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PHYTOCHEMICAL AND ANTIPYRETIC POTENTIAL OF ETHANOLIC LEAF EXTRACT OF HELIOTROPIUM INDICUM L.

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ABSTRACT:

In the present study, the ethanolic leaf extract of *Heliotropium indicum* L (*H. indicum* L) was investigated for phytochemical screening and antipyretic activity in rats using Brewer's yeast induced Pyrexia. The leaves of *H. indicum* were collected from different part of Assam and cut into small pieces and shade dried. The dried powdered leaves (100 gm) were extracted in a Soxhlet apparatus by using 95% ethanol. Phytochemical screening has been done by standard procedure and showed the presence of alkaloids, glycoside, carbohydrate, phytosterol, flavonoids and saponins and absence of fixed oil and gums-mucilage. Albino rats weighing (200-250g) were taken for the experiment divided into four groups of six animals each. Group 1 received 3% aqueous suspension of gum Acacia (1ml/200g) as vehicle orally, group 2 and group 3 received ethanolic leaf extract of *H. indicum* 250 and 500 mg/kg with 3% aqueous suspension of gum Acacia orally and the group 4 served as standard received paracetamol 25 mg/kg with 3% aqueous suspension of gum Acacia orally. The subcutaneous injection of yeast suspension markedly elevated the rectal temperature after 18h of administration. Treatment with *H. indicum* extract at a dose of 250, 500 mg/kg decreased the rectal temperature of the rats in dose dependent manner. This effect was maximal at dose of 500 mg/kg and it caused significant lowering of body temperature ($P< 0.01$) up to 4 hour after its administration. The antipyretic effect started as early as 1h and the effect was maintained for 4h, after its administration. Both the standard drug paracetamol 25mg/kg and tested drug *H. indicum* extract were significantly reduced the yeast elevated rectal temperature, at 2nd, 3rd and 4th hour compared to control group.

INTRODUCTION

Herbal medicines are one type of dietary supplement. They are sold as tablets, capsules, powders, teas, extracts, and fresh or dried plants. Nearly 70% of people taking herbal medicines all around the world. Herbal medicine - also called botanical medicine or phytomedicine - refers to using a plant's seeds, berries, roots, leaves, bark, or flowers for medicinal purposes. Herbalism has a long tradition of use outside of conventional medicine. People use herbal medicines to try to maintain or improve their health. Natural products including plants, animals, and minerals have been the single most productive source of leads for the development of drugs. A number of compounds derived from natural sources are currently undergoing clinical and preclinical studies, particularly as anti-inflammatory, cardiovascular, anti-diabetic, anti-obesity, anti-malarial, anti-viral, and anti-neoplastic agents.[1, 2]

North East India with its rich floristic diversity is also inhabited by a large number of tribes and they lead and intricate life totally dependent on forest plants. Virtually their requirement ranging from food, fuel, fodder, medicine, cordage and various other domestic needs are met from local vegetation. Overall

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tribal population of this region accounts for more than 57% of the total population. However Arunachal Pradesh, Manipur, Meghalaya, Mizoram, Nagaland and Assam compromise more than 73% tribal population of the total population, thus imparting a predominantly tribal character to the region.[3] Pyrexia or fever is caused as a secondary impact of infection, 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 proinflammatory mediator's (Cytokines like interleukin 1β, α, β and TNF- α), which increase the synthesis of prostaglandin E2 (PG E2) near peptic hypothalamus area and thereby triggering the hypothalamus to elevate the body temperature[4.5, 6].

MATERIAL AND METHOD

Collection & authentification of plant material of the Plant: The entire plant of *Heliotropium indicum* Linn. was collected from Nalbari district of Assam during the month of March-April. They were thoroughly washed in running water, segregated from the grass and other extraneous material and the field data of the plant like its height, flower colour and soil condition were noted in the note book. The authentication was carried out by the help of Dr. A.A. Mao (Scientist-E & H.O.O), Botanical Survey of India (BSI), Eastern Regional Centre, Shillong-793003 No: BSI/ERC/2015/Plant identification/151.

Preparation of extracts by hot extraction

500 g of the dried pulverized whole plant of *Heliotropium indicum* L were taken in soxhlet apparatus .After initial defatting with ethanol, hot extraction in a soxhlet apparatus at a temperature not exceeding 70°C were carried out. Then the extract obtained had been filtered and treated at reduced temperature on a rotary vacuum evaporator and concentrated stored at 4°C until further use. The yield was found to be around 7.00% (w/w) with respect to dried whole plant of *Heliotropium indicum*. Dried extract was kept in desiccator and used for further study.

Experimental animals

Healthy young Albino rats weighing between 120 g to 200 g were procured. The animals were individually housed in polypropylene cage and the room condition was maintained at temperature of 25±5 deg C and humidity 45±5 per cent with 12 hr day and night cycle. The animals were fed with Pellet chew feed standard diet and water *ad libitum*. All experimental procedures were conducted with the approval of the Institutional Animal Ethics committee CPCSEA (Reg. No. AdtU/IAEC/2015/006) for the care and use of animals and their guidelines were strictly followed throughout the study.

Preliminary phytochemical test

The ethanolic extract obtained from the extraction process was then subjected to various qualitative tests reported methods to determine the presence of various phytoconstituents such as alkaloids, glycosides, saponin, flavonoids, carbohydrates, amino acids, sterols, gums and mucilage etc. The concentrated extracts were subjected to chemical test as per the methods mentioned below for the identification of the various constituents[7, 8].

- **Detection of alkaloid**

Solvent free extract, 50mg is stirred with few ml of dilute hydrochloric acid & filtered. The filtrate is tested carefully with various alkaloidal reagents as follows:

- MAYER'S TEST: To a few ml of filtrate, a drop or two of Mayer's reagent are added by the side of the test tube. A white or creamy ppt indicates test as positive.
- WAGNER'S TEST: To a few ml of filtrate, few drops of Wagner's reagent are added by the side of the test tube. A reddish-brown ppt. indicates test as positive.
- HAGER'S TEST: To a few ml of filtrate, 1 or 2 ml of Hager's reagent are added by the side of the test tube. A prominent yellow ppt. indicates test as positive.
- DRAGENDORFF'S TEST: To a few ml of filtrate, 1 or 2ml of Dragendorfff's reagent are added by the side of the test tube. A prominent yellow ppt. indicates test as positive.

- **Detection of carbohydrates**

The extract (100mg) is dissolved in 5ml of water & filtered. The filtrate is subjected to the following test.

- MOLISH'S TEST: To 2 ml of filtrate, 2 drops of alcoholic sol of alpha-naphthol are added, the mixture is shaken well & 1ml of cone. H_2SO_4 is added slowly along the side of the test tube & allowed to stand. A violet ring indicates the presence of carbohydrate.
- FELING'S TEST: 1 ml filtrate + 1 ml each of Fehling's solutions A&B heat on water bath for 2 minute and presence of red ppt. indicate the present of sugar.
- BENEDICT'S TEST: To 0.5ml of filtrate, 1ml of benedict reagent is added and the mixture heated on a boiling water bath for 2 minute a characteristic colour ppt indicates the present of sugar.

- BARFOED'S: To 1 ml of filtrate, 1ml of barfoed reagent is added and the mixture heated on a water bath for 2 minutes and red ppt. indicates the present of sugar.

- **Detection of saponin**

The extract 50 mg is diluted with water and made up to 20ml.The saponin is shaken for 15 minutes and a layer of 2 cm of foam indicates the presence of saponin.

- **Detection of phenolic compounds**

- FERRIC CHLORIDE TEST: The extract 50 mg is dissolved in 5 ml of distilled water to this few drops of 5% ferric chloride solution is added. A dark green colour indicates the presence of phenolic compound.
- GELATIN TEST: The extract 50 mg is dissolved in 5 ml of distilled water and 2 ml of 10% sodium chloride solution is added. White ppt. indicates the presence of phenolic compounds.

- **Detection of glycosides and flavanoids**

50mg of extract is hydrolysed with concentrate hydrochloric acid for 2 hr on a water bath, filtered and hydrolysate is subjected to the following test:

- BORNTRAGER'S TEST: To 2ml of filtered hydrolysate, 3ml of chloroform is added and shaken, chloroform layer is separated and 10% ammonia solution is added to it and pink color indicates the presence of glycoside.
- LEAD ACETATE TEST: The extract 50mg is dissolved in distilled water and to this 3ml of 10% lead acetate solution is added. A bulky white ppt. indicates the present of flavanoid.
- MAGNESIUM & HYDROCHLORIC ACID REDUCTION: The extract 50mg is dissolved in 5ml of alcohol and few fragment of magnesium ribbon and HCL acid drop wised is added. If any pink to crimson color develops presents of flavanols glycoside is confirm.
- ALKALINE REAGENT TEST: An aqueous solution of the extract is treated with 10 % of Ammonium hydroxide solution. Yellow fluorescence indicates the present of flavanoid.
- AQUEOUS SODIUM HYDROXIDE TEST: An aqueous solution of the extracts is treated with sodium hydroxide solution it give blue to violet (Anthrocyanine), yellow (flavones) and yellow to orange (flavononones).
- CONCETRATED SULPHURIC ACID TEST: An aqueous solution of the extracts is treated with conc. Sulphuric acid it gives yellowish orange (Anthrocyanines), yellow to orange (flavones), orange to crimson (flavanones).

- **Detection of proteins and amino acids**

- MILLONS TEST: 2 ml of filtrate + few drops of Millon's reagent and white ppt. indicates the present of proteins and amino acids.
- BIURETS TEST: An aliquot of filtrate is treated with conc. Drug of 2% copper sulphate solution. To this 1ml of ethanol of 99% is added followed by excess of potassium hydroxide pellet. Pink color in the ethanolic layer indicates the presents of protein.
- NINHYDRIN TEST: Two drops of ninhydrin solution (10mg of ninhydrin in 200ml of acetone) is added to 2ml of aqueous filtrate. A characteristic colour indicates the presence of proteins.
- LEGAL TEST: 50mg of extracts is dissolved in pyridine, a sodium nitroprusside solution is added and makes alkaline using 10% of NAOH and presence of protein is indicated by pink colour.
- KILLER KILLIANI TEST: To an extract of drug in GAA, few drops of ferric chloride and conc. sulphuric acid were added. A reddish brown colour is form at the junction of two layer and upper layer turns bluish green.

- **Detection of phytosterols**

- LIBBERMANN-BURCHARDS TEST: Extracts 50mg and 2ml acetic anhydride. To this soln. 1-2 drops of conc. Sulphuric acid is added along the side of test tube. And array of color changes shows the presence of phytosterols.

- **Detection of fixed oils and fats**

- SPOT TEST: Pressed a small quantity of extract separately between two filter paper. Oil stains on the paper indicates the presence of fixed oil.
- SAPONIFICATION TEST: Add a few drops of 0.5N alc. KOH to a small quantity of extracts along with a drop of phenolphthalein. Heat the mixture on water bath for 1-2 hour. Formation of soap or partial neutralization of alkali indicates the presence of fixed oil and fats.

- **Detection of gums and mucilage**

Extract 100mg is dissolved in 10ml of distilled water and to this 25ml of absolute alc. is added with constant stirring. White or cloudy precipitation indicates the presence of gums and mucilage.

- **Detection of coumarin**

Extract 50mg is dissolved in 10ml absolute alc. and to this few drops of ferric chloride is added. Greenish fluorescence indicates the presence of coumarin.

ANTIPYRETIC ACTIVITY (Yeast induced pyrexia)

Antipyretic activity on rats was studied with fever induced by 20% Brewer's yeast. Rats (200-250g) were fed uniformly till 24 hours, and food was withdrawn before giving drugs. After measuring rectal temperature of the rats by introducing 1.5 cm of digital thermometer in rectum, pyrexia was induced by injecting subcutaneously, 20% suspension of dried yeast in 2% gum Acacia in normal saline at a dose of 20 ml/kg of body weight. After 18 hour of yeast injection, rats which showed a rise in temperature of at least 1°C were taken for the study. Animals in the various groups were treated as follows:

Group 1: 3% aqueous suspension of gum Acacia (1 ml/200g) as vehicle, orally

Group 2: Ethanolic extracts of leaves of *H. indicum* 250 mg/kg (1ml/200g) with 3% aqueous suspension of gum Acacia, orally.

Group 3: Ethanolic leaf extracts of leaves of *Heliotropium indicum* 500 mg/kg (1ml/200g) with 3% aqueous suspension of gum Acacia, orally

Group 4: Paracetamol 25 mg/kg (1ml/200g) with 3% aqueous suspension of gum Acacia, orally.

Rectal temperature was recorded every hour for four hours after administration of drugs.[4]

RESULT

Results of different chemical tests on the ethanolic extract of *Heliotropium indicum* Linn. showed the presence of alkaloids, glycoside, carbohydrate, phytosterol, flavonoids and saponins and absence of fixed oil and gums-mucilage is presented on Table-1. Effect of ethanolic leaf extract of *H. indicum* on rectal temperature in rats is presented in Table 2. The subcutaneous injection of yeast suspension markedly elevated the rectal temperature after 18h of administration. Treatment with *H. indicum* extract at a dose of 250, 500 mg/kg decreased the rectal temperature of the rats in dose dependent manner. It was found that the extract at a dose of 250 mg/kg caused significant lowering of body temperature at 4 hour following its administration (36.55 ± 0.008). This effect was maximal at dose of 500-mg/ kg and it caused significant lowering of body temperature (P< 0.001) up to 4 hour after its administration (36.25*** ± 0.003). The antipyretic effect started as early as 1h and the effect was maintained for 4h, after its administration. Both the standard drug paracetamol 25 mg/kg and tested drug *H. indicum* extract were significantly reduced the yeast-elevated rectal temperature, at 2nd, 3rd and 4th hour compared to control group.

Table 1: Phytochemical Analysis of Leaf of *H. indicum*, L

Test	Ethanolic extract
Alkaloids	+
Carbohydrates	+
Glycosides	+
Phytosterols	+
Fixed oil and fats	_
Phenolic compound and Tannins	+
Saponins	+
Proteins and Amino acids	+
Gums and Mucilage	_
Flavonoids	+

(+)- Present (-) – Absent

Table 2: Yeast Induced Pyrexia

Treatment	Dose (mg/ kg)	Normal Temperature	Rectal Temperature 18hrs after yeast induced pyrexia, (°C)	Rectal temperature (°C) after treatment with extract			
				1 hr	2 hr	3 hr	4 hr
Control	_	35.20 ±0.004	38.08 ± 0.009	38.09 ± 0.008	38.08 ± 0.007	38.07 ± 0.007	38.08 ± 0.004
H. indicum ethanolic extract	250	35.21 ±0.006	38.07 ± 0.008	37.7 ± 0005	37.03 ± 0.007	36.94 ± 0.004	36.55 ± 0.008
H. indicum ethanolic extract	500	35.21 ±0.006	38.05 ± 0.007	37.8 ± 0.06	37.45 ± 0.003	36.53* ±0.005	36.25* ± 0.003
Paracetamol	25	35.22 ±0.007	38.06 ± 0.006	37.9± 0.007	37.26 ± 0.002	36.65* ± 0.004	35.80* ± 0.003

Values are expressed as Mean ±S.E, n =6 by Anova test (Tukey test); * P< 0.001 Vs control

CONCLUSION

The results of the present study suggest that the ethanolic leaf extract of *H. indicum* in doses of 250 and 500 mg/kg, significantly reduce the temperature of pyretic rats as revealed from the observation that the average percentage of antipyretic activity increased with the concentration of the extracts (500mg/Kg) compared with the control. It is also presumed that the presence of flavonoids may be contributing to antipyretic activities of ethanolic leaf extract of *H. indicum* in addition to the analgesic effect, as in the case of many of the established antipyretics.

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