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Anti-pyretic activity of *Achyranthus aspera* linn. leaves extracts in albino rats

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ABSTRACT

Achyranthus aspera Linn. (Amarantaceae), consist of Achyrol, ecdysterone, linolieic, Oleic palmitic and uses to relieve rheumatic pain, stomachic, bowel complaints, piles, wasps, bee's, piles, snake bites, headache, leprosy, dyspepsia, inflammatory, diuretic, hydrophobia and itching. The evaluation of anti-pyretic activity of plant is carryout by extraction with using solvent system {Petroleum ether (60-80^0 c), Benzene, Chloroform, Ethyl acetate, n-Butanol, and Ethanol} TLC, column chromatography and activity is studies by Yeast induce hyperpyrexia method. Ethanolic extract has good significant ($P < 0.001$) reduction in rectal temperature to the extent of 37.31^{0}c at 180 min. when compared to control group. The n-butanol, chloroform, and benzene have significant reduction in rectal temperature ($P < 0.001$), to the extent of 37.72^{0}c and 37.78^{0}c and 37.35 at 200 mg/kg dose at 180 min. when compared to control group. However, ethyl acetate and pet- ether have reduction in rectal temperature ($P < 0.001$), to the extent of 38.25^{0}c and 38.15^{0}c at 200 mg/kg dose when compared with control group. Where as, Aspirin also has significantly ($P < 0.001$), reduction in rectal temperature to the extent of 37.44^{0}c from 30 min. to 180 min. when compared to control group.

INTRODUCTION

Achyranthus aspera Linn. belonging to family Amarantaceae erect or procumbent annual or perennial herb, 1-2m. in height. It consist Achyrol, ecdysterone, ecdysterone, spooning A, saponin B, Hentricontane, ecdysterone [1]. linolieic, Oleic palmitic [2], Therapeutic it is uses eardrop to relieve pain, rheumatic pain, stomachic & bowel complaints, piles, boils, skin eruptions [3]. poisonous insects, wasps, bee's [4], digestant and dog-bites (Singh and Govil, 2001). piles and snake bites. headache, leprosy & dyspepsia. inflammatory, arcdiatonic, diuretic, hydrophobia & itching [5]. Herbal preparations called "Phyto-pharmaceuticals", "Phytomedicinal" or "Phytomedicine" are preparations made from different parts of herbs of plants. They come in different formulations and dosage forms including tablet, capsule, elixir, powder, extract, cream and parenteral preparations. If an herb is used as therapeutic agent, it should be considered as a drug. The effectiveness, easy availability, less cost and comparatively being devoid of serious toxic effects popularise herbal remedies [6]. The first Pharmacist, Galen, known to have had a number of pains reliving matter including opium in his apothecary shop. For the next few hundred years, the formulation of the medicaments in use changed little. However through this period, the use of herbal extract for medicinal purpose never lost its place [7]. Fever is an elevation of body temperature above the normal circadian range as the result of a change in the thermoregulatory center located in the anterior hypothalamus [8]. Body temperature between 99^{0}F (37.22^{0}C) and 103^{0}F (40.57^{0}C) onward is called pyrexia while rise of body temperature above 107^{0}F (41.66^{0}C) is called hyper pyrexia. A drug that selectively relieves pain by acting in CNS or on peripheral pain mechanisum, without significantly altering consciousness [9].

MATERIALAND METHODS

Collection and size reduction of plant

The leaves of *Achyranthus aspera* Linn were collected from local Area of Toranmal Dist. Nandurbar in winter season and Authenticated from botanical survey of India, Pune. The authentication certificate No. RRG- 1. Dried leaves were reduced to coarse powder using mechanical grinder and passed through a sieve No.40 to obtained powder of desired particle size [10].

Continues hot extraction

About 250gm material was subjected to hot continues

successive extraction with various solvents in increasing order of polarity from Petroleum ether (60-80° c), Benzene, Chloroform, Ethyl acetate, n-Butanol, and Ethanol in a soxhlet extractor at a temp 45° - 50°c up to 40 cycles per batch. The extraction was continuing until the solvent in the thimble becomes clear indicating the completion of extraction. After the complete extraction, the solvent was distilled off and concentrated on a water bath to a dry residue.

The various extracts were subjected to qualitative chemical investigation for the identification of the active principles.

Qualitative chemical investigation of extract [11].

The various Phytoconstituents presence in different extract as

Petroleum ether (40-60) extract	Sterols, Carbohydrates
Benzene extract	Carbohydrates
Chloroform extract	Carbohydrates
n-Butanol extract	Alkaloids, Glycoside
Ethanol extract	Carbohydrates

Identification of active principle by TLC

Detail of thin Layer chromatography

Adsorbent Silica gel G (activated)

Plate size 20 cm x 8 cm

Plate thickness 0.2 mm

Solvent system Chloroform: Methanol (85:15)

Spraying agent Anisaldehyde- sulphuric acid

Developing time At 110°c for 10 min

The spot were visualized as blue and pink [12].

Assessment of anti-pyretic activity

Animal selection

The Ethical clearance was obtained by the Institutional Animal Ethics committee (Registration Number-652/02/a/CPCSEA).

Female albino mice weighting between, 20-25gm were used for acute toxicity study of various extracts. The animals were lasted overnight prior to the experimental products. Albino rats, wistar strain of weighting 150-200gm. were used for anti-pyretic models. Rats were kept in polyprolene cages and led on standard laboratory diet and adlibitum. The animals were exposed to 12 hours of darkness and light each. The bedding materials of cages were changed every day. Rats were divided into group of six.

Material used

A) All extracts

B) Aspirin (Standard)

C) Brewer's yeast (pyretic agent)

Acute toxicity study

Acute toxicity study was carried out according to organization for economic co-operation and development guidelines 423 (15) [13].

Preparation and administration of doses

The test compound i.e. petroleum ether extract, benzene extract, chloroform extract, ethyl acetate extract, n-butanol extract, and ethanol extract was administered orally. Animals are fasted prior to dosing with free access to water. The dose of 1 ml/100gm b.w. of all test materials was given to the mice in stepwise procedure using little doses of 5,50,300 and 2000mg/kg b.w. food was given to the mice 3 to 4 hr. after administering the test materials. Signs and symptoms of toxicity were observed at 2000mg/kg in single animal for all extracts in singing study. The same dose was given to three animals for main toxicity study.

Antipyretic activity

Yeast induce hyperpyrexia method

A 15% suspension of Brewer's yeast in 0.9% saline was prepared eight groups of 6 albino rats of either sex with body weight of 150-200 gm. was used. By insertion of a thermocouple to a depth of 2cm into the rectum the initial rectal temperature were recorded. The animals were fevered by injection of 10 mg/kg of brewer's yeast suspension subcutaneously in the back below the hope of the neck. The sight of injection was massaged in order to spread the suspension beneath the skin. The room temperature was kept at 22-24°c. Immediately after yeast administration, food was withdrawn 18 h. post challenge, the rise in rectal temp the measurement was repeated after 30 min. Only

Table 1:

Extracts	Weight	% yield
Petroleum ether (60-80)	25 gm	2.5%
Benzene	33.5 gm	3.35%
Chloroform	36.8 gm	3.68%
Ethyl acetate	28.9 gm	2.89%
n-Butanol	37.1 gm	3.71%
Ethanol	46.2 gm	4.63%

Table 2:

Extracts	Chemical constituents presence
Petroleum ether (40-60) extract	Sterols, Carbohydrates
Benzene extract	Carbohydrates
Chloroform extract	Carbohydrates
Ethyl acetate extract	Alkaloids, Glycoside
n-Butanol extract	Carbohydrates
Ethanol extract	Sterols, Alkaloids, Glycoside, Saponins

animal with a body temperature of at least 38°c are taken into the test. The animals react the test compound or standard drug by oral administration. Rectal temperature was recorded again 30, 60, 120, and 180 min. post dosing [14].

8 groups are made each contains 6 animals

Group I - Control (200 mg/kg)

Group II - Aspirin as Standard (150 mg/kg)

Group III - Petroleum ether extract (200 mg/kg)

Group IV - Benzene extract (200 mg/kg)

Group V - Chloroform extract (200 mg/kg)

Group VI - Ethyl acetate extract (200 mg/kg)

Group VII - n-Butanol extract (200 mg/kg)

Group VIII - Ethanol extract (200 mg/kg)

STATISTICAL ANALYSIS

Data were subjected to statistical analysis using ANOVA, and statistical comparison was done using the Tukey Kramer multiple comparison test values of $P < 0.01$ were considered statistically significant.

RESULT AND DISCUSSION

Phytochemical Investigation

Table 1: Showing percentage yield of various extracts of *Achyranthus aspera* linn. Leaves

Qualitative chemical test

Table 2: Showing qualitative chemical investigation of various extracts of *Achyranthus aspera* linn. Leaves

Thin layer chromatography of extracts

The ethanolic extract was subjected to thin layer chromatography. Presence of sterols in ethanolic extract was identified by TLC profile. The ethanolic extract on TLC revealed the presence of 2 spots. The R_f value of which were found to be as 0.52 (blue) and 0.92 (Pink) respectively in Chloroform: Methanol (85:15) solvent system.

Pharmacological screening

Acute toxicity study

Acute toxicity study was carried out according to organization of Economic Cooperation and Development guideline in albino mice. The acute toxicity study of various extracts of *Achyranthus aspera* Linn. leaves was showed signs of toxicity like tremour, convulsion and deep breathing at 2000mg/kg b.w. $1/10^{th}$ of the same dose for all these extracts were taken as therapeutic dose i.e. 200mg/kg.b.w.

Antipyretic activity

Yeast induced pyrexia method

Table 4: Showing the results of various extracts of *Achyranthus aspera* linn. Leaves on yeast induced hyperpyrexia method

It indicated that ethanolic extract has good significant ($P < 0.001$) reduction in rectal temperature to the extent of 37.31°c at 180 min. when compared to control group. The n-butanol, chloroform, and benzene have significant reduction in rectal temperature ($P < 0.001$), to the extent of 37.72°c and 37.78°c and 37.35 at 200 mg/kg dose at 180 min. when compared to control group.

However, ethyl acetate and pet- ether have reduction in rectal temperature ($P < 0.001$), to the extent of 38.25°c and 38.38.15°c at 200 mg/kg dose when compared with control group. Whereas, Aspirin also has significantly ($P < 0.001$), reduction in rectal temperature to the extent of 37.44°c from 30 min. to 180 min. when compared to control group.

Hence, to put into nutshell, the active principle/s of leaves of *Achyranthus aspera* Linn. like glycoside, sterols, carbohydrates, saponins may be responsible for the antipyretic activity. However isolation, structural elucidation and screening of the above mentioned active principle/s is needed to pin point the activity of drug.

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Table 3:

Group	Rectal Temp 0c		TttTime after administration tt3rrrs33333dsfdsffdfdf30				
	Initial	18hr after Yeast injection	30 Min	60 Min	90 Min	90 Min	180 min
Control (200 mg/kg)	38.30 ±0.03162	39.35 ±0.0255	39.30 ±0.0070	39.23 ±0.0089	39.20 ±0.0452	39.15 ±0.0070	39.00 ±0.004
Pet. Ether (200 mg/kg)	38.27 ±0.0167	39.54 ±0.0481	38.93 ±0.0219	38.63 ±0.0212	38.40 ±0.0083	38.22 ±0.0122	38.15 ±0.008
Benzene (200 mg/kg)	38.28 ±0.0141	39.48 ±0.0054	38.92 ±0.0122	38.00 ±0.0083	37.92 ±0.020	37.62 ±0.0167	37.35 ±0.007
Chloroform (200 mg/kg)	38.26 ±0.0148	39.44 ±0.0109	38.42 ±0.0212	38.20 ±0.0114	38.08 ±0.0151	37.90 ±0.0109	37.78 ±0.007
Ethyl acetate (200 mg/kg)	38.25 ±0.0122	39.41 ±0.0167	39.10 ±0.0260	38.80 ±0.0207	38.62 ±0.0568	38.40 ±0.0328	38.25 ±0.009
n-butanol (200 mg/kg)	38.28 ±0.0187	39.40 ±0.0158	38.60 ±0.0158	38.80 ±0.0327	38.15 ±0.0182	38.00 ±0.0313	37.72 ±0.018
Ethanol (200 mg/kg)	38.28 ±0.0122	39.37 ±0.0216	37.42 ±0.0230	37.84 ±0.0427	37.51 ±0.0568	37.36 ±0.0260	37.31 ±0.032*
Aspirin (150 mg/kg)	38.30 ±0.0316	39.35 ±0.0255	38.32 ±0.0461	38.09 ±0.0455	37.65 0.0541	37.57 0.0282	37.44 ±0.008

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