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Antipyretic Activity of AOF Herbal Formulation in Brewer's Yeast-Induced Pyrexia in Rats

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The aim of the present study was to investigate the antipyretic activity of AOF herbal formulation by Brewer's yeast-induced pyrexia in Wistar rats. AOF herbal formulation consists of 13 medicinal plant parts and some of which are reputed for antipyretic effect. The acute oral toxicity was carried out in albino mice according to OECD 423-guidelines. It revealed that there is no toxic sign up to the dose level of 2000 mg/kg body weight. Adult albino rats of either sex (200-250 gm) were divided into five groups containing six in each group for antipyretic study. Before yeast injection, the basal rectal temperature of rats were recorded and after that, the rats were given subcutaneous injection of 10 ml/kg of 15% yeast solution. Rectal temperature of each rat was measured again 19 hours after yeast injection. After confirming that those rats were induced pyrexia, the test drugs and standard drug were administered orally into different groups. Three doses of test drug (1, 1.5 and 2 gm/kg body weight) were used. Paracetamol (150 mg/kg) was administered to standard group. Rectal temperatures were measured at 1, 2, 3, 4 and 5 hours after test drug administration. This herbal formulation (2 g/kg body weight) showed significant reduction of yeast-induced pyrexia in rats at 1 hr, 2 hr, 3 hr and 4 hr after administration of test drug ($p<0.001$) when compared to the control group. The present results show that AOF herbal formulation possesses a significant antipyretic effect in Brewer's yeast-induced pyrexia in rats.

Keywords: Antipyretic, Herbal formulation

INTRODUCTION

Traditional medicine have been practicing on different culture regions without the parallel advance of international standards and method for evaluation. Scientific evidence from tests done to evaluate safety and practices is limited while evidence shows that some herbal medicines are effective for specific conditions, further study of products and practices is needed. Requirements and methods for assessment and evaluation are complex. The safety, effectiveness and quality of finished herbal medicine product depend on the quality of their source materials (which can include hundreds of natural constituents) and how elements are handled through production. For many millions of people, often living in rural areas of developing countries herbal medicines, traditional treatments and traditional practitioners are the main, sometimes the only source of healthcare.[1] AOF herbal formulation is produced by private sector, for the treatment of cold, fever, malaria, dizziness, menstrual disorders, cough, diarrhoea, skin infections, muscle cramps, muscle stiffness and neuro-logical disorders due to stress. This herbal formulation consists of 13 medicinal plant parts some of which are reputed for antipyretic effect.[2, 3] Among these 13 plants, *Myristica fragrans*, *Zingiber officinale* Roscoe, *Piper longum* Linn. (or) *Piper cheba* and *Santalum album* Linn. are already shown to have anti-pyretic activity on Brewer's yeast-induced pyrexia in experimental rats.[4-7] The aim of this study was to investigate the antipyretic activity of AOF herbal formulation by Brewer's yeast-induced pyrexia in Wistar rats.

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MATERIALS AND METHODS

Study site

Department of Medical Research (DMR), Ministry of Health and Sports, Myanmar.

Drug

AOF herbal formulation provided from private sector.

Animals

Adult albino mice (ddY strain) (25-30 gm) and adult albino Wistar rats (200-250 gm) were obtained from Laboratory Animal Service Division, DMR. Eighteen albino mice (ddY strain) were used for acute toxicity study. They were maintained under standard condition of temperature (23±1°C), relative humidity (55±10%) and 12 h/12 h light/dark cycle and fed with standard pellet diets with water ad libitum. They were housed in polypropylene cages and each group contained 3 mice.

Thirty albino Wistar rats of either sex were used for antipyretic activity. The body temperature of each rat was recorded by measuring rectal temperature with digital thermometer. They were maintained with standard pellet diet and water ad libitum. Animals were acclimatized to laboratory condition for five days before commencement of any experiment.

Composition of materials used in AOF herbal formulation is shown in Table 1.

Table 1. Composition of AOF herbal formulation

Ingredients (Myanmar name)	Scientific name	Part used	Amount (gram)
Layhnyin	*Syzygium aromaticum* Linn.	Flower	160
Zadeikpho	*Myristica fragrans* Hout.	Fruit	160
Pannoot	*Saussurea sp.*	Root	80
Panma	*Anneslea fragrans*	Bark	32
Samonnet	*Nigella sativa* Linn.	Seed	48
Kun	*Piper betle* Linn.	Leaf	160
Gantgaw	*Mesua ferrea* Linn.	Stamen	96
Kantgyokeni	*Plumbago rosea* Linn.	Stem	128
Nathaphyu	*Santalum album* Linn.	Wood	32
Nathani	*Pterocarpus santalinus* Linn.	Wood	32
Gyin	*Zingiber officinale* Roscoe.	Rhizome	48
Peikchinn	*Piper longum* Linn.	Fruit	96
Ngayokkoung	*Piper nigrum* Linn.	Fruit	112
Payoke	Camphor		112
Theindaw	Natural sodium chloride		80
Zawethar	Ammonium chloride		64
	Total amount		1440

Phytochemical test

Preliminary phytochemical investigation was carried out according to Physicochemical Standards of Unani Formulations.[8]

Acute oral toxicity

The acute oral toxicity was carried out in mice according to Organization for Economic Co-operation and Development (OECD) 423-guidelines.[9]

Before the experiment, the animals were kept fasting overnight for 18 hours but allowed with free access to water. On the experiment day, mice were weighed and test substance (AOF herbal formulation) was administered orally in a single dose by using intragastric needle. One group served as the control and only distilled water was given orally. Six mice were used for each dose level. In this study, starting dose of 300 mg/kg body weight of test substance was given to 6 mice (3 mice per step). Mice were observed after giving dose at least once during the first 30 minutes, periodically during the first 24 hours. Special attention was given during the first 4 hours and daily up to 14 days. Signs of toxicity and mortality of the mice were recorded. Observations included changes in skin and fur, eyes, mucous membranes, respiratory, autonomic, central nervous systems and behavioral pattern. The time of death if any was recorded.

In this study, there were no signs of toxicity and lethality at the dose level of 300 mg/kg body weight. So the test herbal formulation of 2000 mg/kg was administered orally to another 6 mice (3 mice per step). The observation for toxic signs was done as described above. Individual body weight of mice was measured before the test drug was administered and once weekly thereafter. At the end of the test (14 days), the mice were weighed. Three mice each from each dose level of test group (herbal formulation receiving group) and the control group were sacrificed under chloroform anesthesia. Then, gross examination of internal organs was done and recorded.

Antipyretic activity

Adult albino rats of either sex were divided into five groups containing six in each group for this study. The normal body temperature of each rat was recorded by measuring rectal

temperature with digital thermometer. The anti-pyretic activity of AOF herbal formulation was evaluated using Brewer's yeast-induced pyrexia in Wistar rat.[10] Before yeast injection, the basal rectal temperature of rat was recorded and after recording animals were given sub-cutaneous injection of 10 ml/kg of 15% w/v yeast suspended in 0.5% w/v methyl cellulose solution into the animal's dorsum region for elevation of body temperature of rats. Rats were then returned to their cages. Food was withheld and water ad libitum. Rectal temperature of each rat was measured again after 19 hours after yeast injection. Then, those animals which showed a rise in rectal temperature of more than 1°C were included in this study. At the 19 hours after yeast injection, the test drug and standard drug suspended in distilled water were administered into different groups. Distilled water at a dose of 10 ml/kg was administered orally to the control group. Paracetamol (150 mg/kg body weight) was administered orally to standard group of animals. Rectal temperatures were measured at 1, 2, 3, 4 and 5 hours after drug administration.

Statistical analysis

Data were expressed as mean±standard error of mean. The results were analyzed statistically by using Student "*t*" test. Percent reduction in rectal temperature was calculated by using the following formula. The total fall in rectal temperature from the elevated to normal level was considered as 100%.

$$\text{Percent reduction} = \frac{\text{Elevated temperature} - \text{temperature at different intervals}}{\text{Elevated temperature} - \text{initial temperature}} \times 100$$

RESULTS

Phytochemical test

Phytochemical constituents of the test herbal formulation (AOF) are shown in Table 2.

Acute toxicity study of AOF herbal formulation

It was found that no toxic sign and mortality was produced up to the maximal permissible dose 2000 mg/kg body weight in mice. Therefore, according to OECD guidelines 423, the LD_{50} of the AOF herbal formulation was 2500 mg/kg body weight.

Table 2. Phytochemical constituents of the AOF herbal formulation

Phytochemical constituents	
Alkaloids	+
Flavonoids	++
Glycosides	++
Polyphenols	+
Carbohydrate	++
Amino acid	++
Saponins	–
Tannins	++
Steroids/Triterpenes	+
Reducing sugar	++
Cyanogenic glycosides	–

(+)=Presence, (–)=Absence

Antipyretic activity of AOF herbal formulation

The antipyretic activity of AOF herbal formulation at various dose levels of 1 gm/kg, 1.5 gm/kg and 2 gm/kg body weight was studied on Brewer's yeast-induced rat model. The experimental rats showed a marked increase in rectal temperature 19 hours after Brewer's yeast injection.

Table 3. Antipyretic activity of AOF herbal formulation

Groups	Dose	Before yeast	After yeast	Rectal temperature in °C at times (hr) after drug administration				
				1 hr	2 hr	3 hr	4 hr	5 hr
Control	10 ml/kg bw	37.48 ± 0.15	38.97 ± 0.17	38.8 ± 0.12	38.88 ± 0.28	38.47 ± 0.24	38.6 ± 0.12	36.88 ± 0.34
AOF	1.0 gm/kg bw	37.53 ± 0.31	38.78 ± 0.12	38.92 ± 0.08	38.95 ± 0.11	38.9 ± 0.15	38.1 ± 0.39	39.1 ± 0.19
AOF	1.5 gm/kg bw	37.05 ± 0.26	38.65 ± 0.1	36.88 ± 0.62*	7.57 ± 0.38*	37.47 ± 0.23*	37.28 ± 0.26***	36.93 ± 0.45
AOF	2.0 gm/kg bw	37.88 ± 0.16	38.63 ± 0.11	38.02 ± 0.18***	7.72 ± 0.18**	37.65 ± 0.39**	37.43 ± 0.23***	37.53 ± 0.23
Paracetamol	150 mg/kg bw	37.48 ± 0.13	38.45 ± 0.05	37.58 ± 0.11***	7.38 ± 0.1***	36.88 ± 0.35**	36.83 ± 0.37***	37.18 ± 0.19

Values are mean±SEM n=6, *p<0.05, **p<0.01 and ***p<0.001, as compared to the control

In the Table, all time points (2, 3, 4, 5 hours) did not show the antipyretic effect with AOF 1 gm/kg body weight dose. However, significant decrease in rectal temperature was found at the dose of 1.5 gm/kg body weight and 2 gm/kg body weight at 1 hr, 2 hr, 3 hr and 4 hr after administration ($p<0.05$ - $p<0.001$). The maximum antipyretic activity with paracetamol (standard drug) 150 mg/kg body weight produced significant reduction in elevated rectal temperature at 1, 2, 3 and 4 hours after drug administration ($p<0.01$ - $p<0.001$) (Table 3).

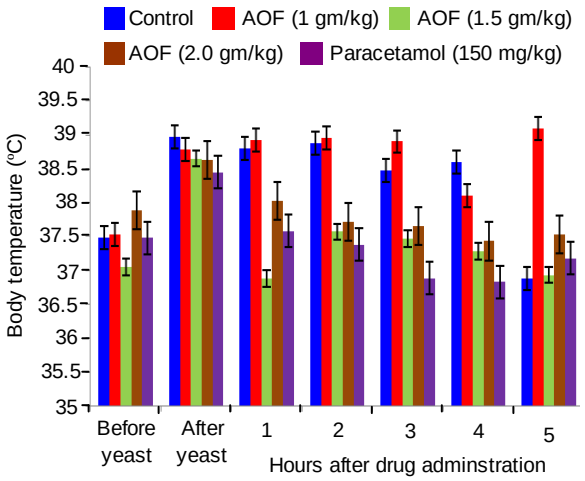

Fig. 1. The effect on body temperature between test drugs and control on yeast-induced fever in albino rats

Comparison of the effect on rectal temperature between test sample (AOF herbal formulation) and paracetamol with control on yeast-induced fever in albino rats is shown in Fig.1.

Table 4. Mean percent reductions of temperature with different doses of AOF herbal formulation and standard drug paracetamol on Brewer's yeast-induced pyrexia in albino rats

Different doses	% reduction of rectal temperature at hourly intervals				
	1 hr	2 hrs	3 hrs	4 hrs	5 hrs
	After treatment				
AOF (1 g/kg)	-16 ±18	-13±10	1±26	10±53	-28±23
AOF(1.5 g/kg)	129±58	81±36	89±29	106±34	126±49
AOF (2 g/kg)	99±46	139±38	132±45	175±28	220±123
Paracetamol (150 mg/kg)	95±16	119±9	180±43	186±44	145±28

All values are expressed as mean±SEM (n=6)

The mean percent reduction of temperature with different doses of AOF herbal formulation and standard drug paracetamol on Brewer's yeast-induced pyrexia in rats is shown in Table 4.

DISCUSSION

In this study, the antipyretic activity was evaluated using Brewer's yeast-induced pyrexia in rat model. Brewer's yeast is a fungi containing lipopolysaccharide which is a cell wall component of gram-negative bacteria. It binds with macrophages, releasing cytokines, interleukin^{-1}, etc., into the blood circulation leading to antigen-antibody reaction. It reduces the blood brain barrier and release arachidonic acid mediated by the enzymes, phospholipase, prostaglandin E_2 synthase and cyclooxygenase. Finally, synthesis and release of PGE_2 into anterior hypothalamus result in pyrexia.[11]

The present study revealed that AOF herbal formulation possessed significant dose-dependent antipyretic activity in experimental rats. In the antipyretic testing model, 1.5 gm/kg and 2 gm/kg of AOF herbal formulation markedly decreased elevated body temperature. Brewer's yeast-induced fever is called pathogenic fever. Its etiology includes production of prostaglandins, particularly PGE2 appears to be a final pathway responsible for fever production induced by several pyrogens.[12] In this study, it is suggested that the antipyretic effect of AOF herbal formulation may be similar to that of paracetamol.

Many studies have shown that flavonoids are mainly responsible for the activities of wound healing, antioxidant, antimicrobial, anti-inflammatory, analgesic and antipyretic activities.[13-15] Therefore, the observed antipyretic activity might be due to the presence of active constituents like flavonoids, alkaloids and triterpenes.

In this study, percent reduction of pyrexia with paracetamol at the dose of 150 mg/kg was in range from 95±16% to 186± 44%. The range of percent reduction of pyrexia were observed from 81±36% to 89±29% and from 99±46% to 220±123% with the doses of 1.5 gm/kg and 2 gm/kg of AOF herbal formulation, respectively. All these doses (1.5 gm/kg and 2 gm/kg) of AOF herbal formulation and standard drug paracetamol at the dose of 150 mg/kg can reduce the fever up to 4 hours after administration. Therefore, the results of the present study revealed that AOF herbal formulation has a significant antipyretic effect in a dose-dependent manner in yeast-induced pyrexia in albino rats.

Conclusion

The present results showed that AOF herbal formulation possessed a significant antipyretic effect in Brewer's yeast-induced pyrexia in rats. This was evidenced as a natural safe remedy for the treatment of pyrexia. And its effect is comparable to that of paracetamol (standard drug).

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Competing interests

The authors declare that they have no competing interests.

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