



ORIGINAL RESEARCH ARTICLE

OPEN ACCESS

Assessment of analgesic and antipyretic activity of traditional formula used in the treatment of seasonal infections

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ABSTRACT

Traditional treatments and medicines are the main sometimes the only source of health care for millions of people living in rural areas of developing countries. The aim of the present study is to investigate the analgesic and antipyretic activity of individual plant extracts and poly-herbal formula [PHF] made in to liquid dosage form of three different doses in albino rats. The plant materials were extracted in boiling distilled water for six hours, filtered, concentrated and dried. The aqueous extracts were prepared in to poly-herbal liquid dosage form by using water, glycerol mixture and acacia 2% in three different preparation of varying extract doses. 1ml of above preparations orally fed for investigation. Pentozocine and paracetamol was used as standard drugs respectively. For individual plants the extracts were suspended in 2% acacia solution and performed. The results of analgesic and anti-pyretic activity of PHF 1 and PHF 2 showed significant results ($P < 0.01$ and $P < 0.05$ respectively) and PHF3 did not show any significant results. In the individual plant extracts slightly significant activity ($P < 0.05$) was observed.

Key Words: Traditional medicine, poly-herbal, formula, extract, glycerol, aqueous.

INTRODUCTION

Traditional medicines is the sum of total knowledge, skills and practices based on the theories, beliefs, and experiences indigenous to different cultures that are used to maintain health, as well as to prevent, diagnose, improve or treat physical and mental illness (WHO, 2008). Traditional medicines have been used in some communities for thousands of years. As traditional medicine practices are adopted by new population there are challenges.

Traditional medicine practices have been adopted on different culture region without the parallel advance of international standards and method for evaluation. Scientific evidence from tests done to evaluate safety and effectiveness of traditional medicine, products 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 processes. 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 (WHO, 2008).

This poly-herbal formula is used by Valayar tribal community of kundry hill area erode district, Tamilnadu, India for the treatment of seasonal infections. This formula consists of *Andrographis paniculata* (Acanthaceae), *Cardiospermum halicacabum* (Sapindaceae) *Evolvulus alsinodes* (Convolvulaceae), *Cynodon dactylon* (Poaceae)

and *Phyllanthus emblica fruit* (Phyllanthaceae). Aim of the present study was to investigate the potency of analgesic and anti-pyretic activity of the poly-herbal formula made into a liquid dosage form in three different doses.

MATERIALS AND METHODS

Plant materials were collected for our work from and around of Bhavani, Erode, Trichi and Thiruchengodu areas of Tamilnadu, India. The plants *Andrographis paniculata* (Acanthaceae), *Cardiospermum halicacabum* (Sapindaceae), *Evolvulus alsinodes* (Convolvulaceae), *Cynodon dactylon* (Poaceae) and *Phyllanthus emblica fruit* (Phyllanthaceae) were identified by the botanical survey of India, Tamilnadu, Coimbatore. Voucher specimen has been preserved (Ref. no. BSI/SRC/5/23/2010-11/Thec. 1813, 8, 9, 12 & 7, dated 03.02.2011). The above plants hereafter designated as plant 1, 2, 3, 4, 5 as per above order.

Collections

The plants were collected by digging the entire plant, cutting and separating the roots from the whole part of the plant. *Phyllanthus emblica fruit* has been collected from kundry hills.

Treatment

The aerial parts were cut into small pieces by means of stainless steel knife and put on polythene sheet and shade dried. *Phyllanthus emblica fruit* was shade dried and the seeds were removed. The dried material was powdered by means of wood grinder.

Preparation of extracts

The coarse powders of 150gm plant material are first macerated for overnight and each were boiled in distilled water for 6 hours separately. It was filtered while hot and concentrated. The resulting extract was dried, percentage yield was noted and kept in a desiccators. The above dried extracts were used to prepare liquid dosage form and pharmacological studies (Cooper and Gunn, 1986).

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Table 1: Formula for liquid dosage form.

Aqueous Extracts	Poly herbal liquid dosage form		
	1	2	3
<i>Andrographis paniculata</i> 500mg/kg	87.5mg	43.75mg	21.87mg
<i>Cardiospermum halicacabum</i> 500mg/kg	87.5mg	43.75mg	21.87mg
<i>Evolvulus alsinodes</i> 500mg/kg	87.5mg	43.75mg	21.87mg
<i>Cynodon dactylon</i> 600mg/kg	105mg	52.5mg	26.35mg
<i>Phyllanthus emblica</i> 600mg/kg	105mg	52.5mg	26.35mg

Table 2: Analgesic activity of liquid dosage form.

Groups	0 min	30 min	60 min	90 min	120 min
Control	3.16±0.30	3.33±0.33	3.0±0.36	3.50±0.22	3.33±0.21
Standard	3.33±0.21	6.83±0.30**	7.83±0.30**	8.0±0.36**	7.50±0.50**
LDF-1	3.33±0.33	5.00±0.36**	7.16±0.60**	7.83±0.47**	6.16±0.30**
LDF-2	3.50±0.42	4.83±0.30*	4.50±0.22*	4.83±0.16*	4.66±0.21*
LDF-3	3.66±0.42	4.16±0.30	4.33±0.21	4.66±0.33	4.33±0.21

Values are mean±S.E.M; n=6; LDF= Liquid dosage form
* P<0.05 and **P<0.01; as compared to the control

Preparation of poly-herbal liquid dosage form

All the weighed quantities of dried extracts has been mixed in water glycerol 70:30 mixture, and stirred at 3000 rpm using Remi high speed mixture for 1 hour along with acacia 2% as suspending agent. Sodium benzoate 0.5% was used as preservative (Mukesh, 2007). Table 1 shows doses for plant extracts 1, 2, 3, 4, 5 that were determined following previously published literatures (Shivaprakash *et al.*, 2011; Datta *et al.*, 2010; Lekshmi and Reddy, 2011; Garg and Paliwal, 2011; Jaijoy *et al.*, 2010).

Experimental animals

Wistar albino rats weigh about 160-175gms of either sex were procured from Govt. Veterinary College, Trishur, Mannuthi, Kerala. Rats were placed in polypropylene cages in a controlled room temperature 22±1°C and relative humidity of 60-70% in registered animal house (87/1999/CPCSEA). They were maintained with standard pellet diet (Amrut brand sangle, India) and water ad libitum. Animal were acclimatized to laboratory condition for seven days before commencement of any experiment. Ethical clearance was obtained from institutional animal ethical committee.

Chemicals and reagents

The standard drug for analgesic activity is pentazocine and for anti-pyretic effect activity paracetamol is used. All the other chemical and reagents were of pure analytical grade and obtained from local supplier.

Acute oral toxicity

The acute oral toxicity was carried out as per OECD 423-guidelines (Acute toxic class method) for both formulations and individual plant extracts. The acute toxicity studies revealed that there is no toxicity signs are produced up to the dose level 2000mg/kg.

Assessment of analgesic activity

Eddy's hot plate method

Albino rats were divided into five different groups of six animals each. Test groups were fed orally with 1ml poly herbal liquid dosage form F1, F2, and F3, respectively. The hot plate was maintained at 55±0.5°C and the animals were placed into the Perspex cylinder on the heated

Table 3: Analgesic activity of individual plants.

Groups	0 min	30 min	60 min	90 min	120 min
Control	3.00±0.36	3.00±0.36	3.33±0.33	3.33±0.33	3.33±0.21
Standard	3.33±0.31	6.00±0.51**	7.00±0.44**	7.66±0.42**	7.00±0.36**
Plant 1	3.50±0.22	4.33±0.21*	4.83±0.30*	5.16±0.40*	4.66±0.33*
Plant 2	3.16±0.30	4.50±0.22*	4.66±0.21*	4.83±0.30*	4.50±0.34*
Plant 3	3.50±0.22	4.00±0.36	4.16±0.30*	4.66±0.42	4.33±0.33
Plant 4	3.00±0.36	3.83±0.30	3.83±0.33	4.00±0.36	3.83±0.40
Plant 5	3.00±0.36	3.66±0.21	3.83±0.33	4.33±0.33	4.16±0.30

Values are mean±S.E.M; n=6; * P<0.05 and **P<0.01; as compared to the control

surface and time to discomfort reaction like licking paws or jumping was recorded as response latency by using a stop watch. The latency was recorded (Mahesh *et al.*, 2009), after 0 min, 30min, 60min, 90min, 120min, after the administration of test and standard drug. The standard drug used was pentazocine at a dose of 10mg/kg.

Individual plants

Rats were divided in to seven groups of six animals each. Test groups were fed orally with 1 ml of plant extracts in acacia suspension. The latency was recorded after 0min, 30min, 60min, 90min, 120min, after the administration of test and standard drug.

Assessment of anti-pyretic activity

Brewer's yeast induced pyrexia model

Anti-pyretic activity was measured by slightly modifying the method described with Albino rats by Venkatesh *et al.* (2012). Before the experiments, for 18 hours, pyrexia was induced by subcutaneously injecting 10ml/kg brewer's yeast into the animals' dorsum region. 19 hour after the injection, the rectal temperature of each rat was measured by digital thermometer. Only rats showing an increase in temperature of at least 0.7°C were used for experiment. Doses of poly herbal liquid dosage form F1, F2, F3 and individual plant extracts were administered orally and temperatures were measured at 1, 2, 3 and 4 hours after administration.

Individual plants

Rats were divided in to seven groups of six animals each. Test groups were fed orally with 1 ml of plant extracts in acacia suspension. The body temperature was recorded after 1, 2, 3 and 4 hours after the administration of test and standard drug.

RESULTS AND DISCUSSION

Analgesic activity

PHF 1 and PHF 2 significantly (P < 0.01 and P < 0.05 respectively) elevated the mean basal reaction time as compared to control group. The highest nociception inhibition was exhibited by PHF 1 at 30, 60, 90, and 120 minutes but PHF 2 produces slightly significant (P < 0.05) effect at the same time intervals so that the activity is in dose dependent manner. While the individual plant extracts 1 and 2 exhibits slightly significant analgesic activity (P < 0.05) at 30, 60, 90, and 120 minutes. Plant extract 3 shows significant activity (P < 0.05) only at 60 minutes.

The maximum nociception inhibition by pentazocine (P < 0.01) as a standard drug was observed at 30, 60, 90 and 120 minutes. PHF 3 and individual drugs 4 and 5 did not produce any significant nociception inhibition as compared to control group. The observations are given in Table 2 & 3 and Figure 1 & 2.

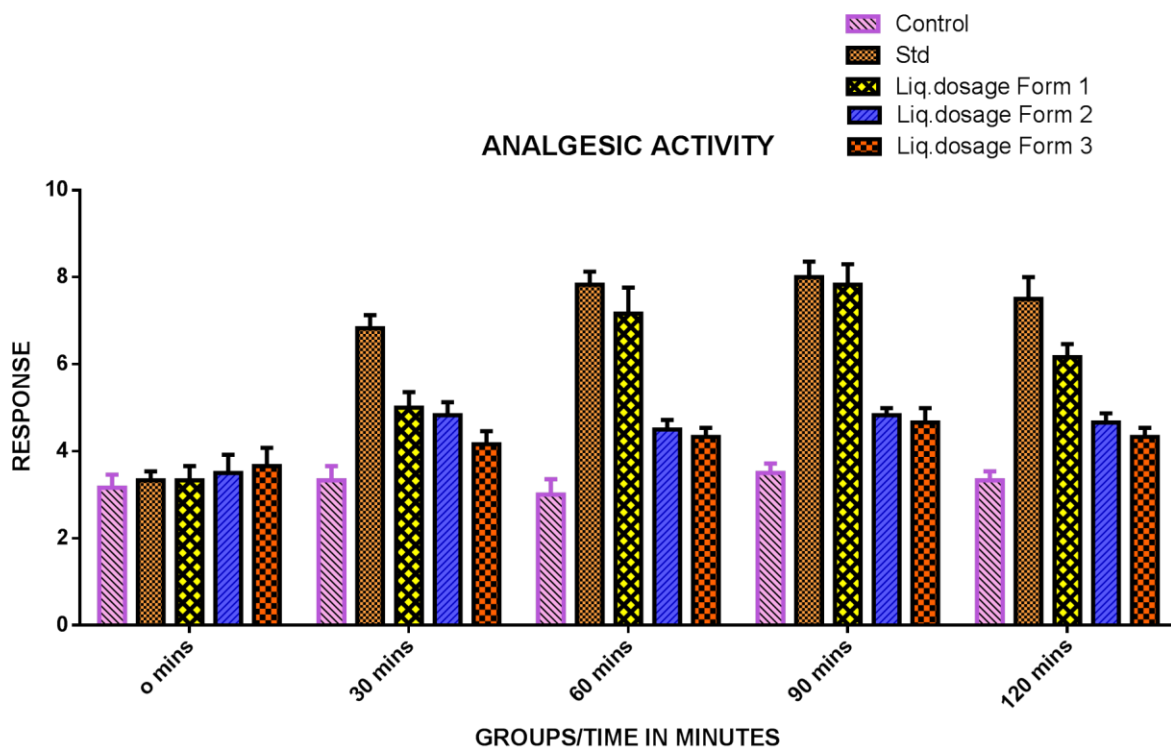


Figure 1: Analgesic activity of liquid dosage form.

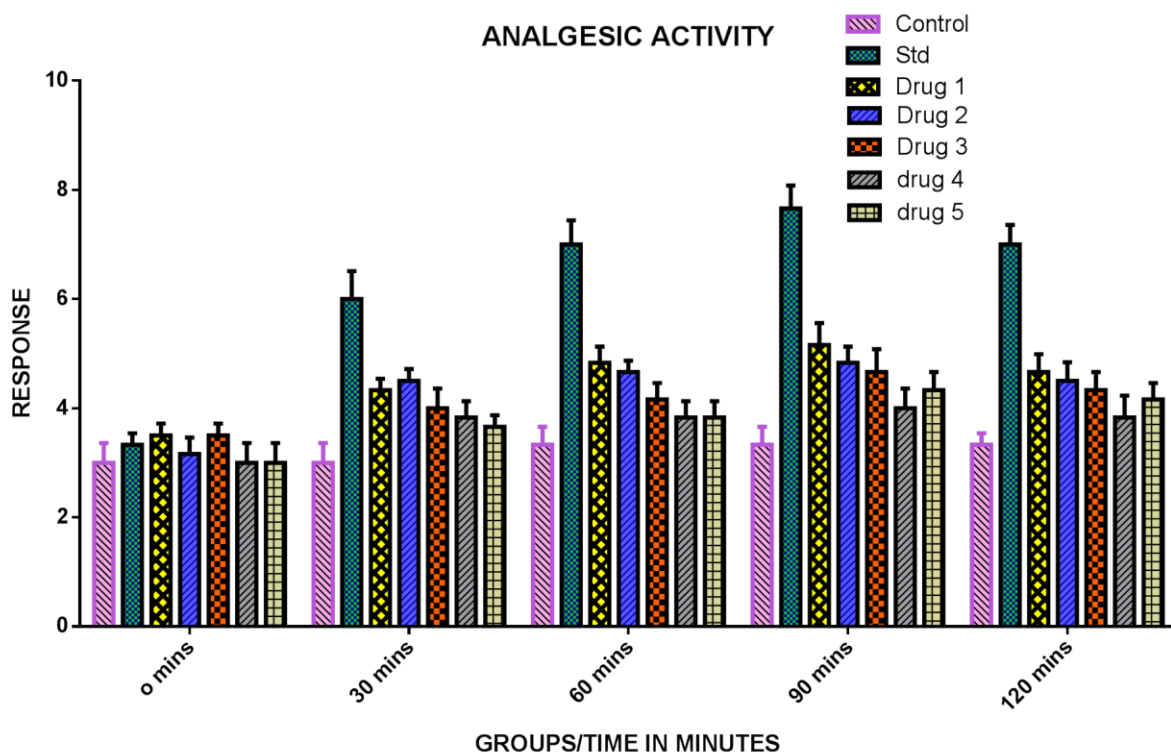


Figure 2: Analgesic activity of individual plants.

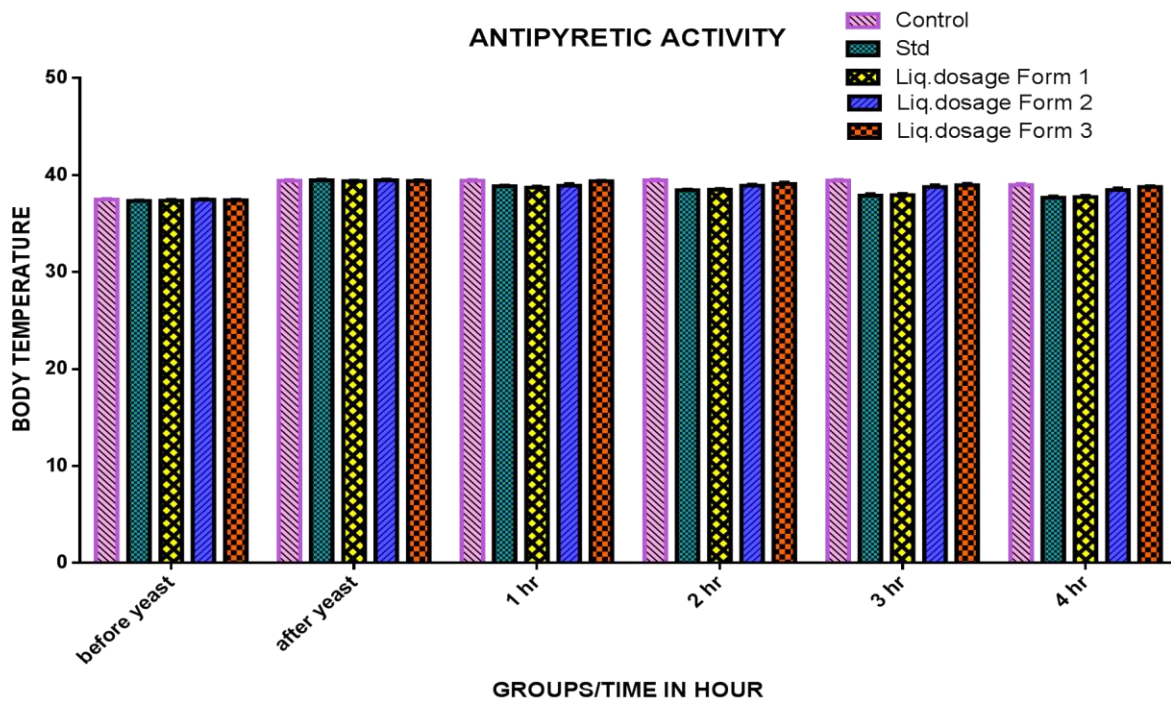


Figure 3: Antipyretic activities of liquid dosage form.

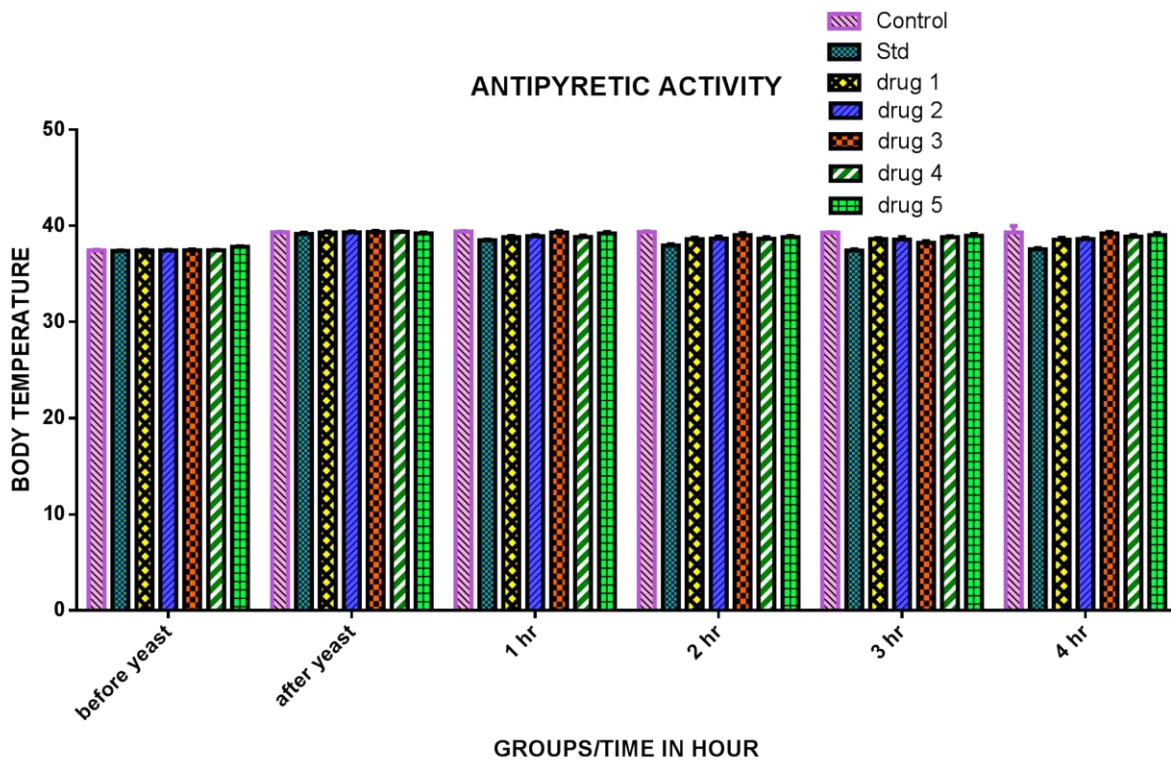


Figure 4: Antipyretic activity of individual plants.

Table 4: Antipyretic activities of liquid dosage form.

Groups	Before yeast	After yeast	1 hr	2 hr	3 hr	4 hr
Control	37.46±0.07	39.40±0.07	39.41±0.09	39.45±0.07	39.43±0.06	38.96±0.11
Standard	37.33±0.04	39.45±0.07	38.85±0.06**	38.43±0.06**	37.88±0.14**	37.66±0.13**
LDF-1	37.35±0.08	39.35±0.08	38.71±0.10**	38.48±0.09**	37.91±0.14**	37.71±0.12**
LDF-2	37.45±0.06	39.45±0.06	38.96±0.16*	38.90±0.10*	38.76±0.17*	38.46±0.16*
LDF-3	37.38±0.07	39.38±0.07	39.35±0.06	39.06±0.17	38.93±0.16	38.75±0.10

Values are mean±S.E.M; n=6; * P< 0.05 and **P< 0.01; as compared to the control; LDF= Liquid dosage form

Table 5: Antipyretic activity of for individual plants.

Groups	Before yeast	After yeast	1 hr	2 hr	3 hr	4 hr
Control	37.45±0.06	39.33±0.04	39.40±0.05	39.35±0.08	39.28±0.06	39.31±0.07
Standard	37.38±0.07	39.16±0.11	38.50±0.07**	37.95±0.14**	37.45±0.10**	37.55±0.13**
Plant 1	37.45±0.06	39.31±0.11	38.81±0.12*	38.61±0.17*	38.63±0.09*	38.56±0.18*
Plant 2	37.45±0.06	39.33±0.09	38.91±0.14*	38.68±0.19*	38.58±0.24*	38.63±0.12*
Plant 3	37.46±0.07	39.35±0.13	39.31±0.14	39.05±0.19	39.25±0.17	38.21±0.15
Plant 4	37.46±0.06	39.38±0.05	38.85±0.13*	38.66±0.17*	38.81±0.10	38.86±0.19
Plant 5	37.83±0.04	39.20±0.10	39.20±0.16	38.81±0.14	38.96±0.18	39.63±0.19

Values are mean±S.E.M; n=6; * P< 0.05 and **P< 0.01; as compared to the control

Antipyretic activity

PHF 1 and PHF 2 significantly ($P < 0.01$ and $P < 0.05$ respectively) decreased the elevated body temperature as compared to control group. The highest inhibition by PHF 1 was produced at 1, 2, 3, and 4 hours but PHF 2 produces slightly significant ($P < 0.05$) activity at the same time intervals so that the activity also is in dose dependent manner. While the individual plant extracts 1 and 2 exhibits slightly significant antipyretic activity ($P < 0.05$) at 1, 2, 3, and 4 hours. Plant extract 4 shows significant activity ($P < 0.05$) only at 1 and 2 hours only.

The maximum antipyretic activity by paracetamol ($P < 0.01$) as a standard drug was observed at 1, 2, 3, and 4 hours. PHF 3 and individual drugs 3 and 5 did not produce any significant reduction in elevated body temperature as compared to control group. The observations are given in Table 4 & 5 and Figure 3 & 4.

CONCLUSION

The results of the present study shows that the poly-herbal formulations possess significant analgesic and antipyretic activities in all the tested experimental animal models indicating inhibition of nociception and reduction in elevated body temperature. The poly-herbal formulation possesses central analgesic activity which was evaluated using hot plate method and antipyretic activity by brewer's yeast induced pyrexia model. According to the data obtained with the various doses of the poly-herbal formulation, it was found that the intensity of the analgesic effect of PHF1 was similar to that of standard drug pentazocine and maximum when compared with individual plant extracts.

The analgesic effect produced by the extract may be via central mechanisms involving opioid receptor systems and regulation of body temperature requires a delicate balance between production and loss of heat, and the hypothalamus regulates the set point at which body temperature is maintained. Yeast induced fever is called pathogenic fever. Its aetiology includes production of prostaglandins, which set the thermoregulatory centre at a lower temperature (Howard, 1993). The present results show that PHF possesses a significant antipyretic effect in yeast-provoked elevation of body temperature in rats, and its effect is comparable to that of paracetamol (standard drug). So inhibition of prostaglandin synthesis could be the possible mechanism of antipyretic action as that of paracetamol (Chandrashekar *et al.*, 2009).

The present results show that PHF1 possesses a significant antipyretic effect in yeast induced elevation of body temperature in rats, and its effect is comparable to that of paracetamol (standard drug). So inhibition of prostaglandin synthesis could be the possible mechanism of antipyretic action as that of paracetamol.

To conclude, it can be stated that the poly-herbal formulation demonstrated potent analgesic and antipyretic effects in a dose dependent manner. This could provide a rationale for the use of this formulation in pain and fever as folk medicine.

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