The Journal of Phytopharmacology 2018; 7(3): 325-333
Online at: www.phytopharmajournal.com

Research Article

ISSN 2320-480X
JPHYTO 2018; 7(3): 325-333
May- June
Received: 14-04-2018
Accepted: 28-05-2018

Amit K Taraphdar
Department of Dravyaguna, Institute of Post Graduate Ayurvedic Education & Research, 294/3/1, Acharya Prafulla Chandra Road, Kolkata, West Bengal - 700 009, India

Arup Mukherjee
Department of Chemical Technology, University College of Technology, University of Calcutta, 92, Acharya Prafulla Chandra Road, Kolkata, West Bengal - 700 009, India

Mradu Gupta
Department of Dravyaguna, Institute of Post Graduate Ayurvedic Education & Research, 294/3/1, Acharya Prafulla Chandra Road, Kolkata, West Bengal - 700 009, India

Correspondence:
Amit K Taraphdar
Department of Dravyaguna, Institute of Post Graduate Ayurvedic Education & Research, 294/3/1, Acharya Prafulla Chandra Road, Kolkata, West Bengal - 700 009, India
Email: mda_amitt[at]rediffmail.com

Antipyretic effect of a polyherbal ayurvedic formulation: A randomized controlled clinical study

Amit K Taraphdar*, Arup Mukherjee, Mradu Gupta

ABSTRACT

The ancient ayurvedic text *Aṣṭāṅgahṛdaya* of *Vāgbhaṭa* (7th Century A.D.) prescribes a specific formulation of four plants having antipyretic properties with minimal side-effects. This polyherbal ayurvedic formulation contains whole plant of *Solanum surratense*, rhizomes of *Zingiber officinale*, stem of *Tinospora cordifolia* and fruits with bracts of *Piper longum*, exhibited significant antipyretic-analgesic properties during rodent experiments without any toxicity may be due to flavonoidic phenolic compounds in it. Present randomized controlled clinical study in sixty eight patients was conducted with this polyherbal ayurvedic formulation using aspirin as standard drug for comparison. The primary outcome measured was reduction in body temperature, while the secondary outcomes measured were assessment of associated symptoms of fever and routine haematological parameters. A representative sample of patients was also studied for reduction in the level of prostaglandin (PGE_2). The clinical study showed that fever was rapidly and substantially reduced after oral administration of the test drug and this antipyretic effect was significant ($p<0.01$) when compared to placebo and more sustained in comparison to aspirin. Many associated symptoms of fever also exhibited significant reductions with this test drug. Prostaglandin levels also registered a substantial decrease during treatment with this polyherbal ayurvedic formulation.

Keywords: Ayurveda, Antipyretic, *Solanum surratense*, *Zingiber officinale*, *Tinospora cordifolia*, *Piper longum*.

INTRODUCTION

Fever is an elevation in the body temperature of warm-blooded animals caused by abnormal functioning of the thermoregulatory mechanism in the central nervous system [1]. The conventional treatment of fever using non-steroidal synthetic antipyretics has been usually associated with gastro-toxicity, nephro-toxicity, hepato-toxicity and affects central nervous system, integumentary system etc. [2] However, polyherbal ayurvedic formulations (PAF) provide treatment of diseases in a holistic approach. The scientific advancement carries with it the improvement in PAF through the study of various phytoconstituents and discovery of useful herbs combinations which work synergistically to produce desirable effect owing to its comparable efficacy, fewer side effects and better acceptability than allopathic drugs [3]. The test drug is a PAF of *Aṣṭāṅgahṛdaya* of *Vāgbhaṭa* for the treatment of *vāta-śleṣmaja jvara*, which may be correlated with acute fever due to upper respiratory tract infection (URTI) and other associated symptoms like uneasy breathing (*śvāsa*), cough (*kāsa*), sinusitis (*pīnas*), headache (*śirośūla*), pain in joints and muscle (*parbaved*) [4]. Characteristic features of *vāta-śleṣmaja jvara* as per *Charakācharya* are cold (*śītaka*) and cough (*kāsa*), heaviness of the body and heavy-eyed (*goūraba*), drowsiness (*tañdṛā*), running nose (*pratīśyaya*), malaise-catarrh (*staīmiṭya*), joint pain (*parbaṛūk*), headache (*śirogṛaha*), cessation of perspiration (*svedāpabartanam*) and moderately high rise of fever (*santāpa madhyavegascha*) [5]. This PAF is comprised of four ayurvedic plants in equal amounts i.e., whole plant of *Kaṇṭakārī* (*Solanum surratense* Burm. f.), rhizomes of *Śuṇṭhī* (*Zingiber officinale* Rosc.), stem of *Guḍūcī* (*Tinospora cordifolia* (Willd.) Miers.) and fruit spikes of *Pippalī* (*Piper longum* Linn.) [4]. This PAF had already been studied for its antipyretic and analgesic efficacy in rodents and found effective [6]. Pharmacognostical, physiochemical, phytochemical screenings including UV-Visible spectroscopic scanning, TLC, HPTLC studies were performed for proper standardization of this formulation [6]. The antipyretic effect was assessed using yeast induced pyrexia model. Hot plate method, tail-flick test and writhing test were used for determining the analgesic properties. Phytochemical analysis revealed the presence of phenols, flavonoids, alkaloids, tannin etc. Significant antipyretic ($P<0.001$) and analgesic ($P<0.01$) properties were noticed in dose dependent manner after aqueous extract administration especially at the dose of 500 mg/kg body weight in rodents. Activities of the test drug were sustained and significantly comparable to the standard drugs without any acute toxicity possibly due to presence of flavonoidic phenolic compounds [6]. There were no scientific documentations found in literature on clinical trials about the antipyretic or analgesic effect of this PAF though a few are

available on individual plant parts of this test drug formulation [7-9]. This research effort was aimed at the clinical evaluation of antipyretic effect of this traditional formulation (*jvarahar yoga*) of four botanicals from *Aṣṭāṅgahṛdayam* for the treatment of fever [4] following scientific methodology. It was, therefore, decided to undertake a clinical study on human subjects to assess the antipyretic efficacy of this PAF by direct measurement of body temperature and assessment of secondary symptoms (like pain, cough etc.) and appropriate supportive laboratory investigations. The objective of this limited study was to confirm and corroborate the overall findings of antipyretic and analgesic effect of this PAF assessed in experimental study [6].

MATERIALS AND METHODS

Preparation of test drug

All the test botanicals in raw form were purchased from local herbal market, Barabazar, Kolkata. These samples were duly authenticated by the office of the Scientist 'F', Central National Herbarium, Botanical Survey of India, Government of India, Botanic Garden, Howrah- 711 103 (Ref No. BSI/CNH/SF/Tech./2015 dated 09.03.2015). A voucher specimen has been deposited in the Dravyaguna laboratory of the Institute of Post Graduate Ayurvedic Education and Research (IPGAER), Kolkata, India for future reference (SVP/PG/72/2015 dated 18.01.2015).

The whole plant of *Solanum surratense*, rhizomes of *Zingiber officinale*, stem of *Tinospora cordifolia* and fruit spikes of *Piper longum* were shade dried in good condition in equal proportion and coarsely powdered up to 40 mesh sizes. Decoction was made with the above mentioned plants parts except *Piper longum* by adding the four times of water and boiling it up to the reduction of one fourth water then added with powder of dried, immature, fruit spikes of *Piper longum* as described in ayurveda [4]. The aqueous extract was filtered through calico cloth and was further concentrated to solid under reduced pressure over water bath in a rotary evaporator below 50^0C. The concentrated extract was collected in petri-dishes and allowed to air-dry for the complete evaporation of water in the absence of sunlight. The whole process was repeated three times and finally blackish green, concentrated extract was obtained (yield 5.91%, w/w). The dry extract after mixing with a non-reactive standard excipient was then capsulated (500 mg/capsule; i.e., 300 mg/capsule, weight of active ingredients) with the help of manual capsule machine in the Pharmacy section of IPGAER, Kolkata in hygienic condition maintaining drug rules. Standardization of test drug capsule was established by doing some studies on physical parameters (i.e., colour of finished powder, texture, smell, taste, weight of active ingredients, disintegration time and dissolution time of capsule etc.) following standard methods [10]. This prepared medicine was termed VSAP4 and used for the clinical study during 2015-16. Similarly aspirin and rice powder were also capsulated (300 mg/capsule, weight of active ingredients) as they looked same with the test drug.

Consent of patients and other ethical aspects

All patients were given verbal and written information about the potential risks and benefits of participation in the study. Written consent was mandatory from each patient before randomization or inclusion in the clinical study of the test drug. The Institutional Ethical Committee for Clinical Research, IPGAER, Kolkata approved the study protocol.

Inclusion and exclusion criteria for selection of patients

Patients aged between 20 to 50 years of both sexes suffering from fever in URTI up to 7 days with associated secondary symptoms (which may or may not be present) i.e., headache *(śirośūla),* pain in joints and muscle *(parbaveḍ)*, malaise-catarrh *(staīmiṭya)*, uneasy breathing *(śvāsa),* cough *(kāsa),* running nose *(pratīśyaya)* and loss of appetite *(arūchi)* [4, 5] were included in the present study.

Patients with temperature more than 39^0C (i.e., >102.2^0F), abnormal pulmonary findings, pleuritis, pneumonia, bronchitis, using warfarin, antibiotics and/or anti-inflammatory drugs, hypersensitivity to NSAIDs, history of trauma, malignancy, diabetes mellitus, high blood pressure, severe systemic and organ disorders, peptic ulcer syndrome, bleeding tendency, major surgery and chronic fever (like malaria, typhoid, tuberculosis etc.), pregnant and lactating females, patients with alcoholism or who were heavy smokers (>20 cigarettes a day) were excluded from the present clinical study [11, 12].

Safety parameters

The investigator recorded adverse effects in the 'Adverse Events Record Form'. Rating the drug safety was based on physical examination, measurement of blood pressure, heart rate, laboratory examination of haematology (leukocytes, erythrocytes, haemoglobin, haematocrit, MCV, MCH), biochemistry (glucose, creatinine, sodium, potassium), routine urine examination (colour, appearance, protein, sugar, urobilinogen, phosphates, RBC, pus cells, parasites, yeast cells etc.) and documentation of adverse effects. Participants were asked about any adverse effects and the answers were recorded by the investigator. The classification of the adverse effects was mild, moderate and severe; the causality of the study medication was determined as definite, probable, possible, unlikely, not related and not possible to judge. All patients were provided with personal telephone number of the investigator for any emergency purposes and also they were instructed to attend nearby health centre if any emergency condition arises. All the patients were regularly contacted through telephone during trial period for monitoring and supervision purposes. No adverse effects were recorded during the study.

Treatment allocation and blinding

Seventy five men and women aged 20 to 50 years with a diagnosis of fever in URTI and related symptoms which may or may not be present, like uneasy breathing, cough, running nose, headache, pain in joints and muscle, malaise-catarrh and loss of appetite were assessed for eligibility for this present study. The eligible patients were 'blinded' to the treatment they received and written informed consent was obtained from every study subject prior to the trial. Ultimately, sixty-eight patients were randomized and allocated into three treatment groups following block randomization method [13] after fulfilling the ethical aspects. This clinical study was a double-blind, randomized, placebo-controlled, parallel-group study involving a total of 68 patients having fever in URTI, while after completion of study 60 patients were analyzed. Randomization and patient flow is shown in Fig.1. The study was carried out at the OPD, IPGAER, Kolkata. The treatment groups included, group S (standard group) received drug aspirin, group D (test drug group) taken VSAP4 and group C (control group) provided with rice powder capsules as placebo. Treatment consisted of oral ingestion of the treatment drug i.e., two capsules three times daily for five days. All patients were advised light diet, plenty of water and rest.

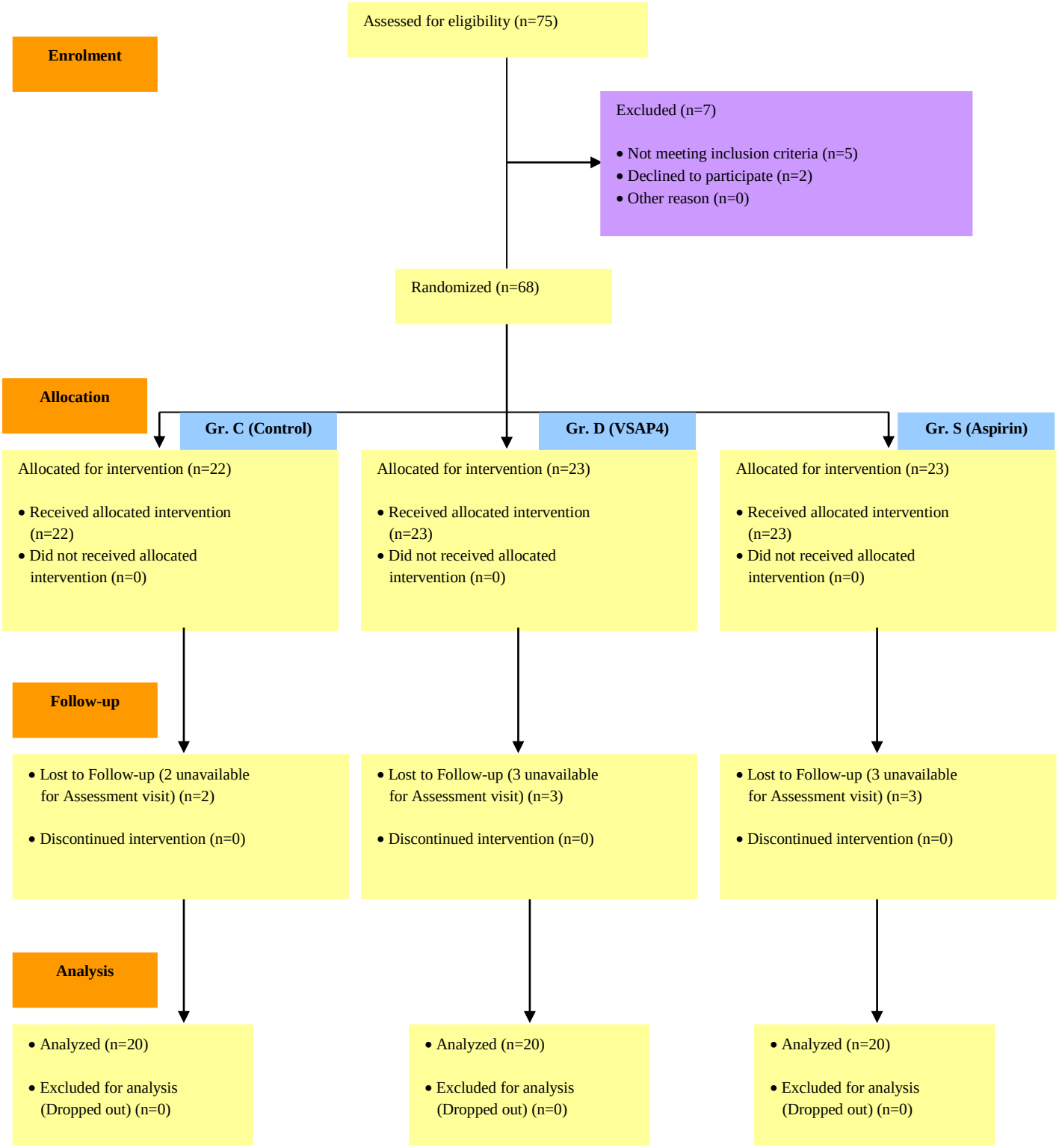

Figure 1: Randomization and patient flow.

The study medication was provided in white paper boxes, numbered consecutively with a medication number. The treatment codes resided with the Professor in-charge of IPGAER, Kolkata and investigator was not aware of treatment assignments. No treatment code was broken before the last follow-up visit was completed or any adverse reaction/ complication arises during clinical study. The pre- and post-treatment data were analyzed on the basis of body temperature measurement, assessment of symptomatic relief of associated symptoms and also haematological parameters of all groups of patients and the results were compared.

Outcome measures

Body temperature measurement

The primary outcome measure in this study was the reduction in body temperature after treatment. This was initially measured and compared on hourly basis and later at progressively larger intervals. The body temperature was measured orally by means of a standard doctor's thermometer in ^{0}F at the start of the treatment and on hourly basis during the first 4 h. It was taken at 4 h intervals during the next 8

h, at 6 h intervals during the subsequent 12 h and at 12 h intervals for the next 2 days. In addition to the temperature measurement done at the OPD of IPGAER, the patients and their attendants were given elaborate instructions to measure the patients' body temperature with standard doctor's thermometer (provided to them free of cost) in ^{0}F and record the same in a blank fever chart supplied to them. Follow-up visits were continued up to fourteen days after the treatment started to record if there was any relapse of fever or complications arises.

Assessment of secondary associated symptoms

The secondary outcome measure was the prevalence of secondary symptoms commonly present in patients suffering from fever in URTI (*vāta-śleṣmaja jvara*) [4, 5]. These symptoms included headache (*śirośūla*), pain in joints and muscle (*parbaveḍ*), cough (*kāsa*), running nose (*pratīśyaya*), uneasy breathing (*śvāsa*), malaise-catarrh (*staīmiṭya*) and loss of appetite (*arūchi*) [4, 5]. All efficacy parameters except the temperature (estimated by clinical digital thermometer) were based on the patient's self-evaluation and recorded by the physician interviewing the patients. The symptoms except temperature ranged from 0 to 4 points (Fig.2). The day of first visit or enlistment of the patient in this clinical trial is considered as visit 1 and subsequent 72 h after 1st visit (i.e., 3rd day of treatment) is considered as visit 2, then 3rd visit on 5th day, 4th visit on 7th day and 5th visit on after another 7 days.

Temperature *(jvara)*	measured orally by clinical digital thermometer in 0^0F at the start	Running Nose *(pratīśyaya)*	0 No 1 Mild 2 Moderate 3 Pronounced 4 Very pronounced
Headache (*śirośūla*)	0 No 1 Mild 2 Moderate 3 Severe 4 Very severe	Uneasy breathing *(śvāsa)*	0 No 1 Mild 2 Moderate 3 Pronounced 4 very pronounced
Pain in joints and muscle *(parbaveḍ)*	0 No 1 Mild 2 Moderate 3 Pronounced 4 Very pronounced	Malaise-catarrhs *(staīmiṭya)*	0 None 1 Slight 2 Moderate 3 Pronounced 4 Very pronounced
Cough, frequency *(kāsa)* 0 No	1 Mild 2 Moderate 3 Pronounced 4 Very pronounced	Loss of appetite *(arūchi)*	0 None 1 Slight 2 Moderate 3 Pronounced 4 Very pronounced

Figure 2: Patient evaluation form.

Assessment of haematological and bio-chemical parameters

As a tertiary outcome measure, the haematological examination (leukocytes, erythrocytes, haemoglobin, ESR) were done to all the patients on 1st and 7th day. Prostaglandin E_2 (PGE_2) is a very potent fever producing autacoids metabolite from arachidonic acid. Most non-steroidal antipyretics such as aspirin and ibuprofen inhibit fever by blocking the biosynthesis of prostaglandins within the endothelium of the hypothalamic vasculature [1, 14]. These findings suggest that the antipyretic effect of VSAP4 can also be estimated by measuring its impact on prostaglandin levels during treatment. Therefore, a representative sample of 18 patients, six each belonging to the three treatment groups, was taken up for measurement of PGE_2. The objective of this limited study was to confirm and corroborate the overall findings of antipyretic effect assessed during the clinical study. The haematological and biochemical investigations of the clinical subjects before and after the clinical trial period were done in a NABL accredited laboratory in Kolkata (*M/s* Suraksha Diagnostics, Kolkata-700054).

Statistical analysis

The results obtained were presented as mean±SEM. Analysis of variance was performed by ANOVA procedures [15, 16]. Values of $p<0.05$ were considered statistically significant, $p<0.01$ were considered very significant and values of $p<0.001$ were taken as highly significant.

RESULTS

Demographic profile

A total of 60 patients (34 female and 26 male) were analyzed after receiving trial medication in this present clinical study. The patients were of an average age of 34.87 years. More than sixty three percent (63.33%) of the patients belonged to the minority Muslim community, while 76% resided in an urban or semi-urban area. There was no significant group difference with regard to distribution of sex, age, community, habitat or occupation. A total of 15 patients who did not

participate in the entire trial or did not turn up for regular follow-up visits or taken other drugs for relief of symptoms during study period were excluded from the study. Trial drug was used among the patients after assessing physical standards of the prepared medicine and found dark brown colour of finished drug powder, sweet-pungent smell, bitter taste, 300 mg/capsule weight of active ingredients (i.e., 60% of 500 mg/capsule total weight), 5.6 min disintegration time and 40 min dissolution time of VSAP4 capsules with minimum six months of self life.

Evaluation of antipyretic effect

The primary outcome measure i.e., symptom fever by measuring the temperature initially hourly and gradually increasing the interval of measuring temperature up to 72 h reveals steady reduction of body temperature up to 4 h and raised during 8 h, again reduced on 18 h, then elevated again on 24 h, once more decreased on 36 h and yet again elevated during 48 h, after that reduced on 60 h. This pattern of body temperature variation was observed in both with test drug VSAP4 and standard drug aspirin up to 60 h. Almost normal body temperature was achieved with VSAP4 and aspirin treated standard group during 4 h, 12 h, 18 h, 36 h and 60 h. During 72 h the body temperature of VSAP4 treated group reached at normal level but the body temperature of the aspirin treated group patients was substantially higher at this time point (Fig.3). In control group the pattern of temperature variation was not significant and persisted up to 72 h and never reached the normal range.

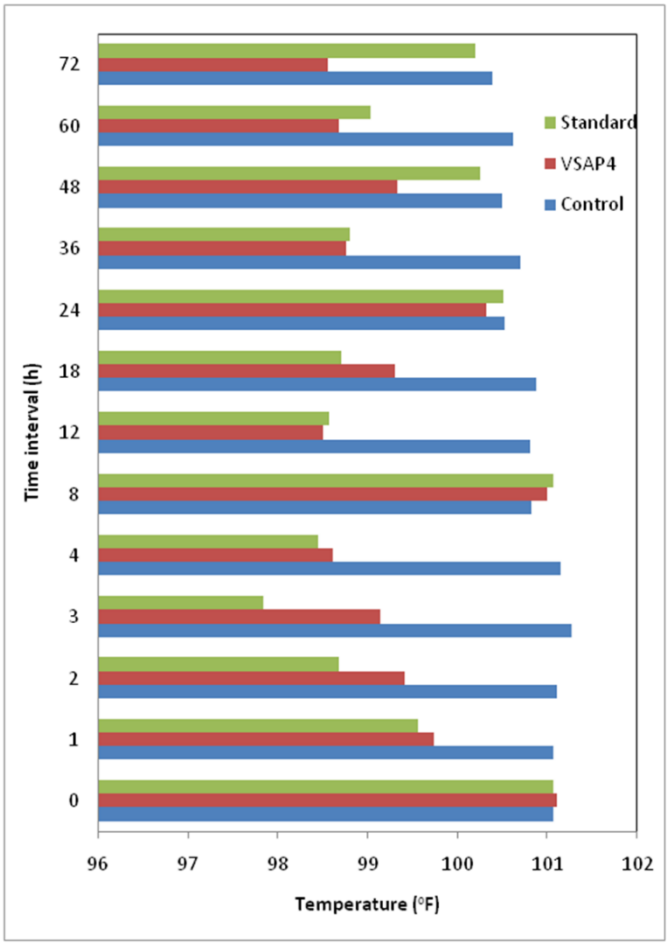

Figure 3: Temperature reading of patients in different groups from 0h to 72h in diverse time interval during treatment.

The mean degrees of fever, defined as the average body temperature in ^{0}F in excess of 98.6^{0}F, recorded in respect of the various groups over the study duration is shown in Table 1. During the clinical study, there was no appreciable change in the level of fever in the control group (group C) during the first 48 h, since the degrees of fever ranged between 1.90 to 2.46^{0}F. Even after 72 h, there was a persistent and substantial level of fever (1.79^{0}F). In case of VSAP4 treated group (group D), the mean degree of fever were 2.50^{0}F at 0 h, 2.40^{0}F at 8 h, and 1.720F at 24 h, 0.69^{0}F at 48 h and almost at zero level at 72 h. The periodic peaking of fever levels just before the drug administration also showed a clear trend of sharp decrease as compared to the initial fever level. In the aspirin treated group (group S), the mean degree of fever decreased more rapidly and substantially within 1 h of the administration of the first drug dose in comparison to VSAP4 treated group and this trend continued up to 4 h and remain almost at zero level. The mean degrees of fever were 2.47^{0}F at 0 h, 2.47^{0}F at 8 h, 1.91^{0}F at 24 h, 1.65^{0}F at 48 h and 1.40^{0}F at 72 h in aspirin treated group. The periodic peaking of fever levels just before the drug administration not showed any clear trend of decrease as compared to the initial fever level as in VSAP4 treated group. The results obtained were found to be significant ($p<0.01$) during ANOVA analysis.

Table 1: Mean degree of fever during clinical study.

Time (h)	Control (Group C)	VSAP4 Group D)	Standard (Group S)
0	2.46 ± 0.18	2.50 ± 0.22	2.47 ± 0.19
1	2.52 ± 0.19	1.13 ± 0.16	0.95 ± 0.16
2	2.56 ± 0.17	0.81 ± 0.13	0.26 ± 0.07
3	2.67 ± 0.13	0.54 ± 0.09	0.08 ± 0.03
4	2.60 ± 0.14	0.14 ± 0.01	0.02 ± 0.01
8	2.22 ± 0.19	2.40 ± 0.15	2.47 ± 0.17
12	2.21 ± 0.18	0.10 ± 0.03	0.01 ± 0.01
18	2.28 ± 0.19	0.63 ± 0.11	0.01 ± 0.002
24	1.92 ± 0.19	1.72 ± 0.18	1.91 ± 0.21
36	2.15 ± 0.17	0.07 ± 0.02	0.01 ± 0.01
48	1.90 ± 0.12	0.69 ± 0.09	1.65 ± 0.21
60	2.01 ± 0.17	0.02 ± 0.01	0.17 ± 0.08
72	1.79 ± 0.17	0.01 ± 0.002	1.40 ± 0.2

Significance related to control; $p<0.01$ (ANOVA test); Values expressed are Mean ± SEM (n = 20)

Evaluation of secondary outcome by assessing associated symptoms

All the patients were assessed for the presence of each of the seven pre-determined associated secondary symptoms namely headache, pain in joints and muscle, cough, running nose, uneasy breathing, malaise-catarrh and loss of appetite before start of treatment (visit 1) and after 72 h or third day during treatment (visit 2). The results of the individual mean scores, as shown in Table 2, clearly demonstrate two things: (1) a significant difference between the test drug treated and control group as well as standard and control group at visit 1 and visit 2, and (2) an low *P* value for the difference between treatment groups and placebo group, i.e., with respect to the improvement between visit 1 and visit 2 ($P<0.01$). It can be clearly seen (Table 2) that the difference in individual mean symptoms between visit 1 and visit 2 was markedly greater for the test drug treatment group (1.46) than for the placebo group (0.43) and standard aspirin treated group (1.07). In order to achieve more detailed information on the study, the mean

values and standard error of mean (SEM) of the individual symptoms were examined (Table 3). Four symptoms namely cough (frequency), running nose, malaise, loss of appetite differ considerably from the aspirin treated group and all seven symptoms differ significantly from control group in respect to VSAP4 treated group. Even a cursory look at these mean values indicates that they are responsible for the earlier observed difference in the mean score value between these groups. Highly appreciable relief from the associated secondary symptoms of headache, pain in joints and muscle, cough, running nose, uneasy breathing, malaise-catarrh and loss of appetite were observed in case of the VSAP4 treated group as compared to the control group. Secondary outcome was also measured with percentage inhibition of associated symptoms among various groups (Table 3). It reveals from the data, that all the associated symptoms were significantly reduced between visit 1 and visit 2 with VSAP4 and aspirin in comparison to control group. Headache and pain in joints and muscle these two associated symptoms were reduced more by aspirin in comparison to VSAP4. Other associated symptoms like cough, running nose, uneasy breathing, malaise-catarrh and loss of appetite were reduced more with VSAP4 in comparison to aspirin.

Table 2: Mean and SEM of individual mean associated symptoms.

Group	Visit 1		Visit 2		Difference visit 1-2	
	Mean	SEM	Mean	SEM	Mean	SEM
Control (C); n=20	1.46	0.08	1.03	0.07	0.43	0.05
Test drug (D); n=20	1.66	0.08	0.20	0.04	1.46	0.07
Standard (S); n=20	1.53	0.09	0.46	0.08	1.07	0.05

Significance related to control; $p<0.01$ (ANOVA test); Values expressed are Mean ± SEM (n=20)

Table 3: Single mean secondary symptoms with percentage inhibition between two visits among the study groups.

	Symptoms	Group	Visit 1		Visit 2		Percentage (%) Inhibition (between 2 visits)
			Mean	SEM	Mean	SEM	
1	Headache *(śirośūla)*	Control (C)	1.90	0.18	1.45	0.19	-
		Test Drug (D)	2.25	0.20	0.35	0.11	47.43
		Standard (S)	2.05	0.25	0.30	0.11	52.42
2	Pain in joints & Muscle *(Parbaveḍ)*	Control (C)	1.15	0.16	0.65	0.15	-
		Test Drug (D)	1.30	0.18	0.15	0.08	39.62
		Standard (S)	1.25	0.23	0.20	0.09	47.72
3	Cough-frequency *(kāsa)*	Control (C)	0.70	0.16	0.65	0.17	-
		Test Drug (D)	0.85	0.19	0.53	0.07	30.51
		Standard (S)	0.90	0.20	0.70	0.19	15.08
4	Running Nose *(pratīśyaya)*	Control (C)	1.05	0.17	0.80	0.16	-
		Test Drug (D)	1.50	0.14	0.49	0.08	43.52
		Standard (S)	1.30	0.15	0.70	0.15	22.34
5	Uneasy breathing *(śvāsa)*	Control (C)	0.85	0.19	0.55	0.15	-
		Test Drug (D)	0.95	0.19	0.15	0.11	48.92
		Standard (S)	0.80	0.16	0.35	0.13	20.96
6	Malaise-catarrh *(staīmiṭya)*	Control (C)	2.20	0.16	1.90	0.16	-
		Test Drug (D)	2.45	0.14	0.85	0.13	51.67
		Standard (S)	2.35	0.13	1.30	0.19	31.04
7	Loss of appetite *(arūchi)*	Control (C)	2.35	0.11	1.20	0.17	-
		Test Drug (D)	2.30	0.18	0.45	0.08	31.49
		Standard (S)	2.05	0.22	0.65	0.26	19.35

Significance related to control; $p<0.05$ (ANOVA test); Values expressed are Mean ± SEM (n = 20)

Assessment of haematological parameters and prostaglandin levels during treatment

The percentage inhibition in the values of haematological parameters taken during the treatment is shown in Table 4. The haematological examination of all the patients suggested that total leucocytes count (/cu mm) and neutrophils (/cu mm) were more inhibited by the test drug VSAP4 and standard drug aspirin in comparison to control group during treatment. ESR (mm) also decreased considerably in case of VSAP4 and aspirin treated group in comparison to control during treatment period. The inhibition of the above haematological parameters was slightly more with VSAP4 in comparison to aspirin treated group. Haemoglobin (mean g%) and total erythrocyte count (mill/cu mm) were less inhibited with VSAP4 in comparison to control and aspirin treated group. Other haematological parameters like lymphocytes, monocytes, eosinophil etc. were not altered considerably during trial.

Table 4: Inhibition (%) in haematology parameters during clinical study.

Parameters		Control (%)	VSAP4 (%)	Standard (%)
1	Mean g(%) of haemoglobin	4.34 ± 0.69	0.51 ± 0.02	2.56 ± 0.08
2	Erythrocyte (mill/cu mm)	3.81 ± 0.55	0.67 ± 0.03	2.48 ± 0.96
3	Leucocytes (/cu mm)	0.98 ± 0.05	17.74 ± 2.77	9.01 ± 1.21
4	Neutrophil (/cu mm)	0.81 ± 0.09	6.15 ± 0.92	4.32 ± 0.18
5	Lymphocyte (/cu mm)	-0.23 ± 0.07	0.51 ± 0.08	0.39 ± 0.07
6	Monocyte (/cu mm)	0.09 ± 0.02	0.31 ± 0.06	0.22 ± 0.10
7	Eosinophil (/cu mm)	1.70 ± 0.07	2.36 ± 0.53	1.90 ± 0.28
8	Basophil (/cu mm)	0.09 ± 0.02	0.05 ± 0.01	0.07 ± 0.02
9	ESR (mm)	-4.91 ± 0.86	7.38 ± 1.72	3.86 ± 0.59

Values expressed are Mean ± SEM (n=20)

The decrease in the PGE_2 levels in the representative sample of patients belonging to the three groups during treatment is shown in Table 5. The overall PGE_2 level registered a decrease of 14.09% in case of the VSAP4 treated group and 13.96% in case of the aspirin treated group, while in case of the control group the decrease was only 3.82%.

Table 5: Prostaglandin E2 (PGE2) level inhibition in patients during clinical study.

Sl. No.	Groups	Inhibition (%)
1	Control	3.82 ± 0.43
2	VSAP4	14.09 ± 0.37
3	Standard	13.96 ± 0.33

Significance related to control; $p<0.05$ (ANOVA test);
Values expressed are Mean ± SEM (n = 6)

DISCUSSION

Present clinical study of antipyretic effect of an ayurvedic polyherbal formulation (VSAP4) from ancient ayurvedic text *Aṣṭāṅgahṛdaya* of *Vāgbhaṭa* (7th Century A.D.), comprising four botanicals namely *Solanum surratense*, *Zingiber officinale*, *Tinospora cordifolia* and *Piper longum* was carried out in patients suffering from fever in upper respiratory tract infection (*vāta-śleṣmaja jvara*) after obtaining significant result in experimental studies in antipyretic, analgesic rodent models including pharmacognostic, phytochemical and chromatographic studies of the test drug [6].

The clinical study showed that fever was rapidly and substantially reduced in case of patients who were administered the test drug ($P<0.01$) in comparison to control. The antipyretic effect of VSAP4 was also more sustained in nature when compared to the standard drug aspirin. The antipyretic action of aspirin is due to the inhibition of the prostaglandin which is responsible for fever and other pharmacological activities [17]. Therefore, the similar trend of temperature reduction with test drug indicates the antipyretic action of VSAP4 may be due to prostaglandin inhibition.

The secondary symptoms like headache, pain in joints and muscle, cough, running nose, uneasy breathing, malaise-catarrh and loss of appetite had been significantly reduced with VSAP4 treated group than the control group. Also all the associated symptoms were more reduced than the aspirin treated group except headache and pain in joints and muscle, which were more reduced with aspirin.

These primary and secondary outcome parameters for assessing the efficacy of VSAP4 from the pre- and post- treatment evaluation were substantiated by changing the biochemical analysis of blood in the study groups. Decrease of leucocytes, neutrophil counts and ESR value indicates the reduction of infections and other aetiological factors responsible to the pyrexia and other secondary symptoms. Observation reveals that test drug produces slightly more inhibition of these parameters than aspirin. Total erythrocyte count, mean g (%) of haemoglobin are also less inhibited by VSAP4 in comparison to control and aspirin treated groups. The symptomatic relief of associated symptoms with the test drug may be due to the changes of the above mentioned biochemical parameters.

Laboratory investigations of PGE_2 level in case of the representative samples registered a substantial decrease during treatment with VSAP4 and aspirin, which is consistent with and corroborates the overall findings regarding the antipyretic efficacy of the test drug during clinical study. The prostaglandin analysis of the pre- and post-treatment of a representative samples were done. This inhibition of PGE_2 indicates the reduction of temperature and pain like other associated symptoms during clinical trial. Prostaglandin E_2 is a very potent fever producing autacoids metabolite from arachidonic acid. Most non-steroidal antipyretics such as aspirin, ibuprofen etc. inhibit fever by blocking the bio-synthesis of prostaglandins within the endothelium of the hypothalamic vasculature [17]. These findings suggest that the antipyretic effect of the test drug can also be estimated by measuring its impact on the prostaglandin levels during treatment.

Significant and sustained antipyretic effect is observed with test drug in this present clinical study in comparison with aspirin. Besides reducing the primary symptom fever, test drug also alleviate the secondary associated symptoms like headache, pain in joints and muscle, cough, running nose, uneasy breathing, malaise-catarrh and loss of appetite without producing any side effects to the patients, assessed by direct measurement of body temperature and evaluation of secondary symptoms associating appropriate laboratory investigations.

As per the modern patho-physiology concept, fever is produced due to exogenous pyrogens that act on the host cells and produce endogenous pyrogens in the form of cytokines, which are regulatory polypeptides. The endothelial cells of anterior hypothalamus release arachidonic acid metabolites when exposed to these endogenous pyrogenic cytokines [18]. Prostaglandin E_2 is one of the arachidonic acid metabolite and a very potent fever producing autacoid that affects the

hypothalamus receptors, raising the thermo-regularity set point and causing hyperthermia [1, 14]. In such an event, management of symptomatic fever ailments is undertaken with nonsteroidal anti-inflammatory and antipyretic chemicals in modern medicine that associate significant risk from gastro-toxicity, hepato-toxicity, nephro-toxicity etc. [2] Polyherbal formulations have been commonly used in ayurveda with the objective of holistic treatment of the disease and its associated symptoms using synergic effect of the constituent medicinal plants [3]. Flavonoids and other phenolic compounds were reported in most of the constituent plants [19-29] of test drug formulation (VSAP4) and were observed present in spot test with test drug extract [6]. Most of these medicinal plants of test drug have been reported to exhibit pharmacological actions such as anti-inflammatory, analgesic, antipyretic, hepato-protective, anti-microbial properties [20-29]. Preliminary phytochemical screening of the test drug extract indicated the presence of phenols, tannins, flavonoids, alkaloids, reducing sugars, saponin and glycosides [6]. Flavonoids have been known to exhibit strong antipyretic properties as well as anti-oxidant properties and are well known for their ability to inhibit pain perception [30-32]. Flavonoids and its related compounds also exhibit inhibition of arachidonic acid peroxidation, which results in reduction of prostaglandin levels thus reducing the fever [33]. Since flavonoids exhibit several biological effects such as anti-inflammatory, anti-microbial, antipyretic, analgesic, anti hepatotoxic and anti-ulcer activities [30-32], it is likely that the antipyretic action of VSAP4 is primarily related to the presence of flavonoidic phenolic compounds.

CONCLUSION

Evaluation of the antipyretic and analgesic action of test drug (VSAP4) during clinical study indicates that this polyherbal preparation exhibits significant antipyretic and peripheral analgesic efficacy that is substantial and sustained in nature and comparable in strength to some of the existing chemical antipyretic and analgesics such as aspirin. The antipyretic and analgesic effects of the test drug was validated and found to be significant following statistical analysis. The use of VSAP4 also led to a substantial reduction in the incidence of associated secondary physical parameters of fever. The antipyretic and analgesic action was also confirmed by a definite lowering of the prostaglandin levels in the representative sample. The antipyretic and analgesic properties of this formula as described in the ayurvedic text *Aṣṭāṅgahṛdayam* [4] were evaluated and assessed using modern scientific techniques in the present clinical study. The overall research findings corroborate and validate the antipyretic and analgesic efficacy of this polyherbal ayurvedic *jvarahar yoga* (antipyretic drug).

Acknowledgments

This work was done by Dr. Taraphdar as a part of PhD research work under the West Bengal University of Health Sciences, Kolkata, India under guidance of Prof. Gupta and Prof. Mukherjee. Authors are thankful to the Director of Ayurveda, Department of Health & Family Welfare, Government of West Bengal for giving necessary permission and facilities to conduct this study.

REFERENCES

1. Kasper DL, Fauci AS, Hauser SL, Longo DL1, Jameson JL, Loscalzo J. Harrison's principles of internal medicine. 19th ed. McGraw Hill Education, New York, 2015, 104-108.
2. Suleyman H, Demircan B, Karagoz Y. Anti-inflammatory and side effects of cyclooxygenase inhibitors. Pharmacol Rep, 2007; 59(3):247-258.
3. Parasuraman S, Thing GS, Dhanaraj SA. Polyherbal formulation: concept of ayurveda. Pharmacogn Rev, 2014; 8(16):73-80.
4. Sastri-Paradakara HS, ed. Astangahridaya of Vagbhata, Chikitsa Sthana; Jvarchikitsitam: Chapter 1, Verse 61. Chaukhamba Sanskrit Santhan, Varanasi, 2015, 555.
5. Kushwaha HCS, ed. Caraka-saṃhitā of Carakāchārya, Vol.II, Chikitsa Sthana; Jvarchikitsitam: Chapter 3, Verse 86-87. Chaukhamba Orientalia, Varanasi, 2009, 87.
6. Taraphdar AK, Mukherjee A, Gupta M. Pharmacological evaluation of antipyretic and analgesic effects of an ayurvedic polyherbal formulation in rodents. International Journal of Current Medical and Pharmaceutical Research. 2016; 2(2):399-406.
7. Govindan S, Viswanathan S, Vijayasekaran V, Alagappan R. A pilot study on the clinical efficacy of *Solanum xanthocarpum* and *Solanum trilobatum* in bronchial asthma. J Ethnopharmacol, 1999; 66(2):205-210.
8. Mustafa T, Srivastava KC. Ginger (*Zingiber officinale*) in migraine headache. J Ethnopharmacol, 1990; 29(3):267-273.
9. Kishore P, Pandey PN, Ruhil SD. Role of *sunthi-guduchi* in the treatment of *amavata*-rheumatoid arthritis. Journal of Research in Ayurveda and Siddha. 1980; 1(3):417-428.
10. World Health Organization. Quality control methods for medicinal plant materials. World Health Organization, Geneva, 1998. (http://www.who.int/iris/handle/10665/41986)
11. Sen SK. Essentials of Clinical Diagnosis, 7th ed. The Standard Book House, Calcutta, 1998, 34-36.
12. Gabrielian ES, Shukarian AK, Goukasova GI, Chandanian GL, Panossian AG, Wikman G, *et al*. A double blind, placebo-controlled study of *Andrographis paniculata* fixed combination *Kan Jang* in the treatment of acute upper respiratory tract infections including sinusitis. Phytomedicine, 2002; 9(7):589-597.
13. Efird J. Blocked randomization with randomly selected block sizes. Int J Environ Res Public Health, 2011; 8(1):15-20.
14. Bennett J, Plum F ed. Cecil Textbook of Medicine (Prism Indian Edition, 20th ed.). WB Saunders Co., Philadelphia, 1996, 1532-1535.
15. Mahajan BK. Methods in Biostatistics. 6th ed. Jaypee Brothers, New Delhi, 2006, 44-143.
16. Pipkin FB. Medical Statistics Made Easy. Churchill Livingstone, Edinburgh London Melpourne and New York, 1984.
17. Winter CA, Porter CC. Effect of alterations in the side chain upon anti-inflammatory and liver glycogen activities of hydrocortisone esters. J Am Pharm Assoc Am Pharm Assoc, 1957; 46(9):515-519.
18. Leon LR. Cytokine regulation of fever: studies using gene knockout mice. J Appl Physiol. 1985, 2002; 92(6):2648-2655.
19. Kusano G, Beisler J, Sato Y. Steroidal constituents of *Solanum xanthocarpum*. Phytochemistry, 1973; 12(2):397-401.
20. Gangwar AK, Ghosh AK, Saxena V. Phytochemical screening and analgesic activity of *Kantkari*. International Journal of Herbal Medicine. 2013; 1(2):177-186.
21. Meena AK, Rao MM, Kandale A, Sharma K, Singh U, Yadav A. Evaluation of Physiochemical and standardisation parameters of *Solanum xanthocarpum* Schrad. & Wendl. International Journal of Chemical and Analytical Science. 2010; 1(3):47-49.
22. Mascolo N, Jain R, Jain SC, Capasso F. Ethnopharmacologic investigation of ginger (*Zingiber officinale*). J Ethnopharmacol. 1989; 27(1-2):129-40.
23. Connell DW. The chemistry of the essential oil and oleoresin of ginger *(Zingiber officinale* Roscoe). Flavour Industry, 1970; 1:677-93.
24. Sinha K, Mishra NP, Singh J, Khanuja SPS. *Tinospora cordifolia* (*Guduchi*), a reservoir plant for therapeutic applications: A review. Indian Journal of Traditional Knowledge. 2004; 3(3):257-270.
25. Singh SS, Pandey SC, Srivastava S, Gupta VS, Patro B, Ghosh AC. Chemistry and medicinal properties of *TINOSPORA CORDIFOLIA* (*GUDUCHI*). Indian Journal of Pharmacology. 2003; 35:83-91.
26. Upadhyay AK, Kumar K, Kumar A, Mishra HS. *Tinospora cordifolia* (Willd.) Hook. f. and Thoms. (*Guduchi*)- validation of the ayurvedic pharmacology through experimental and clinical studies. International Journal of Ayurveda Research. 2010; 1(2):112-121.
27. Mishra P. Isolation, spectroscopic characterization and computational modelling of chemical constituents of *Piper longum* natural product. International Journal of Pharmaceutical Sciences Review and Research. 2010; 2(2):016.
28. Sunila ES, Kuttan G. Immunomodulatory and antitumor activity of *Piper longum* Linn. and piperine. J Ethnopharmacol. 2004; 90(2-3):339-346.
29. Stohr JR, Xiaso PG, Bauer R. Constituents of Chinese piper species and their inhibitory activity on prostaglandin and leukotriene biosynthesis *in vitro*. J Ethnopharmacol. 2001; 75(2-3):133-139.

30. Raj NK, Sripal RM, Chaluvadi MR, Krishna DR. Bioflavonoids classification, pharmacological, biochemical effects and therapeutic potential. Ind J Pharmacol. 2001; 33:2-16.
31. Nijveldt RJ, van Nood E, van Hoorn DE, Boelens PG, van Norren K, van Leeuwen PA. Flavonoids: A review of probable mechanisms of action and potential applications. Am J Clin Nutr. 2001; 74(4):418-425.
32. Sawadogo WR, Meda A, Lamien CE, Kiendrebeogo M, Guissou IP, Nacoulma OG. Phenolic content and antioxidant activity of six acanthaceae from *Burkina Faso*. J Biol Sci. 2006; 6(2):249-252.
33. Baumann J, von Bruchhausen F, Wurm G. Flavonoids and related compounds as inhibition of arachidonic acid peroxidation. Prostaglandins. 1980; 20(4):627-639.

HOW TO CITE THIS ARTICLE
Taraphdar AK, Mukherjee A, Gupta M. Antipyretic effect of a polyherbal ayurvedic formulation: A randomized controlled clinical study. J Phytopharmacol 2018; 7(3):325-333.