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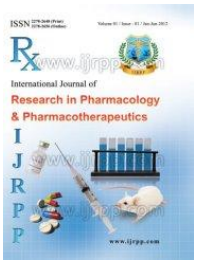

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Evaluation of antipyretic activity of ethanolic extract of *Terminalia pallida*

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ABSRACT

The study was conducted to screen the antipyretic activity of alcoholic extract of the Fruits of *Terminalia Pallida*. *Terminalia Pallida* is a potent medicinal plant in the Indian systems of medicine. Traditionally it is used as a cardioprotective, antihyperlipidemic, antiatherogenic, anti-ulcer, etc. In the present study the alcoholic extract of the seed of *Terminalia Pallida* was studied for their antipyretic activity by milk induced pyrexia in rabbits. It was observed that the alcoholic extract produced significant antipyretic activity ($p < 0.05$). The extract showed marked antipyretic activity in a dose dependent manner.

Keywords: *Terminalia Pallida*, Antihyperlipidemic, Antiatherogenic, Anti-Ulcer, Antipyretic.

INTRODUCTION

The problem of uncontrolled pain led early humans to seek remedies from any materials that they could lay their hands on. In recent times, focus on plant research has increased and non steroidal anti inflammatory drugs constitute one of the most widely used classes of drugs. Herbal drugs are proven as effective as the synthetic drugs with lesser side effects. Herbal Medicines are in line with nature, with less hazardous reactions [1].

Pyrexia or fever is caused as a secondary impact of infection, malignancy or other diseased states. It is the body's natural defense to create an environment where infectious agent or damaged tissue cannot survive [2]. Normally the infected or damaged tissue initiates the enhanced formation of pro-inflammatory mediator's (cytokines like interleukin 1a, a, a and TNF- a), which increase the synthesis of prostaglandin E2 (PGE2) near peptic hypothalamus area and thereby triggering the hypothalamus to elevate the body temperature [3]. 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[4].

The aim of the work is the Evaluation of Antipyretic Activity ethanolic Extract of Terminalia pallida. T*erminalia Pallida* has

documented *to possess* antihyperlipidemic, antiatherogenic activity, but the effect of *Terminalia Pallida* as an Antipyretic agent is still not reported. Hence it was thought worthwhile to screen extract of *Terminalia Pallida* for its Antipyretic activity.

MATERIALS AND METHODS

Animals

The experiment was carried out on albino rabbits. They were 13-15 months old, of both sexes, weighing between 1.5 and 1.6 kg [5]. Considering the group, the rabbits were kept in iron cages [6] to adjust to the environment, and fed with cauliflower, cabbage, banana, and tap water for 40 days before the experiment. Food and water were withdrawn 6 hrs prior to the experiment.

Plant collection and identification

Fruits of *Terminilia pallida* were procured from local market. It was authenticated by the botanist of Dr. K Madhava Chetty, Tirupati, Andhra Pradesh, India. Fruits were powdered with the help of electric grinder and Passed through a sieve for coarse powder. This powder was used for the preparation of ethanolic extract.

Extraction Process

Terminalia Pallida fruit powder was extracted with 7 volumes of 95% ethanol in a Soxhlet apparatus at 60°C-70°C for 6 hrs. The filtrate was distilled and concentrated under reduced pressure at low temperature (40°C) in Buchi rotavapour R□200. A dark brown, semisolid residue was obtained. It was stored at 4°C and used for further studies.

Phytochemical Analysis: The ethanolic extract prepared was analyzed for the presence of alkaloids, saponins, tannins, steroids, flavinoids, anthraquinones, cardiac glycosides and reducing sugars based on the protocols available in the literature [7, 8, 9, 10.]

TESTS FOR ALKALOIDS

Mayer's Test (Potassium Mercuric Iodide)

A fraction of the extract was treated with Mayer's reagent and observed in the formation of a cream-colored precipitate.

Dragendroff's Test

A fraction of the extract was heated with Dragendroff's reagent and observed in the formation of a reddish orange-colored precipitate.

Wagner's Test

A fraction of the extract was treated with Wagner's reagent and observed in the formation of reddish brown -colored precipitate.

Hager's Test

A fraction of the extract was treated with Hager's reagent and observed in the formation of yellow -colored precipitate.

TESTS FOR CARBOHYDRATES

Molisch's test

Fraction of the extract was treated with a solution of 2-napthol and few drops of sulfuric acid was added through the sides of the test tube and observed in the formation of a violet ring between the junction show the presence of carbohydrates.

Fehling's Test

Fraction of the extract was treated with Fehling's A solution and B and they are heated on a water bath for a few minutes and observed in the formation of a red -colored precipitate.

Barfoed's Test

A fraction of the extract was treated with Barfoed's reagent and observed in the formation of a red -colored precipitate.

Benedict's Test

A fraction of the extract was treated with Benedict's reagent and in boiling water bath for a few minutes and observed in the formation of an orange red -colored precipitate.

TEST FOR GLYCOSIDES

Legal test

To the sample 1 ml of pyridine and a few drops of sodium nitroprussside solution was added and then it was made alkaline with sodium hydroxide solution. Appearance of pink color shows the presence of a glycoside.

Kiddes Test

Cardenolides give blue or violet with firs reagent which fades after 1-2 hours. This reagent is prepared by mixing equal volume of 0.21 solution of 3, 5 di nitro benzoic acid in 100 ml of 0.5 N KOH solution on 50% methanol.

Keller killiani test

1 gm of powdered drug extracted with 10 ml of 70% alcohol for a few minutes and filtered. To 5 ml of filtrate add 10 ml of hydrogen peroxide and 0.5 ml of strong solution of lead acetate was added. Precipitate thus obtained was filtered. The filtrate is shaken with 5 ml of chloroform and the layer is separated and to this 1 m l of mixture of volume of

5%ferric sulfate and 99 volumes of glacial acetic acid was added.

To this mixture 1-2 drops of conc. Sulphuric acid is added. Appearance of blue color confirms the presence of deoxy sugars.

Antimony trichloride test

Solution of the extract is heated with antimony trichloride and Tri chloro acetic acid to obtain blue or violet color. Both Cardenolides and bufadienolides give this test.

Borntrager's Test

The extract was treated with chloroform and chloroform layer was separated. To this equal quantity of dilute ammonia solution was added ammonical layer acquires rose pink colour shows the presence of a glycoside.

TEST FOR FIXED OILS

a. Small quantity of extract was separately passed between two filter paper. Appearance of stain on the paper indicates the presence of fixed oil.
b. Few drops of 0.5 alcoholic KOH were added a small quantity of extract along with drops of phenolphthalein. Then the mixture was heated on a water bath for 1-2 hours. Formation of soap neutralization of alkali indicates the presence of fixed oil and fats.

TESTS FOR TANNIONS AND PHENOLIC COMPOUNDS

Ferric chloride test

A fraction of the extract was treated with ferric chloride solution and observed in the formation of brownish colorization.

Lead acetate test

To the extract adds 10% lead acetate solution and observed in the formation of white precipitate.

Gelatin solution test

To the extract, add 1% solution gelatin containing sodium chloride solution and observed in the formation of white precipitate.

TEST FOR SAPONINS

Foam test

The extract was diluted with 20 ml of distilled water and it was agitated on a graduated cylinder for 15 minutes. The formation of 1 cm layer of foam shows the presence of saponins.

TEST FOR PROTEINS

Millon's Test

To the extract, add little amount of water and millon's reagent. The appearance of red color shows the presence of proteins.

Ninhydrin test

To the extract adds, a little amount of Ninhydrin reagent. Appearance of purple color shows the presence of proteins.

TEST FOR FLAVONIODS

Aqueous NaOH Test

To the extract adds a little amount aqueous sodium hydroxide solution and observed in the formation of color.

Blue-violet color (anthocynanine)
Yellow color (flavones)
Yellow-orange (flavones)

Conc. H_2SO_4 Test

To the extract adds a little amount of conc. Sulfuric acid and observed in the formation of color. Yellow- orange (anthocynanine), Yellow color (flavones), Orange-crimson (flavononones).

Schinodo's test

For a small amount of extract adds a piece of magnesium followed by conc., Hydrochloric acid and heated slightly, and then observe the color changes. Dark pink color (flavonoids).

Pharmacological Screening

Depends upon the presence of active constituents in the various extraction pharmacological activities were planned.

Experimental Procedure

Experimental groups

2 groups receiving ethanolic fraction (2 doses; 100 and 200 mg/kg).

Control groups were

1. Aspirin group (+Ve Control): Receiving standard antipyretic agent aspirin.
2. Solvent group (-Ve Control): receiving solvent (used).

The number of rabbits in each group was 6.

Acute toxicity study

An acute toxicity study was carried out by graded doses of each fraction in albino rabbits. Aqueous fraction was 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 mortality[11].

Treatment protocol

Before the experiment, rectal temperatures of the rabbits were recorded by inserting a well lubricated bulb of a thermometer into the rectum. Care was taken to insert it to the same depth each time (about 6 cm). Milk was collected from local cattle. Rabbits were injected with boiled milk at room temperature at the dose of 0.5 ml/kg body weight to induce pyrexia. Induction of fever took about 1 to 2 h [12].Then the solvent (2 ml) was given to the negative control group, the known antipyretic agent, aspirin solution (2 ml) was given to the positive control group and each sample solution (2 ml) was given to the corresponding experimental group. Intraperitoneal route was used to administer boiled milk, aspirin solution, solvent, and sample solutions. Finally, rectal temperatures were recorded at 1 h intervals up to 3 hrs.

RESULTS AND DISCUSSION

Preliminary Phytochemical Screening

The preliminary phytochemical screening of the ethanolic extract showed the presence of plants. phytoconstituents such as carbohydrates, alkaloids, glycosides, flavonoids, tannins and saponins were carried out on the powdered fruits following standard procedure.

In acute toxicity studies, it was found to be safe and no mortality was observed in a dose as high as 200 mg/kg. The results of the effect of ethanolic extract of Terminalia pallida on boiled milk induced pyrexia in rabbits are depicted in Table 2.

The acute toxicity result reveals that this plant might be considered as a broad non-toxic one. The antipyretic activity exhibited that the ethanol extract of Fruits possesses 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 testing animals by the extract at 200 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 fractions showed the presence of tannins and flavonoids. The antipyretic activity observed can be attributed to the presence of flavonoids have been reported to exhibit antipyretic effect [13, 14]. The present study, therefore, supports the claims of traditional medicine practitioners as an antipyretic remedy.

Table 1 Phytochemical Evaluation of different extract of Fruits of *Terminalia pallida*

S.NO.	TESTS	WATER
1.	Alkaloids	+Ve
2.	Carbohydrates	+Ve
3.	Glycosides	+Ve
4.	Fixed Oils	+Ve
5.	Tannins	+Ve
6.	Sterols	+Ve
7.	Saponins	+Ve
8.	Proteins	+Ve
9.	Flavinoids	+Ve

+Ve Indicates Present,-Ve Indicates Absent

Table 2 Antipyretic Effect of EETP on Rectal temperature in Milk induced Pyrexia in rabbits

Groups	Dose	Rectal temperature (ºC)		Rectal temperature after treatment (ºC)		
		Normal	3 h after	1 h (C1)	2 h (C2)	3 h (C3)

					boiling milk admin.			
Solvent				2 ml/rabbit	38.44 ± 0.31	40.16 ± 0.19	40.05 ± 0.12	40.00 ± 0.54	39.88 ± 0.07
Aspirin				10 mg/kg	38.61 ± 0.14	40.11 ± 0.31	39.61 ± 0.32	38.72 ± 0.56	38.72 ± 0.62
EETP				200 mg/kg	38.50 ± 0.09	40.16 ± 0.17	39.89±0.37	39.34±0.34	39.07±0.28
EETP	100 mg/kg	38.55 ± 0.09	40.33 ± 0.40	39.69±0.28	39.40±0.37				

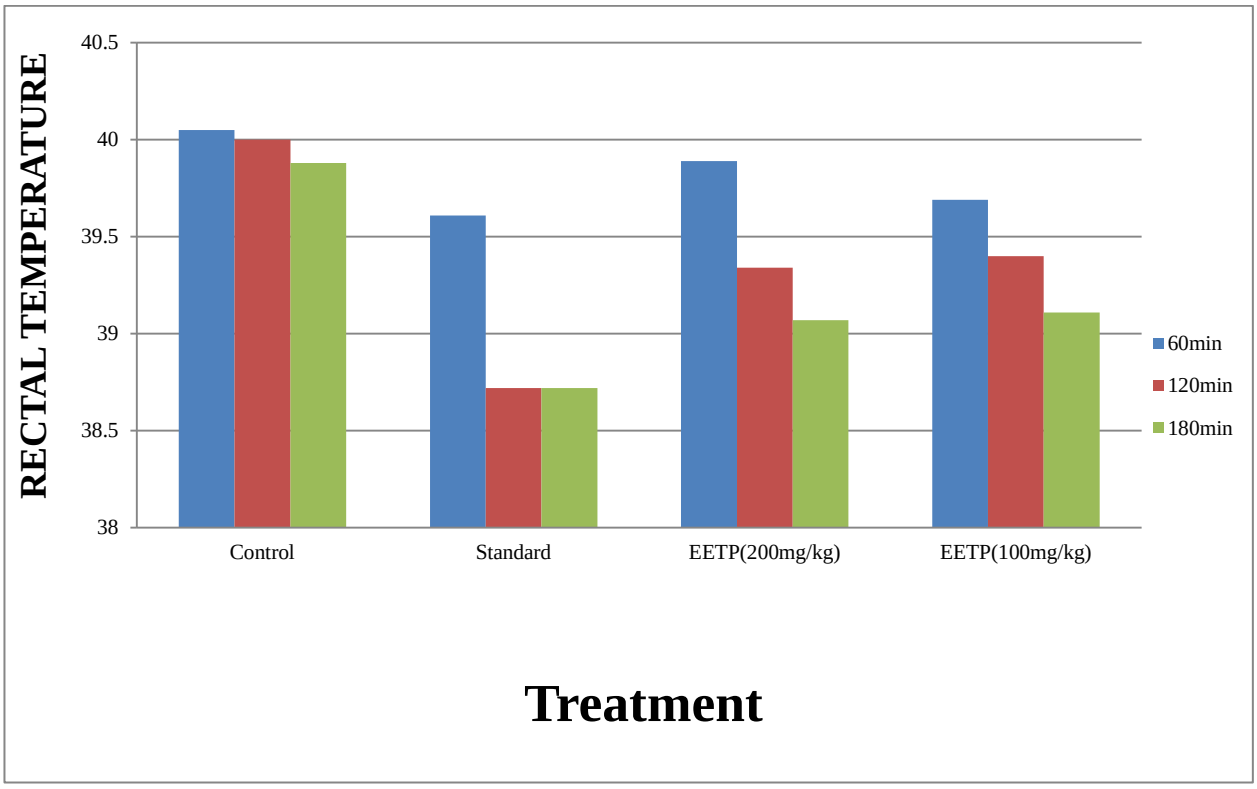

Figure 1 Antipyretic Effect of EETP on Rectal temperature in Milk induced Pyrexia in rabbits

Table 3 Percentage reduction of Rectal temperatures in Milk induced Pyrexia in Rabbits

Groups	**Dose**	**% Rectal temperature after treatment (°C)**		
		1 h (C1)	**2 h (C2)**	**3 h (C3)**
Solvent	2 ml/rabbit	6.4 ± 0.27	9.3 ± 0.12	16.3 ± 0.74
Aspirin	10 mg/kg	33.3 ± 0.13	92.6 ± 0.71	92.6 ± 1.52
EETP	200 mg/kg	33.1 ± 0.51	86.7 ± 0.64)	90.4 ± 0.65

EETP	100 mg/kg	15.7 ± 0.15	70. 4 ± 0.35	71.6 ± 0.34

% reduction = B-C_n÷ B-A× 100; where n = 1, 2 and 3.

CONCLUSION

The ethanolic extract of *Terminalia Pallida* has antipyretic effect supporting the ethno pharmacological use as antipyretics. This effect may be explored with the use of the plant in the management of some other diseases.

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