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Antipyretic activity investigations on various extracts and fractions of Jwarnashak Panch Kashya (Polyherbal formulation)

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Abstract
Jwarnashak Panch Kashya polyherbal formulation contained equal mixture of five selected plants such as *Swertia chirata*, *Tinospora cordifolia*, *Zingiber officinale*, *Fumaria parviflora*, and *Cyperus rotundus* has been used in the treatment of pyrexia. But the polyherbal formulation has not ever been evaluated scientifically to validate its traditional claims. Therefore, the present investigation was planned to evaluate antipyretic activity of polyherbal formulation in rats using brewer's yeast-induced pyrexia model. Powdered polyherbal formulation was extracted successively and exhaustively using Soxhlet apparatus solvents to prepare *n*-hexane, chloroform, methanol and water extracts. Various extracts of polyherbal formulation were subjected to antipyretic activity evaluation in rats using brewer's yeast-induced pyrexia model. The results of antipyretic activity showed that chloroform and methanol extracts possess significant antipyretic activity with respect to control and statistically similar to paracetamol because these extracts contain therapeutically active classes of phytoconstituents. *n*-hexane and water extracts did not show any sign of decline in temperature with respect to control. The ethyl acetate fraction was prepared from bioactive methanol extract to get flavonoids and phenolic compounds rich fraction. The ethyl acetate fraction and remaining bioactive extract were subjected to antipyretic activity. The ethyl acetate fraction possesses significant antipyretic activity at the dose of 162 mg/kg with respect to control**,** and statistically similar to paracetamol. The remaining bioactive extract did not exhibit antipyretic activity with respect to control. Finally, it can be concluded that bioactive chloroform extract and ethyl acetate fraction will be further subjected to column chromatography to extract bioactive fraction which contains antipyretic constituents act synergistically.

Keywords: antipyretic activity, Jwarnashak Panch Kashya, paracetamol, polyherbal formulation

1. Introduction
Fever usually occurs when any infectious agent or foreign particle enters into human body then human body triggers the immunological response. In such situation the COX (cyclooxygenase) enzymes activates which act on the arachidonic acid and produce prostaglandin which increase the normal temperature of the human body then that infectious agent which enters into our body cannot resist the higher temperature and does not survive into our body but sometimes the temperature becomes too high then we use the antipyretic agents to reduce the temperature of human body [4]. A large number of synthetic antipyretic drugs are available in local market such as naproxen, ibuprofen, nimesulide, ketoprofen, aspirin, choline salicylate and paracetamol [1]. All the antipyretic drugs inhibits the COX enzymes then there will be no production of prostaglandin which increase the temperature but these drugs are associated with side effects like the NSAIDs cause gastric lesions and some shows the signs of anemia, nausea, clotting problems, kidney problems, throat and skin infection and also shows the liver cytotoxicity [2]. Therefore, well planned research is needed to explore more efficacious, safer and with lower side effects as better alternatives to synthetic drugs. Investigating plants, based on their use in traditional system and medicine, is a sound, viable and cost effective strategy to develop new drugs.
Jwarnashak Panch Kashya polyherbal formulation has been tradionally used in the treatment of fever. The ayurvedic formulation was prepared by mixing equal amount of each selected medicinal plants such as *Tinospora cordifolia* aerial parts (Guduci; Menispermaceae), *Swertia chirata* aerial parts (Chirata; Gentianaceae), *Fumaria parviflora* whole plant (Papra; Fumariaceae), *Zingiber officinale* rhizomes (Ginger; Zingiberaceae) and *Cyperus rotundus* rhizomes (Nagarmotha; Cyperaceae) [12].
An exhaustive scrutiny of literature suggested that no scientific report on antipyretic activity of Jwarnashak Panch Kashya polyherbal formulation to validate its traditional claims till date.

Thus, it was planned to investigate antipyretic activity of Jwarnashak Panch Kashya polyherbal formulation.

2. Material and Methods

2.1 Collection and identification of plant material

The selected Indian traditional plants used in present investigations were purchased from Himalaya Herb Stores, Madhav Nagar, Saharanpur, Uttar Pradesh, India in 2015. The identity of each plant was confirmed by Dr. Avneet Pal Singh, Assistant Professor, Department of Botany, Punjabi University, Patiala, India (Reference No. SPL-111/Bot, dated 25-12-2015).

2.2 Chemicals, solvents and reagents

Various chemicals, solvents and reagents of LR grade were used in present investigations were purchased from S.D. Fine Chemicals, Mumbai, India and E Merck, Delhi, India.

2.3 Preparation of various extracts and fractions

The selected medicinal plants were properly dried and powdered separately. The ayurvedic formulation was prepared by properly mixing of similar amount of each plant. Powdered polyherbal formulation (2.5 kg) was extracted successively and exhaustively using Soxhlet apparatus solvents to prepare *n*-hexane extract (HE), chloroform extract (CE), methanol extract (ME) and water extract (WE). Water extract of polyherbal formulation was prepared by decoction method. Various extracts were concentrated under reduced pressure using rotary vacuum evaporator (BUCHI, Switzerland) and stored in desiccator.

The bioactive extract (100 g) was uniformly dispersed in water (100 ml) and transferred to round bottom flask (1 L capacity) and heating with ethyl acetate (250 ml) with continuous stirring at 50ºC for 30-45 minute. The ethyl acetate layer was separated by separating funnel and water layer was again treated with ethyl acetate. Similar procedure was repeated until ethyl acetate dose not dissolve phytoconstituents from water layer. The separated ethyl acetate layer were concentrated under reduced pressure using rotary vacuum evaporator to get ethyl acetate fraction (EAF) and remaining bioactive extract (RBE) and stored in desiccator. Various extracts and fractions were further subjected to qualitative chemical tests to determine various classes of therapeutically active phytoconstituents using standard procedures [6].

2.4 Animals

Wistar albino rats (either sex) of body weight 150-200 g were purchased from Lala Lajpat Rai University of Veterinary and Animal Science, Hisar, India for present research work. The animals were placed under ideal ambient conditions in the animal house facility of Punjabi University, Patiala, Punjab, India. The present investigations were approved by IAEC of Punjabi University, Patiala (Ref. No. 107/99/CPCSEA/2016-32, dated 27/5/2016). The rats were placed in laboratory for 10-20 days (2 h) to adopt laboratory conditions before the start of experiment. Overnight fasted rats were selected for present investigations. Various test doses of extracts and fractions were given orally to rats with oral cannula attached on a tuberculin syringe. The animals were reused in the present investigation after washing.

2.5 Evaluation of antipyretic activity by using brewer's yeast-induced pyrexia model

The initial rectal temperature of the Wistar albino rats was recorded by inserting a lubricated digital thermometer (external diameter 6 mm) 3 cm into the rectum of the rat. The values displayed were manually recorded. The animals with almost uniform body temperature were selected for present investigations. Pyrexia (hyperthermia) was induced in selected rats with normal saline (0.8%) suspension of brewer's yeast (12.5%) at the dose of 1 ml/100 g, *s.c*. The rectal temperature changes were recorded for 12 h at interval of one hour to determine stress related to handling. These animals were handled carefully so as to minimize the possible stress. After 18 h administration of brewer's yeast, the rectal temperature of rats was reached at its maximum value. Similarly, the rectal temperature of brewer's yeast treated rats was recorded again. The rats having rectal temperature raised at least 2°F were selected for further investigation. The test doses of various extracts, fraction, paracetamol and vehicle were administrated orally to the selected rats and rectal temperature of rats was recorded at 1 h interval for 3 h [13].

2.6 Vehicle and Standard Drugs

Pyrexia was induced in rats with normal saline (0.8%) suspension of brewer's yeast (12.5%). Various test doses of extracts were prepared in vehicle (2% tween 80 in distilled water) in according to body weight of animals. Paracetamol was used as standard antipyretic drug at the dose of 150 mg/kg, *p.o.*, to compare the antipyretic activity of test groups.

2.7 Experiment design

Experimental design comprises two experimental protocols. A total number of 12 groups of rats were made, and each group comprised 6 rats.

Experimental protocol 1: comprising groups 1 to 10, was designed to assess antipyretic activity of various crude extracts of polyherbal formulation.

Group 1 – Control group received vehicle (*p.o.*); Group 2 – Standard group received paracetamol (150 mg /kg, *p.o.*); Group 3 and 4 – Test groups received 400 and 800 mg/kg, *p.o.*, respectively; Group 5 and 6 – Test groups received 400 and 800 mg/kg, *p.o.*, respectively; Group 7 to 8 – Test groups received 400 and 800 mg/kg, *p.o.*, respectively; Group 9 and 10 – Test groups received 400 and 800 mg/kg, *p.o.*, respectively.

Experimental protocol 2: comprising groups 11 and 12, was designed to assess antipyretic activity of various fractions of bioactive extract. The data of control and standard was reused as in the experiment protocol 1.

Group 11 – Test group received 162 mg/kg, *p.o.* and Group 12 – Test group received 235 mg/kg, *p.o.*

2.8 Statistical analysis

The results are presented as mean ± S.D. The difference between the groups was statistically analyzed using one way ANOVA followed by Student-Newman-Keuls test [11].

3. Result

The percentage yields of HE, CE, ME and WE of ayurvedic formulation were calculated as 1.81, 2.28, 9.37 and 12.58% w/w respectively.

Various extracts of polyherbal formulation were subjected to antipyretic activity evaluation in rats using brewer's yeast-induced pyrexia model. It is evident from table 1 CE and ME possesses antipyretic activity (400 or 800 mg/kg, *p.o.*) with respect to control and statistically similar to paracetamol because these extracts contain therapeutically active classes of

phytoconstituents. HE and WE did not show any sign of decline in temperature with respect to control. Rats treated with CE and ME (400 or 800 mg/kg, *p.o.*) showed significant decrease in temperature in relation to control group and statistically similar to the paracetamol.

Table 1: Antipyretic activity of various crude extracts of polyherbal formulation.

Treatment	Dose (mg/kg)	Rectal temperature (-18 h) ºF	0 h	1 h	2 h	3 h
Control	Vehicle	98.69 ± 0.11	101.69 ± 0.56	101.65 ± 0.51[a]	101.65 ± 0.50[a]	101.64 ± 0.47[a]
Paracetamol	150	98.45 ± 0.22	101.75 ± 0.66	100.58 ± 0.58[*]	97.44 ± 0.59[*]	97.01 ± 0.42[*]
HE	400	98.65 ± 0.23	101.50 ± 0.44	101.44 ± 0.65[a]	101.42 ± 0.70[a]	101.44 ± 0.16[a]
	800	98.28 ± 0.21	101.49 ± 0.40	101.41 ± 0.49[a]	101.40 ± 0.65[a]	101.35 ± 0.48[a]
CE	400	98.45 ± 0.30	101.65 ± 0.55	101.50 ± 0.57[a]	98.55 ± 0.45[*a]	97.30 ± 0.36[*]
	800	98.35 ± 0.35	101.70 ± 0.43	101.10 ± 0.54[a]	98.01 ± 0.35[*a]	97.25 ± 0.45[*]
ME	400	98.62 ± 0.40	101.56 ± 0.50	101.26 ± 0.45[a]	98.44 ± 0.45[*a]	97.20 ± 0.55[*]
	800	98.45 ± 0.19	101.60 ± 0.51	101.21 ± 0.58[a]	97.88 ± 0.48[*]	97.16 ± 0.19[*]
WE	400	98.55 ± 0.65	101.58 ± 0.54	101.56 ± 0.45[a]	101.52 ± 0.14[a]	101.52 ± 0.16[a]
	800	98.48 ± 0.44	101.75 ± 0.40	101.60 ± 0.67[a]	101.55 ± 0.55[a]	101.53 ± 0.18[a]

n=6; data is expressed as Mean ± S.D.; [*]P<0.05 vs. Control; [a]P<0.05 vs. Paracetamol; one way ANOVA followed by Student-Newman-Keuls Test.

The bioactive ME was further partitioned with ethyl acetate as solvent to prepare ethyl acetate fraction (EAF) and remaining bioactive extarct (RBE). The percentage yields of EAF and RBE were calculated as 40.52 and 58.88%, respectively. Qualitative chemical tests for major classes of phytoconstituents showing presence of steroids, triterpenoids, alkaloids, flavonoids, coumarins, tannins in EAF and anthraquinone glycosides, cardiac glycosides, cyanogenetic glycosides in RBE. The test doses of EAF and RBE were designed on the basis of their percentage yields with respect to bioactive methanol extract from which these two fractions were obtained. EAF and RBE were subjected to antipyretic activity evaluation in rats using brewer's yeast-induced pyrexia model. It is evident from table 2 the rectal temperature of rats treated with EAF significantly decreased in relation to control and statistically similar to paracetamol at the dose of 162 mg/kg, *p.o.*, whereas RBE did not exhibit antipyretic activity with respect to control.

Table 2: Antipyretic activity of EAF and RBE obtained from ME of polyherbal formulation.

Treatment	Dose (mg/kg)	Rectal temperature (-18 h) ºF	0 h	1 h	2 h	3 h
Control	Vehicle	98.69 ± 0.11	101.69 ± 0.56	101.65 ± 0.51[a]	101.65 ± 0.50[a]	101.64 ± 0.47[a]
Paracetamol	150	98.45 ± 0.22	101.75 ± 0.66	100.58 ± 0.58[*]	97.44 ± 0.59[*]	97.01 ± 0.42[*]
EAF	162	98.69 ± 0.40	101.79 ± 0.55	101.46 ± 0.36[a]	97.66 ± 0.36[*]	97.05 ± 0.64[*]
RBE	235	98.75 ± 0.69	101.76 ± 0.51	101.70 ± 0.35[a]	101.66 ± 0.42[a]	101.64 ± 0.53[a]

n=6; data is expressed as Mean ± S.D.; [*]P<0.05 vs. Control; [a]P<0.05 vs. Paracetamol; one way ANOVA followed by Student-Newman-Keuls Test.

4. Discussion

Antipyretic activity of various extracts and fractions of polyherbal formulation was screened using standardized model, i.e., brewer's yeast-induced pyrexia model in rats. This model was selected for present study because require no sophisticated instrumentation, minimum financial support and time, highly effective and easy to carry out. The available review of literature suggested that brewer's yeast induces fever in animals via increased level of prostaglandin E2 by cyclooxygenase enzymes [13].

Various extracts of polyherbal formulation were subjected to antipyretic activity evaluation in rats using brewer's yeast-induced pyrexia model at the doses of 400 and 800 mg/kg, *p.o.* The results of antipyretic activity showed that CE and ME possesses significant antipyretic activity with respect to control and statistically similar to paracetamol at the dose of 400 or 800 mg/kg, *p.o.*, respectively, because these extracts contain therapeutically active classes of phytoconstituents. HE and WE did not show any sign of decline in temperature with respect to control at any tested doses. The EAF was prepared from bioactive ME to get flavonoids and phenolic compounds rich fraction. EAF and RBE were subjected to antipyretic activity evaluation in rats at the doses of 162 and 235 mg/kg, *p.o.*, respectively, using brewer's yeast-induced pyrexia model. The EAF possesses significant antipyretic activity at the dose of 162 mg/kg with respect to control**,** and statistically similar to paracetamol. RBE did not exhibit antipyretic activity with respect to control at tested dose.

The review of literature revealed that antipyretic agents act by inhibition of cyclooxygenase enzyme, decreased concentration of prostaglandin E2 in brain, decreased levels of inflammatory mediators, increased anti-inflammatory response at injurious area and increased antipyretic signal in the brain. Therefore, it is suggested that bioactive extract and fraction of polyherbal formulation may be act via abovementioned mechanism of actions [4].

The close scrutiny of literature suggested that steroids – *β*-sitosterol [7]; flavonoids – flavonoidal fractions [10]; triterpenoids – cordepressic acid, friedelin [3, 14]; alkaloids – berberine, berbamine, palmatine, matrine [5, 9] and phenols - 2-(1-oxopropyl)-benzoic acid [8] have been scientifically reported to exhibit antipyretic activity. The qualitative chemical tests of polyherbal formulation showed presence of steroids, triterpenoids, alkaloids, flavonoids and phenols as major therapeutically active classes of phytoconstituents in bioactive extract and fraction. Thus, these constituents may be responsible for antipyretic activity of bioactive extract and fraction of polyherbal formulation.

Finally, it can be concluded that the bioactive extract and fraction will be subjected to antipyretic activity-guided fractionation using column chromatography to extract bioactive fractions which contain mixture of antipyretic constituents act by synergistically.

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6. Declaration of interest
The authors report no declaration of interest.

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