Antipyretic Activity of *Alstonia macrophylla* Wall ex A. DC: An Ethnomedicine of Andaman Islands

Debprasad Chattopadhyay[1], Ganeshan Arunachalam[1], Lopamudra Ghosh[2], Rajendran K.[3], Asit B. Mandal[4], S. K. Bhattacharya[1, 3]

[1]ICMR Virus Unit, Infectious Diseases & Beliaghata General Hospital, Beliaghata, Kolkata
[2]Division of Pharmacognosy, Department of Pharmaceutical Technology, Jadavpur University, Kolkata
[3]National Institute of Cholera & Enteric Diseases, Beliaghata, Kolkata
[4]Biotechnology Laboratory, Central Agricultural Research Institute, Andaman & Nicobar Island, India

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ABSTRACT. Purpose. *Alstonia macrophylla* Wall ex A. DC. Leaf, used in different ailments by the Onge tribes of Little Andaman Island, India, was investigated for its antipyretic potential. **Methods**. The methanol extract and its fractions were tested on normal body temperature and yeast-induced pyrexia in Wistar Albino rats. **Results**. The leaf extract at oral doses of 200 and 300 mg/kg, and the *n*-butanol fractions of the extract at 50 mg/kg showed significant reduction in normal body temperature and yeast-provoked elevated temperature in a dose-dependent manner comparable to that of standard antipyretic drug paracetamol. The antipyretic effect was started at 1h and extended for at least 5h after the drug administration. **Conclusions.** The antipyretic effect was more pronounced when the fraction A and B was administered together, indicating that both the fractions may contain antipyretic compounds which produce an additive effect in combination. Phytochemically these fractions contain β-sitosterol and ursolic acid.

INTRODUCTION

Alstonia macrophylla Wall ex A. DC. (Apocynaceae), locally known as Chuharoi by Onge and Tachoroi in Nicobarese is a panatropical tree native to Malaysia and stretching to the Bay Islands. Ethnobotanical literature indicate that the decoctions of *A. macrophylla* leaves (AML) and stem bark is widely used by the Onge and Nicobarese of Little Andaman Islands, India, to treat stomachache, skin diseases and urinary infections (1). Leaves are reported to have anticholeretic and vulnerary effect, and are greased with hot coconut oil for sprains, bruises and dislocated joints as poultice and used as febrifuge (2). Recently we have reported the moderate antibacterial, limited antifungal (3) and strong antiinflammatory activity (4) of AML extract. Although the plant is wildly used for remission of several ailments related to fever (Authors personal experience with the Onge tribes), its antipyretic potential has not been explored yet. Therefore, the present study, for the first time, aims to evaluate the antipyretic activity of the methanolic extract of AML. Pyrexia or fever is caused as a secondary impact of infection, tissue damage, inflammation, graft rejection, malignancy or other diseased states. It is the body's natural defence to create an environment where infectious agent or damaged tissue cannot survive. Normally the infected or damaged tissue initiates the enhanced formation of pro-inflammatory mediator's (cytokines like interleukin 1β, α, β and TNF-α), which increase the synthesis of prostaglandin E2 (PGE2) near preoptic hypothalamus area and thereby triggering the hypothalamus to elevate the body temperature (5). As the temperature regulatory system is governed by a nervous feedback mechanism, so when body temperature becomes very high, it dilate the blood vessels and increase sweating to reduce the temperature; but when the body temperature becomes very low hypothalamus protect the internal temperature by vasoconstriction. High fever often increases faster disease progression by increasing tissue catabolism, dehydration, and existing complaints, as found in HIV, when fever during seroconversion results faster disease progression (6). Most of the antipyretic drugs inhibit COX-2 expression to reduce the elevated body temperature by inhibiting PGE2 biosynthesis (7). Moreover, these synthetic agents irreversibly inhibit COX-2 with high selectivity but are toxic to the hepatic cells, glomeruli, cortex of brain and heart muscles, whereas natural COX-2 inhibitors have lower selectivity with fewer side effects (7). A natural antipyretic agent with reduced or no toxicity is therefore, essential. As AML is a century old medicaments used in ailments that caused fever and have antiinflammatory potential (4), so it will be a cost effective alternative approach to study this plant for the development of an effective antipyretic agent.

Corresponding Author: D. Chattopadhyay, Senior Research Officer, ICMR Virus Unit, Infectious Diseases & Beliaghata General Hospital, GB 4, 1st Floor, 57, Beliaghata Main Road, Kolkata 700 010. debprasadc@yahoo.co.in; icmrvubd@vsnl.net

MATERIAL AND METHODS

Plant material

The leaves of *Alstonia macrophylla* Wall ex A. DC were collected from the rain forests of Middle and South Andamans, India, during April, June and October 1999 and February, August and November 2000. The voucher specimens have been identified (Herbarium No. 9220, 9221, 9227 and 9228 respectively) and deposited at the Herbarium Section of the Botanical Survey of India, Andaman & Nicobar Circle, Port Blair, India.

Preparation of Extract

Extraction and fractionation

Coarsely powdered dry leaves (1 kg) were successively extracted with 95% MeOH at room temperature for 72 h. The whole extract was filtered and the solvent were evaporated to dryness in vacuo to a residue with an Eyela Rotary Evaporator (Japan) at 40^{o}-45^{o}C. The percentage yield of the prepared extract (residue) was 8.9% ± 0.21 (w/w). The preliminary analysis of the residue was performed by the phytochemical group tests for alkaloids, flavonoids, triterpenoids and steroids, reducing sugars, tannins, saponins and amino acids [3, 8]. The presence of alkaloids was detected by Mayer, Dragendorff's, Wagner's and Hager's tests. Flavonoids was tested by dissolving the extract in ethanol and hydrolyzed with 10% H_2SO_4, cooled and further extracted with diethyl ether, and finally treated separately with diluted $NaCO_3$, 0.1 M NaOH, and diluted NH_3 solution for the development of yellow colour. Triterpenoids and steroids was identified by Libermann-Burchard reaction and Salkowski test and reducing sugars by Benedict's and Fehling's test. For tannins the extract was dissolved in distilled water, filtered and filtrate were treated separately with 10% potassium dichromate for yellowish brown precipitate, 10% lead acetate for yellow precipitate and 5% ferric chloride for greenish black colouration. The detection of saponin was made by dissolving the extract (residue) in distilled water and shaken in a graduated cylinder for 15 min to see the formation of stable foam; and amino acids was identified by ninhydrin test [8]. The chemical tests of residue revealed the presence of tannins, triterpenoids, flavonoids, alkaloids, sterols and sugars. For separation of the major bioactive compounds the residue was partitioned between *n*-BuOH and water. The *n*-butanol part of the extract (25 g) was then subjected to Si-gel column chromatography eluting with petroleum ether (PE), PE: $CHCl_3$ (at different ratio), $CHCl_3$, $CHCl_3$: MeOH (at different ratio) and MeOH. All eluted fractions were monitored by TLC. The fractions A (9 g), B (5 g) and C (6 g) were repeatedly chromatographed with Si-gel column. The presence of β-sitosterol, ursolic acid and β-sitosterol glucoside in fractions A, B and C respectively was pointed out by TLC, IR, MS, NMR and crystallography (3). The lyophilized aqueous portion (32 g) revealed the presence of inorganic salts and sugars and was not tested further. The whole extract and the fractions are insoluble in water and hence, we used propylene glycol as vehicle. The extract residue (18, 36 and 54 mg) or fractions (9 mg) were dissolved in 0.5 ml propylene glycol and diluted with 4.5 ml distilled water 30 min before each experiment, and administered orally to each animals (body weight 180 gm) to get a desired concentration of 100, 200, 300 and 50 mg/kg respectively, otherwise the unused dried extract and fractions were stored in a vacuum dessicator for future use.

Animal used

Adults Wistar albino rats of either sex weighing 180-200 g each were used. The animals were kept in the standard metal cages in groups of 10 per cage, with free access to standard diet and water *ad libitum* in the animal house of the Pharmaceutical Technology Department, Jadavpur University, Kolkata, and maintained at room temperature under suitable nutritional and environmental conditions throughout the experiment. The Institutional Animal Ethics Committee reviewed the entire animal protocols prior to conducting the experiments.

Study on normal body temperature

Rats of either sex were divided into seven groups, comprising six in each group. The body temperature of each rat was measured rectally at predetermined time intervals before and for 5h after the administration of either propylene glycol (vehicle control) or AML extract at doses of 100, 200 and 300 mg/kg, fractions A and B at 50 mg/kg and a combination of fractions A and B (50 + 50 mg/kg body weight) orally.

Induction of fever by Yeast-induced pyrexia

Yeast induced pyrexia was used to evaluate the antipyretic activity of the extract. The rats were divided into eight groups of six animals each and the body temperature of each rat was recorded by measuring rectal temperature at predetermined time intervals. Fever was induced by injecting 15% suspension of Brewer's yeast (*Saccharomyces*

cerevisiae), following a standard method (9). In brief, the rats were allowed to remain quiet in the cage for sometimes. A thermister probe was inserted 3-4 cm deep into the rectum, after fastened the tail, to record the basal rectal temperature. The animals were then given a subcutaneous injection of 10 ml/kg of 15% w/v Brewer's yeast suspended in 0.5% w/v methylcellulose solution and the animals were returned to their housing cages. 19h after yeast injection, the rats were again restrained in individual cages to record their rectal temperature. Immediately the AML extract was administered orally at doses of 100, 200 and 300 mg/kg to the first three groups of animals, the fourth and fifth group received 50 mg/kg of fraction A and B respectively, while the sixth group received a combination of both the fractions A (50 mg/kg) and B (50 mg/kg), the seventh group received 5 ml/kg of propylene glycol as vehicle control and the last group was administered with 150 mg/kg of paracetamol (for each rat 27 mg of paracetamol was dissolved in 0.5 ml propylene glycol and diluted with 4.5 ml distilled water) as drug control. Rectal temperature of all the rats was recorded at 19 h, immediately before extract or vehicle or paracetamol administration, and again at 1h interval upto 23h, after yeast injection (10).

Statistical analysis

The statistical analysis was carried out with SPSS 13.0 (Windows) software. Difference of the parametric data of body temperature(s) was examined by two-way analysis of variance (ANOVA) with Dunett's Post hoc pair wise multiple comparison t test, to compare a set of experimental data against control mean.

RESULTS

The effect of AML extract on normal body temperature in rats is presented in Figure 1. The results showed that the leaf extract at doses of 200 mg/kg caused significant lowering of the body temperature up to 3 h following extract administration, as the normal mean temperature 36.5^0C at 0 h was reduced to 36.0^0C at 3 h. While maximum lowering of body temperature was noticed at 300 mg/kg of the leaf extract, as the mean temperature of 36.5^0C was reduced to 35.4^0 C within 3-5h period in a dose-dependent manner.

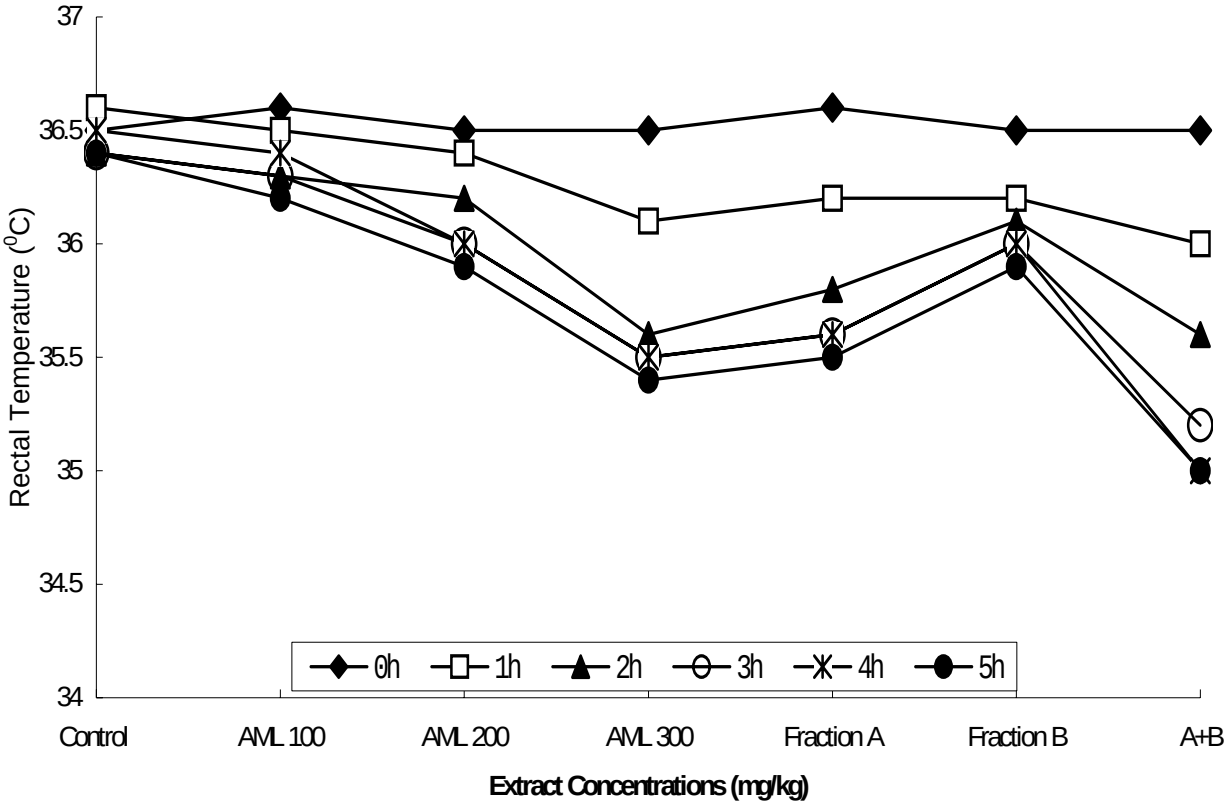

Figure 1. Effect of different concentrations of *A.macrophylla* extract on normal body temperature of Rats.

Table 1. Statistical Analysis by SPSS 13.0 on Normal Body temperature

Body Temp	2-WAY ANOVA Dunnett t (2-sided) Post Hoc test, compare with vehicle control group (|MD| ±SE)					
	Methanol extract of *Alstonia macrophylla* leaf			Fractions		
	100 mg/kg	200 mg/kg	300 mg/kg	A (50 mg/ kg)	B (50 mg/ kg)	A+B
0hrs	0.100 ± .0414	0.000 ± .0414	0.000 ± .0414	0.100 ± .0414	0.000 ± .0414	0.000 ± .0414
1hrs	0.100 ± .0390	0.200 ± .0390*	0.300 ± .0390*	0.400 ± .0390*	0.400 ± .0390*	0.600 ± .0390*
2hrs	0.100 ± .0451	0.200 ± .0451*	0.833 ± .0451*	0.600 ± .0451*	0.300 ± .0451*	0.800 ± .0451*
3hrs	0.100 ± .0414	0.400 ± .0414*	0.900 ± .0414*	0.800 ± .0414*	0.400 ± .0414*	1.200 ± .0414*
4hrs	0.100 ± .0436	0.500 ± .0436*	1.000 ± .0436*	0.900 ± .0436*	0.500 ± .0436*	1.500 ± .0436*
5hrs	0.200 ± .0390*	0.500 ± .0390*	1.000 ± .0390*	0.900 ± .0390*	0.500 ± .0390*	1.400 ± .0390*

**p=<0.001, MD=Mean difference, SE= Standard Error.*

The interesting finding is the effect of combination of fraction A and B at 50 mg/kg each, showing maximum lowering of temperature as the initial temperature 36.5^0C is reduced to 35.0^0C within 4-5h period, as shown in Figure 1. The results of 2-way ANOVA showed that there was significant differences among the extract treated groups ($F=765.15$, $p<0.001$), body temperatures ($F=722.30$, $p<0.001$ and interaction of different concentration of the extract and fractions ($F=48.33$, $p<0.001$) in maintaining normal body temperature (Table 1).

The effect of AML extract on yeast-induced pyrexia is presented in Figure 2. The data revealed that the rectal temperature of 37.3^0C at 0h was markedly elevated to 39.6^0C for vehicle control and 39.0^0C for paracetamol control group 19h after the subcutaneous injection of yeast suspension. The animals treated with AML extract at 100, 200 and 300 mg/kg doses showed a decrease in the rectal temperature by 0.5, 0.6 and 1.3^0C respectively within 1h. On the otherhand, in 21h the temperature was reduced by 0.6^0C for all the groups of animals received AML extracts; and at 22h the temperature was reduced by 0.8 to 1.0^0C. While the recorded temperature 38.5^0C, 38.0^0C and 37.5^0C for 100, 200 and 300 mg/kg group in 23rd h showed that the extract can significantly reduced the temperature by 0.9, 1.2, 1.5^0C respectively.

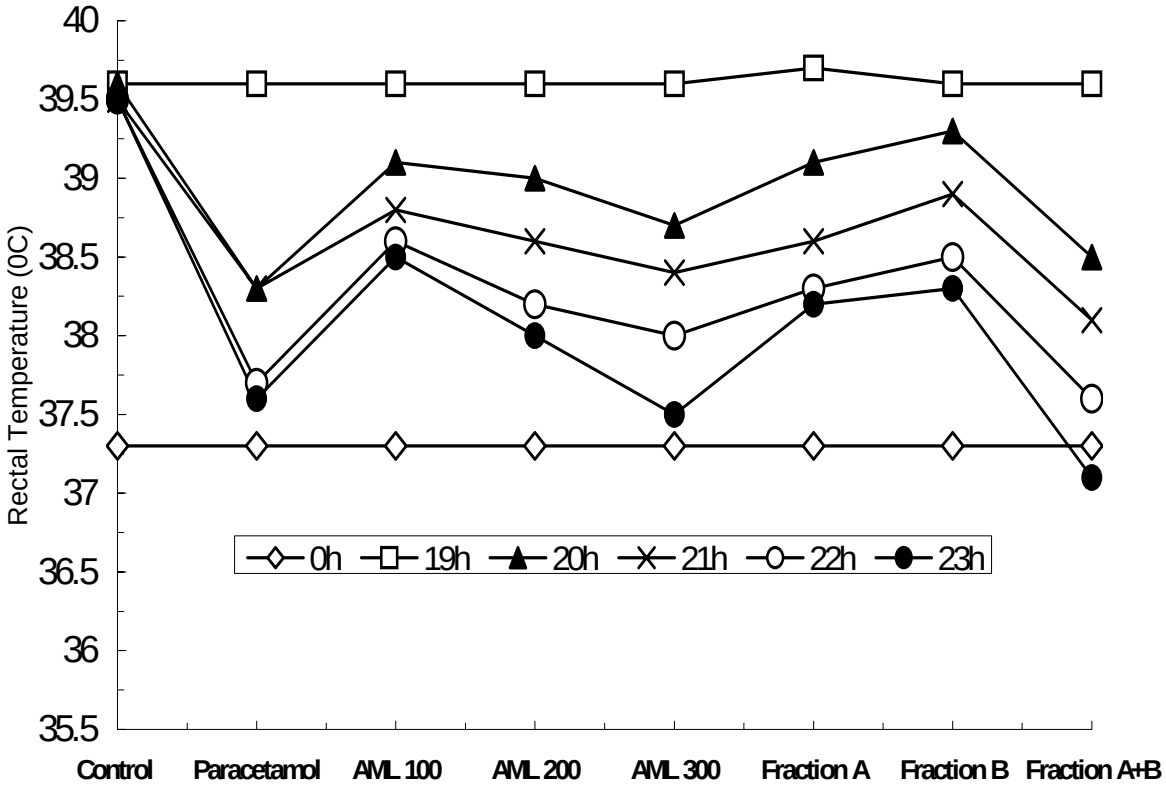

Figure 2. Effect of different concentrations of *A.macrophylla* leaf extract on yeast-induced pyrexia in rats.

Table 2. Statistical Analysis by SPSS 13.0 on Yeast-induced pyrexia

Rectal Temp	2-WAY ANOVA Dennett t (2-sided) Post Hoc test, compare with vehicle control group (|MD| ±SE)						
	Methanol extract of *Alstonia macrophylla* leaf			Fractions			Paracetamol (150 mg/kg)
	100 mg/kg	200 mg/kg	300 mg/kg	A (50 mg/kg)	B (50 mg/kg)	A+B	
0hrs	0.0 ± .0408	0.0 ± .0408	0.0 ± .0408	0.0 ± .0408	0.0 ± .0408	0.0 ± .0408	0.0 ± .0408
19hrs	0.0 ± .0447	0.0 ± .0447	0.0 ± .0447	0.0 ± .0447	0.0 ± .0447	0.0 ± .0447	0.0 ± .0447
20hrs	0.500 ± .0396*	0.600 ± .0396*	0.900 ± .0396*	0.500 ± .0396*	0.317 ± .0396*	1.100 ± .0396*	1.300 ± .0396*
21hrs	0.700 ± .0365*	0.900 ± .0365*	1.100 ± .0365*	0.900 ± .0365*	0.600 ± .0365*	1.400 ± .0365*	1.200 ± .0365*
22hrs	0.900 ± .0465*	1.300 ± .0465*	1.500 ± .0465*	1.200 ± .0465*	1.000 ± .0465*	1.900 ± .0465*	1.800 ± .0465*
23hrs	1.000 ± .0387*	1.500 ± .0387*	2.000 ± .0387*	1.300 ± .0387*	1.200 ± .0387*	2.400 ± .0387*	1.900 ± .0387*

**p=<0.001, MD=Mean difference, SE= Standard Error.*

However, the paracetamol (150 mg/kg) treated group showed that the rectal temperature of 39.0^0C was reduced by 0.7^0C at 21h and 1.4^0C at 23h respectively. The antipyretic effect of leaf extract at 300 mg/kg is similar to the paracetamol group. Interestingly the fraction A and B in combination, showed maximum reduction in temperature, from 39.0^0C to 37.1^0C within 5h of extract administration. This signifies that rectal temperature of the treated rats was decreased in a dose-dependent manner by AML extract. Furthermore, the antipyretic effect was started within 1h of extracts administration, and maintained for at least 5 h after the administration of the extract. The statistical analysis also revealed that the temperature difference was significant among the extract treated groups ($F=909.02$, $p<0.001$), body temperatures ($F=5831.97$, $p<0.001$) and different concentration of the extract and fractions interaction ($F=115.83$, $p<0.001$) to reduce the elevated temperature.

To determine the role of extract and or fractions in restoring normal body temperature or reducing the elevated body temperature of the treated animals, the Dunett's Post hoc pair wise when compared with the control group, the analysis indicated that the mean differences were statistically significant on treatment groups of AML 200 and 300 mg/kg (Table 1 and Table 2). Moreover, the highest reduction in body temperature was found with 300 mg/kg dose of AML extract; and the administration of both the fraction (A and B) in combination yielded highest significance level in comparison with paracetamol group.

DISCUSSIONS & CONCLUSIONS

Search for herbal remedies with potent antipyretic activity received momentum recently as the available antipyretics, such as paracetamol, nimusulide etc. have toxic effect to the various organs of the body (11). The body's ability to maintain a natural balance of COX 1 and 2 that regulate inflammatory response play a crucial role in supporting cardiovascular, immune, neurological, and joint and connective tissue systems (7). A number of plant extracts modulate enzymes of cyclooxygenase pathway, as reported with the rosmarinic acid of *Rosmarinus officinalis* that inhibit leukotriene and prostaglandins synthesis, while COX-1 and COX-2 was inhibited by cirsilineol, cirsimaritin, apigenin, rosmarinic acid and eugenol of *Ocimum sanctum* similar to ibuprofen, naproxen, and aspirin (12).

The results showed that the methanol extract of AML possesses a significant antipyretic effect in maintaining normal body temperature and reducing yeast-induced elevated body temperature in rats in a dose dependent manner and its effect is comparable to that of the standard antipyretic drug paracetamol. Furthermore, the significant reduction of yeast provoked elevated temperature of the tested animals by the extract at 200 mg/kg dose or more and 50 mg/kg of fractions appears to be due to the action of ursolic acid, β-sitosterol and its glucoside alone or in combination, as the maximum antipyretic effect was found in combination of fraction A (β-sitosterol) and fraction B (ursolic acid). Moreover, the statistical analysis with two-way ANOVA showed that the methanol extract of AML decreases both the normal and yeast elevated body temperature in a short span

of time in a dose dependent manner, when compared with control group. The interesting finding in yeast-induced experiment is that the fraction A and B in combination was more effective than the paracetamol. The bioactive fraction A contains β-sitosterol, a phytosterol with an extra alkyl group at C-24 in the side chain. The β-sitosterol is a plasminogen activator and promotes the formation of essential polyunsaturated fatty acids from linoleic acid, required for prostaglandin and leukotriene synthesis (13). Beta-sitosterol and its glycoside possess potent anti-inflammatory and antipyretic activity (14), by reducing the secretion of pro-inflammatory cytokines and TNF-α (14, 15). In vitro, animal, and human studies have shown that β-sitosterol and its glucosides in combination selectively enhance the activity of helper-T cells with a significant rise in interleukin 2 (IL-2) and gamma interferon (IFN-γ) level, with enhanced natural killer (NK) cell activity and thus, are therapeutically useful in immune dysfunction diseases (15). These phytosterols can enhance "adaptive" immunity through the stimulation of "innate" immune system, and hence termed as the 'adaptogen' which promote overall health without the side effect and rapid response of drugs (16). On the otherhand, fraction B of AML extract contains ursolic acid as major constituent (3). The ursolic acid, a pentacyclic triterpene, has diverse pharmacological actions including antiinflammatory (4, 17-19), antihistaminic (20) and analgesic (17, 21). As a potent antiinflammatory agent it inhibit human leukocyte elastage (22), 5-lipooxygenase and cyclooxygenase (12, 23) and thereby prostaglandin biosynthesis (24). Ursolic acid is also a potent and highly selective inhibitor of cyclic AMP-dependent protein kinase and phosphodiestarase and thereby regulates metabolism, cell division and gene expression (25, 26).

It was evident from the study that the observed antipyretic effects of the extract were similar in both magnitude and time course. However, to know the exact mechanism of action of *Alstonia macrophylla* leaf extract further study with purified fractions is warranted.

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REFERENCES

1. Bhargava N. Ethnobotanical studies of the tribes of Andaman and Nicobar Islands, India. I. Onge. *Econ Bot*, 37: 110-119, 1983.
2. Asolkar, LV.; Kakkar, KK.; Chakre, OJ., (ed.). Second supplement to Glossary of Indian Medicinal Plants with active principles, Part 1, Publications & Information Directorate, CSIR, New Delhi, India, pp. 51-52, 1992.
3. Chattopadhyay D, Maiti K, Kundu AP, Chakrabarty MS, Bhadra R, Mandal SC, Mandal AB. Antimicrobial activity of *Alstonia macrophylla*: A folklore of Bay Islands. *J Ethnopharmacol*, 71: 49-55, 2001.
4. Arunachalam G, Chattopadhyay D, Chatterjee S, Mandal AB, Sur TK, Mandal SC. Evaluation of antiinflammatory activity of *Alstonia macrophylla* Wall ex A. DC. Leaf extract. *Phytomedicine*, 9(7): 632-635, 2002.
5. Spacer CB, Breder CD. The neurologic basis of fever. *New England J Med*, 330: 1880-1886, 1994.
6. Veugelers PJ, Kaldor JM, Strathdee SA, Page-Shafer KA, Schechter MT, Coutinho RA, Keet IP, van Griensven GJ. Incidence and prognostic significance of symptomatic primary human immunodeficiency virus type 1 infection in homosexual men. *J Infect Dis*, 176: 112-117, 1997.
7. Cheng L, Ming-liang H, Lars B. Is COX-2 a perpetrator or a protector? Selective COX-2 inhibitors remain controversial. *Acta Pharmacologica Sinica*, 26 (8): 926-933, 2005.
8. Trease, GE.; Evans, WC., Pharmacognosy, 12th Edn, ELBS Publication, Baillier Tindall, East Bourne, pp. 344-352, 539, 1996.
9. Murugesan T, Mandal SC, Bhakta T, Das J, Pal M, Saha BP. Evaluation of anti-pyretic potential of *Jussiaea suffrutucosa* Linn. Extract in rats. *Phytomedicine*, 7**:** 231-234, 2000.
10. Chattopadhyay D, Arunachalam G, Mandal AB, Mandal SC. Evaluation of antipyretic activity of leaf extracts of *Mallotus peltatus* (Geist) Muell. Arg. var *acuminatus*: A Folk medicine. *Phytomedicine*, 9: 727-730, 2002.
11. Guyton, AC.; Hall, JE., Textbook of Medical Physiology. 9th Edn. W.B.Saunders Company, Philadelphia, pp. 920-922, 1998.
12. Kelm MA, Nair MG, Strasburg GM, DeWitt DL. Antioxidant and cyclooxygenase inhibitory phenolic compounds from *Ocimum sanctum* Linn. *Phytomedicine*. 7(1):7-13, 2000.

13. Kinsella JE, Lokesh B, Broughton S, Whelan J. Dietary polyunsaturated fatty acid and eicosanoids; potential effects on the modulation of inflammatory and immuned cells: an overview. *Nutrition*, 6: 24-44, 1990.
14. Gupta MB, Nath R, Srivastava N, Shankar K, Kishor K, Bhargava KP. Antiinflammatory and antipyretic activities of β-sitosterol. *Internat J Immunopharmacol*, 18: 693-700, 1996.
15. Bouic PJD. Plant sterols and sterolins: a review of their immune-modulating properties. *Altern Med Rev*, 4:170-177, 1999.
16. Wagner, H., Immunostimulants and adaptogens from plants. Amason JT, Mata R, Romeo JT (eds), Phytochemistry of Medicinal Plants. *Plenum Press,* New York, pp. 1-18, 1995.
17. Chattopadhyay D, Arunachalam G, Sur TK, Bhattacharya SK, Mandal AB. Analgesic and Antiinflammatory Activity of *Alstonia macrophylla* and *Mallotus peltatus* leaf extracts: Two popular Ethnomedicines of Onge, A Nigrito Tribes of Little Andaman. *Oriental Pharmacy and Experimental Medicine* 5(2) in press, 2005.
18. Liu J. Pharmacology of oleanolic and ursolic acid. *J Ethnopharmacol*, 49:57-68, 1995.
19. Chattopadhyay D, Arunachalam G, Mandal AB, Sur TK, Mandal SC, Bhattacharya SK. Antimicrobial and antiinflammatory activity of folklore *Mallotus peltatus* leaf extract. *J Ethnopharmacol*, 82, 229-237, 2002.
20. Tsuruga T, Chun Y, Ebizuka Y, Sankawa U. Biologically active constituents of *Melaleuca leucadendron*; inhibitors of induced histamine release from rat mast cells. *Chem Pharmacol Bull*, *3*9: 3276-3278, 1991.
21. Kosuge T, Yokota M, Sugiyama K, Mure T, Yamazawa H, Yamamoto T. Studies on bioactive substances in crude drugs used for arthritic disease in trational Chinese medicine. III. Isolation and identification of anti-inflammatory and analgesic principles from the whole herb of *Pyrola rotundifolia* L. *Chem Pharmaceut Bull*, 33: 5355-5357, 1985.
22. Ying QL, Rinehart AR, Simon SR, Cheronis JC. Inhibition of human leucocyte elastase by ursolic acid. *Biochemical J*, 277: 521-526, 1991.
23. Najid A, Simon A, Cook J, Chable-Rabinobitch H, Delage C, Chulia A, Riguad M. Characterization of ursolic acid as a lipoxygenase and cyclooxygenase inhibitor using macrophages, platelets and differentiated HL 60 leukemic cells. *FEBS*, 229: 213-217, 1992.
24. Ringbom T, Segura L, Noreem Y, Perera P, Bohlin L. Ursolic acid from *Plantago major*, a selective inhibition of cyclooxygenase-2 catalyzed prostaglandin biosynthesis. *J Nat Prod*, 61: 1212-1215, 1998.
25. Wang BH, Polya GM. Selective inhibition of cyclic AMP-Dependent protein kinase by amphiliphilic triterpenoids and related compounds. *Phytochemistry*, 41: 55-63, 1996.
26. Karin M, Smeal T. Control of transcription factors by signal transduction pathways: the beginning of the end. *Trends Biochem Sciences*, 17: 418-422, 1992.