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Evaluation of Antipyretic Effect of a Traditional Polyherbal Preparation: A Double-Blind, Randomized Clinical Trial

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Abstract: The ancient Ayurvedic text *Charak samhita* of Indian medicine prescribes a specific group of ten plants having antipyretic properties with minimal side-effects. The aqueous extract of polyherbal ayurvedic preparation PD-10 (from the roots of *Hemidesmus indicus* R. Br. (Asclepiadaceae), *Rubia cordifolia* L. (Rubiaceae), *Cissampelos pareira* L. (Menispermaceae), fruits of *Terminalia chebula* Retz. (Combretaceae), *Emblica officinalis* Gaertn. (Euphorbiaceae), *Terminalia bellirica* Roxb. (Combretaceae), *Vitis vinifera* L. (Vitaceae), *Grewia asiatica* L. (Tiliaceae), *Salvadora persica* L. (Salvadoraceae) and granules of *Saccharum officinarum* L. (Poaceae)) exhibited significant antipyretic-analgesic properties during rodent experiments while exhibiting low toxicity and ulcerogenicity. The presence of flavonoids, tannins and polyphenols in this extract prompted this double-blind, randomized clinical trial on 60 patients using Aspirin (60 mg kg^{-1} body weight per day) as the standard drug for comparison. The primary outcome measured was reduction in body temperature, while the secondary outcomes measured were prevalence of associated symptoms of fever and routine blood and urine parameters. A representative sample of patients was also studied for reduction in the level of Prostaglandin (PGE_2). The clinical trial showed that fever was rapidly and substantially reduced after oral administration of PD-10 and this antipyretic effect was more sustained and highly significant ($p<0.001$) when compared to Aspirin. Many associated symptoms of fever also exhibited significant reductions when PD-10 was administered as compared to Aspirin. Prostaglandin levels also registered a substantial decrease during treatment with the test drug.

Key words: Polyherbal preparation, antipyretic, clinical trial, prostaglandin

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 (Fauci *et al*., 1998). The conventional treatment of fever using non-steroidal synthetic antipyretics has been usually associated with gastro-toxicity, nephro-toxicity, hepato-toxicity, central nervous system and dermatological effects (Suleyman *et al*., 2007). However, traditional Indian systems of medicine like Ayurveda are based on comprehensive treatment of ailments relying only on natural herbal drugs which lead to fewer side-effects. Ayurvedic literatures like Charak Samhita provide a rich text for common herbal drugs that have been in traditional usage for centuries for treatment of many diseases. According to Ayurveda, pyrexia originates from a combination of indigestion, seasonal variations and significant alterations in daily routine (Sharma and Dash, 1998). Charak Samhita has classified the medicinal plants into 50 *Mahakashays* on the basis of their actions, each *Mahakashay* having ten plants (Sharma and Dash, 1999). The group of antipyretic drugs called *Jwarhar Mahakashay* consists of *Sariva* (*Hemidesmus indicus* R. Br.), *Manjistha* (*Rubia cordifolia* L.), *Patha* (*Cissampelos pareira* L.), *Haritaki* (*Terminalia chebula* Retz.), *Amala* (*Emblica officinalis* Gaertn.), *Vibhitak* (*Terminalia bellirica* Roxb.), *Draksha* (*Vitis vinifera* L.), *Parushak* (*Grewia asiatica* L.), *Peelu* (*Salvadora persica* L.) and *Sharkara* (*Saccharum officinarum* L.) plants, which have been ascribed with antipyretic action (Sharma and Dash, 1999).

Some of these medicinal plants have been reported to exhibit pharmacological actions such as anti-inflammatory, analgesic, hepato-protective, anti-microbial and antiulcer

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properties (Gupta *et al*., 2008). Besides various sugars, these plants were reported having triterpenoids, flavonoids, flavonidic glycosides and polyphenolic compounds (Sharma *et al*., 2001; Kirtikar and Basu, 1984; Baltenweck-Guyot *et al*., 2000; Foo *et al*., 1998; Kundu and Mahato, 1993). Flavonoids and polyphenolic compounds are known for biological effects that include anti-inflammatory, anti-microbial, anti-hepatotoxic and anti-ulcer activities (Narayana *et al*., 2001; Oweyele *et al*., 2005).

Preliminary phytochemical analysis of the aqueous extract of a homogenous mixture of these ten plants revealed the presence of tannins, flavonoids, glycosides and salicyclates (Gupta *et al*., 2008). Experimental studies on rodents indicated very low toxicity (LD_{50}>>8000 mg kg^{-1}) and low ulcerogenicity of this polyherbal preparation as compared to aspirin. The antipyretic-analgesic effect of this polyherbal preparation mentioned in standard Ayurvedic texts was evaluated during experimental trials on rats and mice and found to be very significant (Gupta *et al*., 2008). However, there were no scientific evaluations based on clinical trials about the antipyretic efficacy of this medicinal preparation.

This research effort was aimed at the clinical evaluation for antipyretic efficacy of a traditional combined formulation of *Jwarhar mahakashay* drugs of Charak Samhita by direct measurement of body temperature, assessment of secondary symptoms and appropriate supportive laboratory investigations.

MATERIALS AND METHODS

This clinical trial was a double-blind, randomized, placebo-controlled, parallel-group study involving a total of 60 patients having fever. The study was carried out at the Institute of Post Graduate Ayurvedic Education and Research at S.V.S.P. Hospital, Kolkata and the Department of Chemical Technology, Calcutta University, Kolkata.

Preparation of research drug and its administration: The roots of *Sariva* (*Hemidesmus indicus* R. Br.), *Manjistha* (*Rubia cordifolia* L.) and *Patha* (*Cissampelos pareira* L.), fruits of *Haritaki* (*Terminalia chebula* Retz.), *Amala* (*Emblica officinalis* Gaertn.), *Vibhitak* (*Terminalia bellirica* Roxb.), *Draksha* (*Vitis vinifera* L.), *Parushak* (*Grewia asiatica* L.) and *Peelu* (*Salvadora persica* L.) and *Sharkara* granules (*Saccharum officinarum* L.) were obtained from the Apothecary Department of the Institute of Post Graduate Ayurvedic Education and Research, Kolkata during May 2006. These were authenticated and identified by the Department of Ethnobotany, Botanical Survey of India, Shibpur, Howrah and a voucher specimen was deposited in the herbarium before their utilization. All plant parts were dried in shade and coarsely powdered up to 40 mesh size. Equal portions by weight of all ingredients were homogenously mixed and subjected to soxhlet extraction in refluxing distilled water. The extraction was continued for 48 h using distilled water four times by weight of the crude drug mixture. The aqueous extract was filtered through calico cloth and was further concentrated to solid under reduced pressure over water bath in a rotary evaporator. The extract yield from 300 g of plant powder mixture was 75 g. This Jwarhar Mahakashay preparation was termed PD-10 for all systematic evaluation studies and used for the clinical trials during 2006-07.

Patients: Patients were selected after general examination from the Outdoor Patients Department of the Institute of Post Graduate Ayurvedic Education and Research (S.V.S.P. Hospital), Kolkata using the following inclusion criteria: (i) Age: 15-60 years; (ii) Sex: both male and female; and (iii) History of fever: duration of fever up to 7 days.

Patients were excluded if they reported any history of trauma, malignancy, severe systemic and organ disorders, major surgery and chronic fever. Pregnant and lactating females were excluded from the study (Sen, 1994).

Ethical aspects: All patients were given verbal and written information about the potential risks and benefits of participation in the study. Written consent was required before randomization. The institutional clinical research committee of the participating center approved the study protocol.

Treatment allocation and blinding: Initially 75 patients were selected for the purpose of this study, out of which 60 patients were finally followed up for this study. These patients were randomly allocated to one of the three equal-sized treatment groups and received the treatment dose of 60 mg kg^{-1} body weight per day. The treatment-groups included: Group A (standard group): the drug Aspirin (60 mg kg^{-1} body weight per day), Group D (test drug group): the test drug PD-10, Group C (control group): rice powder as placebo. Treatment consisted of oral ingestion of the treatment drug 3 times daily for 5 days. All patients were advised light diet, plenty of water and rest.

The study medication was provided in white paper boxes, numbered consecutively with a medication number. The treatment allocation schedule was based on computer-generated random numbers. The treatment codes resided with the principal investigator and the local investigators were not aware of treatment assignments. No treatment code was broken before the last follow-up visit was completed.

The body temperature was measured orally by means of a standard doctor's thermometer in °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 measurements done at the Outdoor Patients Department, the patients and their attendants were given elaborate instructions to measure the patients' body temperature and record the same in a diary. Follow-up visits were continued for five days after the treatment period to record if there was any relapse of fever.

Outcome measures: 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 secondary outcome measure was the prevalence of secondary symptoms commonly associated with fever among the patients. These symptoms included body-ache, headache, running nose, nausea, indigestion, weakness and anorexia (Sharma and Dash, 1998).

As a tertiary outcome measure, the hematological examinations of blood (TC, DC, Hb%, ESR, malaria parasite) and urine (colour, appearance, protein, sugar, urobilinogen, phosphates, RBC, pus cells, parasites, yeast cells, etc.) were done for all the patients before and after treatment. Prostaglandin E_2 (PGE_2) is a very potent fever producing autacoids metabolite from arachidonic acid (Fauci *et al*., 1998; Bennett and Plum, 1996). Most non-steroidal antipyretics such as Aspirin and Ibuprofen inhibit fever by blocking the biosynthesis of Prostaglandins within the endotheliun of the hypothalamic vasculature. These findings suggest that the antipyretic effect of the research drug can also be estimated by measuring its impact on the 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 Prostaglandin E_2 (PGE_2). The estimation of PGE_2 level was done following HPLC method (Gatti *et al*., 1995) at the pathological laboratory of Rama Krishna Mission Seva Pratisthan Hospital, Kolkata, a reputed medical institution having facilities for this specialized test. The objective of this limited study was to confirm and corroborate the overall findings of antipyretic effect assessed during the detailed study as described earlier.

Statistical analysis: The results obtained were presented as mean±SEM. Analysis of variance was performed by ANOVA procedures (Pipkin and Livingstone, 1984). 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

General information: A total of 60 patients (36 female and 24 male) were randomized and received trial medication after providing written agreement of their participation in the trial. The patients were of an average age of 36.67 years. Seventy seven percent of the patients belonged to the minority Muslim community, while 72% 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 were excluded from the study.

Evaluation of antipyretic effect: The mean degrees of fever, defined as the average body temperature in °F in excess of 98.6°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 1.96 to 2.66°F. Even after 72 h, there was a persistent and substantial level of fever (1.34°F).

In case of the test drug group (group D), the level of fever decreased rapidly and substantially within 2 h of the administration of the first drug dose. The subjects had practically no fever until very close to the time of the second dose and this trend continued for the subsequent doses as well. 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. Thus, the average degrees of fever were 2.77°F at 0 h, 1.61°F at 8 h, 0.85°F at 24 h and 0.1°F at 36 h, the level of fever subsequently remaining at zero level.

In the standard group (group A), the level of fever decreased substantially within 3 h of the administration of the first drug dose. Thereafter, the subjects had almost no fever until very close to the time of the second dose and this trend continued for the subsequent doses as well.

Table 1: Mean degrees of fever during clinical study

Time (h)	Group C	Group D	Group A
0	2.41±0.19	2.77±0.19[a]	2.79±0.19[a]
1	2.42±0.14	0.82±0.22[a]	1.19±0.21[a]
2	2.60±0.12	0.19±0.09[a]	0.38±0.09[a]
3	2.61±0.12	0	0.08±0.04[a]
4	2.66±0.15	0	0
8	2.22±0.14	1.61±0.15[a]	1.75±0.16[a]
12	2.21±0.10	0	0.48±0.10[a]
18	2.26±0.16	0	0.05±0.04[a]
24 h	1.96±0.15	0.85±0.10[a]	0.92±0.09[a]
36 h	2.02±0.11	0.1±0.06[a]	0.18±0.07[a]
48 h	2.07±0.11	0	0.07±0.04[a]
60 h	1.77±0.08	0	0.08±0.03[a]
72 h	1.34±0.10	0	0.03±0.02[a]

Significance related to control; [a]$p<0.001$, [b]$p<0.01$, [c]$p<0.1$, (ANOVA test); Values expressed are Mean±SEM (n = 20)

Table 2: Decrease in incidence of associated secondary symptoms during clinical study

Symptom	Group C (%)	Group D (%)	Group A (%)
Body ache	22.2±6.4	75.0±8.30	75.0±3.80
Headache	28.6±5.9	83.3±7.60	80.0±6.90
Running nose	20.0±6.6	77.8±11.8	75.0±7.20
Nausea	0.0±0.0	100.0±0.00	33.3±8.10
Indigestion	37.5±9.7	83.3±2.40	60.0±10.5
Weakness	40.0±8.9	83.3±3.40	66.7±7.70
Anorexia	-33.3±8.0	75.0±4.80	66.7±9.40
Total	24.5±6.6	80.9±3.50	66.7±7.80

Values expressed are Mean±SEM (n = 20)

Table 3: Increase in routine hematological parameters during clinical study

Parameters	Group C (%)	Group D (%)	Group A (%)
Mean g (%) of hemoglobin	5.52±1.69	7.12±1.35	7.36±0.58
Erythrocyte (mill/cu mm)	2.81±0.55	2.15±0.43	4.48±1.06
Leucocytes (mill/cu mm)	-3.10±0.77	-1.61±0.85	3.29±1.21
Neutrophil (cu mm)	2.23±0.39	6.15±0.92	-0.32±0.18
Lymphocyte (cu mm)	-12.03±1.57	-15.57±2.08	-15.98±1.67
Monocyte (cu mm)	-13.04±2.51	-23.81±1.86	-36.00±3.06
Eosinophil (cu mm)	-46.70±1.97	-56.36±2.53	-50.00±6.28
Basophil (cu mm)	0.00±0.00	0.00±0.00	0.00±0.00
ESR (mm h^{-1})	-16.89±2.86	-17.64±1.72	-14.67±0.59
Malarial parasite	0.00±0.00	0.00±0.00	0.00±0.00

Values expressed are Mean±SEM (n = 20)

Table 4: Prostaglandin E2 (PGE2) levels in patients during clinical study

Groups	Decrease (%)
C	2.35±0.76
D	13.19±2.11[a]
A	11.32±2.58[a]

Significance related to control; [a]p<0.001; [b]p<0.01; [c]p<0.1 (ANOVA test); Values given are Mean±SEM (n = 6)

The periodic peaking of fever levels just before the drug administration also showed a clear decreasing trend as compared to the initial fever level. For example, the degrees of fever were 2.79°F at 0 h, 1.75°F at 8 h, 0.92°F at 24 h and 0.18°F at 36 h, the level of fever subsequently remaining at negligible levels.

The results obtained were found to be highly significant ($p<0.001$) during ANOVA analysis.

Evaluation of associated secondary symptoms: All the patients were assessed for the presence of each of the seven pre-determined associated secondary symptoms, namely body ache, headache, running nose, nausea, indigestion, weakness and anorexia before and after treatment. The decrease in the incidence of each of these secondary symptoms in the three groups is presented in Table 2.

The overall incidence of secondary symptoms in patients showed a decrease of 24.5% in terms of number of symptom-patients in case of Group C during the treatment period, while the decrease was 80.9% in case of Group D and 66.7% in case of Group A patients. Highly appreciable relief from the associated secondary symptoms of nausea, indigestion, anorexia, body ache, running nose and headache was observed in case of the test drug group (Group D) as compared to the control group (Group C).

Laboratory investigations regarding secondary parameters: The increase in the values of different hematological parameters during the treatment is shown in Table 3.

The routine hematological examination of all the patients suggested that none of the patients were suffering from malaria. No other substantial difference among the groups was noticed in respect of the other hematological parameters studied.

The routine pathological examination of urine samples of patients revealed no appreciable changes in the colour, appearance, pH, protein, sugar, ketones, bile salts, bile pigments, urobilinogen, phosphates, occult blood, R.B.C., yeast cells and parasites during the treatment period. The level of epithelial cells and pus cells reduced by 20-25% in case of both Group D and Group A, while no appreciable changes were noticed in case of Group C.

Estimation of prostaglandin levels: The decrease in the Prostaglandin E_2 (PGE_2) levels in the representative sample of patients belonging to the three groups during treatment as estimated by HPLC method is detailed in Table 4.

The overall Prostaglandin E_2 (PGE_2) level registered a decrease of 13.19% in case of the test drug group and 11.32% in case of the standard drug group, while in case of the control group, the decrease was only 2.35%.

DISCUSSION

The clinical study showed that fever was rapidly and substantially reduced in case of patients who were administered the test drug. The antipyretic effect of the research drug was also more sustained in nature when compared to the standard drug, Aspirin. ANOVA tests conducted to establish the statistical significance of the clinical findings showed that the results were highly significant ($p<0.001$). The secondary symptoms, especially body ache, running nose, anorexia and headache also exhibited a drastic reduction when the test drug (PD-10) was administered as compared to Aspirin. Laboratory investigations in the form of routine blood examination, malarial testing and routine urine tests did not show significant difference in the impact on these parameters between various groups. However, the overall Prostaglandin E_2 (PGE_2) level in case of the representative sample registered a substantial decrease during treatment

with PD-10, which is consistent with and corroborates the overall findings regarding the antipyretic efficacy of PD-10 during clinical study.

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 (Leon, 2002). One of the arachidonic acid metabolite prostaglandin E_2 (PGE_2) is a very potent fever producing autacoid that affects the hypothalamus receptors, raising the thermo-regulatory set point and causing hyperthermia (Fauci *et al*., 1998; Bennett and Plum, 1996). In such an event, management of symptomatic fever ailments was undertaken with nonsteroidal anti-inflammatory and antipyretic chemicals in modern medicine that associate significant risk from gastro-toxicity to hepato-toxicity to nephro-toxicity (Suleyman *et al*., 2007).

Traditional Ayurvedic text Charak Samhita describes a specific group of 10 drugs (called Jwarhar mahakashay) that possess antipyretic properties. Polyherbal formulations have been commonly used in this Indian system of medicine with the objective of holistic treatment of the disease and its associated symptoms using synergic effect of the constituent medicinal plants. The aqueous extract of homogenous mixture of the dried parts of these ten plants mixed in equal quantities by weight was termed PD-10 for analysis. Flavonoids and flavonidic glycosides were reported in most of these plants and were observed present in spot test with PD-10 preparation (Baltenweck-Guyot *et al*., 2000; Foo *et al*., 1998; Kundu and Mahato, 1993). Flavonoids have been known to exhibit strong antipyretic properties as well as anti-oxidant properties (Narayana *et al*., 2001; Nijveldt *et al*., 2001). Most of these medicinal plants have been reported to exhibit pharmacological actions such as anti-inflammatory, analgesic, hepato-protective, anti-microbial and anti-ulcer properties (Gupta *et al*., 2008).

Preliminary phytochemical screening of the extract indicated the presence of tannins, reducing sugars, flavonoids, glycosides and salicyclates (Gupta *et al*., 2008). Flavonoids are well known for their ability to inhibit pain perception (Sawadogo *et al*., 2006) and to exhibit anti-inflammatory properties due to their inhibitory effects on enzymes involved in the production of the chemical mediator of inflammation (Oweyele *et al*., 2005). Flavonoids and its related compounds also exhibit inhibition of arachidonic acid peroxidation, which results in reduction of prostaglandin levels thus reducing the fever (Baumann *et al*., 1980). Since flavonoids exhibit several biological effects such as anti-inflammatory, anti-microbial, anti-hepato-toxic and anti-ulcer activities (Narayana *et al*., 2001; Nijveldt *et al*., 2001), it is likely that the antipyretic action of PD-10 preparation is primarily related to the presence of flavonoids.

Evaluation of the antipyretic action of PD-10 during clinical trials indicates that this polyherbal preparation from a mixture of the ten *Jwarhar Mahakashay* drugs exhibits significant antipyretic efficacy that is substantial and sustained in nature and comparable in strength to some of the existing chemical antipyretics such as Aspirin. The antipyretic effect of the test drug was validated and found to be significant following statistical analysis. The use of PD-10 also led to a substantial reduction in the incidence of associated secondary physical parameters of fever. The antipyretic action was also confirmed by a definite lowering of the Prostaglandin levels in the representative sample. The antipyretic properties of *Jwarhar Mahakashay* drugs as described in the traditional Ayurvedic text (*Charak samhita*) were evaluated and assessed using modern scientific techniques in this clinical study. The overall research findings corroborate and validate the antipyretic efficacy of these drugs, which has been traditionally ascribed to them.

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