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EVALUATION OF ANTIPYRETIC AND ANALGESIC ACTIVITY OF PARUSAKA (*GREWIA ASIATICA* LINN.): AN INDIGENOUS INDIAN PLANT

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ABSTRACT
In the modern medical system, pyrexia is not only considered as a disease, but Jwara or pyrexia has been depicted as a cardinal disease from the period of Brihatrayee (2000B.C – 7th Century A.D.) due to its severeity and worsening prognosis. The vivic classification of the disease, Jwara has been acertained in different traditional Ayurvedic texts and the treatment are being elaborately described in these texts, but most are the compond formulation pertaining to endangered species. Considering this, Parusaka (*Grewia asiatica* Linn.) has been selected as a drug having the Jwaraghna (antipyretic) action and Vedananasaka (analgesic). The basis for the pharmacological action is based on panachabhautika composition. This study was carried out to evaluate its analgesic and antipyretic activities in experimental animal model.
The aqueous extract of Parusaka was administered in different doses to swiss albino mice / rats and compared for analgesic – antipyretic activity by administering aspirin. The extract (doses of 200-300mg / kg body weight) showed good analgesic effect due to its inhibitory effects on pain induced by acetic acid in writhing test (Chemical stimulation) and tail immersion test (Thermal stimulation). The trial drug (300mg /kg body weight) also produced significant increase in the hot plate reaction time (when compared to aspirin 400mg/kg body weight in mice) indicating its analgesic effects. The extract containing 400mg /kg body weight dose had significant effect than aspirin (100mg / kg body weight) on reducing pyrexia, induced by administering lipopolysaccharide extract from *E. Coli*. This study therefore support the use of Parusaka as indigenous analgesic and antipyretic agent in India and further investigations is required to elucidate its pharmacodynamic action.
Keywords : *Grewia asiatica* Linn., analgesic, antipyretic activity

INTRODUCTION

Jwara (fever) is produced due to the improper function of the thermoregulatory center i.e. hypothalamus. The pathogenesis of the disease is followed by the inflammation and manifested by temperature, non-sweating and pain all over the body. The ingestion of incompatible food material produces some toxins or the external bacteriological organisms which are the causative factor for the production of disease. The produced toxins when accumulated in the body and not expelled in proper time then this accumulation of the toxins termed as 'Ama', due to the impaired condition of the function of Jatharagni, gets lodged into the different srotas specifically Swedabaha srotas and Rasabaha srotas[1]. The vivid classification of the disease Jwara has been ascertained in Ayurvedic Samhitas and its classified treatment is also found in these texts but the specific treatment is important now a day as prior to the identification of the specific pyrogen. This very methodology to such the antipyretic effect is classically categorized in Charak Samhita in context to Jwaraghna mahakasaya[1]. The drugs were included in the specific mahakasaya (compound formulation in decoction form) and the importance of this mahakasaya cannot be ignored in context to carry out any research work in the present days also[2]. However keeping the view of availability of all indigenous drugs, cost and efficacy of the most common plant among them needs to be explored for cost effectiveness. Considering this, Parusaka has been taken as a drug having the Jwaraghna action and Vedana nasaka effect. The basis of the Ayurvedic pharmacological action is based on panchabhautika composition due to traditional use since centuries.

Parusaka is commonly used as a natural food additive. It is a good source of vitamin A and vitamin C, and fair source of calcium, phosphorus, and iron, vitamin B complex[3]. Ripe fruit juice used in fever[1] and heart disease, madatyaya (alcoholism) and yoni roga (gynecological disorders), vatapitta nasaka, mutradosa nasaka, pipasa nasaka, ruchikara[4], malakaraka[5], kshata kshaya, daha, rakatakshaya[6] and sotha nasaka[7]. The roots are effective in rheumatism and leaves are effective against *S.aureus* and *E.coli*. It has also anti-tubercular action[8]. The principal active constituents isolated from fruits are also reported [9-11].

Aims Of The Study

To carry out the study with the indigenous plants, Parusaka (*Grewia asiatica* Linn.) for evaluation of its antipyretic and analgesic action in experimental model.

MATERIAL AND METHODS

All chemicals including acetic acid used for the present study were of analytical grade and were purchased from local market. The animal model used for the observation of analgesic effect was Swiss albino mice (male). Swiss albino rats (male) were used as animal model for the observations of antipyretic effect.

Drug selection

In the Vedic literature, traditional practice and modern books, it is commonly described that Parusaka has an antipyretic activity. To validate this claim, this herb was selected for the present research work. Drug, aspirin is gifted by Reckit and Colman India, Calcutta. Fruit of

Grewia asiatica was procured from the local market and was authenticated by the taxonomist as per usual norms.

Collection of the drugs and selection of their parts

Parusaka is warm climate fruit plant. In India, this plant grows satisfactory and produces well up to an elevation of 1,000 m. Adequate sunlight and warm or hot temperatures are required for fruit ripening, development of appropriate fruit colour, good eating and proper medicinal quality. Summer season is best for fruit quality and therefore Parusaka fruits were collected in a very ripening state and voucher specimen no. NRIADD/Kol/04/2010 was deposited in the Department of Pharmacognosy, National Research Institute of Ayurvedic Drug Development, Kolkata, India

Preparation of aqueous extract

The shed dried course powder of fruit (# 20) of the Parusaka was taken in a round bottom flask. The distilled water (4 times) was added to it. The bottle was kept upon thermal regulating heater. After 48 hrs, it was put for filtration using filter paper at least three times. Filtrate of aqueous extract was placed upon the steam bath. The most part of water was evaporated and formed a semi-solid substance. For complete removal of the water, it was kept into the increased temperature with vacuum. Then it was dried and collected in powder form. The weight of the extracted materials was 40 gm which was collected from 500 gm crude dried Parusaka powder. This extracted material was preserved in the freezing condition with sealing.

Dose selection

Five consecutive doses were selected for 5 separate groups of animals to observe the activity. In case of analgesic activity, the lowest dose was 100 mg /kg. body weight and the highest was 300 mg / kg. body weight.

In case of antipyretic activity test, the lowest dose was 100mg/kg. body weight and the highest was 500 mg /kg. body weight.

Experimental animals

Swiss male albino rats and mice were collected from the authentic animal supplier. Each animal was collected from the same breeding colony and batch. The weight of the albino mice varied from 20g to 25g and 130g-160g weight in case of albino rats.

All the animals were divided into five groups each containing 5 in numbers. The groups of animals were kept in their respective cages marked for them. The cages were marked as group-I , group-II , group-III , group-IV , group-V to indicate Parusaka*'s* aqueous extract was administered in the dose of 100 mg /kg, 150 mg/ kg, 200 mg/ kg, 250mg / kg, and 300 mg/ kg of body weight respectively to observe the analgesic activity.

In case of antipyretic activity test the cages were marked as group-I , group-II , group-III, group-IV , group-V to indicate Parusaka*'s* aqueous extract is administered in the dose of 100 mg/ kg, 200 mg/ kg, 300 mg/ kg , 400 mg/ kg and 500 mg/ kg body weight respectively.

They were supplied with autoclaved pellets food and sterile water in order to avoid any probable contamination. Every day food and water was supplied in sufficient amount. The temperature was controlled. The cages were cleaned properly in every week by detergent, savlon etc. The ambient temperature was tried to maintain through 24 hrs.

The IAEC approved for the study: letter No.138A/CRI/2009-2010 and Registration No. 694/a/CPCSEA/03.

Number of experiments

Acetic acid induced Writhing Test, Tail Immersion method, Hot Plate method were performed for observing analgesic activity and a separate experiment was done to observe the antipyretic activity and the results obtained from the experiments were verified by various statistical analysis.

Procedure of drug administration

The aqueous extract of *Grewia asiatica* was administered in intra-peritoneal route. Prior to the administration of the extracted drugs the animals were kept without food for 12 hrs, whereas adequate water was continued. During administration, they were carefully handled by which traumatic injury was avoided.

Experiment

Before 12 hrs of the experiment the food was withdrawn but water was being continued. In the morning, drugs were administered intra-peritoneal in standard, controlled and treated groups. Intra-peritoneal administration is chosen as because it is one of the most frequently-used parenteral routes for better drug absorption and maintenance the accurate dose in experimental animals.

Observation of analgesic activity

Analgesic activity test is done three processes as given such below

Acetic acid –induced writhing test (Chemical stimulation)

Group wise the animals received i.p. in various doses of aqueous extract of Parusaka (100,150,200,250 and 300 mg/kg) an hour before i.p. injection of 0.6%v/v acetic acid (10ml/kg). Control group received 0.2 ml normal saline and the standard controlled group received aspirin in 400mg/kg body weight[12].

Tail immersion method (Thermal stimulation)

The albino mice were divided into groups of 5 animals each. normal saline (control), 100, 150, 200, 250 and 300 mg/ kg. Parusaka and 400 mg/ kg. aspirin were administered i.p. The tail (up to 5cm) was then dipped in a water bath at 55$\pm$0.7°C. Pain threshold in thermal induced pain were measured after 10, 30, and 60 minutes of i.p. injection of Parusaka[13].

Hot plate method (Thermal stimulation)

The animals were placed gently on a plate maintained at 53 $\pm$0.5°C. Reaction time was taken as the intermediate time between the instant of the animal reaches the hot plate and the moment of the animal licks its forepaws or jumps out. Measurements were carried out after i.p. injection of Parusaka aqueous extract and aspirin. In every case aspirin was used as standard drug and was administered in the dose of 400 mg/ kg body weight by i.p.[14].

Observation of antipyretic activity

Swiss albino rats were fasted for 24 hrs before the experiments. At the beginning of the study, the rectal temps were measured. Fever was induced by an i.p. injection of 0.01 mg/ml lip polysaccharide (LPS) extracted with ether from E. coli. Rectal temperature of

each animal was recorded at 30, 60 and 90 min. The control animals received 100 mg/ kg of aspirin, served as the standard drug. Each result was calculated as the mean of three readings[14].

Table 1 : **Effect of *Grewia asiatica* on acetic acid induced pain (writhing test)**

	Average# number of writhing ± SE (30 minute)					
Sample	**A_1**	**A_2**	**A_3**	**A_4**	**A_5**	**Average**
Group-I	9.00 ± 0.707	10.20 ± 0.73	10.00 ± 0.71	11.00 ± .71	10.60 ± 0.92	2.56 ± 1.14
Group-II	8.20 ± 0.8	8.20 ± 0.86	8.20 ± 0.48	9.40 ± 0.81	8.20 ± 0.73	3.08 ± 1.37
Group-III	5.60 ± 0.67	4.80 ± 0.73	5.00 ± 0.44	5.20 ± 0.58	4.20 ± 0.48	3.68 ± 1.64
Group-IV	2.40 ± 0.24	2.40 ± 0.50	2.60 ± 0.40	2.20 ± 0.48	2.00 ± 0.31	1.66 ± 0.74
Group-V	0.00 ± 0.00	0.2 ± 0.08	0.2 ± 0.08	0.20 ± 0.08	0.00 ± 0.00	0.55 ± 0.25
Aspirin	0.40 ± 0.4	0.20 ± 0.08	0.00 ± 0.00	0.20 ± 0.08	0.00 ± 0.00	0.88 ± 0.39
Acetic acid	19.00 ± 1.70	20.40 ± 1.806	19.20 ± 1.463	20.40 ± 1.50	21.60 ± 2.293	20.12 ±0.471

Group-I to Group-V indicate *G. asiatica* in the dose of 100 mg, 150 mg, 200 mg, 250 mg and 300 mg per kg body weight respectively. Aspirin used in the dose of 400 mg/kg body weight. $A_1 – A_5$ indicate number of experimental animals.
Average (number of writhing) of 5 animals, SE = Standard error (n = 5).

Table 2 : Effect of *G. asiatica* on pain threshold in thermal induced pain by hot plate method (53± 0.5°C)

Incubation period	Sample	Average # duration of pain threshold (minute) ± SE				
		A_1	**A_2**	**A_3**	**A_4**	**A_5**
10 Minute	Blank	3.1 ± 0.33	3.7 ± 0.37	2.9 ± 0.33	3.5 ± 0.45	4.8 ± 0.37
	Group-I	3.8 ± 0.57	3.5 ± 0.22	2.7 ± 0.37	3.7 ± 0.30	3.6 ± 0.4
	Group-II	3.6 ± 0.40	4.1 ± 0.40	4.5 ± 0.22	4.4 ± 0.29	3.9 ± 0.33
	Group-III	3.9 ± 0.33	4.5 ± 0.22	3.7 ± 0.54	4.2 ± 0.374	3.8 ± 0.37
	Group-IV	3.9 ± 0.50	3.6 ± 0.25	4.3 ± 0.53	5.3 ± 0.56	4.9 ± 0.40
	Group-V	7.4 ± 1.07	6.3 ± 0.54	7.4 ± 1.17	5.1 ± 0.51	5.3 ± 0.54
	Aspirin	2.12 ± 0.42	1.92 ± 0.38	2.37 ± 0.47	2.28 ± 0.45	2.00 ± 0.40
	Group-I	4.5 ± 0.38	3.3 ± 0.44	2.9 ± 0.12	3.4 ± 0.55	4.5 ± 0.39
	Group-II	4.4 ± 0.24	4.1 ± 0.89	4.9 ± 0.33	5.3 ± 0.67	4.1 ± 0.74
	Group-III	4.1 ± 0.89	4.2 ± 0.25	3.0 ± 0.27	4.3 ± 0.30	3.3 ± 0.30
	Group-IV	3.7 ± 0.62	3.8 ± 0.37	4.5 ± 0.22	7.4 ± 1.16	5.6 ± 1.47
	Group-V	7.7 ± 0.54	8.1 ± 1.03	8.4 ± 0.75	6.6 ± 0.43	7.4 ± 0.62
	Aspirin	2.78 ± 0.55	2.45 ± 0.49	2.17 ± 0.44	2.37 ± 0.47	2.12 ± 0.43
	Group-I	4.8 ± 0.37	3.5 ± 0.44	2.9 ± 0.33	3.7 ± 0.2	4.4 ± 0.24
	Group-II	6.2 ± 0.37	4.7 ± 0.84	5.1 ± 0.33	5.12 ± 0.34	4.6 ± 0.51
	Group-III	4.6 ± 0.4	4.5 ± 0.45	3.7 ± 0.30	4.4 ± 0.509	32.0 ± 0.46
	Group-IV	4.5 ± 0.67	4.1 ± 0.33	4.6 ± 0.43	7.8 ± 0.663	5.6 ± 0.48
	Group-V	9.2 ± 0.73	9.6 ± 1.12	8.8 ± 0.72	8.0 ± 0.65	9.3 ± 0.64
	Aspirin	2.86 ± 0.57	2.45 ± 0.49	2.78 ± 0.56	2.69 ± 0.54	2.50 ± 0.50

Group-I to Group-Vindicates *G. asiatica* in the dose of 100 mg, 150 mg, 200 mg, 250 mg and 300 mg per kg body weight respectively. Aspirin used in the dose of 400 mg/kg body weight. $A_1–A_5$ indicate number of experiment set.
average duration of 5 animals. SE = Standard error (n=5).

Table 3 : Effect of *G. asiatica* on pain threshold in thermal induced pain by tail immersion method (55±0.7°C)

Incubation Period	Sample content	Average # duration of pain threshold (minute) ± SE				
		A_1	**A_2**	**A_3**	**A_4**	**A_5**
10 Minute	Group-I	3.0 ± 0.273	2.6 ± 0.187	2.7 ± 0.123	2.9 ± 0.187	2.5 ± 0.223
	Group-II	2.5 ± 0.158	2.8 ± 0.123	3.2 ± 0.254	3.4 ± 0.51	3.5 ± 0.224
	Group-III	3.2 ± 0.717	3.6 ± 0.187	3.8 ± 0.25	4.1 ± 0.244	3.7 ± 0.3
	Group-IV	5.3 ± 0.538	5.0 ± 0.474	4.3 ± 0.4898	4.7 ± 0.339	4.2 ± 0.254
	Group-V	4.5 ± 0.387	5.2 ± 0.463	5.2 ± 0.4636	5.2 ± 0.514	6.0 ± 0.3535
	Aspirin	4.98 ± 0.85	4.58 ± 0.83	5.14 ± 0.88	5.64 ± 0.91	5.16 ± 0.79
30 Minute	Group-I	3.0 ± 0.158	2.4 ± 0.187	2.7 ± 0.255	3.1 ± 0.187	2.5 ± 0.223
	Group-II	3.1 ± 0.244	3.1 ± 0.292	3.6 ± 0.187	3.2 ± 0.3	3.4 ± 0.2915
	Group-III	4.3 ± 0.435	4.2 ± 0.254	5.0 ± 0.316	5.0 ± 0.651	5.1 ± 0.678
	Group-IV	5.0 ± 0.651	6.0 ± 0.689	5.4 ± 0.291	5.9 ± 0.5099	5.3 ± 0.3
	Group-V	6.5 ± 0.316	6.4 ± 0.484	6.4 ± 0.6204	7.4 ± 0.2915	7.5 ± 0.5567
	Aspirin	5.34 ± 1.09	5.52 ± 1.03	5.74 ± 1.08	5.84 ± 1.09	6.00 ± 1.21
60 minute	Group-I	3.2 ± 0.273	2.3 ± 0.2	2.4 ± 0.187	2.9 ± 0.418	2.5 ± 0.273
	Group-II	3.7 ± 0.254	3.8 ± 0.255	4.1 ± 0.43	4.3 ± 0.538	4.0 ± 0.524
	Group-III	5.2 ± 0.3391	4.7 ± 0.561	5.3 ± 0.784	5.2 ± 0.7	5.4 ± 0.291
	Group-IV	6.8 ± 0.734	6.8 ± 0.751	7.5 ± 0.851	7.2 ± 0.969	6.1 ± 0.484
	Group-V	7.6 ± 0.484	7.3 ± 0.681	6.6 ± 0.4847	8.6 ± 0.7483	8.5 ± 0.3535
	Aspirin	6.20 ± 1.27	6.56 ± 1.41	6.50 ± 1.05	6.62 ± 0.99	6.68 ± 1.15

Group-I to Group-V indicates *G. asiatica* in the dose of 100 mg, 150 mg, 200 mg, 250 mg and 300 mg per kg body weight respectively. Aspirin used in the dose of 400 mg/kg body weight. $A_1–A_5$ indicate number of experiment set.
average duration of 5 animals. SE = Standard error (n=5).

Table 4 : **Effect of *Grewia asiatica* on pain threshold in thermal induced pain (55 ± 0.7°C) (tail immersion method) ANOVA and multiple comparison data**

Incubation period (min)	F ratio		Critical difference at 5% level with ranked sample mean #		
	F_1 (between sample sets)	F_2 (between animal sets)	LSD[+]	SRP[++]	DNMP[+++]
10	41.43[a] (df 5, 20)	0.672[b] (df 4, 20)	14.73 Group-I, Group-II, Group-III, Group-IV, Group-V	21.1806 Group-I, Group-II, Group-III, Group-IV, Group-V	21.884 (Group-I, Group-II, Group-III, Group-IV, Group-V
30	27.62[a] (df 5, 20)	2.0345[b] (df 4, 20)	27.4723 Group-I, Group-II, Group-III, Group-IV, Group-V	39.485 Group-I, Group-II, Group-III, Group-IV, Group-V	30.2656 Group-I, Group-II, Group-III, Group-IV, Group-V
60	57.0172[a] (df 5, 20)	0.3065[b] (df 4, 20)	29.1650 Group-I, Group-II, Group-III, Group-IV, Group-V	41.918 Group-I, Group-II, Group-III, Group-IV, Group-V	32.1303 Group-I, Group-II, Group-III, Group-IV, Group-V

Significance levels of *F* values : a < 0.005; b > 0.10, df=degree of freedom

Group-I to Group-V indicate *Grewia asiatica* in the dose of 100 mg, 150 mg, 200 mg, 250 mg and 300 mg/kg body weight respectively. As indicate aspirin in the dose of 400 mg/kg body weight. #Two means not included in the same parenthesis are statistically significant different at $P < 0.05$. +Least significant difference procedure, ++Student range procedure, +++Duncan's new multiple range procedure.

Table 5: **Relative percentage changes[ψ] in temperature by *Grewia asiatica* on lip polysaccharide induced hyperpyrexia**

Incubation period (min)							
	Pyrogen	Aspirin	P_{100}	P_{200}	P_{300}	P_{400}	P_{500}
30	-22.50[a]	- 18.75[a]	-22.19[a]	-21.56[a]	-16.56[a]	-11.88[a]	-12.50[a]
60	-	-10.83[a]	-18.30[a]	-16.13[a]	-7.41[a]	-6.48[a]	-5.54[a]
90	-	- 4.17[a]	-13.81[a]	-11.01[a]	-1.68[b]	-0.50[b]	-0.50[b]

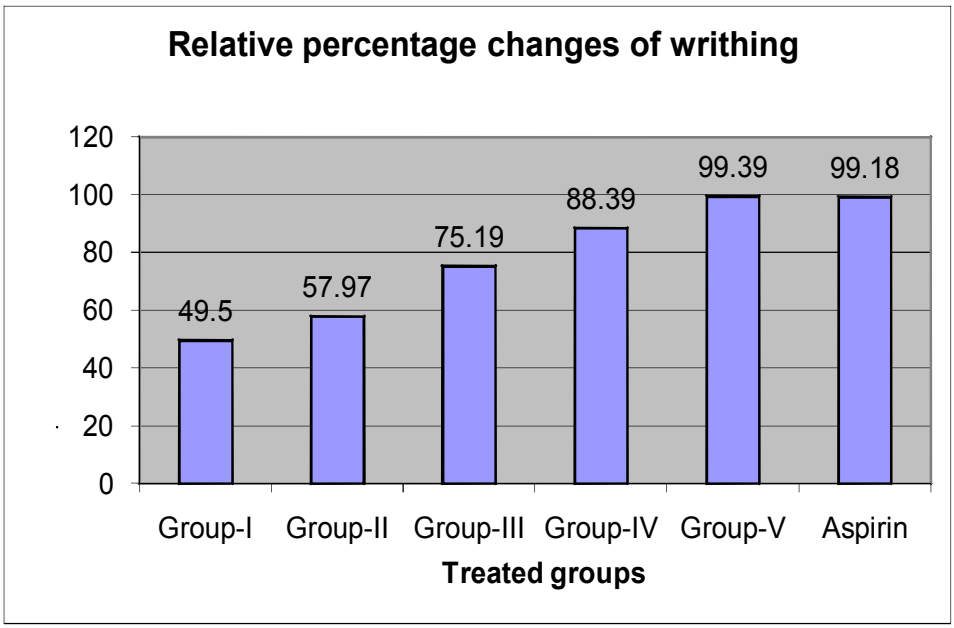

Figure 1: Relative percentage changes of writhing by *G. asiatica* on acetic acid induced writhing test.

Group-I to group-V indicate *G. asiatica* in the dose of 100 mg, 150 mg, 200 mg, 250 mg and 300 mg per kg body weight respectively. Aspirin used in the dose of 400 mg/kg body weight.

RESULTS AND DISCUSSION

Grewia asiatica Linn. has been evaluated as potent analgesic and antipyretic agent by taking various doses of aqueous extract of its, by comparing with the standard drug, i.e. aspirin. The results, verified by statistical analyses ('t' test and confident level), are shown in appropriate tables. The analysis of variance (ANOVA) in two ways (between the samples and between the animal sets) and ranked means are also shown. Analgesic activity of *G. asiatica* was observed in 5 animal sets and antipyretic activity in one animal set.

Analgesic activity was observed by writhing test[12] on administrating acetic acid (0.6%) in the dose of 10ml/kg, inducing pain. The standard drug, aspirin was used in the dose of 400mg/kg, to arrest the acetic acid induced pain. Results were listed in Table 1 and the relative percent changes with respect to acetic acid along with statistical data are listed in the Figure 1.

From the findings it is appeared that *G.asiatica* in the dose of 100 mg to 250mg /kg body weight showed significant inhibitory effect on acetic acid induced pain. 300mg/kg body weight of *G.asiatica* has also shown good

inhibitory effect which is similar to aspirin. Analgesic activity observed by hot plate method[14] and tail immersion method[13] is listed in the Table 2 and 3. The relative percent changes with respect to control (blank) in various periods, along with statistical analysis are listed in Table 4. From the findings it was observed that *G. asiatica* in the dose of 100 to 300 mg/kg body weight have significant inhibitory effect on thermal induced pain. 300mg/kg body weight has greater inhibitory effects and is more potent than aspirin in the dose of 400 mg/kg. The analgesic action of *G. asiatica* was started within 10 minutes.

The antipyretic activity[14] was observed by administering lipopolysaccharide to induce hyperpyrexia. The standard drug, aspirin was used in the dose of 100 mg/kg body weight. Results are shown in Table 5.

From the findings it is appeared that *G. asiatica* in the doses of 300 to 500 mg /kg having greater antipyretic activity than aspirin (100mg/kg) which was observed within 30 minutes after administration of *G. asiatica.*

These analgesic and antipyretic observations show that the *G.asiatica* in the higher dose may be acting in a similar fashion as NSAIDs, which exhibit all these two activities simultaneously.

CONCLUSION

The working hypothesis behind this project has been the evaluation of analgesic and antipyretic activities. To test this hypothesis, *G.asiatica* was chosen to consider it as a potent analgesic –antipyretic agent. To observe the analgesic activity, writhing test and thermal induced pain method has been adopted. *G.asiatica* in the dose of 300 mg /kg body wt has significant analgesic effect on the basis of the result of the experimental methods.

For observation of antipyretic activity, lipopolysaccharide was administered to increase body temperature. It was observed that *G.asiatica* in the dose of 300 to 500 mg /kg have potent anti pyretic activity. In both the cases, the possible mechanism of action of *G.asiatica* is similar to NSAIDs.

In the nut shell it is stated that Parusaka (*G.asiatica* Linn) has significant analgesic and antipyretic effect in selected dose schedule when compared with the standard drug i.e. Aspirin. The traditional knowledge and practice has been revalidated in this study by using Parusaka as Jwaraghna and Vedanasthapaka, which is being used since Vedic period.

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