Evaluation of the Anti-Pyretic Effects of Ethanolic Extract of *Sonchus wightianus DC* using wistar rats

Abstract
This study aimed to investigate the ethanolic extract of *Sonchus wightianus DC* for its pharmacological activities, including anti-pyretic, behavioral, and immunomodulatory effects. The extract was prepared via Soxhlet extraction, and its chemical composition was analyzed for bioactive compounds. The extract demonstrated significant antipyretic effects in both yeast-induced and vaccine-induced hyperpyrexia models in rats. Furthermore, behavioral tests revealed an increase in ambulation and rearing frequency compared to the negative control group. Additionally, a notable reduction in white blood cell (WBC) count was observed, suggesting potential immunomodulatory properties. These results support the medicinal potential of *Sonchus wightianus DC* in treating fever and modulating immune responses.

Keywords: *Sonchus wightianus DC*, ethanolic extract, anti-pyretic, behavioral effects, immune modulation, Soxhlet extraction, phytochemical analysis.

1. Introduction

Sonchus wightianus DC, a member of the Asteraceae family, is a plant native to India and various parts of Asia. It has been used in traditional medicine for centuries, particularly for its anti-inflammatory, analgesic, and antipyretic properties. *Sonchus wightianus* is believed to possess a wide array of medicinal effects, including the treatment of fever, digestive disorders, and respiratory issues, making it an important plant in traditional therapeutic practices. The leaves, which are commonly used in herbal formulations, have been reported to show significant bioactivity, though scientific validation of these claims remains limited.

The therapeutic potential of *Sonchus wightianus* DC is largely attributed to its rich phytochemical profile, which includes a variety of bioactive compounds. These include flavonoids, alkaloids, terpenoids, phenols, saponins, tannins, and steroids. Flavonoids and alkaloids, in particular, have been recognized for their anti-inflammatory, antioxidant, and antimicrobial properties. Terpenoids are known for their ability to modulate inflammatory pathways, while phenolic compounds are celebrated for their antioxidant and potential immune-boosting effects. Given this diverse range of bioactive compounds, *Sonchus wightianus* is believed to offer a synergistic approach to managing fever and inflammation, which are commonly encountered in various infectious and non-infectious diseases.

Despite the plant's extensive use in folk medicine, scientific studies supporting its efficacy, particularly for anti-pyretic activity, are relatively scarce. Fever, a common symptom of numerous diseases, is typically treated with anti-pyretic medications like paracetamol or ibuprofen. However, concerns regarding side effects, long-term use, and drug resistance have driven the search for natural alternatives with fewer adverse effects. Plants like *Sonchus wightianus* that possess promising anti-pyretic and anti-inflammatory properties could provide safer, more sustainable therapeutic options for managing these symptoms.

The present study aims to evaluate the anti-pyretic activity of the ethanolic extract of *Sonchus wightianus* DC in animal models. Specifically, the study investigates the effect of the plant extract on fever induced by both yeast and vaccine administration in Wistar rats. Furthermore, the study explores the behavioral effects of the extract, including its impact on motor activity, and examines its potential immunomodulatory effects by assessing changes in white blood cell count. By focusing on these key areas, the study seeks to provide scientific validation for the traditional use of *Sonchus wightianus* in the treatment of fever and related inflammatory conditions. The findings of this research could pave the way for future studies and potentially contribute to the development of plant-based anti-pyretic formulations for clinical use [1-15].

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2. Materials and Methods

Plant selection:

Drug discovery from medicinal plants involves a wide range of fields of study and analytical techniques. According to the intensive literature survey, *Sonchus wightianus DC* was used for the present study [16].

Collection and Identification:

Collection and identification of plant include the plant material of leaves of *Sonchus wightianus DC* was collected from nainital uttarakhand and the plant samples were authenticated by Dr. S.N. Dwivedi A.P.S. College Rewa. Voucher Specimen Number: J/Bot./2024-044. The plant specimen was washed thoroughly under the tape water to discard dust and other unwanted materials. Then plant part was dried under shade with usual sun- air drying.

Preparation of Extract:

The leaves of *Sonchus wightianus DC* were thoroughly washed under the tap water and then with distilled water to remove any physical impurities. The cleaned leaves were allowed to dry in the shade. After drying, grind it into fine powder using an mixer. Then fine powder stored in an airtight container for further investigation [16].

Extraction Procedure:

The dried plant material was ground into fine particles using a mixer. A total of 500g of the powdered plant material was placed in a Soxhlet apparatus and subjected to continuous hot percolation for 8 hours, using 50% ethanol as the solvent. The resulting extract was filtered through Whatman filter paper No. 1, and the ethanol was evaporated using a heating mantle. Finally, the extract was further dried in a tray dryer [16].

Preliminary Phytochemical Screening of Ethanolic Extract of *Sonchus wightianus DC* [17]:

The ethanolic extract of *Sonchus wightianus DC* was subjected to preliminary phytochemical screening to identify the presence of various bioactive compounds, including alkaloids,

flavonoids, saponins, tannins, steroids, phenols, and terpenoids. The tests were performed according to standard protocols as follows:

- **Test for Terpenoids (Salkowski's Test):** To 0.5 g of the extract, 2 ml of chloroform was added, followed by the careful addition of 3 ml of concentrated sulfuric acid (H_2SO_4). A reddish-brown color at the interface indicates the presence of terpenoids.
- **Test for Flavonoids:** A portion of the aqueous filtrate was treated with 5 ml of dilute ammonia solution, followed by the addition of 1 ml of concentrated sulfuric acid. A yellow color, which fades upon standing, confirms the presence of flavonoids.
- **Test for Saponins:** To 0.5 g of the extract, 5 ml of distilled water was added in a test tube. The mixture was shaken vigorously and observed for stable, persistent froth. The froth was then mixed with 3 drops of olive oil and shaken again. The formation of an emulsion indicates the presence of saponins.
- **Test for Tannins:** Approximately 0.5 g of the extract was boiled in 10 ml of water and filtered. A few drops of 0.1% ferric chloride were added to the filtrate. The appearance of a brownish-green or blue-black color confirms the presence of tannins.
- **Test for Alkaloids:** 0.5 g of the extract was dissolved in 10 ml of acid alcohol, boiled, and filtered. To 5 ml of the filtrate, 2 ml of dilute ammonia solution and 5 ml of chloroform were added. After gently shaking the mixture, the chloroform layer was separated and extracted with 10 ml of acetic acid. The extract was divided into two portions, to which Mayer's reagent and Dragendorff's reagent were added separately. A cream-colored precipitate (with Mayer's reagent) or a reddish-brown precipitate (with Dragendorff's reagent) indicates the presence of alkaloids.
- **Test for Phenols:** The total phenolic content was determined using the method of Bray and Thorpe [17]. A standard curve of caffeic acid was prepared in 80% ethanol. From the stock solution (100 µg/ml), 0.1 to 0.9 ml was transferred into separate test tubes, and the volume was adjusted to 1 ml with 80% ethanol. To each tube, 1 ml of Folin-Ciocalteu reagent (diluted 1:2 with distilled water) and 2 ml of 20% sodium carbonate (Na_2CO_3) solution were added. The mixture was shaken, boiled for 1 minute, cooled, and diluted to 25 ml with distilled water. The optical density was measured at 750 nm using a spectrophotometer against a blank.
- **Test for Steroids:** To 0.2 g of the extract, 2 ml of acetic acid was added. The solution was cooled in ice, and concentrated sulfuric acid (H_2SO_4) was carefully added. A color change from violet to blue or bluish-green indicates the presence of steroidal compounds, which is characteristic of the aglycone portion of cardiac glycosides.

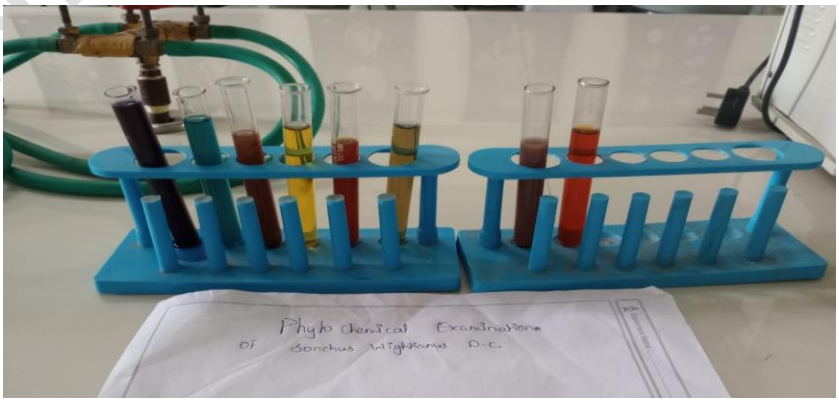

Figure 1:- Phytochemical Test of Extract

Experimental Animals

Male Wistar rats are easy to breed, low-cost, and simple to handle, making them an ideal model for inflammation studies due to their similarities to humans. These rats are also suitable for both pharmacokinetic and pharmacodynamic studies. Based on these criteria, rats were selected for the study. Brewer's yeast and Tab vaccine can be easily induced in rats.

Male Wistar rats, weighing between 220 and 270 g, were obtained from the animal house at Swami Vivekananda College. All animals were housed under standard controlled conditions with a temperature maintained at 24 ± 2°C, humidity at 50% ± 5%, and a 12-hour light: 12-hour dark cycle. The rats were kept individually in large, spacious, and hygienic cages throughout the experimental period. They had free access to food (standard commercial rat chow) and water, and received proper care.

The animals were allowed to acclimatize to the laboratory conditions for one week before the experiment began. The experimental protocol was approved by the Institutional Animal Ethical Committee of our organization (Approval No: IAEC/SVCP/2024/01) and adhered to the guidelines set by the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), New Delhi [18].

Acute oral toxicity:

The plant *Sonchus wightianus DC* was found to have a safe ethanolic extract when given at doses of 100mg and 200mg per kg of body weight, according to the literature. No mortality was observed at both doses. Therefore, it was reported that the LD50 of the plant extract is 2g/kg of body weight [19].

Experiment Method

Table 1: Animal Group Design-

A Total number of 25 healthy adult Wistar rat were divided into 5 groups each:

S. No.	**Groups**	**No. of Animals**
1.	Normal Control	5
2.	Negative Control	5
3.	Low dose of extract (100mg/kg)	5
4.	High dose of extract (200mg/kg)	5
5.	Standard drug : paracetamol (100mg/kg)	5
	Total	25

Figure 2:- Animal Group Design

For assessing antipyretic activity, Brewer's yeast-induced hyperpyrexia and TAB (Typhoid) vaccine-induced pyrexia models were employed, based on the literature survey.

Brewer's Yeast-Induced Hyperpyrexia:

The animals were fasted overnight prior to the experiment, with water provided ad libitum. The rats were randomly divided into five groups. Pyrexia was induced by subcutaneous injection of a 20% (w/v) brewer's yeast suspension (10 ml/kg) into the dorsum of the rats. Seventeen hours after the injection, the rectal temperature of each rat was measured using a thermometer. Only rats showing an increase in temperature of at least 0.7°C were selected for the study.

The rats in two test groups received 100 mg/kg and 200 mg/kg of *Sonchus wightianus DC* extract orally. The control group was treated with 2 ml/kg of saline, while the standard group received paracetamol (100 mg/kg). The negative control group was administered brewer's yeast. The initial rectal temperature of the rats was recorded, and temperatures were subsequently measured at 60, 90, and 120 minutes post-extract administration. The mean temperature of each group was recorded [20].

Figure 3:- Inducing Brewer's Yeast Suspension

TAB (Typhoid) Vaccine-Induced Pyrexia:

In this method, the rats were divided into five groups, including two test groups. The control group received 2 ml/kg of saline. The normal rectal temperature of the rats was recorded using a thermometer at hourly intervals over a 4-hour period. The TAB vaccine was administered intravenously into the marginal ear vein of the rats at a dose of 0.5 ml/rat. *Sonchus wightianus DC* leaf extract was administered orally at doses of 100 mg/kg and 200 mg/kg, 60 minutes after the TAB vaccine administration, when significant pyrexia was expected. The rectal temperature was recorded every hour for up to 3 hours. Paracetamol (100 mg/kg) was used for comparison in the standard group [21].

Figure 4:- Vaccine Used in Experiment

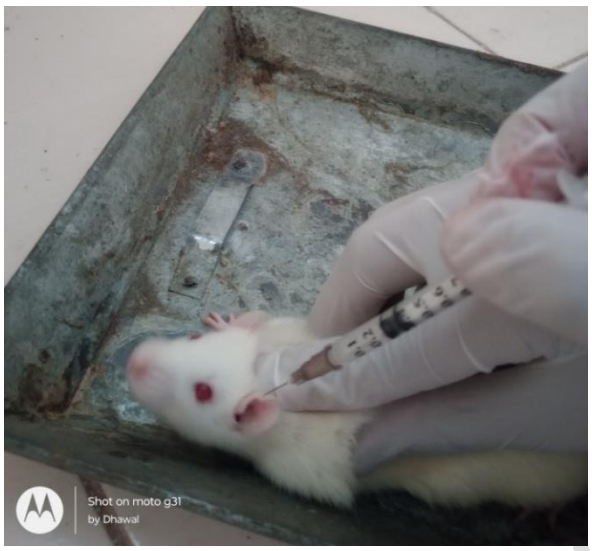

Figure 5:- Inducing TAB Vaccine

Figure 6:- Inducing Extract Orally

Figure 7:- Inducing Standard Drug (Paracetamol) Orally

Parameters to Be Assessed

The following parameters were assessed:

Body Temperature:

The body temperature of all rats was measured before and after the experiment by placing a thermometer in the rectal cavity, as pyrexia induces an increase in body temperature.

Behavioral Studies:

- **Open Field Test:** The open field test was conducted to assess locomotion, exploration, and anxiety. Each rat was placed in the center of an open field apparatus, a circular wooden box with a diameter of 72 cm and a height of 36 cm, with the floor divided into 16 regions. Rats were observed individually for 5 minutes, and three parameters were recorded[22]:
  (i) **Ambulation:** The number of grid lines crossed by the rat with all four paws.
  (ii) **Rearing:** The number of times the rat stood on its hind paws.

WBC Count:

One milliliter (1 ml) of blood was carefully drawn from the tail vein of a rat using a sterile needle and syringe, and the sample was subsequently transported to the laboratory for analysis. The purpose of this procedure was to conduct a white blood cell (WBC) count, which serves as a crucial diagnostic test for evaluating the activity of the immune system and the overall health of the animal. The blood sample was handled with the utmost care to preserve its integrity, and appropriate steps were taken to ensure its preservation during transit, guaranteeing accurate results in the laboratory.

Figure 8:- Collecting Blood Sample

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3. Result

Extraction:

The ethanolic extract was prepared by soxhlet extraction technique and the % yield was calculated, its physical characteristics were shown in (table no. 3)

Table No. 2: Percentage Extractive Value and Physical Characteristic of Extract

Extract	**%Dry Weight(w/w)**	**Colour**	**Odor**	**Consistency**
Ethanolic extract	65%	Brownish green	Characteristic	Smooth(semi solid)

% yield of extracted compound:

The % of extracted compound was found to be 65 % by using formula shown below & after calculating % yield extract. The extract use to make formulation.

% yield = Practical yield/theoretical yield ×100

% yield = 65/100 × 100

% yield = 65%

8.3 Phytochemical screening test:

Table No. 3: Preliminary phytochemical analysis *Sonchus Wightianus DC* extract:

Phytochemical groups	Presence/absence
Terpenoids	++
Flavonoids	++++
Saponins	++
Tannins	+++
Alkaloids	+
Phenols	+++
Steroids	+++

- Absent; + Present; ++ Low concentration; +++ Moderate concentration; ++++ High concentration.

Evaluation of Anti-pyretic activity:

Table No. 4: Effect of ethanolic extract of *Sonchus wightianus DC* on yeast induced hyperpyrexia in rats

Groups	Pre-drug temp (℃)	Post-drug temp (60min)	Post-drug temp (90min)	Post-drug temp (120min)
Normal Control	39.4± 0.3	38.7±0.1	38.3±0.2	37.4±0.3
Negative Control	40.2± 0.4	39.5± 0.3	39.4± 0.3	38.7± 0.3
Low dose of extract	39.5± 0.3	38.8± 0.4	38.1± 0.5	37.2± 0.2*
High dose of extract	39.2± 0.4	38.5± 0.3*	37.2± 0.2**	36.5± 0.3**
Standard drug : paracetamol	39.5± 0.2	38.3± 0.4*	37.3± 0.4*	36.5± 0.2*

Each value is the mean ± S.E.M. of 5 rats. * P < 0.05; **p < 0.01 compared with control; student's t-test.

In the brewer's yeast induced hyperpyrexia model, artificial hyperthermia was induced by administration of exogenous pyrogens in the form of yeast. General reduction of the rectal temperature was observed 60 minutes, 90 minutes and 120 minutes after oral administration of the highest dose (200 mg / kg) of the extract. The observed antipyretic effect of the extract may be due to the flavonoids and alkaloids contents of the leaves. These flavonoids and alkaloids may act by blockage of the synthesis of prostagladins E2 (– a peripheral fever mediator) through the inhibition of prostaglandins synthetase. Therefore the extract could be mediating it analgesic and antipyretic effects like the non steroidal anti-inflammatory drugs.

Table No. 5: Effect of ethanolic extract of *Sonchus wightianus DC* on vaccine induced hyperpyrexia in rats

Groups	Pre-drug temp (°C)	Post-drug temp (1st hr)	Post-drug temp (2nd hr)	Post-drug temp (3rd hr)
Normal Control	39.3±0.2	37.8±0.2	37.4±0.3	37.1±0.3
Negative Control	40.8±0.3	39.4± 0.4	39.2± 0.2	38.8± 0.3
Low dose of extract	39.6±0.4	38.8±0.6	37.5±0.4	36.3±0.4*
High dose of extract	39.3±0.7	37.9±0.5	37.0±0.2*	36.3±0.4**
Standard drug : paracetamol	40.5±0.2	37.6±0.3	36.9±0.3*	35.9±0.5**

Values are expressed as mean ± SEM of five animal per group (n=5). * P < 0.05; **p < 0.01 significant when compared with reference drug paracetamol.

When the extract was administered to rats with established TAB vaccine-induced fever, the fever was significantly reduced and the body temperature was normalized by administration of 100 and 200 mg/kg dose intraperitoneally. The response in higher doses was almost comparable to that of paracetamol.

Behavioral Studies:

- **Open field test:**

(a) Ambulation frequency

The current findings revealed a marked (P<0.001) reduction in ambulation frequency in the negative control group compared to the vehicle group. In contrast, administration of Extract 1, Extract 2, and the standard drug resulted in a significant increase in ambulation frequency relative to the negative control. All data are presented as mean ± SEM, based on two separate models with 5 animals per group (n=5).

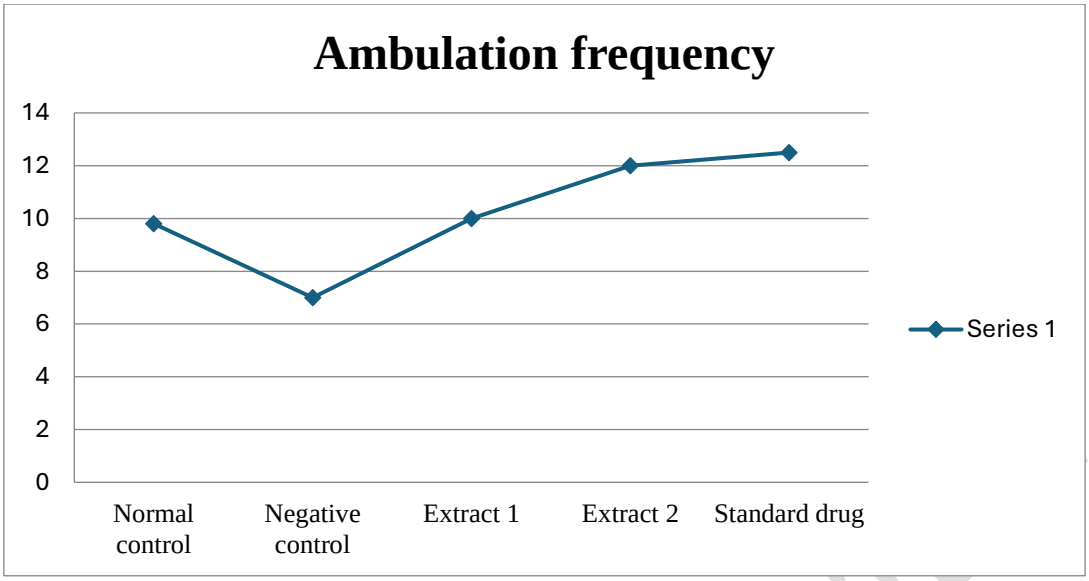

Chart 1:- Amulation Frequency from Brewer's Yeast Model

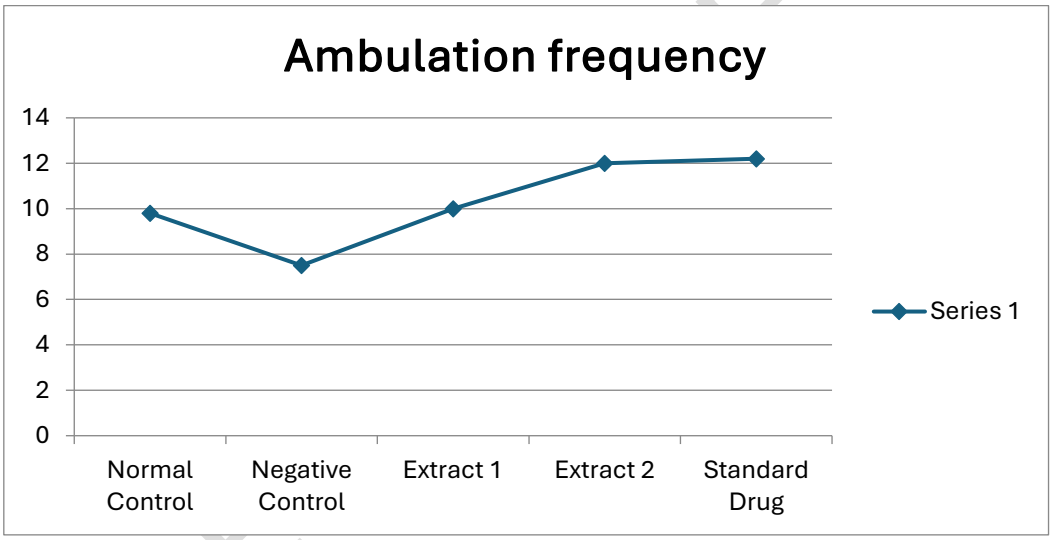

Chart 2:- Amulation Frequency from TAB Vaccine Model

(b) Rearing frequency

The current results demonstrated a substantial ($P<0.001$) reduction in rearing frequency in the negative control group when compared to the vehicle group. Conversely, treatment with Extract 1, Extract 2, and the standard drug led to a significant increase in rearing frequency relative to the negative control. Data are presented as mean ± SEM, derived from two independent models, each consisting of 5 animals per group (n=5).

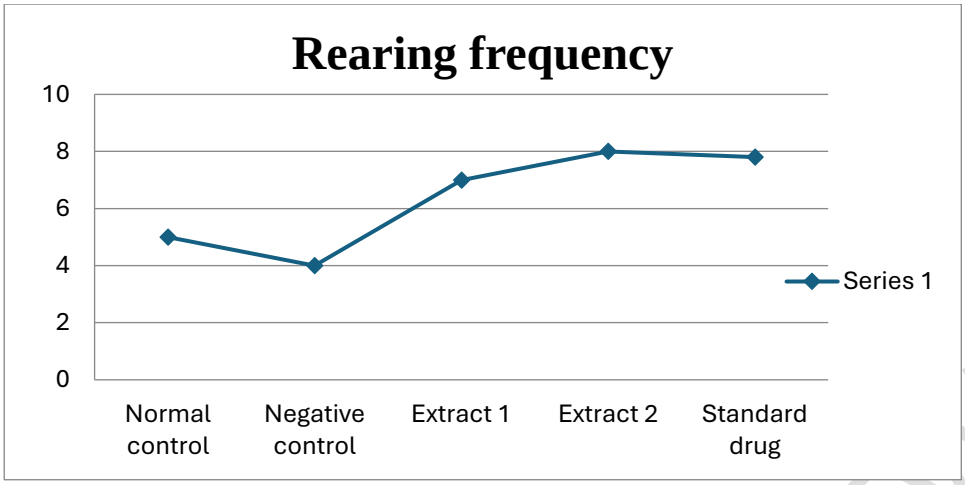

Chart 3:- Rearing Frequency from Brewer's Yeast Model

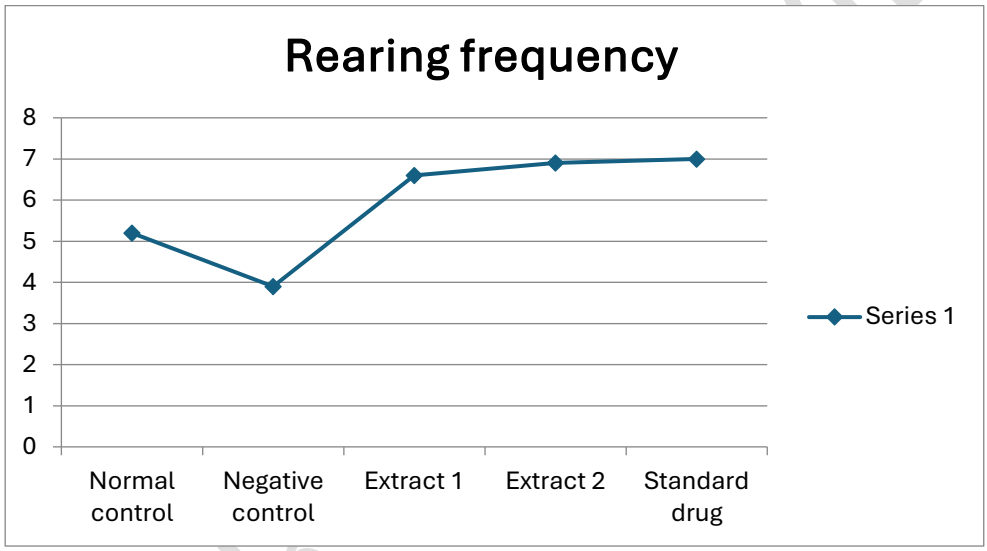

Chart 4:- Rearing Frequency from TAB Vaccine Model

WBC Count:

The current results revealed a significant ($P<0.001$) elevation in white blood cell (WBC) count in the negative control group compared to the vehicle-treated group, indicating a potential inflammatory or immune response disruption. In contrast, treatment with Extract 1, Extract 2, and the standard drug resulted in a marked reduction in WBC count relative to the negative control group, suggesting that these interventions may help modulate immune function or alleviate inflammation. This reduction in WBC count highlights the possible therapeutic benefits of these treatments in regulating immune responses. All data are expressed as mean ± SEM, with values derived from two distinct experimental models, each consisting of 5 animals per group (n=5).

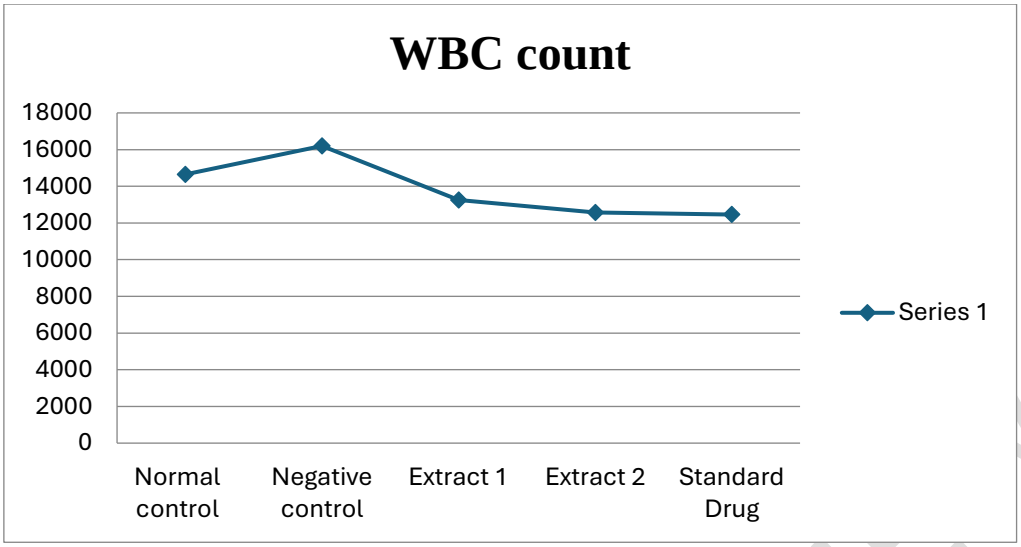

Chart 5:- WBC Counts from Brewer's Yeast Model

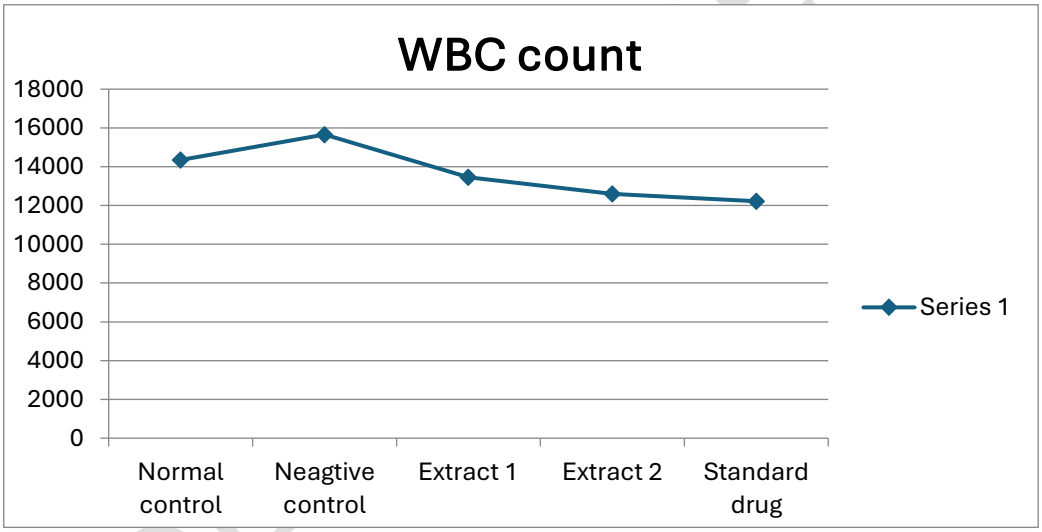

Chart 6:- WBC Counts from TAB Vaccine Model

4. Discussion

The results of this study indicate that *Sonchus wightianus DC* ethanolic extract exhibits significant anti-pyretic, behavioral, and immunomodulatory activities in animal models. The observed anti-pyretic effect may be attributed to the flavonoids and alkaloids in the extract, which likely inhibit the synthesis of prostaglandins, a known mediator of fever. The increase in ambulation and rearing frequencies suggests that the extract has an analgesic effect, relieving discomfort associated with fever. The reduction in WBC count further supports the

potential of the extract to modulate immune responses, making it a candidate for therapeutic applications in inflammatory and fever-related conditions.

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5. Conclusion

The ethanolic extract of *Sonchus wightianus DC* demonstrated significant anti-pyretic, behavioral, and immunomodulatory effects in experimental models. These findings suggest that the extract has potential as a natural remedy for fever and immune modulation. Further studies are warranted to isolate and identify the specific bioactive compounds responsible for these effects and to evaluate the safety and efficacy of the extract in clinical settings.

COMPETING INTERESTS DISCLAIMER:

Authors have declared that they have no known competing financial interests OR non-financial interests OR personal relationships that could have appeared to influence the work reported in this paper.

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