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BOILED MILK INDUCED PYREXIA IN RABBITS- ANTIPYRETIC ACTIVITY VERNONIA CINEREA ROOTS

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ABSTRACT

Antipyretic effect of petroleum ether and chloroform soluble fractions of ethanol extract of the roots of *Vernonia cinerea* was investigated. Intraperitoneal administration of boiled milk at a dose 0.5 ml/kg body weight in albino rabbit leads to pyrexia. Intraperitoneal (*i.p. route*) administration of petroleum ether and chloroform soluble fractions of ethanol extract of the roots of *Vernonia cinerea* at a dose 250 mg/kg body weight were shown significantly reduce the elevated body temperature of rabbit which was compared with standard aspirin (market product) and solvent used.

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INTRODUCTION: *Vernonia cinerea*, (Fam. Asteraceae), locally known as Sahadevi, is an annual plant^{24, 21} that is widely distributed to India, Bangladesh, Srilanka and Malay island¹⁰. The roots of the plant used traditionally for the treatment of all types of eruptive boils, the juice of sahadevi is used for quicker healing of accidental wounds, filariasis, in toxic viral fevers¹⁸ Although the plant is widely used for remission of several ailments related to fever, its antipyretic potential has not been explored yet. Therefore, in the present study an attempt was made to establish the antipyretic effect of petroleum ether and chloroform soluble fraction of ethanol extract of the roots of *Vernonia cinerea*.

Pyrexia or fever is caused as a secondary impact of infection, malignancy or other diseased states^{13, 14}. It is the body's natural defense to create an environment where infectious agent or damaged tissue cannot survive^{19, 8}. Normally the infected or damaged tissue initiates the enhanced formation of pro-inflammatory mediator's (cytokines like interleukin 1 α , 1 β , 1 γ and TNF- α), which increase the synthesis of prostaglandin E2 (PGE2) near peptic hypothalamus area and thereby triggering the hypothalamus to elevate the body temperature^{23, 17}.

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 become 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^{9, 4}. Most of the antipyretic drugs inhibit COX-2 expression to reduce the elevated body temperature by inhibiting PGE-2 biosynthesis¹⁵. Moreover, these synthetic agents irreversibly

inhibit COX-2 with high selectivity but are toxic to the hepatic cells, golmeruli, cortex of brain and heart muscles, whereas natural COX-2 inhibitors have lower selectivity with fewer side effects¹⁵. A natural antipyretic agent with reduced or no toxicity is therefore, essential. As roots of *Vernonia cinerea* is a old medicaments used in ailments that caused fever¹⁸, so it will be a cost effective alternative approach to study this plant for the development of an effective antipyretic agent.

MATERIALS AND METHODS:

Plant Materials: The roots of *Vernonia cinerea* was collected from various part of Nellore district of Andhra Pradesh and identified by Dr. K. Manivannan, Professor and Head, Department of Horticulture, Annamalai University, Chidambaram, India and the specimen no., Hort/56/2009, the roots were cut, air-dried and ground into powder.

Preparation of Petroleum Ether and Chloroform Fractions of Ethanol Extract: Powdered dried roots (900 g) of the plant were extracted (cold) with ethanol (5 L) in three flat bottom glass containers, through occasional shaking and stirring for 10 days¹⁶. The whole extract was filtered and the solvent were evaporated to dryness *in vacuo* with an Rotary Evaporator at 40°-50°C to afford a blackish green mass (45 g) which was further extracted with petroleum ether (3 x 50 ml), chloroform (3 x 50 ml) and methanol (3 x 50 ml) to afford petroleum ether, chloroform and methanol fractions, respectively^{1, 7}. The preliminary phytochemical screening of the different fractions was carried out by chemical tests and thin layer chromatographic methods³.

Preparation of Sample and Standard Solutions: 2.5 percent ethanol in distilled water (autoclaved) was used as solvent to prepare sample and standard solutions. The sample solutions of petroleum ether and chloroform fractions were prepared by dissolving each dried fraction in the solvent to

obtain 120 mg per 2 ml solution. To facilitate dissolution few drops of tween 80 was added. The each fraction was administrated at a dose of 250 mg/kg body weight²². Aspirin as Disprin soluble tablet was collected from local market in Andhra Pradesh was used as known antipyretic agent. The standard solution was prepared by dissolving the tablet in the solvent to obtain 15 mg aspirin per 2 ml solution. The dose of aspirin was maintained 10 mg/kg body weight².

Animals: The experiment was carried out on albino rabbits. They were 13-15 months old of both sexes weighing between 1.5-1.6 kg²⁰. They were collected from Sashtra College of Pharmacy, Nellore. The rabbits were kept in iron cages⁶ (considering group), were fed with cauliflower, cabbage, banana and tap water for 40 days before experiment to adjust with environment. Food and water were withdrawn 6 hours prior to the experiment². The animals were grouped as;

- Experimental groups- Two groups-one group receiving petroleum ether and other group receiving chloroform fraction.
- Control groups were:

- Aspirin group (+ve Control) - receiving standard antipyretic agent aspirin.
- Solvent group (-ve Control) - receiving solvent (used).

Number of rabbits in each group was four.

Acute Toxicity Study: Acute toxicity study was carried out by using graded doses of each fraction. Both petroleum ether and chloroform fractions were administered intraperitoneally in graded doses (200 to 1000 mg/kg body weight). They were observed continuously for the first 2 h for toxic symptoms and up to 24 h for mortality¹¹.

Treatment Protocol: Before experimentation rectal temperature of rabbits were recorded by inserting a well lubricated bulb of a thermometer in the rectum. Care was taken to insert it to the same depth each time (about 6 cm)². Milk was collected from local cow had been boiled. When temperature of the boiled milk equilibrates to room temperature then rabbits were injected boiled milk at the dose of 0.5 ml/kg body weight, to induce pyrexia. Induction of fever was taken about one to two h².

RESULTS:

TABLE 1: EFFECT OF PETROLEUM ETHER AND CHLOROFORM FRACTIONS OF *VERNONIA CINEREA* ROOTS ON BOILED MILK INDUCED PYREXIA IN RABBIT

Groups	Dose	Rectal temperature (°F)		Rectal temperature after admin. of fraction (°F)		
		Normal (A)	3 h after boiled milk administered in (B)	1 h (C ₁)	2 h (C ₂)	3 h (C ₃)
Solvent	2 ml/rabbit	101.0 ± 0.2	104.1 ± 0.23	103.9 ± 0.24 (3.84 ± 0.10)*	103.9 ± 0.23 (3.84 ± 0.10)*	103.7 ± 0.33 (11.5 ± 0.26)*
Aspirin	10 mg/kg	101.4 ± 0.11	104.1 ± 0.11	103.7 ± 0.20 (16.0 ± 0.12)*	101.5 ± 0.10 (96.3 ± 0.10)*	101.5 ± 0.10 (96.3 ± 0.10)*
Pet. Ether	80mg/kg	101.4 ± 0.1	101.1 ± 0.20	103.9 ± 0.18 (7.4 ± 0.18)*	101.7 ± 0.13 (88.9 ± 0.23)*	101. ± 0.16 (88.9 ± 0.23)*
Chloroform	80mg/kg	101.5 ± 0.1	104.1 ± 0.20	103.7 ± 0.28 (40.0 ± 0.10)*	101.7 ± 0.23 (92.3 ± 0.20)*	101.7 ± 0.22 (92.3 ± 0.20)*

All values are expressed as mean ±SE (n=4), percentage reduction in rectal temperature is give within parentheses; *P< 0.05 Significant compared to control (solvent).

% reduction $\frac{B-C_n}{B-A} \times 100$, where $n = 1, 2$ and 3 .

Then solvent (2 ml) was given on negative control group, known antipyretic agent aspirin solution (2 ml) was given on positive control group and two sample solutions (each 2 ml) were given to two experimental groups (**Table 1**). Intraperitoneal route was used to administer boiled milk, aspirin solution, solvent and sample solution. Finally, rectal temperatures were recorded 1 h intervals up to 3 h.

DISCUSSIONS AND CONCLUSION: The preliminary phytochemical screening of the petroleum ether and chloroform fraction showed the presence of steroids, tannins and flavonoids. In acute toxicity study, both fractions were found to be safe and no mortality was observed to a dose as high as 800 mg/kg. The results of effect of two fractions of roots of *Vernonia cinerea* on boiled milk induced pyrexia in rabbits are depicted in Table 1. Both petroleum ether and chloroform fractions produced significant ($P < 0.05$) antipyretic effect. At a dose of 250 mg/kg body weight, chloroform fraction reduced (92.3 ± 0.20) % of elevated rectal temperate compared to aspirin (96.3 ± 0.10) % followed by petroleum ether fraction (88.9 ± 0.23) % after 3 hours. It was also observed that solvent have no effect on the reduction of pyrexia of rabbit.

Search for safe herbal remedies with potent antipyretic activity received momentum recently as the available antipyretic, such as paracetamol, aspirin, nimesulide etc. have toxic effect to the various organs of the body¹². The acute toxicity result reveals that this plant might be considered as a broad non-toxic one. The antipyretic activity exhibited that the both petroleum ether and chloroform fraction of ethanol extract of roots possess a significant antipyretic effect in maintaining normal body temperature and reducing boiled milk induced

elevated rectal temperature in rabbits and their effect are comparable to that of standard antipyretic drug aspirin. Such reduction of rectal temperature of tested animals by the both fractions at 250 mg/kg appears to be due to the presence of a single bioactive principles or mixture of compounds in them. The phytochemical analysis of the petroleum ether and chloroform fractions showed the presence of steroids, tannins and flavonoids. The antipyretic activity observed can be attributed to the presence of steroids, tannins and flavonoids¹⁷. The present study, therefore, supports the claims of traditional medicine practitioners as an antipyretic remedy. However, to know the exact mechanism of action of *Vernonia cinerea* root extract further study with purified fractions/ bioactive compounds are warranted.

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