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Evaluation Of The Antipyretic Activity Of Traditional Forms Of *Enicostema Axillare* (Poir. Ex Lam.) A. Raynal In An Animal Model

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ABSTRACT

Enicostema axillare (Poir. ex Lam.) A. Raynal of the family Gentianaceae is a folk medicinal herb with excellent properties in various diseases and uses for ages. In Indian systems of medicine, it is widely used under various names viz. *Mammajjak*, *Nagajihva*, *Nahi*, *Trunapatra*, and *chhota chirayata* and which found in Maharashtra, Gujarat, Andhra Pradesh, Madras, and coastal parts. In rural India, decoction or juice of *Nahi* drink for fever, anorexia, loss of appetite, worms, and skin diseases with miraculous effects. In snake bite, the local rural people apply the paste of *Nahi*. Hence, they call it 'Maay' (mother of remedies for all diseases). This study assessed the antipyretic activity of the whole plant of *Nahi* (*E. axillare*). *Nahi* (*Enicostemma axillare* (Poir. ex Lam.) A. Raynal) was self-collected from the natural habitat and authenticated by Agharkar Research Institute (ARI) [AUTH - 44], Pune. Preparation of *Nahi churn*, *Kwath*, and *Swaras* was done by standard Ayurvedic method and Physicochemical and phytochemical analyses were also carried out. Antipyretic activity tests using 20% Brewer's yeast-induced hyperthermia in Rats were assessed. The drug formulations i.e. *Panchang Swaras*, *Kwath*, *Churn* & Aq. Extract have shown a high degree of antipyretic action.

INTRODUCTION

Globally, inflammation, pyrexia, and pain have been treated with NSAIDs. However, they often produce major side effects such as kidney failure, hepatotoxicity, and increased risk of bleeding. Hence, the search for affordable, suitable drugs with antipyretic and analgesic activities without side effects is needed.¹

Indian medicinal plants provide suitable sources for the development of new drugs. *Enicostema axillare* (Poir. ex Lam.) A. Raynal of the family Gentianaceae is a folk medicinal herb with excellent properties in various diseases and uses for ages.² In Indian systems of medicine, it is widely used under various names viz. *Mammajjak*, *Nagajihva*, *Nahi*, *Trunapatra*, and *chhota chirayata* and which

found in Maharashtra, Gujarat, Andhra Pradesh, Madras, and coastal parts. In rural India, decoction or juice of *Nahi* drink for fever, anorexia, loss of appetite, worms, and skin diseases with miraculous effects. In snake bite, the local rural people apply the paste of *Nahi*. Hence, they call it 'Maay' (mother of remedies for all diseases).^{3,4} Several studies have been conducted on aqueous extract of *Nahi* to determine anti-diabetic, anti-cancer, and anti-arthritis activity. Very little research work is carried out on antipyretic activity. Similarly, there is no previous study on the *Panchang* (whole Part) of *Nahi* for pyrexia. Thus, this study assessed the antipyretic activity of the whole plant of *Nahi* (*E. axillare*).^{5,6}

MATERIALS AND METHODS

Collection of material: The plant material used in this study was *Nahi* (*Enicostemma axillare* (Poir. ex. Lam.) A. Raynal) was self-collected from the natural habitat and authenticated by Agharkar Research Institute (ARI) [AUTH – 44], Pune. Preparation of *Nahi churna* was done by standard Ayurvedic method as described in *Sharangdhar Samhita*. Physicochemical and phytochemical analyses were carried out at Bhide Foundation, Pune.^{7,8,9}

Preparation of extracts:

- 1) *Swarasa* (Juice): fresh plant parts were Crushed to extract the juice, filtered to remove solids
- 2) *Churna* (Powder): the whole plant was dried, ground into a fine powder, and sieved to ensure uniform particle size
- 3) *Kwath* (Decoction): The whole plant was chopped and boiled in water, reduced the volume by half, and filtered to obtain the decoction.¹⁰

Experimental animal: Wister albino rats (150–250 gm) of either sex were used for the antipyretic activity test. Animals were maintained on a standard animal pellet diet and water ad libitum at the animal laboratory of Crystal Biological Solutions, Handewadi, Pune (CPCSEA 2030/PO/RcBiBt/S/18/CPCSEA Ministry of Environment and Forests, Government of India) The animals were acclimatized for seven days before the study. Permission and approval for animal studies were obtained from the Animal Ethical Committee (approval number IAEC CRY/2223/089).

Antipyretic activity test using 20% Brewer's yeast induced hyperthermia in Rats:¹¹ The activity was assessed with pyrexia induced by Brewer's yeast. The normal temperature was noted before injections of Brewer's yeast using the rectal route using a digital probe thermometer for rats. Fever was induced by subcutaneous injection of 20% w/v suspension of Brewer's yeast at a dose of 1 ml/100gm body weight. After 18 hours, the temperature rise was recorded.

A sample of 42 rats was selected by stratified random sampling and divided into seven groups of rats, consisting of six rats in each. Group 1 (Normal), Group 2 (Disease), Group 3 (Standard), Group 4, 5, 6, and 7 were treated with *Churn*, *Swaras*, *Kwath*, and Aq. Extract of *Nahi* respectively.

The dose calculations were decided by standard procedure with the formula used Rat dose = Human dose × 0.018 (Conversion factor of 200 gm Rat) The Human dose used is as per the guidelines from Ayurvedic Pharmacopeia of India (A.P.I.), *Sharangdhar Samhita* and Research Paper of antipyretic activity of Aq. Extract.²³ c) The rectal temperature was recorded by using a digital thermometer before and after induction and at hourly intervals for an initial 4 hours followed by 24 hours. All the treatments were administered orally.

Treatment protocol

Table 1: The treatment received by grouped animal

Group	Induction	Treatment
Group 1 (Normal Control)	NIL	NIL
Group 2 (Disease Control)	20% of Brewer’s yeast solution (S.C.)	NIL
Group 3 (Standard)		Paracetamol 100mg/Kg in Carboxy Methyl Cellulose (CMC)
Group 4 (Test -1)		<i>Panchang swarasa</i> 1.8 ml/Kg
Group 5 (Test-2)		<i>Panchang churna</i> 270 mg/Kg in 1% CMC
Group 6 (Test-3)		<i>Panchang kwath</i> 2ml/kg
Group 7 (Test-4)		Aqueous extract 200mg/kg

Data analysis: Results were expressed as mean ± SME. ANOVA test were used for statistical analysis. As effect was considered to be significant at the P < 0.05 level.

RESULTS

The Macroscopic and Microscopic, Organoleptic characters, Physicochemical analysis, Phytochemical analysis, and TLC of this whole plant match as per the API. The result of the preliminary phytochemical analysis showed the presence of Glycosides, Saponins, Tannins, Phenolic compounds, Alkaloids, Flavonoids, Steroids, etc. in the powder of *E. axillare* (Poir.ex.Lam.) A. Raynal.

Effect of *E. axillary* on Brewer’s yeast–induced pyrexia in rats: As the temperature was increased in all groups except normal control animals after subcutaneous injection of brewer’s yeast. The mean temperature reduction produced by *Panchang swarasa*, *Panchang churn*, *Panchang kwath*, and aqueous extract of *E. axillary* were significant as compared to that of Disease Control and Normal control (Table 2). *Panchang swarasa* group showed a significant decrease in rectal temperature at 2, 4, and 24 hrs. *Panchang churn* group showed a significant decrease in rectal temperature at 2, 3, 4, and 24 hrs. *Panchang kwath* group showed a significant decrease in rectal temperature at 4 and 24 hrs. **Aq. Extract** group showed a significant decrease in rectal temperature at 2, 3, 4, and 24 hrs.

Table 2: Effect of *E. axillary* on Brewer’s yeast–induced pyrexia in rats (Value present Mean (° C))

Treatment	Before induction	After induction yeast				Before dosing	After dosing				
		1hr	2hr	3hr	4hr		1hr	2hr	3hr	4hr	24hr
Normal control	35.3	35.33	35.33	35.28	35.40	35.23	35.42	35.23	35.32	35.28	35.32
Disease Control	35.23	35.38	35.53	35.70	35.85	37.15	37.62	37.62	37.47	37.72	37.88
standard	35.40	35.50	35.68	35.85	36.03	37.20	36.58	36.45	36.92	36.85	36.05
<i>Panchang Swaras</i>	35.35	35.45	35.68	35.82	36.07	37.43	37.08	36.93	36.87	36.73	36.25
<i>Panchang churna</i>	35.40	35.55	35.65	35.82	36.00	37.30	37.22	37.10	37.02	36.92	36.18
<i>Panchang kwath</i>	35.43	35.53	35.63	35.80	35.98	37.40	37.35	37.18	36.93	36.65	36.05
Aq. extract	35.35	35.47	35.65	35.78	35.98	37.15	36.93	36.82	36.77	36.57	36.20

In all groups except normal and disease control groups at 4 and 24 hrs, we found that all the animals showed a significant decrease in rectal temperature at 4 and 24 hrs.(table 3)

Table 3: Percent temperature reduction potential of Panchang swaras, Panchang churn Panchang kwath and Aqueous extract of *E. axillary* (Value present Mean ($^{\circ}$ C), P <0.005 it is judged as significant)

Treatment	Follow up	Before dosing	After dosing	X	% of improvement	t value	P value
Normal control	1 hr	35.23	35.42	-0.19	-0.53%	-1.655	0.159
	2hr	35.23	35.23	0	-0.01%	0	1
	3hr	35.23	35.32	-0.09	-0.25%	-0.85	0.434
	4hr	35.23	35.28	-0.05	-0.15%	-0.808	0.456
	24hr	35.23	35.32	-0.09	-0.25%	-0.714	0.507
Disease control	1 hr	37.15	37.62	-0.47	-1.26%	-2.591	0.049
	2hr	37.15	37.62	-0.47	-1.26%	-2.59	0.049
	3hr	37.15	37.47	-0.32	-0.85%	-1.906	0.115
	4hr	37.15	37.72	-0.57	-1.53%	-3.248	0.023
	24hr	37.15	37.88	-0.73	-1.97%	-3.841	0.012
Standard control	1 hr	37.2	36.58	0.62	1.66%	2.052	0.095
	2hr	37.2	36.45	0.75	2.02%	2.601	0.048
	3hr	37.2	36.92	0.28	0.76%	1.336	0.239
	4hr	37.2	36.85	0.35	0.94%	1.612	0.168
	24hr	37.2	36.05	1.15	3.09%	5.411	0.003
<i>Panchang swaras</i>	1 hr	37.43	37.08	0.35	0.93%	1.685	0.153
	2hr	37.43	36.93	0.5	1.33%	2.565	0.05
	3hr	37.43	36.87	0.56	1.51%	2.371	0.064
	4hr	37.43	36.73	0.7	1.86%	2.941	0.032
	24hr	37.43	36.25	1.18	3.15%	4.692	0.005
<i>Panchang chrna</i>	1 hr	37.3	37.22	0.08	0.21%	1.185	0.289
	2hr	37.3	37.1	0.2	0.54%	2.928	0.033
	3hr	37.3	37.02	0.28	0.76%	3.576	0.016
	4hr	37.3	36.92	0.38	1.03%	4.839	0.005
	24hr	37.3	36.18	1.12	2.99%	5.519	0.003
<i>Panchang kwath</i>	1 hr	37.4	37.35	0.05	0.13%	0.197	0.852
	2hr	37.4	37.18	0.22	0.58%	0.859	0.43
	3hr	37.4	36.93	0.47	1.25%	2.064	0.094
	4hr	37.4	36.65	0.75	2.01%	2.622	0.047
	24hr	37.4	36.05	1.35	3.61%	2.8	0.038
Aqueous extra	1 hr	37.15	36.933	0.22	0.58%	2.137	0.086
	2hr	37.15	36.817	0.33	0.90%	3.492	0.017
	3hr	37.15	36.767	0.38	1.03%	4.213	0.008
	4hr	37.15	36.567	0.58	1.57%	3.063	0.028
	24hr	37.15	36.2	0.95	2.56%	5.27	0.003

DISCUSSION

The Physicochemical and phytochemical analysis shows that *E. axillare* (*Nahi*) has Glycosides, Saponins, Tannins, Phenolic compounds, Alkaloids, Flavonoids, Steroids, etc. Pyrexia may be due to injury, infection, or other disease states. Yeast-induced pyrexia is termed a pathogenic fever and therefore all the above chemical compounds may play an important role in antipyretic activity. *E. littorale* may inhibit IL-1, IL-6, and TNF- α production or activity. Otherwise may inhibit COX activity and exhibit antipyretic activity.

CONCLUSION

Standard allopathy Paracetamol is effective for a typical window as usual. The traditional forms of *Nahi* do not have such window effects. It is an important outcome—the drug formulations i.e. *Panchang Swaras, Kwath, Churn & Aq. Extracts* have shown a high degree of antipyretic action. Test items' antipyretic effect is comparable with reference standard paracetamol. Although the exact mechanism of antipyretic activity of *Enicostema axillare* (Poir. ex Lam.) A. Raynal E. is not entirely implicit, it's identified anti-inflammatory, antioxidant, and possible COX- inhibitory properties suggest several potential pathways.

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