

## Antipyretic Activity of Methanolic and Ethyl Acetate Extract of *Holostemma ada* Kodien Schult on Wistar Rats.

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### ABSTRACT

Investigation on the antipyretic activity of the traditionally used medicinal plant leaf extracts of *Holostemma ada* Kodien Schult (Asclepiadaceae); the methanolic (ME-HAKS) and ethyl acetate leaf (EAE-HAKS) extract (200 and 400mg/kg) was challenged against the acetylsalicylic acid (300mg/kg) as positive control for the assessment antipyretic activity on wistar rats; on subcutaneously treated with aqueous suspension 15 ml/kg of 20% w/v brewer's yeast. Preliminary phytochemical investigation showed the presence of flavonoids; tannins, saponines, anthocyanins, steroids, alkaloids and phenols were the major component in the methanolic extract and ethyl acetate extracts. The methanolic showed the dose – dependence reduction in hyperpyrexia when compared with the ethyl acetated extract and positive control. Hence further investigation on the separation and isolation of active principle will lead to a potent anti-pyretic agent.

**Keywords:** antipyretic activity, *Holostemma ada*

### INTRODUCTION

India is an ironic source of therapeutic flora and a number of plant derived oils and extracts are used against various ailments related to human health by traditional healers by different systems of medicine such as Ayurveda, Unani and Siddha. Only a few of them have been scientifically explored. Secondary metabolites derived from plants as natural products such as flavonoids, terpenes, phenols and alkaloids<sup>1,2</sup> have increased significant consideration by the researcher in recent years due to their diverse multi pharmacological properties these plants still represent an enormous cradle of natural antioxidants that might serve as leads for the development of novel drugs. Numerous anti-inflammatory, neuroprotective, , antipyretic, analgesic activities digestive, hepatoprotective, anti-cancer, anti-diabetic and antinecrotic medicines have lately been exposed to have an antioxidant and/or radical scavenging mechanism as part of their activity<sup>3-5</sup>.

*Holostemma ada* Kodien schult (Asclepiadaceae) important medicinal plant and widely distributed in tropical forest in India IUCN Red List of Threatened species<sup>6</sup>. The plant is used as antidiabetic, rejuvenative, aphrodisiac, expectorant, galactagogue, stimulant, Orchitis, pain, stomach ache, gonorrhoea and in ophthalmic disorders<sup>7, 8</sup>. Traditionally in the ayurvedic system of medicine they used for the treatment of fever, antidiabetic and anti-inflammatory activity. The root and leaves of Arkaparni is used in the form of powder and juice to treat

spider-poisoning, Fever with burning sensation: Decoction of jivanti root mixed with ghee removes burning sensation and fever. (40-60 ml). Eye diseases: In case of Defects of vision leaves of jivanti is used as a vegetable. Wound: Paste of jivanti should be applied locally for three days. It presses the wound due to sliminess and thus promotes its healing. Hence in the present work an attempted was made to perform in-vivo antipyretic activity of leaf extract of *Holostemma ada* Kodien schult.

### MATERIALS AND METHODS:

Plant material: Leaves of *Holostemma ada* Kodien Schult were collected from Tirumala hills, Chittoor District Andhra Pradesh, India; during June – July 2012 and the plant was identified and authenticated by Dr. Madavashetty, Professor, Department of Botany, S.V. University, Tirupathi (A.P), and India. The materials were washed thoroughly and shade dried.

Preparation of Extraction and phytochemical screening of plant material: The leaves of *Holostemma ada* Kodien Schult after shade drying were pulverized by a power-driven grinder and the powder were passed through sieve (40-mesh), to get a fine powder. The powder material (2kg), were subjected for sequential extraction with increase in the polarity of the solvents n-hexane, ethyl acetate, methanol, and hydro-alcohol (1:1) at 60-70°C by Soxhlet extractor. Each solvent were extract with the

Table 1: Antipyretic activity of methanolic and ethyl acetate leaf extracts of *Holostemma ada Kodiensis* Schult.

Treatment	Control (2% Tween 80 solution)	Positive control ASA	EAE-HAKS 200	EAE-HAKS 400	ME-HAKS 200	ME-HAKS 400
Dose mg/kg	-	300	200	400	200	400
Before yeast injection	37.10±0.60	36.27±0.43	36.97±0.71	37.30±0.33	36.42±0.64	36.63±0.64
19h	38.87±0.32	37.22±0.17 <sup>a</sup>	38.05±0.41	38.77±0.12	37.82±0.08	38.05±0.29
20h	39.18±0.16	36.50±0.19 <sup>a</sup>	39.20±0.54	39.35±0.26	37.77±0.30 <sup>b</sup>	37.13±0.18 <sup>b</sup>
21h	39.48±0.16	36.48±0.21 <sup>a</sup>	39.32±0.12	39.63±0.08	36.92±0.31 <sup>b</sup>	36.35±0.10 <sup>b</sup>
22h	39.20±0.17	36.35±0.23 <sup>a</sup>	38.92±0.10	38.75±0.16	36.85±0.27 <sup>b</sup>	36.27±0.14 <sup>b</sup>
23h	38.97±0.12	35.90±0.14 <sup>a</sup>	38.75±0.16	38.62±0.19	36.43±0.20 <sup>b</sup>	35.93±0.18 <sup>b</sup>
24h	38.77±0.15	35.93±0.16 <sup>a</sup>	38.38±0.15	38.37±0.24	36.98±0.12 <sup>b</sup>	35.95±0.53 <sup>b</sup>
27h	38.70±0.08	36.17±0.16 <sup>a</sup>	37.37±1.26	37.68±0.33	36.97±0.08	36.68±0.21

Control ((2% Tween 80 solution), Positive control acetyl salicylic acid ASA(300mg/kg), 200 and 400 mg/kg {EAE-HAKS(ethyl acetate) and ME-HAKS(methanolic)} leaf extracts of *Holostemma ada Kodiensis* Schult.

Data representing mean±SEM.(n=6), aP<0.001, bP<0.01 as compared with the control group performed by one-way ANOVA multiple comparison by Dunnett's test

material until the solvent used shows colourless in the syphon; and the solvents were collected separately and the material were air dried at room temperature and the same materials were used for extraction with other solvents; this step was repeated for all the solvent according to their polarity. All the extracted solvents were concentrated under reduced pressure using Heidolph rotary evaporators. These extracts were subjected for phytochemical screening by employing standard phytochemical tests<sup>9</sup>.

Experimental animals: Experimental animals Healthy Swiss albino mice (25–30g) and adult Wistar rats (140–180 g) (M) housed and maintained (23 ±4°C, relative humidity (60–70%) in the animal house facility on a standard diet with water ad libitum, were acclimatized for two weeks before the experimentations. All animal experiments were carried out in accordance with the approval CPCSEA APPROVED REG. NO. 1230/a/08/CPCSEA, and guidelines of the Institutional Animal Ethics Committee IAEC I.D. NO: - IAE/SKIPS/2012/08/I.

Acute toxicity studies: The acute oral toxicity (p.o) at 0–2000 mg/kg study was performed for methanolic and hydro-alcoholic extract in adult swiss albino mice of both sexes. This method was carried out according to OECD guide (line no 423)<sup>10</sup>, by adopting fixed dose method. Six animals per treatment group at different dose range 100, 300, 1000, 2000 mg/kg respectively (n=6) and observed at 30 mins time interval for 4h and 6, 12, 18 and 24h and then daily for next 14 days to record symptoms of toxicity and death<sup>11</sup>. No acute toxic effects (agility, muscular tonus, tremors, convulsions, and problem in breathing, body weight, urination, and water or food intake) or mortality was observed following treatment, so the procedure was repeated upto 3.5gm/kg p.o. None of the treated groups displayed any significant change of behavior as compared with the untreated controls.

#### Antipyretic

Yeast-induced pyrexia<sup>12,13</sup>: Thirty-six male Wistar rats (140–180 g) were randomly divided into 6 groups; a lubricated thermometer probe was inserted 3–4 cm deep into the rectum and fastened to the tail by adhesive tape were followed for measuring the normal body temperature

of each rat was measured rectally at predetermined intervals and recorded. After measuring the basal rectal temperature, animals were injected subcutaneously with 15 ml/kg of 20% w/v brewer's yeast in aqueous suspension in the dorsum of the rats. Eighteen hours after brewer's yeast injection, the animals were again restrained for rectal temperature recording, as described previously. Only rats that showed an increase in temperature of at least 1°C were used for this study. *Holostemma ada Kodiensis* Schult. (EAE-HASK and ME-HASK) extracts at the doses of 200 and 400 mg/kg were administered orally to four groups of animals. The control group received an equivalent volume of vehicle (2% Tween 80 solution) and the positive control group received acetylsalicylic acid (ASA, 300 mg/kg) orally; change in the body temperature were measured (19 to 27h) respectively. Statistical studies were performed by one-way ANOVA multiple comparison by Dunnett's test, all the value were expressed in mean ± SEM by using graphpad prism 6.1 versions.

#### RESULTS AND DISCUSSION

Pyrexia (fever) is triggered on impact of infection, malignancy or other ailments. Any infection will damage the tissue initiates numerous chemical which form the pro-inflammation mediators, which increases the synthesis of PEG 2;<sup>14</sup> which triggers the hypothalamus to elevate the body temperature; which increases the body temperature are reduced by regulating the nerve feedback mechanism by dilation of blood vessels and increasing the sweating. Yeast-induced hyperpyrexia is called as pathogenic fever. Thus, induction of the fever by the yeast triggers the production of the prostaglandins which stimulates the release of the phagocytic endogenous substance pyrogen which gets circulated in the blood and act on thermoregulatory center hypothalamus (cAMP- secondary messenger-mediated) mechanism<sup>15</sup>. The hyperpyrexia was appears to be reducing in the dose-dependent methanolic extracts of extract of *Holostemma ada Kodiensis* Schult. for both the concentration 200 and 400mg/kg with the P value 0.01, when compared with acetyl salicylic acid (ASA) shown in table:1; but the ethyl acetate extract did to show

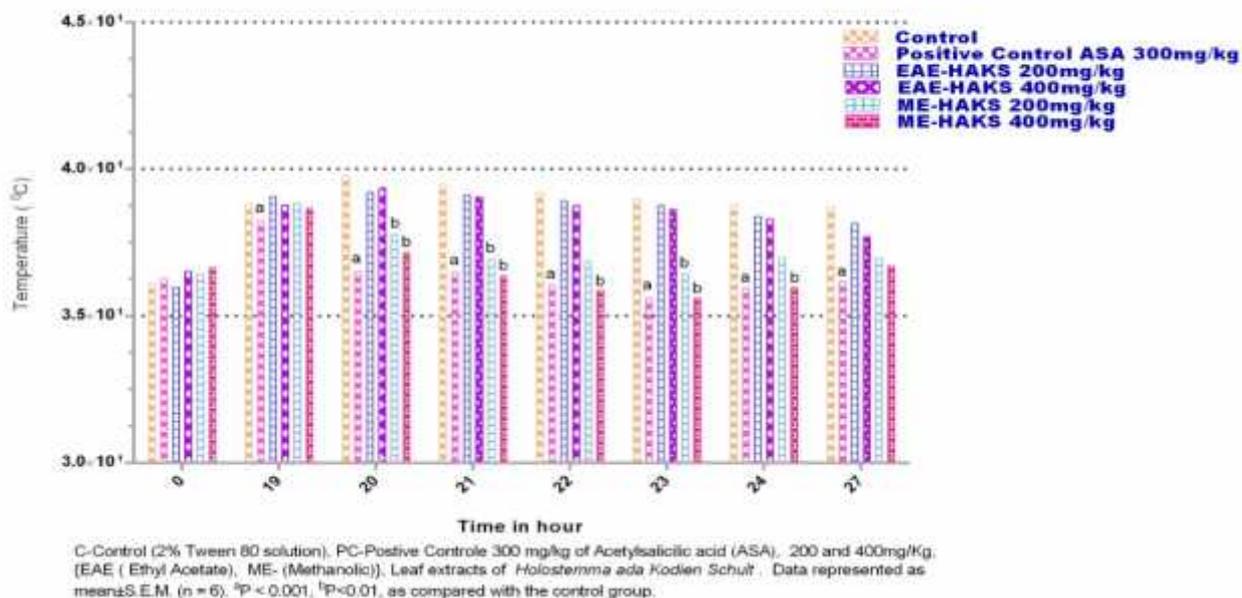
Yeast induced Antipyretic activity for leaf extracts of *Holostemma ada-kodien schultes*.

Fig 1: Yeast induced pyrexia and Antipyretic activity for the leaf extract of *Holostemma ada Kodien Schult*.

any significant reduction of pyrexia when compared with methanolic and reference standard. Thus; the previous studies suggested for the antipyretic activity ASA were suggested that the mechanism of action might be on the inhibition of PGE<sub>2</sub>, the primary mode of action of these drugs as antipyretics appears at present to be the inhibition of cyclo-oxygenase and a consequent reduction of prostanoid material in pyrogen-sensitive areas of the brain<sup>16</sup>. Hence the methanolic extract (ME-HAKS) might be working in the inhibition of the synthesis of prostaglandin pathway which is similar to that of aspirin. Thus; the traditional finding of the leaf being used for the fever was evaluated and the methanolic extract of *Holostemma ada Kodien Schult*. possess the antipyretic activity Figure:1.

### CONCLUSION

The antipyretic studies were evaluated for the various extract of *Holostemma ada Kodien Schult*. among them methanolic extract possess an potent antipyretic activity; from the preliminary phytochemical studies shows the presence of flavonoids; tannins, saponines, anthocyanins, steroids, alkaloids and phenols. From our previous investigation made on the various extract studies showed that the methanolic extract possess a potent antioxidant activity, total phenol and flavonoids content (data not included)<sup>17</sup>. Natural phenolic and related classes of secondary phytoconstituents were extensively studied for the antipyretic, anti-inflammation and analgesic activity. Hence investigation should be made on the bioactive secondary metabolites which could be possibly acting as antipyretic activity for the extracts of *Holostemma ada Kodien Schult*.

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