75 mg/kg, 150 mg/kg and 300 mg/kg) of TMF-76 and standard drug, paracetamol (150 mg/kg). In the acute toxicity study, there were no toxic signs and mortality in mice at the various doses of TMF–76 (175 mg/kg, 550 mg/kg, 1750 mg/kg and 5000 mg/kg) up to 14 days observation period. Therefore, the LD_{50} (the dose which has proved to be lethal causing death to 50% of the tested group of animals) value of TMF-76 was found to be more than 5000 mg/kg body weight. In the antipyretic activity study, the significant antipyretic activity was observed in three different doses of TMF-76 ($p<0.05$-0.001) after administration of the test drug. In this study, percent reductions of rectal temperature with paracetamol (150 mg/kg) were ranged from 29±28% to 97±20%. Percent reductions of rectal temperature with TMF-76 (75 mg/kg, 150 mg/kg and 300 mg/kg) were ranged from (69±33% to 95±49%), (60±20% to 105±32%) and (52±58% to 170±67%), respectively. According to the present findings, this study proved scientifically that TMF-76 possessed significant antipyretic activity on Brewer's yeast induced pyrexia in albino rat model and TMF-76 was nontoxic.

Keywords: Traditinal medicine, Acute toxicity, Antipyretic activity, Paracetamol, Brewer's yeast

INTRODUCTION

Body heat is generated by basal metabolic activity and muscle movement, and lost by conduction, convection, evaporation and radiation. Body temperature is controlled in the hypothalamus, which is directly sensitive to changes in core temperature and indirectly responds to temperature-sensitive neurons in the skin. The normal set-point of core temperature is tightly regulated within the range 37±0.5°C, which is necessary to preserve the normal function of many enzymes and other metabolic processes.[1]

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Fever or pyrexia is a condition that may be suffered by almost all of the people.

In Myanmar Traditional Medicine Hospitals and clinics, there are many patients suffer from malaria, seasonal fever and fever some inflammatory diseases including rheumatic arthritis, heat illness (heat exhaustion, heat stroke), malignant hyperpyrexia) every year. Antipyretic medications have the effect of lowering the body temperature, which result in a reduction of fever. Varieties of Myanmar traditional herbomineral antipyretic drugs are used in fever. Among them, TMF-76 was more popular and useful because it has been recorded as antipyretic drug in ancient literature and used in traditional medicine hospital and clinic since 1982.[2] TMF-76 is composed only 4 kinds of ingredient namely, *Mesua ferrea* Linn. (ကုံကော်ဝတ်ဆံ), *Sepia esculenta* (ပင်လယ်ရေမြုပ်), Purified Sulphur and Glucose.

In Myanmar traditional medicine, although TMF-76 is used among Myanmar population in treating pyrexia for many years and it is described in curriculum according to the experience of traditional medicine practitioners and ancient literatures, there is lack of scientific information on the safety and efficacy profile. This study is aimed to find out physico-chemical properties of TMF-76 (*Apu-Kja-Hsei:*) and to determine acute toxicity and antipyretic activity of TMF-76 (*Apu-Kja-Hsei:*) on animal models.

MATERIALS AND METHOD

Study design was laboratory based experimental animal study. Study site was Research Division, University of Traditional Medicine (Mandalay) and Pharmacology Research Division, Department of Medical Research (Yangon). Study period was 1st October 2019 to 30th September 2020. For acute toxicity test, study population was adult female Albino mice (DDY strain) 6 in numbers, weighing between 25-30 g. For antipyretic test, study population was adult Albino rat (Wistar strain) 30 in numbers, weighing between 180-220 gm from Laboratory Animal Services Division, Department of Medical Research (Yangon).

Purification of Sulphur

Sulphur was purified by the *Sodhana* method.[3] In this purification method, equal amounts of ghee, sulphur, and milk were used. First, 500 gm of ghee was added to a stainless-steel pot and gradually heated until melted. Then, 500 gm of sulphur was added until it reached boiling point. Another pot 500 ml of raw cow milk was covered with thin cloth and tied. The melted sulphur was gradually added to the raw cow milk pot, the sulphur solidified. After that, solid sulphur was removed from the milk and washed with warm water. The process was repeated three times. Each time, when the sulphur was gradually melted, fresh ghee and fresh milk were used. Finally, 468 gm of solid sulphur was obtained, made into a fine powder, and stored in a glass bottle.

Preparation of TMF-76

The raw materials of TMF-76 were collected from Zay-Cho Market, Mandalay. Stamen of *Mesua ferrea* Linn (ကုံကော်ဝတ်ဆံ) and *Sepia esculenta* (ပင်လယ်ရေမြုပ်) were carefully cleaned to remove dust and foreign materials. Then, the 80 g of purified sulphur (သုဒ္ဓပြုပြီးကန့်), 160 g of glucose, 24 g of stamen of *Mesua ferrea* Linn and 24 g of *Sepia esculenta* were made into fine powder with grinding machine and mixed all. The powder of TMF-76 was stored in air tight bottle.

Physico-chemical analysis of TMF-76

Physico-chemical properties of TMF-76 were analyzed at Research Division, University of Traditional Medicine, Mandalay by the methods of WHO, 2011.[4]

Acute toxicity study

For acute toxicity test, healthy adult six female albino mice (ddy strain), were kept in the cages for at least 5 days prior to dosing to allow for acclimatization to the laboratory conditions. The animals were maintained in standard laboratory conditions with unlimited supply of food and water.[5]

TMF-76 was dissolved in 1% methyl cellulose for required concentration to be administered. Animal was fasted food but not water for 18 hours prior to dosing. The fasted animals were weighed and the dose was calculated according to body weight. By using intragastric needle, a single dose of the test substance was administered. Then, food was withheld for 1 to 2 hours.[5] Animals were observed individually once during the first 30 minutes after dosing, periodically during the first 24 hours (with special attention given during the first 4 hours) and daily thereafter, for a total of 14 days. Animals were observed for toxic signs and mortality. All observations were systemically recorded with individual records being maintained for each animal. LD_{50} was calculated by the AOT 425 Stat-Program (version 1) prepared for the United States Environmental Protection Agency.

Antipyretic activity study

The determination of antipyretic activity of TMF-76 was done by using the method described in Screening of Herbal Drugs for Antipyretic Activity in Herbal Drug Technology[6] and the method of Chattopadhyay, *et al*., (2005).[7] Yeast nduced pyrexia in albino rats was commonly used animal model for the determination of antipyretic activity of herbal extracts and modern drugs. TMF-76 was dissolved in 1% methylcellulose (drug vehicle) before administration to the rats.

Thirty numbers of rats were divided into five groups of six rats in each such as group I (control group), group II (low dose of test group, 75 mg/kg), group III (medium dose of test group, 150 mg/kg), group IV (high dose of test group, 300 mg/kg) and group V (standard drug paracetamol 150 mg/kg). They were kept in separate cages in constant room temperature (23±2°C). Animals were provided with standard rodent pellet diet and had free access of food and water. The normal body temperature of each rat was measured rectally by using a digital clinical thermometer inserting 3 cm depth into the rectum before the experiment. The body weight for each rat was recorded and the dose of freshly prepared brewer's yeast to be injected was calculated. Pyrexia was induced at the back of the neck by subcutaneous injection of 15% w/v of brewer's yeast suspended in 0.5% w/v methyl cellulose solution (10 ml/kg). The site of injection was massaged in order to spread the suspension beneath the skin. Then, rats were returned to their housing cages.

Immediately after yeast administration, animals were fasted to the end of the experiment for 24 hours but given free access of water during the experiment. After 19 hours of yeast injection, the rectal temperature for each rat was measured and recorded again.[6] Only animals showing an increase in the rectal temperature at least 1°C or more were used for the study. The rats from group I were administered orally with drug vehicle (1% methyl cellulose) and served as control. TMF-76 was administered orally with three doses (75 mg/kg, 150 mg/kg and 300 mg/kg) to group II, III and IV, respectively. The fifth group (group V) was orally given standard drug, paracetamol (150 mg/kg).[8] The rectal temperature for each rat was measured hourly up to 5 hours (1 hr, 2 hr, 3 hr, 4 hr and 5 hr) after administration of drug vehicle and test drugs, (i.e., 20 hours, 21 hours, 22 hours, 23 hours and 24 hours after injection of yeast).[9]

Percent reduction of rectal temperature was calculated by using the following formula. The total fall in rectal temperature from the elevated temperature to baseline level is considered as 100%.[10] In antipyretic activity study, standard statistical methods were used in the calculation of arithmetic mean ($\bar{X}$), Standard Deviation (SD) and Standard Error (SE). Unpaired "t" test was used to observe the significance of difference between means of both control and experimental groups. P value <0.05 was considered significant.

$$\text{Percent reduction} = \frac{\text{Elevated temperatre - temperature at different interval}}{\text{Elevated temperature - initial temperature}} \text{ x } 100$$

RESULTS AND DISCUSSIONS

In the present study, physico-chemical analysis, acute toxicity and antipyretic activity of TMF-76 were determined.

Physico-chemical analysis of TMF-76

WHO (2011) stated that physicochemical parameters were determined for the quality and purity of the drug. The results of physico-chemical analysis of TMF-76 were shown in (Table-1).

Table 1. Physico-chemical properties of TMF-76

No.	Physico-chemical parameters	Results
1	Water and volatile matter content	5.53%
2	pH (1% solution) (w/v)	7.4
3	pH (10% solution) (w/v)	7.5
4	Total ash	10.2%
5	Acid insoluble ash	9.7%
6	Water soluble ash	3.2%
7	Water soluble matter	39.8%
8	Ethanol soluble matter	29.76%
9	Swelling index	Nil
10	Foaming index	<100

Acute toxicity study

Acute toxicity test of TMF-76 was investigated on female albino mice by using main test of OECD guideline 425 according to the considerations of animal welfare. In the acute toxicity study, there were no toxic signs and mortality in mice at the various doses of TMF–76 (175 mg/kg, 550 mg/kg, 1750 mg/kg and 5000 mg/kg) up to 14 days observation period. Therefore, the LD_{50} value of TMF-76 was found to be more than 5000 mg/kg body weight.

Antipyretic activity study

In this study, *in vivo* antipyretic activity was carried out by using oral administration of three different doses of TMF-76 (75 mg/kg, 150 mg/kg and 300 mg/kg) and standard paracetamol in brewer's yeast induced pyrexia in albino rat model. After oral administrations, the results of mean rectal temperatures of rats in the different doses of TMF-76 and standard paracetamol groups were shown in (Table 2).

Table 2. Mean rectal temperatures of albino rats with different doses of TMF-76 and Paracetamol

Different groups of treatments	Rectal temperatures at hourly interval (in ºC) (Mean±SD)						
	Before treatment		After treatment				
	0 hr	19 hr after yeast	1 hr	2 hr	3 hr	4 hr	5 hr
Control	36.95±0.49	38.43±0.45	38.80±0.42	38.95±0.34	38.92±0.25	38.98±0.21	39.08±0.16
TMF-76 75 mg/kg	37.25±0.30	38.83±0.33	39.17±0.16	38.67±0.57	38.45±0.52	37.87±0.42***	37.48±0.74***
TMF-76 150 mg/kg	37.08±0.96	38.77±0.12	39.15±0.44	38.72±0.34	38.42±0.46*	37.85±0.41***	37.20±0.37***
TMF-76 300 mg/kg	37.57±0.27	38.63±0.23	38.55±0.77	38.22±0.65*	37.80±0.51**	37.40±0.49***	37.02±0.58***
Paracetamol 150 mg/kg	37.30±0.32	38.58±0.26	37.58±0.26***	37.42±0.24***	37.45±0.30***	37.95±0.2***	38.27±0.37***

*p< 0.05, **p<0.01, ***p<0.001

After oral administration, significant reduction of rectal temperature of test drug groups were observed when compared with those of the control group. In group II, significant reductions of rectal temperature were observed at 4 hours ($p<0.001$) and 5 hours ($p<0.001$). In group III, significant reductions of rectal temperature were observed at 3 hours ($p<0.05$), 4 hour and 5 hours ($p<0.001$). In group IV, significant reductions of rectal temperature were observed at 2 hours ($p<0.05$), 3 hour ($p<0.01$), 4 hour and 5 hours

($p<0.001$). In group V, significant reductions of rectal temperature were observed started at 1 hour up to 5 hours ($p<0.001$).

In comparison with different doses of TMF-76 and standard paracetamol, the percent reductions of rectal temperature of standard paracetamol were 83±21% at 1hr, 97±20% at 2 hr, 93±38% at 3 hr, 52±28% at 4 hr and 29±28% at 5 hr. Significant percent reductions of rectal temperature of 75 mg/kg and 150 mg/kg of TMF-76 were 95±49% ($p<0.05$) and (105±32%) ($p<0.01$) at 5 hr, respectively. Significant percent reductions of rectal temperature of 300 mg/kg of TMF-76 were 145±89% ($p<0.05$) at 4 hr and 170±67% ($p<0.001$) at 5 hr, respectively. The normal set-point of core temperature is tightly regulated within the range 37±0.5°C.[1]

Significant percent reduction of rectal temperature of group III at 5 hr and those of group IV at 4 hr and at 5 hr were more than 100%. This is because the temperature measurement at these hours were lower than their respective basal temperature at 0 hr and reached nearly the normal set point. The results are shown in (Table 3) and (Fig. 1).

Table 3. Mean percent reductions of rectal temperature with different doses of TMF-76 and standard drug, Paracetamol on brewer's yeast-induced pyrexia in albino rats

Different groups of treatment	Mean percent reductions of rectal temperature at hourly interval after treatment (Mean±SD)				
	1 hr	2 hr	3 hr	4 hr	5 hr
TMF-76 (75 mg/kg)	-28±26%	16±26%	30±29%	69±33%	95±49%*
TMF-76 (150 mg/kg)	-29±26%	1±17%	18±15%	60±20%	105±32%**
TMF-76 (300 mg/kg)	20±65%	52±58%	95±58%	145±89%*	170±67%***
Paracetamol (150 mg/kg)	83±21%	97±20%	93±38%	52±28%	29±28%

*$p<0.05$, **$p<0.01$, ***$p<0.001$. All values are expressed as mean±SD (n=6). Statistical comparison was made between paracetamol (150 mg/kg) and different doses of TMF-76.

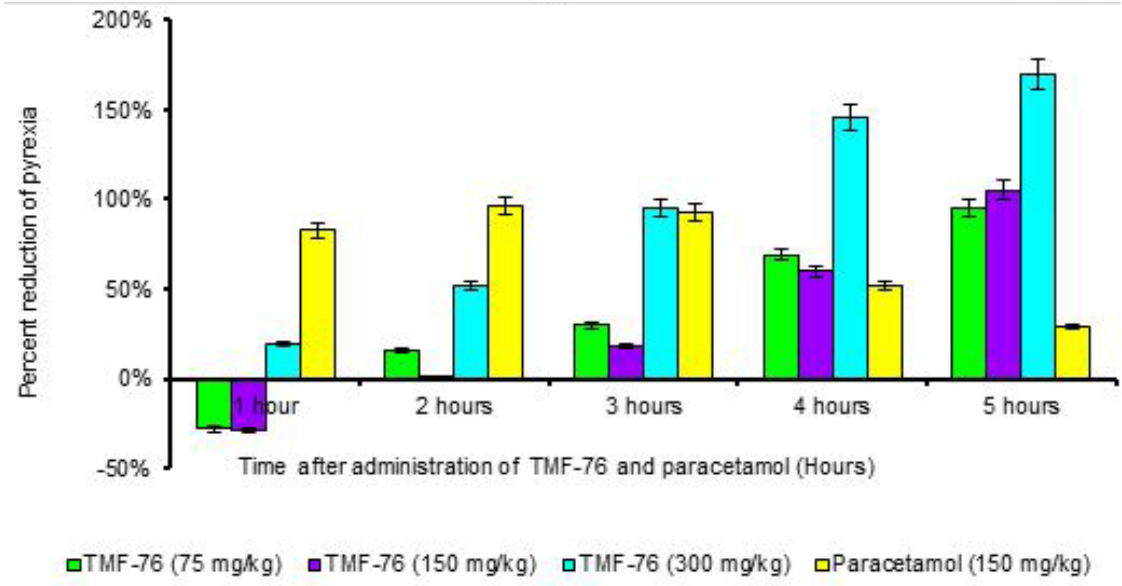

Fig. 1. Comparison of the effects on mean percent reductions of pyrexia in rats between the different doses levels of test drugs, TMF-76 receiving groups with the standard drug, paracetamol (150 mg/kg) receiving group. Values are in mean±SD, n=6.

According to this findings, 300 mg/kg of TMF-76 possesses significant antipyretic effect at 2 hr, 3hr, 4hr and 5 hr compare with standard paracetamol group. Therefore, 300 mg/kg of TMF-76 was therapeutic dose which can give significant antipyretic effect on Brewer's yeast induced pyrexia in albino rat model and it was safe.

Traditional medicine is the medical system based on the concept of *Mahabhuta*. According to Myanmar traditional medicine, almost all of the fever is due to excessive of *ushna tejo* in body. Therefore, the drugs that are excessive of *sita tejo* may quench *ushna tejo* and which result in reduce fever by lowering the body temperature.[11] In the *Rasa* (taste) of TMF-76, *Sita* quench *Ushna Tejo* heat, *Akāsa* can break excessive external *Prithvi* and sweet taste of *Prithvi* which may cause strengthen the body. Therefore, the *Rasa* of TMF-76 (*Apu-Kja-Hsei:*) is assumed to control pyrexia.[12]

In Myanmar Traditional Medicine, *Mesua ferrea* Linn. (ကံကော်ဝတ်ဆံ) can be used in fever, insomnia, indigestions, cold, asthma, as carminative, cardiotonic, antipyretic agent, dyspepsia and in cosmetics. [13, 14] *Sepia esculenta* (ပင်လယ်ရေမြုပ်) is used to treat indigestion, fever, cough and to relieve thirst.[15] Glucose is the main source of energy for neural activity, changes in its availability might alter local cerebral metabolism.[16] Purified Sulphur, is the *rasayana* drugs and it had been stated in ancient literatures that it was very effective in fever and chronic disease.[17] TMF-76 was formulated by the ingredients mentioned above which are effective for pyrexia. Therefore, this TMF-76 was found to possess antipyretic effect.

This TMF-76 contained Sulphur. Hemolytic anemia is encountered in patients with G6PD deficiency after taking Sulphur drugs.[18] Therefore, people who are allergic to Sulphur drug or who has history of G6PD deficiency should not take this TMF-76 for treatment.

Conclusion

In this study, three different doses of TMF-76 showed significant reductions in rectal temperature of yeast induced pyrexia rats and this drug was found to be nontoxic. The high dose of TMF-76 possesses antipyretic activity compare with standard paracetamol. Therefore, this study scientifically proved that safe and significant antipyretic activity of TMF-76 that had been used for a long time according to the experiences of Myanmar Traditional Medicine Practitioners. The results indicated that TMF-76 can be safely used for the treatment of pyrexia.

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