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Evaluation of antipyretic activity of ethyl acetate extract of *Adenema hyssopifolium G. Don* in a rat model

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ABSTRACT

Objective: To evaluate the effect of ethyl acetate extract of *Adenema hyssopifolium* (AHEAE) on normal body temperature and brewer's yeast-induced pyrexia rats. **Methods:** Preliminary phytochemical tests, acute toxicity tests and antipyretic evaluation were carried out in ethyl acetate extract of *Adenema hyssopifolium*. Two doses of the extract (300 or 600 mg/kg orally) and standard antipyretic agent, paracetamol at a dose of 150 mg/kg were administered to various group of the rats. Mean rectal temperature before and after treatment was noted. **Results:** The phytochemical analysis of AHEAE revealed the presence of flavonoid and iridoid glycosides as major phytoconstituents. The administration of AHEAE at a dose of 300 or 600 mg/kg produced significant reduction ($P<0.001$ and $P<0.01$) of the body temperature in normal and pyrexia rats on a dose dependent manner. The antipyretic influence of AHEAE was comparable to that of standard antipyretic agent, paracetamol (150 mg/kg), and onset of action and reduction in pyrexia towards normal body temperature was delayed when compared to paracetamol treatment. At dose of 600 mg/kg, AHEAE reduced pyrexia to normal body temperature at 4 h after its administration compared to reduction of pyrexia to normal body temperature at 2 h by standard drug. The reduction of fever was consistent in paracetamol group from 2 to 4 h after its administration to normal body temperature compared to AHEAE treatments. **Conclusions:** Our present results corroborate with the traditional notion of *Adenema hyssopifolium G. DON* that is being used as an effective cure of fever and add authenticity to claim of indigenous healers that the taxon is a potential antipyretic agent.

1. Introduction

Adenema hyssopifolium G. Don (Gentianaceae) (AHEAE) is a slender perennial herb of great medicinal value in India. It was reported to possess antidiabetic[1-3], antimalarial[4], hepatoprotective[5,6], antitumor[7] and antibacterial activities[8]. The iridoid glycoside swertiamarin isolated from *Adenema hyssopifolium* holds promise as an antihyperlipedimic agent[9]. Some of the important constituents are betuline, a triterpene sapogenin isolated, secoiridoid glycoside swertiamarin and monoterpene alkaloids enicoflaavin and gentiocrucine[10-13]. Ethnomedicinal studies of north Gujarat (India) revealed the use of hot aqueous extract of *Adenema hyssopifolium* by tribal inhabitants for the treatment of diabetes. It is also useful to cure fever, stomach ache, dyspepsia and malaria in interior part of Gujarat[14]. The whole plant is powdered and used traditionally in India for treating tumors, which is referred in the treatise of Siddha system of medicine namely "Siddha Vaidhya Pathartha Guna Vilakam"[15]. The dried *Adenema hyssopifolium* and pepper can be ground well and mixed in the ratio of 10 : 1 and be advocated at a 1 g to assist the retention of foetus[16]. This combination was effective in treating seasonal fever in winter by traditional practitioners of Madurai district, Tamil Nadu, India. This form of therapy although prevalent had not yet been subjected to any scientific validation. Owing to the paucity of data, the present investigation focuses on evaluating the antipyretic activity of ethyl acetate extract of whole plant of *Adenema hyssopifolium* in brewer's yeast-induced pyrexia rat model.

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2. Materials and methods

2.1. Plant material

Adhering to traditional practices, whole plants of *Adenema hyssopifolium G. Don* were collected from Madurai, Tamil Nadu, India. The plant material was authenticated by Dr. D. Stephen, Taxonomist American College, Madurai, India. The voucher specimen was preserved in our institute herbarium for future reference (R.A. No.15/05). The study material was washed well and air dried for 10 days under shade. The dried plant material was pulverized to coarse material and passed through a No. 40 mesh sieve. The sieved materials were stored in an air tight container.

2.2. Preparation of AHEAE

The dried plant materials (1.3 kg) were submitted to sequential maceration with petroleum ether (60-80℃), chloroform, ethyl acetate and ethanol (90%) at room temperature. After each step, the extracts were collected and the solvents were evaporated under reduced pressure using rotary evaporator. The yield of chloroform, ethyl acetate and ethanolic extracts were 3.62, 10.36 and 14.72 w/v respectively. Preliminary phytochemical tests showed the presence of iridoid glycosides[17], phenols, saponins, tannins and flavonoids[18] in ethyl acetate extract of *Adenema hyssopfolium G. Don* and hence it was used for the antipyretic evaluation.

2.3. Experimental animals

Swiss albino female mice (20-25 g) and Wistar female rats (150-200 g) were obtained from the animal house of Pharmacology Department, Arulmigu Kalasalingam College of Pharmacy, Tamil Nadu, India. The Experimental protocol was approved by the institutional animal ethics committee and entire study involving treatments were according to the norms of committee-for-the-purpose-of-control-and-supervision-of-experiments-on-animals (CPCSEA), New Delhi, India. Animals were maintained under standard laboratory conditions and received standard food pellets with water *ad libitum*.

2.4. Chemicals

Brewer's yeast procured from Himedia Laboratories Pvt. Ltd., Mumbai and pure form of paracetamol obtained from Shasun Chemicals and Drugs Ltd., Pondicherry as gift sample. All other chemicals used for the study were of analytical grade and obtained commercially.

2.5. Acute toxicity study

Acute toxicity was performed with different doses of AHEAE according to the method described by Ghosh[19]. Consequent to dosing, the animals were observed with vigil continuously for the first 2 h for behavior and then checked at a regular interval of 6 h through a term of 14 days after AHEAE treatment. Toxic symptoms and mortality were promptly recorded[20]. AHEAE was suspended with 5% gum acacia before administration.

2.6. Study design to assess the effect of AHEAE on normal body temperature in rats

Sutar *et al*[21] method was adopted for the evaluation of AHEAE on normal body temperature. Female rats were divided into three groups of six for each. The initial rectal temperature (0 h) reading was taken from all groups of animals. The body temperature of each rat was measured rectally at predetermined intervals after the administration of 5% v/v aqueous gum acacia to control group and AHEAE (suspended with 5% v/v gum acacia) to the treatment group. Readings were taken up to a duration of 4 h in control and AHEAE (300 or 600 mg/kg) treated animals.

2.7. Brewer's yeast-induced pyrexia

Zhao Yongna *et al*[22] method was adopted for evaluation of antipyretic activity. The temperature was measured using digital thermometer (BD™ Rapid Flex, Becton Dickinson India Pvt. Ltd., New Delhi). After measuring the basal rectal temperature, pyrexia was induced in animals by subcutaneous injection of 15% brewer's yeast (10 mL/kg) suspended in saline solution into back side of below the nape of the neck and injected site is massaged in order to spread. After 19 h of yeast injection, the rectal temperature of each rat was measured using a digital thermometer. Female rats that showed rectal temperature of 38 ℃ and above were selected for the antipyretic evaluation.

2.8. Study design to assess the effect of AHEAE on brewer's yeast-induced pyrexia rats

Female rats were divided into four groups of six animals each. Group 1 rats received 5% gum acacia served as normal control, group 2 rats received paracetamol (150 mg/kg) served as standard, group 3 rats received AHEAE at dose of 300 mg/kg, and group 4 rats received AHEAE at dose of 600 mg/kg. The rectal temperature of all groups of rats was recorded up to 4 h using digital thermometer.

2.9. Statistical analysis

The data were analyzed for significance using the unpaired two-tailed student's *t*-test. P values <0.05 were considered as significant.

3. Results

3.1. Acute toxicity study

Acute toxicity results revealed that ethyl acetate extracts up to a dose of 3 000 mg/kg did not show any mortality and toxicity. Hence, to carry out antipyretic evaluation, 300 and

600 mg/kg doses of AHEAE were selected.

3.2. Effects of AHEAE on normal body temperature of rats

In normal rats, AHEAE 300 mg/kg produced more significant (P<0.001) reduction in normal temperature at 1, 3 and 4 h compared to control animals (Table 1).

Administration of AHEAE after 2 h produced less significant reduction in temperature compared to control animals (P<0.01). Meanwhile, the treatment of AHEAE with the dose of 600 mg/kg showed more significant reduction in normal body temperature at 2 to 4 h compared to control animals (P<0.001). Both doses of AHEAE effectively reduced normal body temperature from 1 to 4 h after administration.

3.3. Effects of AHEAE on yeast-induced pyrexia rats

Table 2 showed the significant elevation of body temperature in yeast-induced pyrexia rats at 19 h compared to rats of the control group at 0 h.

Table 1

Effect of AHEAE on normal body temperature (n=6) (℃) (mean ± SE).

Group	Rectal temperaturebefore and after treatment				
	0 h	1 h	2 h	3 h	4 h
Control (5% gum acacia)	37.40±0.05	37.30±0.06	37.30±0.06	37.30±0.07	37.20±0.02
AHEAE (300 mg/kg)	37.20±0.07	36.90±0.05[a]	36.10±0.31[b]	36.50±0.05[a]	36.30±0.04[a]
AHEAE (600 mg/kg)	37.30±0.12	36.20±0.18[b]	36.20±0.25[a]	36.40±0.18[a]	36.60±0.06[a]

[a]P<0.001, [b]P<0.01 compared to control animals.

Table 2

Effect of AHEAE on brewer's yeast-induced pyrexia rats (n=6) (℃) (mean ± SE).

Group	Rectal temperature before and after treatment					
	Before yeast injection	1 h	2 h	3 h	4 h	19 h
Control (5% gum acacia)	37.50±0.07	39.30±0.05	39.30±0.03	39.30±0.15	39.10±0.11	39.20±0.18
Paracetamol (150 mg/kg)	37.70±0.06	38.40±0.12[a]	37.70±0.06[a]	37.10±0.06[a]	37.00±0.06[a]	39.70±0.03
AHEAE (300 mg/kg)	37.60±0.15	39.20±0.10[b]	38.80±0.09[b]	38.50±0.11[a]	38.20±0.10[a]	39.60±0.06
AHEAE (600 mg/kg)	37.40±0.10	39.00±0.04[b]	38.50±0.16[b]	38.10±0.10[a]	37.70±0.05[a]	39.70±0.09

[a]P<0.001, [b]P<0.01 compared to control animals.

The reduction of elevated body temperature in AHEAE treatment groups both at the doses of 300 or 600 mg/kg were observed from 1 to 4 h after administration when compared to control animals. AHEAE at dose of 300 mg/kg reduced body temperature more significantly at 3 to 4 h (P<0.001) compared to 1 to 2 h (P<0.01) after administration than control animals. At the same time, treatment of AHEAE at dose of 600 mg/kg showed more significant reduction in elevated temperature at 2 to 4 h (P<0.001) compared to 1 h (P<0.01) than control animals. The standard drug and paracetamol reduced pyrexia more significantlyas well as consistently from 1 to 4 h compared to control animals (P<0.001). Thus the treatment of AHEAE showed dose dependent effect in the brewer's yeast-induced pyrexia rats.

4. Discussion

Pyrexia may be due to the infection or one of the sequence of tissue damage, inflammation, graft rejection or other diseased states[23]. The enhanced formation of pro-inflammatory mediators (cytokines like interleukin 1β, α, β and TNF-α) increases the synthesis of prostaglandin E_2 (PGE_2) near the pre-optic hypothalamus area, and it leads to triggering the hypothalamus to elevate the body temperature due to the infected or damaged tissue[24]. Currently, paracetamol and non-steroidal anti-inflammatory drugs (NSAIDs) are commonly prescribed to treat fever. These agents inhibit cyclooxegenase 2 (COX-2) to decrease the body temperature by inhibiting PGE_2 biosynthesis. These drugs causes toxic effects to the liver cells, glomeruli, cortex of brain and heart muscles due to inhibition of COX-2, but natural COX-2 inhibitors have lower selectivity with fewer side effects[25]. Recently, search for new drugs from plant sources or herbal formulations with potent antipyretic activity have received more attention due to the toxic nature of the existing antipyretic and anti-inflammatory agents[26]. Therefore, there is an urge for the search of a natural antipyretic agent with reduced or no toxicity to treat fever effectively.

The study results showed that ethyl acetate extract of *Adenema hyssopifolium* possess significant reduction in normal body temperature and brewer's yeast-induced pyrexia in rats on a dose dependent manner. At a dose of AHEAE 600 mg/kg showed significant temperature reduction in pyrexia rats from 2 to 4 h. Also, administration of AHEAE at dose of 300 mg/kg reduced elevated body temperature more significantly at 3 and 4 h. The antipyretic action of AHEAE may be due to the inhibition of the synthesis of PGE_2 in pyrexia rats. Flavonoids were reported for its antipyretic activity and iridoid glycoside was reported for its analgesic and antiphlogistic activities[27, 28].

In conclusion, for the first time, the present finding revealed that AHEAE possess significant antipyretic activity

in yeast-induced pyrexia rats and it supports the folklore use of *Adenema hyssopifolium* in the treatment of fever. The antipyretic action may be due to the presence of flavonoids and iridoid glycoside in the ethyl acetate extract of this plant. Further detailed studies are in progress to determine the mechanism of action of this plant for its antipyretic activity.

Conflict of interest statement

We declare that we have no conflict of interest.

References

[1]Vishwakarma SL, Sonawane RD, Rajani M, Goyal RK. Evaluation of effect of aqueous extract of *Enicostemma littorale* Blume in streptozotocin-induced type 1 diabetic rats. *Indian J Exp Biol* 2010; **48**(1): 26-30.

[2]Mansuri KJ, Goyal BR, Upadhyay UM, Sheth J, Goyal R. Effect of long term treatment of aqueous extract of *Enicostemma littorale* in Type 2 diabetic patients. *Orien Pharm Exp Med* 2009; **9**(1): 39-48.

[3]Bhatt NM, Barua S, Gupta S. Protective effect of *Enicostemma littorale* Blume on rat model of diabetic neuropathy. *Am J Infect Dis* 2009; **5**(2): 99-105.

[4]Sanket S, Sarita G. *In vitro* Anti plasmodial activity of *Enicostemma littorale*. *Am J Infect Dis* 2009; **5**(3): 259-62.

[5]Goyal RK, Vishwakarma SL. Hepatoprotective activity of *Enicostemma littorale* in carbon tetrachloride induced liver damage. *J Nat Rem* 2004; **4**: 120-26.

[6]Rajasekaran A, Arivukkarasu R, Murugesh S. Hepatoprotective effect of *Adenema hyssopifolium G. Don* (Gentianaceae) in carbon tetrachloride-induced hepatotoxicity in rats. *Trop J Pharm Res* 2010; **9**(2): 157-63.

[7]Mahajan Rita P, Bharambe Shailendra M, Mahulikar Pramod P, More Dhananjay H. Evaluation of *in vitro* antimicrobial activity of phytochemicals and extracts of *Enicostemma littorale*. *J Pure Appl Micorbiol* 2010; **4**(1): 379-85.

[8]Pavithra PS, Janani VS, Charumathi KH, Indumathy R, Sirisha P, Verma RS. Antibacterial activity of plants used in Indian herbal medicine. *Int J Green Pharm* 2010; **4**(1): 22-8.

[9]Hitesh V, Mandapati R, Vasudevan S, Harish P, Ramesh G. Swertiamarin: A lead from *Enicostemma littorale* Blume for anti-hyperlipidaemic effect. *Eur J Pharmacol* 2009; **617**(1-3): 108-12.

[10]Vishwakarma SL. Rajani M, Bagul M, Goyal R. A rapid method for the isolation of. Swertimarin from *Enicostemma littorale*. *Pharm Biol* 2004; **42**: 400-3.

[11]Mayurkumar BP, Shrihari MM. Aldose reductase inhibitory activity of a C-glycosidic flavonoid derived from *Enicostemma hyssopifolium*. *J Complem Integr Med* 2009; **6**(1): 22-7

[12]Ghosal S, Singh AK, Sharma PV, Chaudhuri RK. Chemical constituents of gentianaceae Ⅸ:Natural occurrence of erythrocentaurin in *Enicostemma hyssopifolium* and *Swertia lawii*. *J Pharm Sci* 2009; **63**(6): 944-5.

[13]Arivukkarasu R, Rajasekaran A, Murugesh S. Anti-inflammatory activity of alcoholic extract of *Adenema hyssopifolium G. Don* in acute and chronic experimental models in albino rats. *J Appl Biosci* 2009; **19**: 1049-53

[14]Goyal RK, Vishwakarma SL. Hepatoprotective activity of *Enicostemma littorale* in Carbon tetrachloride induced liver damage. *J Nat Rem* 2004, **4**: 120-6.

[15]Mani Senthilkumar KT, Rajkapoor B, Kavimani S. Protective effect of *Enicostemma littorale* against CCl_4-induced hepatic damage in rats. *Pharm Biol* 2005; **43**(5): 485-7.

[16]Loganathan N. *Poorveegam thoguppu nool kazheenziyam, mooligaikal, parampariya maruthuvam-Nallanool*. Poorveegam Aivu Arakattallai, Puducherri; 2007.

[17]Dinda B, Debnath S, Harigaya Y. Naturally occurring secoiridoids and bioactivity of naturally occurring iridoids and secoiridoids. *Chem Pharm Bull* 2007; **55**: 689-728.

[18]Vaijanathappa J, Badami S. Antiedematogenic and free radical scavenging activity of swertiamarin isolated from *Enicostemma axillare*. *Planta Medica* 2009; **75**(1): 12-7.

[19]Ghosh MN. Toxicity studies. In: *Fundamentals of experimental pharmacology*. 4th ed. Kolkata, India: Mc Graw-Hill; 2008.

[20]Akhila JS, Deepa S, Alwar SC. Acute toxicity studies and determination of median lethal dose. *Current Sci* 2007; **93**(7): 917-20.

[21]Sutar NG, Patil VV, Deshmukh TA, Jawle NM, Patil VR, Bhangale SC. Evaluation of anti-pyretic potential of seeds of *Moringa oleifera* Lam. *Int J Green Pharm* 2009; **3**(2): 148-50.

[22]Zhao Y, Reanmongkol W, Bouking P, Li Z, Zhan R. Analgesic and antipyretic activities of the aqueous extract of *Urtica macrorrhiza* in experimental animals. *Fitoterapia* 2005; **76**: 91-5.

[23]Mahesh SP, Swati P, Sachin RP, Ravi Kumar M, Patil MB. Evaluation of the analgesic and antipyretic activities of ethanolic extract of male flowers (inflorescences) of *Borassus flabellifer* L. (arecaceae). *Int J Pharmacy Pharm Sci* 2009; **1**(2): 98-106.

[24]Sanjib B, Bodhisattva R. Preliminary investigation on antipyretic activity of cuscuta reflexa in rats. *J Adv Pharm Tech & Res* 2010; **1**(1): 83-7.

[25]Chattopadhyay D, Arunachalam G, Ghosh L, Rajendran K, Mandal AB, Bhattacharya SK. Antipyretic Activity of Alstonia macrophylla Wall ex A. DC: An ethnomedicine of Andaman Islands. *J Pharm Pharmaceut Sci* 2005; **8**(3): 558-64.

[26]Gupta M, Mazumder UK, Kumar RS, Gomathi P, Rajeshwar Y, Kakoti BB, et al. Antiinflammatory, analgesic and antipyretic effects of methanol extract from *Bauhinia racemosa* stem bark in animal models. *J Ethnopharmacol* 2005; **98**(3): 267-73.

[27]Sutar NG, Patil VV, Deshmukh TA, Jawle NM, Patil VR, Bhangale SC. Evaluation of anti- pyretic potential of seeds of *Moringa oleifera* Lam. *Int J Green Pharm* 2009; **3**(2): 148-50.

[28]Jothimanivannan C, Kumar RS, Subramanian N. Anti-inflammatory and analgesic activities of ethanol extract of aerial parts of *Justicia gendarussa* Burm. *Int J Pharmacol* 2010; **6**: 278-83.