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Antipyretic Activity Assessment Of Justicia Adhatoda L. Whole Plant Extract

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ABSTRACT
Objective: In the present investigation was Anti-pyretic activity Assessment of *Justicia adhatoda L.* Whole part of methanol extract (JAWME) against Brewer's yeast suspension induces rats.
Methods: To produce fever, a 20% (w/v) suspension of brewer's yeast (10 ml/kg) was administered subcutaneously into the animal's back area. The temperature in the rectum of each participant was measured using a thermometer 17 hours after the injection. The experiments were carried out just on rats that showed a temperature rise of at least 0.7°C. The test samples were given orally after a duration of 17 hours, and the temperature was monitored at 1, 2, 3, 4, 5, and 6 hours after the administration of the medicine. Each group employed six rodents . The dose of Normal control (DW) 5 ml/kg, Brewer's yeast suspension 10ml/kg,Standard (Aspirin)150 mg/kg,Extract JAV 200 mg/kg and Isolated compound ICFJ 40 mg/kg
Result: Phytochemical screening result revealed the presence of saponins, flavonoids, alkaloids, etc. The stipulated plant *Justicia adhatoda L.* Whole part of methanol extract (JAWME) possess significance antipyretic property as comparable to standard drug Aspirin
Conclusion: The methanolic extract of *Justicia adhatoda L* reveals the reduction in the body temperature level as compare to Standard drug (Aspirin). The result suggests that *Justicia adhatoda L* possesses anti-pyretic property.

Key Words: Brewer's yeast suspension, Aspirin, *Justicia adhatoda L*

1. INTRODUCTION

Plants play a unique and essential function on Earth since they serve as the fundamental foundation for life. They are the main organisms responsible for producing food in all food systems. [1] Plants are responsible for directly supplying 90% of the calories that humans consume and around 80% of the protein that humans ingest. Throughout history, plants and herbs have served as essential medicinal foundations for medicine. Approximately 80% of the Indian populace lives in tiny and isolated regions, where the per capita income is quite little and inadequate to cover the expenses of expensive allopathic therapies. India has a wide range of plants with medicinal characteristics that are deliberately grown for use in traditional medical therapies.[2] Hence, it is crucial that this priceless natural asset be harnessed and enhanced in line with technology advancements and human need. Presently, the objective is to achieve therapy without any adverse consequences by employing nutraceuticals.
These studies offer crucial insights into the medicinal potential of plants and aid researchers in selecting which plants to further investigate. [3,4]
A considerable proportion of individuals prefer natural therapies because they believe that these treatments are less likely to have adverse effects or disturb the body's natural balance. [5]

1.1 Antipyretic Mechanisms

The primary mechanism by which antipyretics operate is as follows:
Prostaglandin Synthesis Inhibition: The production of prostaglandins is reduced by the inhibition of the enzyme cyclooxygenase (COX) by a variety of antipyretics, including NSAIDs like aspirin, ibuprofen, and naproxen. By acting on the hypothalamus to elevate the body's temperature set point, prostaglandins are lipid compounds that are essential for the induction of fever and inflammation. [6]

Central Nervous System Action: Certain antipyretics, including paracetamol (acetaminophen), exert their antipyretic effects by directly acting on the hypothalamus to disrupt prostaglandin synthesis locally within the central nervous system. This action assists in the regulation of body temperature without the substantial anti-inflammatory effects associated with NSAIDs. [7]

1.2 Types of Antipyretics

1.2.1 NSAIDs: Aspirin, ibuprofen, and naproxen are effective antipyretics because they have the capacity to inhibit the synthesis of prostaglandins and COX enzymes. Their versatility in managing febrile conditions associated with inflammation and pain is further enhanced by their anti-inflammatory and analgesic properties.

Paracetamol (Acetaminophen) is a medication that is extensively employed for its analgesic and antipyretic properties. It acts centrally on the hypothalamus to reduce fever without causing significant anti-inflammatory effects. It is generally regarded as safe when administered at the recommended dosages; however, liver toxicity may result from excessive or prolonged use. [8]

1.2.2 Corticosteroids: In certain autoimmune diseases or inflammatory conditions, corticosteroids such as prednisone or dexamethasone may be employed to suppress fever by reducing inflammation and modulating immune responses. They are more frequently employed for their anti-inflammatory properties, although they may also possess antipyretic properties.

A diverse array of botanical species that have been traditionally used across various cultures for their ability to reduce fever and ameliorate associated symptoms are included in plants with antipyretic activity. Willow bark (Salix spp.) is a notable example.[9] It has been historically regarded as a valuable source of salicin, a compound that is metabolized in the body to salicylic acid. This acid is a precursor to aspirin, a well-known nonsteroidal anti-inflammatory drug (NSAID) that is renowned for its antipyretic, analgesic, and anti-inflammatory properties. In the same vein, Ginger (Zingiber officinale) has been employed for centuries in traditional medicine systems such as Ayurveda and Chinese medicine due to its anti-inflammatory properties, which contribute to its role in fever management. Echinacea (Echinacea spp.) is an additional plant of note that is notably valued in Native American traditions for its immune-boosting properties and its capacity to alleviate fevers during infections.[10] In modern pharmacology and healthcare, the significance of investigating botanical sources is underscored by the potential alternatives or complements to synthetic medications for fever reduction that these plants offer, exemplifying nature's pharmacopeia.

2. MATERIAL AND METHODS

2.1 Plant collection and preparation

Plant material was collected locally from medicinal garden of Veer Bahadur Singh Purvanchal University, Jaunpur-222003, India. Identified and authenticated was done by scientist-E Arti Grag, Botanical Survey of India, Central Regional Centre, Praygraj, U.P. The samples are preserved in the institutional herbarium with accession numbers *Justicia adhatoda* L. accession no. 104530 for future reference [11].

2.2 Extraction of plant material

Plant material was extracted by using cold maceration method; plant samples were collected, washed, rinsed and dried properly. Powder form of plant sample was extracted with different organic solvents (petroleum ether, ethyl acetate, and methanol) and allows standing for 4-5 days each. The extract was filtered using filter paper to remove all unextractable matter, including cellular materials and other constituents that are insoluble in the extraction solvent. Extract was transferred to beaker and evaporated; excessive moisture was removed and extract was collected in air tight container. Qualitative analysis of extracts of different solvents was carried out to find out the presence of various phytoconstituents [11]. Extraction yield of all extracts were calculated using the following equation below:

$$\textbf{Percentage Yield} = \frac{\textbf{Actual yield}}{\textbf{Theoretical yield}} \times 100$$

2.3 Qualitative phytochemical estimation of extracts

Detailed phytochemical testing was performed to identify presence or absence of different phytoconstituents in extracts by using standard procedures**.** The extracts were subjected to differeent tests

2.4 Antipyretic activity

The capacity of a substance to reduce fever is referred to as antipyretic activity. An elevated body temperature is the hallmark of fever, a prevalent symptom of inflammation and infection. The hypothalamus, the region of the brain that regulates body temperature, is the target of antipyretic agents, including acetaminophen (paracetamol) and nonsteroidal anti-inflammatory drugs (NSAIDs) like ibuprofen. These agents prevent the synthesis of prostaglandins, which are composed of compounds that are involved in the production of fever and the inflammatory response. Antipyretics reduce fever by decreasing the body's temperature set point by reducing prostaglandin levels. This activity is essential for the management of febrile conditions and the enhancement of patient welfare, particularly in the context of inflammatory diseases and infections. Continuous research into novel antipyretic compounds is crucial for the development of safer and more effective treatments.[12,13]

2.5 Animals

The creatures The Swiss albino mice were housed in standard, capacious, hygienic polypropylene enclosures and were maintained at a temperature of 22 ± 2 ◦C with a 12/12-h light and dark cycle. The mice were procured from the in-house animal facility of PBRI. Water was administered ad libitum until the conclusion of the research, and all animals were

fed rat normal pellet diet (NPD) that was commercially available and purchased from Keval Sales Corporation, Vadodara. The experimental protocols were approved by Institutional Animal Ethics Committee (1337/PO/Re/S/10/CPCSEA).

2.6 Experiment

Prior to the studies, male rats were deprived of food for an extended period and given unrestricted access to water. The basal rectal temperature of each mouse was measured by inserting a thermistor probe approximately 1 cm into the rectum using a digital thermometer. To produce fever, a 20% (w/v) suspension of brewer's yeast (10 ml/kg) was administered subcutaneously into the animal's back area. The temperature in the rectum of each participant was measured using a thermometer 17 hours after the injection. The experiments were carried out just on rats that showed a temperature rise of at least 0.7°C. The test samples were given orally after a duration of 17 hours, and the temperature was monitored at 1, 2, 3, 4, 5, and 6 hours after the administration of the medicine. Each group employed six rodents. The selection of the dosages was based on the acute toxicity research that was previously explored in several literatures.[14,15]

2.7 Animal Grouping:

Table 2.1: Animal Grouping and dose for Antipyretic activity

S. No.	TREATMENT	DOSE	No. of Animal
1.	Normal control (DW)	5 ml/kg	06
2.	Brewer's yeast suspension	10ml/kg	06
3.	Standard (Aspirin)	150 mg/kg	06
4.	Extract JAV	200 mg/kg	06
5.	Isolated compound ICFJ	40 mg/kg	06

2.8Statistical Analysis

All the measurements were done in triplicate and results are expressed in terms of mean ± standard deviation and IC_{50} values were calculated using Graph Pad Prism 5 version 5.01 (Graph pad software, Inc., La Jolla, CA, USA.) statistical software.

3- RESULTS AND DISCUSSIONS

The extraction of *Justicia adhatoda L* whole part was performed and yield of extract was found to be 8.1%. The colors of all extracts were dark brown, methanolic extract of *Justicia adhatoda L* whole part was semisolid.

3.1- Phytochemical screening test

Phytochemical screening tests were performed using methanolic extract of *Justicia adhatoda L* whole part (JAWME) and was found to be JAWME extract contains Glycosides, Alkaloids, Amino acids, Carbohydrates, Glycosides, Phenolic compounds, Tannins, Saponins, Flavonoids, Proteins. The bioactive compounds provide semi-qualitative information on the active constituents of the extract. Phytochemical investigation of PAMW revealed the presence of higest numbers of phytoconstituents in methanolic extract Table 1. Therefore JAWME was taken further in-vivo study.

Table 3.1: Results of phytochemical screening test

S. No.	Experiment	Result		
		Pet. Ether Extract	Ethyl Acetate	Methanol
Test for Carbohydrates				
+1.	Molisch's Test	-	-	+
2.	Fehling's Test	-	-	+
3.	Benedict's Test	-	-	+
4.	Bareford's Test	-	-	+
Test for Alkaloids				
1.	Mayer's Test	-	+	+
2.	Hager's Test	-	+	+
3.	Wagner's Test	-	+	+
4.	Dragendroff's Test	-	+	+
Test for Terpenoids				
1.	Salkowski Test	-	-	+
2.	Libermann-Burchard's Test	-	-	+

Test for Flavonoids				
1.	Lead Acetate Test	-	+	+
2.	Alkaline Reagent Test	-	+	+
3.	Shinoda Test	-	+	+
Test for Tannins and Phenolic Compounds				
1.	$FeCl_3$ Test	-	+	+
2.	Lead Acetate Test	-	+	+
3.	Gelatine Test	-	+	+
4.	Dilute Iodine Solution Test	-	+	+
Test for Saponins				
1.	Froth Test	+	-	-
Test for Protein and Amino acids				
1.	Ninhydrin Test	-	-	+
2.	Biuret's Test	-	-	+
3.	Million's Test	-	-	+
Test for Glycosides				
1.	Legal's Test	-	-	+
2.	Keller Killani Test	-	-	+
3.	Borntrager's Test	-	-	+

+ = Components present

3.2 *In-vivo* Antipyretic activity

In-vivo antipyretic activity refers to the ability of a substance to reduce fever in a living organism, typically tested using animal models. This evaluation is crucial in pharmacological research for developing treatments for febrile conditions. The procedure often involves inducing fever in animals, commonly rats or mice, using agents like lipopolysaccharides (LPS) or yeast, which trigger an immune response leading to elevated body temperature. The test substance is then administered, and its effects on body temperature are monitored and compared to control groups. Successful antipyretic agents will demonstrate a significant reduction in fever compared to controls. This type of study helps in understanding the mechanisms of fever reduction and the efficacy of potential therapeutic agents in managing fever in clinical settings.

3.3 Model: Brewers' yeast Pyrexia

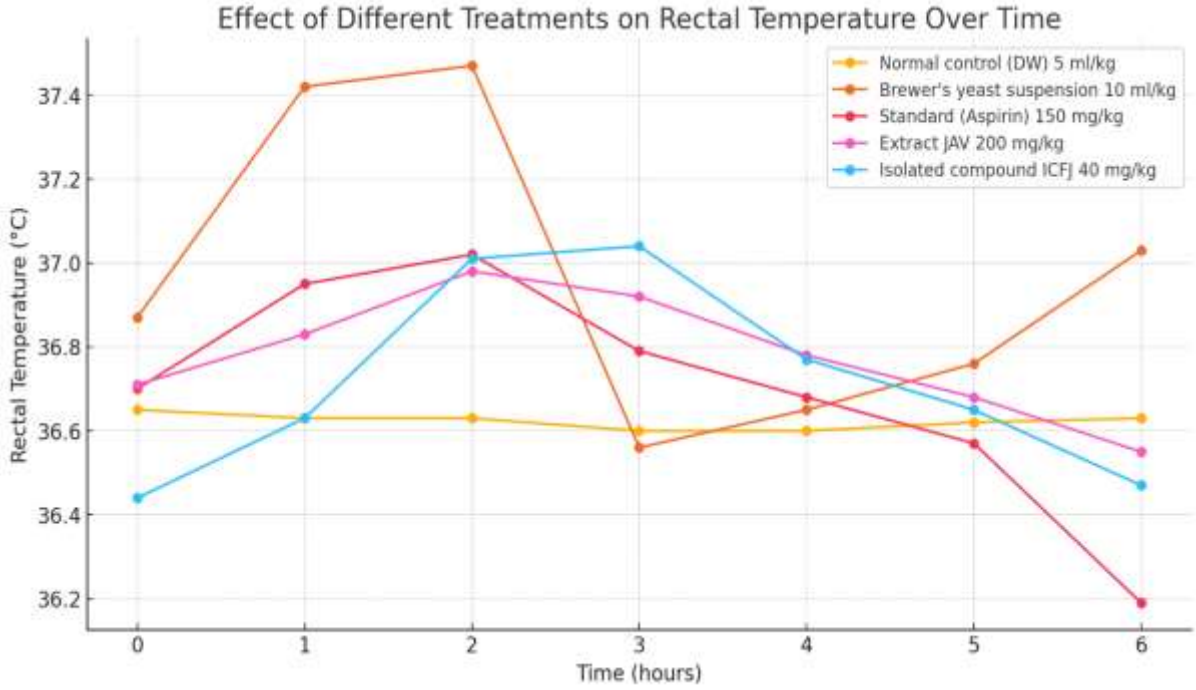
The Brewer's yeast pyrexia model is an established method for evaluating the antipyretic activity of compounds in vivo. In this model, fever is induced in animals, typically rodents, by administering a suspension of Brewer's yeast (Saccharomyces cerevisiae) intraperitoneally. The yeast acts as a pyrogen, stimulating the immune system to produce endogenous pyrogens like interleukin-1 (IL-1) and tumor necrosis factor-alpha (TNF-α), which elevate the body temperature, mimicking a febrile state. After fever induction, the test compound is administered, and the rectal temperature of the animals is measured at regular intervals to assess its efficacy in reducing the elevated body temperature. This model is widely used due to its simplicity, reliability, and relevance to human febrile conditions. It helps in screening potential antipyretic agents and understanding their mechanisms of action in controlling fever.

Table 3.2: Brewers' yeast Pyrexia for in-vivo Antipyretic activity

S. No.	Treatment	Dose	Initial rectal temperature	0 hrs	1 hrs	2 hrs	3 hrs	4 hrs	5 hrs	6 hrs
1	Normal control (DW)	5 ml/kg	36.59±0.112	36.65±0.166	36.63±0.106	36.63±0.093	36.60±0.083	36.60±0.011	36.62±0.115	36.63±0.084
2	Brewer's yeast suspension	10ml/kg	36.42±0.027	36.87±0.091	37.42±0.111	37.47±0.121	36.56±0.147	36.65±0.148	36.76±0.100	37.03±0.101
3	Standard (Aspirin)	150 mg/kg	36.19±0.077	36.70±0.197	36.95±0.420*	37.02±0.449	36.79±0.400	36.68±0.391	36.57±0.390	36.19±0.323*
4	Extract JAV	200 mg/kg	36.30±0.067	36.71±0.185	36.83±0.178*	36.98±0.203	36.92±0.325	36.78±0.379	36.68±0.430	36.55±0.476
5	Isolated compound ICFJ	40 mg/kg	36.12±0.163	36.44±0.085*	36.63±0.096*	37.01±0.388	37.04±0.394	36.77±0.260	36.65±0.268	36.47±0.328*

The results are reported as the mean value plus or minus the standard deviation, with a sample size of 6. The data was evaluated using a statistical method called one-way analysis of variance (ANOVA), followed by a Bonferroni t-test. The value of P is less than 0.05, indicating statistical significance.
Antipyretics are substances that lower abnormally high body temperature. Yeast-induced pyrexia is referred to as pathogenic fever, and its cause includes the creation of prostaglandins, which reduce the temperature at which the thermoregulatory center is set. Both the extract and compound exhibited statistically significant ($p < 0.05$) antipyretic action and effectively reduced the high rectal temperature after 6 hours of therapy. The rectal temperatures (°C) in the group treated with a dose of 200 mg/kg body weight of the extract and a dose of 40 mg/kg body weight of the isolated compound were measured to be 36.55 ± 0.476 and 36.47 ± 0.328, respectively. These temperatures were compared to the yeast-induced temperature of 37.03 ± 0.101, the normal temperature of 36.63 ± 0.084 in other groups, and the temperature of 36.19 ± 0.323 in the group treated with aspirin (reference drug).

Figure 3.1: Brewers' yeast Pyrexia for in-vivo Antipyretic activity



Here's the graph showing the effect of different treatments on rectal temperature over time. The plot compares the normal control, Brewer's yeast suspension, standard (Aspirin), extract JAV, and isolated compound ICFJ treatments. This visualization helps in understanding the antipyretic effects of each treatment by observing the changes in rectal temperature across the hours following administration

4-DISCUSSION & CONCLUSION:

The capacity of a substance to reduce fever in a living organism, known as in-vivo antipyretic activity, is typically assessed using animal models. This assessment is essential in the development of therapies for febrile conditions in the context of pharmacological research. After inducing pyrexia with brewer's yeast, the plant extracts were administered to rodents. The antipyretic efficacy of the extract is demonstrated by a substantial decrease in body temperature compared to the control group, as evidenced by the regular monitoring of the animals' body temperature. It is hypothesised that bioactive compounds in Adhatoda vasica, including alkaloids and flavonoids, contribute to this effect by regulating the production of pyrogenic cytokines and influencing the central thermoregulatory pathways.. Effects of various interventions on rectal temperature over time. The normal control, Brewer's yeast suspension, standard (Aspirin), extract JAV, and isolated compound ICFJ treatments are compared in the plot. This visualization aids in comprehending the antipyretic effects of each treatment by monitoring the fluctuations in rectal temperature in the hours following administration.
A volume of 10 milliliters of distilled water was employed to dissolve 10 milligrams of gallic acid. Distilled water was added to 1, 2.5, 5, 7.5, 12.5, and 25 ml of this solution in a volumetric flask, resulting in a total volume of 46 ml. After adding 1.0 ml of Folin-Cocteau reagent, the contents of the vial were stirred vigorously. The mixture was allowed to stand for a duration of 2 hours with periodic agitation, and 3.0 ml of a 2% solution of sodium carbonate was added 3 minutes thereafter. The measurement of the absorbance of the blue hue that formed was conducted at a wavelength of 760 nm. The standard table of gallic acid is depicted in Table and Figure, respectively.

The antipyretic effect of the Methanolic extract of adhatoda vasica was evaluated in Wistar strain albino rats using Brewer's yeast-induced pyrexia. The antipyretic efficacy of the methanolic extract was evaluated using doses of 100 mg/kg and 200 mg/kg. Outcome - The extract derived from the Adhatoda vasica plant had a dose-dependent antipyretic activity in laboratory mice that were stimulated with yeast, leading to a noteworthy ($P < 0.01$) rise in body temperature. Conclusion: Adhatoda vasica nees demonstrates the presence of a methanolic extract. In-vivo antipyretic activity refers to the ability of a chemical to decrease fever in a living creature. This is usually studied by utilizing animal models. The plant has shown significant antipyretic efficacy in comparison to the usual medication. This assessment is crucial for the advancement of treatments for feverish diseases within the realm of pharmaceutical research. Following the induction of fever using brewer's yeast, the plant extracts were fed to rats.

The findings suggest that the methanolic extract of Adhatoda vasica leaves has a significant antipyretic effect in experimental rats with elevated body temperature caused by yeast. The results indicate that the extract exhibited antipyretic activity that was dose-dependent. It exhibited substantial antipyretic activity at a dosage of 200mg/kg. This resulted in the maintenance of a consistent body temperature for an extended period. Flavonoids are recognized for their ability to target prostaglandins, which are implicated in the development of pyrexia. Therefore, the antipyretic activity of the methanolic extract of Adhatoda vasica leaves may be influenced by the presence of flavonoids.

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