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Evaluation of Antipyretic and Anti-Inflammatory Activity of Aqueous Extract of *Leptadenia Reticulata* in Animal Models

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Abstract

Objective: To study the antipyretic and anti-inflammatory effects of the Aqueous Extract of *Leptadenia Reticulata* (AELR) in different animal models. **Materials and Methods:** Acute toxicity studies were performed and extract was found to be safe upto 2000 mg/kg. Two doses were selected for the "evaluation of antipyretic and anti-inflammatory activity of aqueous extract of *Leptadenia reticulata* in animal models" *i.e* 200 mg/kg and 400 mg/kg b. w. Antipyretic activity was evaluated using baker's yeast induced pyrexia in albino rats and cow milk induced pyrexia in albino rabbits. Anti-inflammatory activity was evaluated using carrageenan induced paw edema and turpentine oil induced paw edema in albino rats. **Results:** In all the animal models AELR at the dose of 200 mg/kg b.w and 400 mg/kg b.w showed significant ($P<0.01$) antipyretic and anti-inflammatory activity. **Conclusion:** These finding could justify the inclusion of aqueous extract of *Leptadenia reticulata* in the management of pyrexia and inflammation.

Keywords: Baker's Yeast, Cow Milk, Carrageenan, *Leptadenia reticulata*, Turpentine Oil

1. Introduction

Pyrexia or fever may be the result of infection, tissue damage, inflammation or other disease states. A common feature of these conditions is the enhanced formation of cytokines such as interleukin-1, interleukin-6, interferon-α and β and tumor necrosis factor. The cytokines increase the synthesis of PGE2 by activating arachidonic acid pathway. PGE2 triggers the hypothalamus to elevate the body temperature by promoting increase in heat generation and decrease in heat loss[1]. Inflammation is a protective attempt by the organism to remove the injurious stimuli and to initiate healing process[2]. Many synthetic drugs are used to treat fever and inflammation, but these drugs have several side effects. Therefore, many medicinal plants are used to cure diseases with lesser side effects.

Leptadenia reticulate (Retz.) Wight & Arn. is a much branched twining shrub of family Asclepiadaceae. It is commonly called as 'jiwanti". This plant is distributed in the Southern parts of India. Traditionally this plant has stimulant and restorative properties. The leaves and roots are used in skin infections such as ring worm, wounds, nose and ear disorders, asthama and fever. The main chemical constituents reported are: α-amyrin, β-amyrin, ferulic acid, luteolin, diosmetin, rutin, β-sitosterol, stigmasterol, hentriacontanol, a triterpene alcohol simiarenol, apigenin, pregnane glycosides[3]. Aerial parts of *Leptadenia reticulata* is reported to contain tocopherol and possess several pharmacological activities such as galactogogue, antimicrobial and anti-inflammatory

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activity[4]. The present study was designed to evaluate the effect of aqueous extract of the whole plant of *Leptadenia reticulata* on pyrexia and inflammation.

2. Materials and Methods

2.1 Plant Extract

Aqueous extract of the whole plant of *Leptadenia reticulata* was procured from GR Herbals extractions (Dl. No. MP/25D/11/134) Indore, Madhya Pradesh.

2.2 Preliminary Phytochemical Studies

The preliminary phytochemical screening of the aqueous extract of *Leptadenia reticulata* revealed the presence of sterols, triterpenoids, flavonoids, proteins and carbohydrates[5].

2.3 Experimental Animals

Wistar albino rats (150–200 g) and albino rabbits (1-2 kg) were maintained on a standard pellet, cabbage and carrot diet respectively and water *ad libitum*. They were housed in polypropylene cages and maintained under standard conditions (12 h light–dark cycle; 23–25 °C; 35–60 % relative humidity). All the experimental protocols for animal care procedures were approved by the ethical committee of Gokaraju Rangaraju College of Pharmacy. Principles of laboratory animal care guidelines were followed and prior permission was sought from the Institute Animal Ethics Committee (IAEC) for conducting the experiments. Present study was carried out in CPCSEA approved animal house (Reg. no. 1175/ac/08/CPCSEA) of Gokaraju Rangaraju College of Pharmacy, Bachupally, Hyderabad, India.

2.4 Acute Toxicity Study

Acute toxicity studies were performed as per OECD guidelines 425. According to these guidelines a limit test was carried out using 5 female mice. A test dose of 2000 mg/kg of aqueous extract of *Leptadenia reticulata* was administered orally to the first mice and it was observed for 48 hrs. When there was no mortality seen, other four mice were given the same dose and were observed for 14 days.

The oral administration of aqueous extract of *Leptadenia reticulata* did not exhibit any signs of toxicity and mortality at 2000 mg/kg bd. wt. All animals were safe even after 14 days of observation.

2.4.1 Selection of Dose

From the above toxicity studies, 2000 mg/kg bd. wt was identified and the working dose was considered as $1/10^{th}$ i.e., 200 mg. To perform this study two doses were selected *i.e.* 200 mg/kg bd. wt and 400 mg/kg bd. wt[6].

2.5 Evaluation of Antipyretic Activity

2.5.1 Baker's Yeast Induced Pyrexia in Albino Rats

Antipyretic activity was assessed using baker's yeast (containing *Saccharomyces cerevisiae*) induced pyrexia in Wistar albino rats. The rectal body temperature was measured by introducing a digital thermometer coated with glycerine, inserted into the rectum to a depth of 3 cm. After measuring the basal rectal temperature, animals were given a *subcutaneous* injection of 20% baker's yeast suspended in 0.9 % saline (10 mL/kg bd. wt). Rats were then returned to respective cages. After 18 h of yeast administration, group-I served as disease control, received saline, group-II received AELR (200 mg/kg bd. wt), group-III received AELR (400 mg/kg bd. wt) and group-IV paracetamol (150 mg/kg bd. wt) *p.o*. The temperature was measured with a thermometer hourly upto 4 h[7,8].

2.5.2 Cow Milk Induced Pyrexia in Rabbits

Antipyretic activity was assessed using cow milk induced pyrexia in rabbits, as per the method described by M. Thirumal et al[9]. Group-I served as disease control, received cow milk and saline, group-II received cow milk and AELR (200 mg/kg bd. wt), group-III cow milk and AELR (400 mg/kg bd. wt) and group-IV received cow milk and paracetamol (10 mg/kg bd. wt) *p.o*. The temperature was measured with a thermometer hourly upto 4 hours after cow milk injection.

2.5.3 Carrageenan Induced Paw Edema in Rats

Anti-inflammatory activity was carried out using carrageenan induced paw edema in rats, as per the method described by Kalabharathi H L et al[2]. Group-I served as disease control, received carrageenan (1% w/v) and saline, group-II received carrageenan (1% w/v)

and AELR (200 mg/kg bd. wt), group-III carrageenan (1% w/v) and AELR (400 mg/kg bd. wt) and group-IV received carrageenan (1% w/v) and indomethacin (10 mg/kg bd. wt) *p.o*. Each rat was administered *orally* with the respective drug 1 h prior to the administration of the carrageenan. 0.05 mL of 1% carrageenan was injected aseptically into the sub-plantar surface of left hind paw of each rat. Paw edema was measured by mercury plethysmometer hourly upto 4 h to study the effect of aqueous extract the plant on inflammation. The difference between the '0' and '3rd' h reading gives the actual edema.

2.5.4 Turpentine Oil Induced Paw Edema in Rats

Anti-inflammatory activity was carried out using turpentine oil induced paw edema in rats, as per the method described by Surender Singh et al[10]. Group-I served as disease control, received turpentine oil and saline, group-II received turpentine oil and AELR (200 mg/kg bd. wt), group-III turpentine oil and AELR (400 mg/kg bd. wt) and group-IV received turpentine oil and indomethacin (10 mg/kg bd. wt) *p.o*. Paw edema was measured by mercury plethysmometer hourly upto 4 hours. The difference between the '0' and '3rd' h reading gives the actual edema.

2.6 Statistical Analysis

All the results are expressed as mean ± SEM of six animals in each group. The data was evaluated using ANOVA followed by Dunnet's 't' test. A significant value of $p<0.01$ was considered statistically significant.

3. Results

In baker's yeast induced pyrexia in rats, AELR (at the doses 200 mg/kg bd. wt and 400 mg/kg bd. wt) significantly ($P<0.01$) reduced the body temperature at 19 h, 20 h, 21 h and 22 h of yeast administration when compared to the control group.

In cow milk induced pyrexia in rabbits AELR (at the doses 200 mg/kg bd. wt and 400 mg/kg bd. wt) significantly ($P<0.01$) reduced the body temperature in

Table 1: Effect of AELR on baker's yeast induced pyrexia in albino rats

Compound	Initial Temp in (°C) 0 h	Temp. at 18 h after yeast adm.	Temp. (°C) at different hours after treatment			
			19 h	20 h	21 h	22 h
Control	36.5±0.1	38.8±0.1	38.7±0.1	38.6±0.1	38.5±0.1	38.4±0.1
AELR (200 mg/kg bd. wt)	36.4±0.1	38.7±0.1	37.6±0.1*	37.0±0.1*	36.7±0.1*	36.5±0.1*
AELR (400 mg/kg bd. wt)	36.7±0.2	38.9±0.1	37.6±0.1*	36.9±0.1*	36.4±0.1*	35.8±0.1*
Paracetamol (150 mg/kg bd. wt)	36.5±0.1	39.0±0.1	37.8±0.1*	37.1±0.1*	36.3±0.1*	35.4±0.1*

Values are expressed as mean ± SEM; n=6. *P<0.01 compared with the control

Table 2: Effect of AELR on cow milk induced pyrexia in albino rabbits

Compound	Initial Temp in (°C) 0 h	Temp. (°C) at 2 h after cow milk adm.	Temp. (°C) at different hours after treatment			
			3 h	4 h	5 h	6 h
Control	38.4±0.1	40.2±0.1	40.1±0.1	40±0.1	39.9±0.1	39.8±0.12
AELR (200 mg/kg bd. wt)	38.5±0.1	40.2±0.1	39.65±0.1*	39.5±0.1*	39.3±0.1*	38.9±0.09*
AELR (400 mg/kg bd. wt)	38.4±0.1	40.2±0.1	39.5±0.1*	39.3±0.1*	39±0.1*	38.6±0.1*
Paracetamol (10 mg/kg bd. wt)	38.6±0.1	40.3±0.1	39.43±0.1*	39.2±0.1*	38.9±0.1*	38.61±0.1*

Values are expressed as mean ± SEM; n=6. *P<0.01 compared with the control

rabbits at 3 h, 4 h, 5 h and 6 h of cow milk administration when compared to the control group.

In carrageenan and turpentine oil induce inflammation in rats AELR (at the doses 200 mg/kg bd. wt and 400 mg/kg bd. wt) significantly (P<0.01) reduced paw edema in rats when compared to the control group.

4. Discussion

Pyrexia or fever may be the result of infection, tissue damage, inflammation or other disease states. A common feature of these conditions is the enhanced formation of cytokines such as interleukin-1, interleukin-6, interferon-α and β and tumor necrosis factor. The cytokines increase the synthesis of PGE2 by activating arachidonic acid pathway. PGE2 triggers the hypothalamus to elevate the body temperature by promoting increase in heat generation and decrease in heat loss. Antipyretic drugs act by inhibiting the enzyme cyclooxygenase and reduce the levels of PGE2 within the hypothalamus[1]. In baker's yeast induced pyrexia in rats and cow milk induced pyrexia in rabbits, AELR at the doses of 200 mg/kg bd. wt and 400 mg/kg bd. wt significantly reduced the body temperature.

Carrageenan induced paw edema is a commonly used primary test for the screening of new anti- inflammatory agents and it is believed to be biphasic. The first phase (1- 2 hr) is due to the release of histamine or serotonin and the second phase of edema is due to the release of prostaglandin[2].

Turpentine oil induced paw edema is characterized by a triphasic release of inflammatory mediators. The initial phase is mediated by histamine and serotonin, intermediate phase by kinin like substance and the late phase by cycloxygenase and lipoxygenase products[7].

In carrageenan and turpentine oil induced paw edema in rats, AELR at the doses of 200 mg/kg bd. wt and 400 mg/kg bd. wt significantly inhibits the edema induced by carrageenan and turpentine oil in rats.

The earlier reports of Krishnamoorthi Mahalakshmi et al.[11], supports the role of flavonoids, triterpenoids and

Table 3: Effect of AELR on paw edema induced by carrageenan in albino rats

Compound	Change in paw volume (mL) at different hours				% of Inhibition at 3 h
	1 h	2 h	3 h	4 h	
Control	1.2±0.01	1.20±0.012	1.16±0.01	1.03±0.033	0.0
AELR (200 mg/kg bd. wt)	0.92±0.01*	0.87±0.01*	0.79±0.01*	0.66±0.01*	31.8
AELR (400 mg/kg bd. wt)	0.83±0.01*	0.77±0.01*	0.70±0.01*	0.58±0.02*	39.6
Indomethacin (10 mg/kg bd. wt)	0.78±0.01*	0.71±0.01*	0.62±0.01*	0.54±0.01*	46.5

Values are expressed as mean ± SEM; n=6. *P<0.01 compared with the control

Table 4: Effect of AELR on paw edema induced by turpentine oil in albino rats

Compound	Change in paw volume (mL) at different hours				% of Inhibition at 3 h
	1 h	2 h	3 h	4 h	
Control	1.7±0.042	1.6±0.047	1.4±0.051	1.1±0.05	0.0
AELR (200 mg/kg bd. wt)	1.3±0.04*	0.8±0.06*	0.7±0.01*	0.7±0.01*	46.42
AELR (400 mg/kg bd. wt)	01.1±0.04*	0.8±0.03*	0.6±0.02*	0.5±0.01*	52.1
Indomethacin (10 mg/kg bd. wt)	1.0±0.03*	0.7±0.03*	0.6±0.01*	0.5±0.01*	55

Values are expressed as mean ± SEM; n=6. *P<0.01 compared with the control

steroids in antipyretic and anti-inflammatory activity. Since these chemical constituents are also present in AELR, may be responsible for antipyretic and anti-inflammatory activity.

5. Conclusion

Antipyretic activity was performed using two models *i.e.* baker's yeast induced pyrexia in albino rats and cow milk induced pyrexia albino rabbits. AELR at 200 mg/kg bd. wt and 400 mg/kg bd. wt significantly decreased the elevated body temperature in both the animal models.

Anti-inflammatory activity was performed using two models *i.e.* carrageenan induced paw edema in rats and turpentine oil induced paw edema in rats. AELR at 200 mg/kg bd. wt and 400 mg/kg bd. wt significantly reduced the paw edema in both the animal models.

These finding could justify the inclusion of aqueous extract of *Leptadenia reticulata* in the management of pyrexia and inflammation.

The present study therefore supports the claims of traditional use of the plant for antipyretic and anti-inflammatory activities. To know the exact mechanism of action of AELR, further study with purified fractions of the bioactive compound is needed.

6. References

1. Hatapakki BC, Hukkeri VI. Antipyretic activity of root of *Marsdenia tenacissima* in rats. Journal of Natural Remedies. 2011; 11(2):98–102.
2. Kalabharathi HL, Suresh RN, Pragathi B, Pushpa VH, Satish AM. Anti-inflammatory activity of fresh tulsi leaves (*Ocimum sanctum*) in albino rats. International Journal of Pharma and Bio Sciences. 2011; 2(4):45–9.
3. Bawra B, Dixit M, Chauhan NS, Dixit VK and Saraf DK. *Leptadenia reticulata* a Rasayana herbs. Asian Journal of Plant Sciences. 2010; 9(6):314–9.
4. Rani S, Manavalan D, Killimozhi K, Balamurugan. Preliminary study on the anti-implantation activity of *Leptadenia reticulata* in female rats. International Journal of Pharma Technical Research. 2009; 1(4):1403–5.
5. Khandelwal KR. Practical pharmacognosy techniques and experiments. 2005. p. 30–149.
6. OECD Guideline for testing of chemicals (425). 2001. p. 1–26.
7. Olorunfemi OJ, Nworah DC, Egwurugwu JN, Hart VO. Evaluation of anti-inflammatory, analgesic and antipyretic effect of *Mangifera indica* leaf extract on fever-induced albino rats (Wistar). British Journal of Pharmacology and Toxicology. 2012; 3(2):54–7.
8. Emdad Hossain, Subhash Mandal C, Gupta JK. Phytochemical screening and *in-vivo* antipyretic activity of the methanol leaf-extract of *Bombax malabaricum* DC (Bombacaceae). Tropical Journal of Pharmaceutical Research. 2011; 10(1):55–60.
9. Thirumal M, Surya Srimanthula, Kishore G, Vadivelan R, Anand Kumar AVS. Analgesic and antipyretic effects of aqueous extract from *Clerodendrum inerme* (L.) Gaertn. leaves in animal models. Der Pharmacia Lettre. 2013; 5(2):315–23.
10. Singh S, Nair V, Gupta YK. Antiarthritic activity of Majoon Suranjan (a polyherbal Unani formulation) in rat. Indian Journal of Medical Research. 2011; 134:384–8.
11. Mahalakshmi K, Natesan SK, Parthiban KG, Sekar G. Anti-inflammatory and antipyretic activity of *Fioria vitifoli*. International Current Pharmaceutical Journal. 2013; 2(7):119–21.