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Investigation of the Synergistic Anti-pyretic and Anti-inflammatory Activity of *Sonchus wightianus* DC, Paracetamol and Indomethacin Combination in Rat Models

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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ABSTRACT

Introduction: In conventional Indian medicine, several plants have long been utilized to treat various ailments. Among these herbal species, *Sonchus wightianus* DC is known for anti-inflammatory and antipyretic effects attributed to compound found in its leaves such as fatty acid methyl esters, sterol and triterpenoids. Induced pyrexia and inflammation in animal models serves to investigate the potential synergistic interactions between traditional herbal remedies and standard medication.

Aims and Objectves: Thus, this research aimed to evaluate the anti-pyretic and anti-inflammatory properties of methanolic extract obtained from *Sonchus wightianus* DC leaves tested alone and in combination with classic anti-inflammatory agents using the Carrageenan paw edema and anti-pyretic agent using yeast-induced pyrexia in Wistar rats.

Methods: Soxhlet extraction was used to obtain the methanolic extract. Inflammation was induced by a 1% (w/v) carrageenan injection, while 20% Brewer's yeast triggered pyrexia in male Wistar rat. The antipyretic activity was evaluated by taking the body temperature at various hours, while the anti-inflammatory activity was assessed by measuring the paw and cytokines (TNF-α, IL-6, IL-1β).

Results: The research investigation discovered that the extract of *Sonchus wightianus* DC along with varying doses of Indomethacin and Paracetamol, considerably reduced raised body temperature and avoided inflammation, exhibiting percentage inhibition. The study further revealed that at a dosage of 300 mg/kg of the plant extract, 300 mg/kg plant extract combined with Paracetamol (150 mg/kg), and 300 mg/kg plant extract combined with Indomethacin (10 mg/kg), there was a marked reduction in paw edema and pyrexia in the experimental models. Moreover, the extract was found to substantially lower serum levels of key inflammatory mediators, including IL-1β, IL-6, and TNF-α.

Conclusion: These findings indicates that *Sonchus wightianus* DC extract has potential therapeutic benefits in managing inflammation and fever, especially when combined with standard drugs like Paracetamol and Indomethacin.

Keywords: Anti-inflammatory; antipyretic; edema; pyrexia; Sonchus wightianus DC.

1. INTRODUCTION

Pyrexia: A multifaceted change in the body's regular temperature, known as pyrexia, can be caused through contagious, non-contagious inflammatory reactions, malignant growths, and other unspecified influences [1]. Raising set-point temperature in the hypothalamus caused by increasing prostaglandin E2 production results in a medical symptom known as pyrexia [2]. The production of inflammatory agents, such as TNF-α, interleukin 1β, α, and β, is increased in contagious or impaired tissue. This leads to an increase in prostaglandin components (PGE2) in the hypothalamus, causing increased body temperature [3]. Antipyretic medications, such as synthesized NSAIDS, are used to lower body temperature during excessive heat by reducing COX-2 expression. They suppress PGE2 generation, a fever facilitator, but can induce ulcers in the stomach, anemia, nausea, coagulation issues, kidney problems, infection of the skin, and liver cytotoxicity on long term use. Further study is required to discover medications that are more reliable, safe, trustworthy, with fewer negative effects [3].

Inflammation: The human immune system uses inflammation as a defensive mechanism in reaction to potentially harmful stimulation like irritants and/or damage to cells [4]. Many infectious agents, including viral and bacterial agents, poisons and harmful chemicals, as well harm to cells, may result in inflammation [5].

As a form of protection, inflammation includes elements such as granulation development, leukocyte infiltration, and fluid retention, all of which can cause or exacerbate numerous responses [6]. When adverse events occur, an immune-mediated signalling pathway is set off, which activates leukocytes and releases inflammatory mediators like TNF-α, interleukin 1β (IL-1β), and interleukin-6. The specific receptors (IL-6R, TNFR-1, TNFR-2, TLR4, GM-CSFR, etc.) that these mediators bind with and stimulates, generating transcription elements and signalling substances to become phosphorylated. This generates inflammation-producing cells from the bloodstream and controls the amounts of inflammatory mediators in tissues. By reestablishing equilibrium, sudden injury acts as a defense mechanism. On the other hand, unnoticed inflammation can cause

long-term conditions like cancers and neurological illnesses. Basic paths include transcription elements transporting inside the nucleus, elements of the signalling cascades in signalling organelles, and receptors on the membranes of cells [5]. The inflammatory reaction seeks to remove damaging substances and damaged tissue components thus promoting recovery. This includes the secretion of cytokines by neutrophils and macrophages, which regulate how inflammation resolves. The method of resolve is influenced by recruiting monocyte and anti-inflammatory cytokines. Aspirin, Ibuprofen, and Indomethacin are among nonsteroidal anti-inflammatory medications that helps in lowering inflammation and pain [7]. Although NSAIDs differ in molecular structure, they work in essentially the same way. They involve many doses, which might cause medication concentration variations and patient non-compliance. Since NSAIDs have adverse consequences, testing for anti-inflammatory medications is essential. Native herbal remedies provide us a more integrated strategy to therapy because they are less poisonous, biodegradable, sourced from renewable resources, and frequently less expensive than artificial drugs [8,9,10].

The plant *Sonchus wightianus* DC, often known as Dudhe, belongs to the Asteraceae family [11] and is highly regarded for its antibacterial and diuretic qualities. Folklore medicine claims that it can be used to treat a wide range of conditions, such as tonsillitis, appendicitis, deafness, gout, cough, bronchitis, asthma, jaundice, and sore throats [12]. The plant contains fatty acid methyl esters, sterol and triterpenoids molecules. which can be useful in anti-inflammatory and anti-pyretic studies [10,13,14,15]. Therefore, this project lays its special emphasis on using herbal plant (*Sonchus Wightianus* DC) based approach for deteriorating the negative repercussions of pyrexia and inflammation which has been known to be multifaceted.

2. METHODOLOGY

Collection and Authentication of plant: From the Eastern Himalayan region of Kalimpong, West Bengal, India, fresh leaves of *Sonchus wightianus* DC were gathered in an area devoid of pesticides and other contamination. Central Ayurveda Research Institute, Government of India, Ministry of AYUSH, Central Council for Research in Ayurvedic Science, Bengaluru, carried out the process of authentication. (Dr. V. Rama Rao), Research Officer, Botany, and (Dr. S.H. Doddamani), Assistant Director In-Charge, identified and verified the plant material with the authentication number:

SMPU/CARI/BNG/2023-24/2035:

Sonchus wightianus* DC *extract preparation: The cleaned, air dried and crushed leaves went through extraction in Soxhlet using methanol. The extract was dried by storing in water bath below 70°C [16]. After the extraction process the solvent were removed by using rotatory evaporator [13]. The extraction was done by Green Chem Pvt. Ltd. (2030, 1st Cross Rd, HAL 2nd Stage, Kodihalli, Bengaluru, Karnataka 560008) a Batch code: SWE/RD/01.

Initial phytochemical assessment: The methanolic extract of *Sonchus wightianus* DC leaves was analyzed for various phytoconstituents, including alkaloids, volatile oils, steroids, terpenoids, flavonoids, emodins, anthraquinones, fatty acid, phenols, and glycosides, utilizing established methodologies as stated by Harborne and Kolkate [17,18].

Drugs and chemicals: The commercial formulations of Paracetamol (Paracip, Cipla), Indomethacin (Indocap, Jagsonpal Pharmaceuticals Pvt Ltd) were used. The supplier of Brewer's yeast and Carrageenan was Sigma-Aldrich.

Experimental animals: The studies were conducted on Albino Wistar rats weighing between 150 and 200 grams [19]. The animals were provided by the Central Animal House of Krupanidhi College of Pharmacy, Bengaluru. Under standard experimental conditions, animals were housed in sanitized polypropylene cages with sterile paddy husk bedding, maximum six per cage [20]. Animals were kept in a laboratory room for at least a week, with food and water availability prior to testing [19]. The methods described were assessed and given approval by the Institutional Animal Ethical Committee of the Krupanidhi College of Pharmacy, Bengaluru, India. (IAEC NUMBER: KCP/IAEC/ PCOL/137/AUG-2023). The studies followed the criteria for the care and use of laboratory animals specified by the Institutional Animal Care Committee, CPCSEA, New Delhi, India [20].

2.1 Selection for the Dosage

A review of the literature revealed that the methanolic extracted extract of *Sonchus*

wightianus DC was considered harmless, with a dosage of 2000 milligrams per kilogram being regarded as LD50 cut off value (safe dose) [21]. Animal consumption of the plant is another indication that it is less poisonous. Therefore, as the submaximal and maximum doses for the experiment, 1/20 of this dose, or 100 milligrams per kilogram, and three times of this dose or 300 milligrams per kilogram were chosen [22,23].

2.2 Study Design

2.2.1 For evaluation of anti-pyretic activity

Model for pyrexia caused by brewer's yeast: Forty two Wistar rats selected were separated into seven distinct groups and treated as follows: Normal groups i.e. Group I were treated with (saline 10 ml/kg body weight), while positive control were treated with 10 ml/kg body weight of 15% water-based subcutaneous suspension of Brewer's yeast in normal saline denoted as Group II, animals in Group III were treated with Paracetamol 150 milligrams per kilogram body weight as standard, while animals in Group IV & V were given the extract of *Sonchus* leaves [lower dosage 100 milligrams per kilogram & higher dosage 300 milligrams per kilogram] body weight and the animals in Group VI & VII were administered with combined regimen of plant leaf extract + Paracetamol [lower dosage 100 milligrams per kilogram + 150 milligrams per kilogram & higher dosage 300 milligrams per kilogram + 150 milligrams per kilogram]. A digital tele-thermometer was placed into the rat's rectal canals and left there for around two minutes to take their initial body temperatures. The stable state temperature values occured were noted as the pre-temperatures and fever was produced in all groups except normal group by treating 10 ml/kg body weight of 15% water-based subcutaneous suspension of Brewer's yeast in normal saline into the dorsum area of animal's and after 18 hours of yeast treatment, the rectal temperatures were reassessed again [20]. For a maximum of six hours, each rat's rectal temperature was measured sixty minutes after the medication was administered [19].

2.2.2 Parameters assessed in the study

Rectal temperature: For a maximum of six hours, the rectal temperature of each rat was taken every sixty minutes after the drug was administered [19].

2.2.3 For evaluation of anti-inflammation activity

Model of paw edema resulting from Carrageenan: Seven distinct groups of Wistar rats (6 rats each group) were selected. Before standard (Indomethacin) was administered, the right hind paw's initial volume was measured. Forty two Wistar rats chosen were separated into seven groups and treated as follows: Normal saline 10 ml/kg body weight were given to Group I, while injections of 1% (w/v) Carrageenan (produced in 0.9% NaCl) were sub-plantarly delivered to Group II right paw, Group III were treated with Indomethacin 10 milligrams per kilogram body weight, Group IV&V received *Sonchus* plant leaf extract [lower dosage 100 milligrams per kilogram & higher dosage 300 milligrams per kilogram] body weight and Group VI & VII were treated with Indomethacin 10 milligrams per kilogram + *Sonchus* Extract 100 milligrams per kilogram & Indomethacin 10 milligrams per kilogram + *Sonchus* Extract 300 milligrams per kilogram [lower dosage & higher dosage] [24]. Wistar rats of each group except the normal control were given the medications one hour prior to the subcutaneous injection of 1% (w/v) Carrageenan (produced in 0.9% NaCl) into the right hind paw's sub-plantar area. Using a plethysmometer, the volume displacement method was used to determine the hind paw volume at different periods (0, 1, 2, 3 and 4 hours) following the injection of Carrageenan. Paw volume in millilitres changes following administration were compared with the initial readings in order to assess the outcomes [25].

2.2.4 Parameters assessed in the study

- Paw volume
- Percentage of inhibition
- Inflammatory modulators such as cytokines like IL-1β, IL-6 and TNF-α

2.2.5 Calculating the percentage inhibition [26]

By calculating the percentage inhibition of inflammatory processes, the relative effectiveness of the medication under research was determined. It was determined using the subsequent formula:

$$\% \text{ Inhibition } = \frac{Vc - Vt}{Vc} \times 100$$

Where, **Vc** = Edema volume of Control group
Vt = Edema volume of Test group

Investigation of the serum concentrations of TNF-α, IL-6, and IL-1 β*:* Once the Carrageenan-induced paw edema study was completed, the rats were anesthetized and specimens of blood were taken via the orbital sinus. After centrifugation and enabling the blood to coagulate, the serum was separated, and the obtained samples were kept at -20°C until needed. The serum levels of TNF-α, IL-6, and IL-1β were determined using commercial ELISA kits (Kaushik Laboratory) [27].

2.2.6 Histopathology of paw

Following the completion of the study involving paw edema produced by Carrageenan, the Wistar rats were euthanized by overdoing of carbon dioxide and the paws from the standard (Indomethacin), control (Carrageenan), and *Sonchus wightianus* DC extract treatment groups were meticulously spread through a wax-coated metallic tray. After fixing for at least 24 hours in a 10% neutrally buffer formalin solution, they were cleaned with xylene, drained by washing in progressively higher grades of ethanol and embedded into paraffin wax. After using a microtome to segment the samples, hematoxyline and eosin (H and E) staining was applied. Under a light microscope with magnifications of x100 each segment was inspected. Histopathologic spots were observed and documented, and images of the spots were obtained [28].

2.3 Statistical Analysis

Results were provided in the form of mean ± SEM. The data set was examined using a one-way Analysis of Variance and Dunnet test. The P value of <0.05 was considered significant by graph pad prism.

3. RESULTS

Initial phytochemical assessment: The Initial Phytochemical Assessment showed presence of Steroids, Terpenoids, fatty acid Emodins, anthraquinone and Phenol in leaves of SWME.

3.1 Assessment of Anti-Pyretic Activity

Impact of SWME extract and SWME extract combined with Paracetamol on pyrexia caused by Brewer's yeast: By delivering experimental animals Brewer's yeast at 20% (w/v) suspended in normal saline (0.9%), temperature to induce fever, studies were performed for determining the antipyretic and synergistic effects of the SWME and Paracetamol. The results were assessed against the control group and varying oral doses of the SWME and Paracetamol were given. Following the initial recording at 0 hours, the rectal temperature was taken at 1, 2, 3, 4, 5, and 6 hours later. In contrast to the group under control, the SWME administered groups at amount of 100 milligrams per kilogram & 300 milligrams per kilogram, as well as SWME + Paracetamol combination treated groups at amount of 100 milligrams per kilogram SWME + Paracetamol (150 milligrams per kilogram) & 300 milligrams per kilogram SWME + Paracetamol (150 milligrams per kilogram), respectively, showed a decrease in raised body temperatures from one to six hours after treatment. When compared to the control group, the 100 milligrams per kilogram dosing of SWME considerably lowered body temperature three to six hours after administration displayed in a Table 1. The Table 1 also indicates that the groups that received 300 milligrams per kilogram dosage of SWME and those that received 100 milligrams per kilogram dosage SWME + Paracetamol at 150 milligrams per kilogram and had a greater reduction in high temperature after two to six hours in comparison to the control group. Comparing the treated animals to the control group, the standard medication Paracetamol and SWME at 300 milligrams per kilogram + Paracetamol at 150 milligrams per kilogram consistently and more significantly reduced pyrexia from one to six hours displayed in a Table 1. In rats with pyrexia caused by Brewer's yeast, medication with SWME alone or when combined with Paracetamol demonstrated a dependent on dose effects. as seen in the Table 1.

3.2 Assessment of Anti-Inflammatory Activity

Impact of SWME extract and SWME extract combined with Indomethacin on paw edema caused by Carrageenan: The most often used technique to assess the anti-inflammatory impact of plant-based substances is paw edema caused by Carrageenan as an in vivo model for inflammation. Wistar rats from the control group given a sub plantar injection of Carrageenan demonstrated a substantial rise in paw thickness over a period in comparison to the normal group,

Table 1. Impact of SWME extract and SWME extract combined with Paracetamol on pyrexia caused by Brewer's yeast

Groups	Treatment	Initial rectal tempeture (18 hrs before yeast injection °C)	Rectal temperature 18 hrs after yeast injection (°C)						
			0 Hr	1 Hr	2 Hr	3 Hr	4 Hr	5 Hr	6 Hr
1	Normal group (saline treated)	36.95±0.46	36.95± 0.46	36.95± 0.47	36.95± 0.47	36.95± 0.46	36.95± 0.46	36.95± 0.47	36.95± 0.47
2	Control group (Carrageenan)	36.91±0.51	38.27± 0.49	38.51± 0.47	38.76± 0.52	38.93± 0.42	39.14± 0.42	39.04± 0.44	38.97± 0.48
3	Standard group (Indomethacin)	36.91±0.51	38.27± 0.49	38.31± 0.35**	38.23± 0.37**	37.93± 0.39**	37.68± 0.42**	37.42± 0.43**	37.19± 0.50**
4	*Sonchus wightianus* Extract (low dose)	36.90±0.52	38.30± 0.48	38.50± 0.46	38.48± 0.46	38.41± 0.42	38.29± 0.47	38.03± 0.46	37.92± 0.51
5	*Sonchus wightianus* Extract (high dose)	36.91±0.51	38.29± 0.49	38.44± 0.45	38.39± 0.52*	38.19± 0.50*	37.93± 0.53*	37.77± 0.50*	37.51± 0.50*
6	Indomethacin + *Sonchus wightianus* Extract (low dose)	36.91±0.51	38.27± 0.49	38.29± 0.53**	38.22± 0.50**	37.86± 0.52**	37.59± 0.49**	37.33± 0.52**	37.15± 0.61**
7	Indomethacin + *Sonchus wightianus* Extract (high dose)	36.97±0.45	38.27± 0.49	38.25± 0.50**	38.19± 0.54**	37.79± 0.59***	37.42± 0.63***	37.24± 0.57***	37.08± 0.49***

*P-Values are expressed as (Mean ± SEM), n = 6. followed by a one-way ANOVA and statistical evaluation done utilizing the Dunnett's test. All the groups were compared with a Positive Control Group. **significant at P<0.05 in contrast with the Control Group, otherwise non-significant, or ns*

Table 2. Impact of SWME extract and SWME extract combined with Indomethacin on paw edema caused by Carrageen

SL NO.	Groups	Paw volume					Percentage of inhibition			
		0Hr	1Hr	2Hr	3Hr	4Hr	1Hr	2Hr	3Hr	4Hr
1	Normal group (saline treated)	1.31± 0.007	1.30± 0.005	1.30± 0.005	1.30± 0.005	1.31± 0.005				
2	Control group (Carrageenan)	1.33± 0.012	1.87± 0.032	2.06± 0.022	2.20± 0.005	2.24± 0.021				
3	Standard group (Indomethacin)	1.32± 0.014	1.83± 0.022**	1.94± 0.010**	1.85± 0.023**	1.73± 0.019**	2.13%	5.82%	15.90%	22.76%
4	*Sonchus wightianus* Extract (low dose)	1.33± 0.018	1.87± 0.019	1.96± 0.016	2.13± 0.005	2.03± 0.014	-	4.8%	3.18%	9.37%
5	*Sonchus wightianus* Extract (high dose)	1.34± 0.018	1.86± 0.014	1.95± 0.021*	1.91± 0.017*	1.82± 0.014*	0.5%	5.33%	13.18%	18.75%
6	Indomethacin + *Sonchus wightianus* Extract (low dose)	1.34± 0.018	1.85± 0.021*	1.98± 0.030*	1.80± 0.011**	1.66± 0.014***	1.06%	3.88%	18.18%	25.89%
7	Indomethacin + *Sonchus wightianus* Extract (high dose)	1.32± 0.013	1.81± 0.016**	1.91± 0.005**	1.74± 0.021***	1.61± 0.014***	3.20%	7.28%	20.90%	28.12%

*P-Values are expressed as (Mean ± SEM), n = 6. followed by a one-way ANOVA and statistical evaluation done utilizing the Dunnett's test. All the groups were compared with a Positive Control Group. **significant at P<0.05 in contrast with the Control Group, otherwise non-significant, or ns*

Table 3. Impact of SWME extract and SWME extract combined with Indomethacin on inflammatory cytokines levels of serum

SL NO	Groups	Parameters		
		Interleukin-1 β (ng/l)	Interleukin-6 (pg/ml)	Tumor necrosis factor-α (pg/ml)
1	Normal group (saline treated)	17.87±0.12	201.92±0.45	221.34±0.54
2	Control group (Carrageenan)	57.45±0.92	450.81±0.33	422.28±0.54
3	Standard group (Indomethacin)	26.15±0.52**	215.18±0.46**	235.84±0.64**
4	*Sonchus wightianus* Extract (low dose)	40.18±0.44	250.95±0.69	261.27±0.46
5	*Sonchus wightianus* Extract (high dose)	33.12±0.61*	232.12±0.74*	243.76±0.53*
6	Combination 1 Indomethacin 10 mg/kg + Plant extract100 mg/kg	23.19±0.57**	211.22±0.51**	230.10±0.60**
7	Combination 2 Indomethacin 10 mg/kg + Plant extract 300 mg/kg	20.53±0.72***	207.12±0.29***	225.85±0.72***

*P-Values are expressed as (Mean ± SEM), n = 6. followed by a one-way ANOVA and statistical evaluation done utilizing the Dunnett's test. All the groups were compared with a Positive Control Group. **significant at P<0.05 in contrast with the Control Group, otherwise non-significant, or ns.*

as illustrated in the Table 2. In contrast to the group under normal, there is a noticeable rise in paw volume at one hour and it continues to rise throughout the duration of the experiment. The standard group (Indomethacin 10 milligrams per kilogram), the SWME treatment groups (100 milligrams per kilogram & 300 milligrams per kilogram), and the combination of SWME & Indomethacin at a dosage of SWME (100 milligrams per kilogram) + Indomethacin (10 milligrams per kilogram) & SWME (300 milligrams per kilogram) + Indomethacin (10 milligrams per kilogram) administered groups all showed no significant difference in paw volume at one hour. (Indomethacin 10 mg/kg) i.e. the standard group, the combined use of SWME along with Indomethacin at dosage SWME (100 milligrams per kilogram) + Indomethacin (10 mg/kg) & combined use of SWME along with Indomethacin at dosage SWME (300 milligrams per kilogram) + Indomethacin (10 milligrams per kilogram) were significant at two hours. However, SWME with dosage of 100 milligrams per kilogram as well as 300 milligrams per kilogram did not show any meaningful effects. Notable variations were seen in standard group (Indomethacin 10 milligrams per kilogram) at three and four hours, the SWME treatment groups at 100 & 300 milligrams per kilogram, and the combination of SWME & Indomethacin at a dosage of SWME 100 milligrams per kilogram + Indomethacin (10 milligrams per kilogram) & SWME 300 milligrams per kilogram + Indomethacin (10 milligrams per kilogram). For SWME 100 milligrams per kilogram, the percentage of the paw volume inhibited was 4.8%, 3.18%, and 9.54% at two, three, and four hours, respectively; for SWME 300 milligrams per kilogram, it was 0.5%,5.33%, 13.18% and 18.75% for SWME (100 milligrams per kilogram) + Indomethacin (10 milligrams per kilogram) combination, it was 1.60 %, 3.88%, 18.18%,25.89% for SWME (300 milligrams per kilogram) + Indomethacin (10 milligrams per kilogram) combination, it was 3.20%,7.28%,20.90%, 28.12% and for the standard group (Indomethacin 10 milligrams per kilogram), it was 2.13%, 5.82%,15.90%, 22.76% at one, three, and four hours, respectively. For SWME 300 milligrams per kilogram as well as SWME 300 milligrams per kilogram + Indomethacin 10 milligrams per kilogram, the percentage of inhibition of paw volume was more noteworthy.

Impact of SWME extract and SWME extract combined with Indomethacin on inflammatory cytokines levels of serum: The results of this research indicates that those group that were treated with Carrageenan had higher amounts of cytokines (TNF α, IL-6, and IL-1 β) than that of the group under normal, whereas the introduction of SWME extracts, both alone as well as in combination, substantially decreased the amounts of inflammation-associated mediators produced in the serum in comparison to the control group Table 3. In comparison with the other groups, the effects of SWME at dosage 300 milligrams per kilogram, SWME combined with Indomethacin at dosage 100 milligrams per kilogram + 10 milligrams per kilogram & at dosage 300 milligrams per kilogram + 10 milligrams per kilogram were the most significant as showed in Figs. 1,2,3 and Table 3.

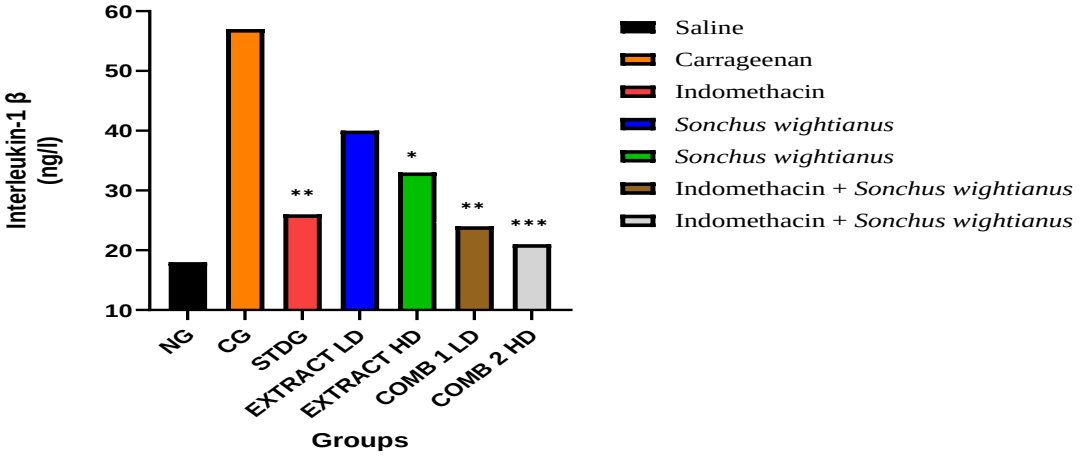


Fig. 1. Impact of SWME extract and SWME extract combined with Indomethacin on IL 1- β

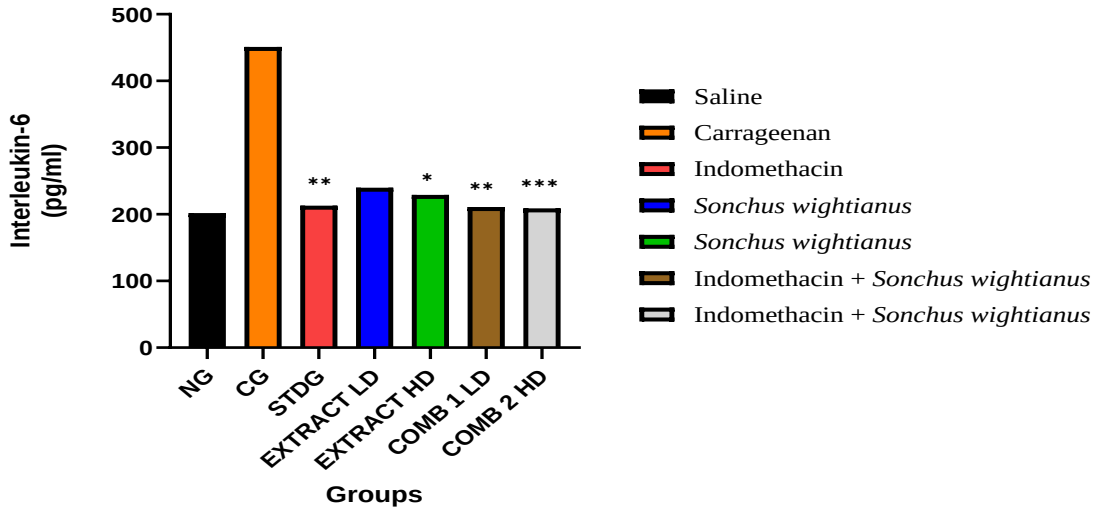


Fig. 2. Impact of SWME extract and SWME extract combined with Indomethacin on IL-6

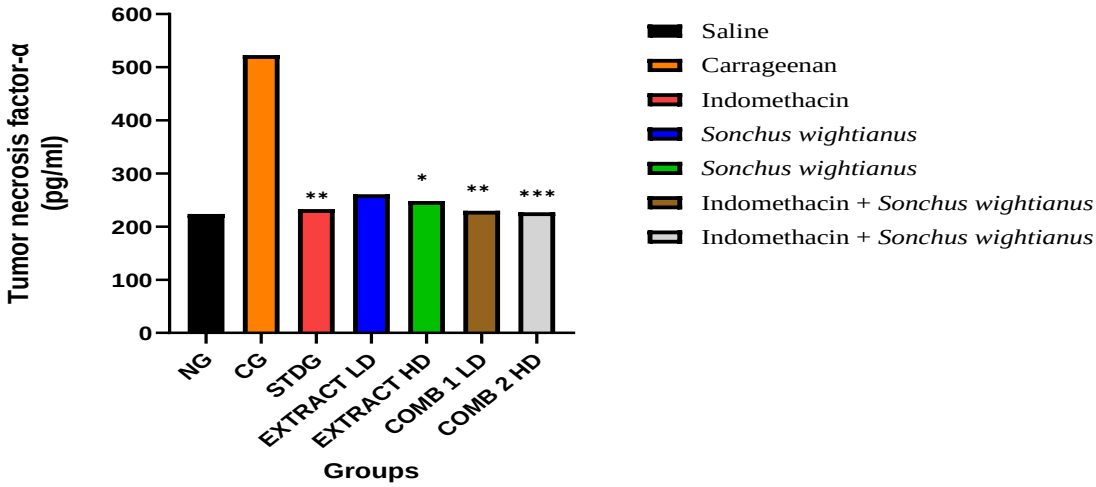
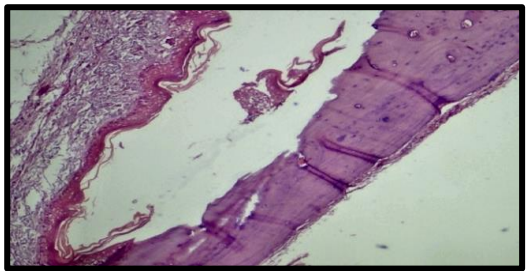


Fig. 3. Impact of SWME extract and SWME extract combined with Indomethacin on TNF-α

3.3 Histopathological Examination

The main side effects of Carrageenan administration include epidermal edema, proliferating epithelium, and infiltration of inflammatory cells. The normal control group's paw showed no signs of inflammation or no substantial inflammation-related cell infiltration. The subcutaneous and sub epidermal layers have been found to be undamaged and in normal architecture as indicated in Fig. 4. The administration of Carrageenan in positive control group resulted in infiltrating the cells associated with inflammation and manifestation of acute inflammation within both epidermis and dermis, accompanied by widespread extravasations of PMN leucocytes and a decrease in lymphocytes. Additionally, PMN deposition around dermal capillaries, resulting in subcutaneous and edematous edema as indicated in Fig. 5. The Indomethacin induced rats paw sections only displayed a mild-inflammatory response, mild hyperplasia of the epithelium and sub epidermal edema and there was inhibition of PMN infiltration. The infiltration pattern was focal rather than diffuse in the positive control group as indicated in Fig. 6. In the paw tissues of the groups administered with *Sonchus* extract 100 milligrams per kilogram and 300 milligrams per kilogram resulted mild to moderate edema, mild to moderate inflammation, and a decrease in PMN infiltration as indicated in Figs. 7 and 9. In the groups that received combined treatment of 100 milligrams per kilogram *Sonchus* extract +

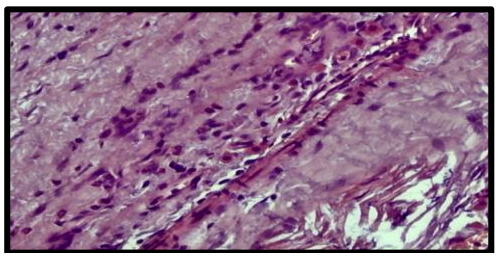
10 milligrams per kilogram Indomethacin as well as 300 milligrams per kilogram *Sonchus* extract + 10 milligrams per kilogram Indomethacin, there was a noteworthy decrease in PMN infiltration, Mild inflammation (MI), and Mild Sub Epidermal Edema (MSEE) as indicated in Figs. 8 and 10. Not only there was minimal PMN infiltration in these groups, but there was also no congestion seen. It is more likely that the paw tissues in these groups were normal tissues than damaged ones. Thus, it was discovered that SWME could reduce the histological changes caused by Carrageenan.



NORMAL CONTROL

- No sign of inflammation
- No inflammatory cell infiltration

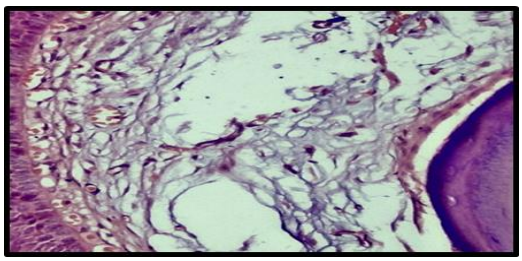
Fig. 4. Normal Control



POSITIVE CONTROL

- Severe inflammatory cell infiltration mainly PMN (Poly Morpho Nuclear Cells I Inflammatory cells)
- Severe acute inflammation in the dermis and epidermis

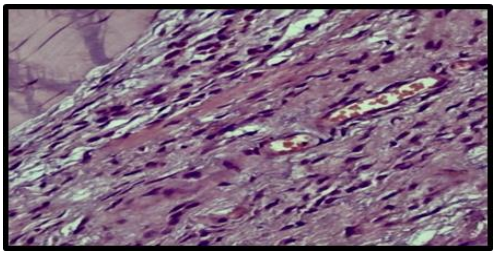
Fig. 5. Positive Control



STANDARD GROUP

- Mild infiltration of PMN (Poly Morpho Nuclear Cells I Inflammatory cells)
- Mild epithelial hyperplasia (EHP) and Sub epidermal edema (SEE)

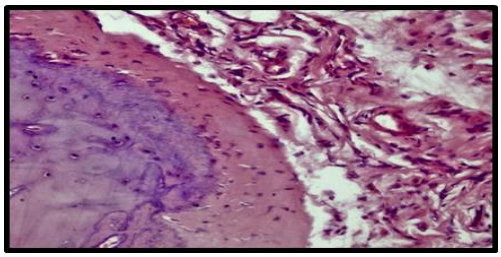
Fig. 6. Standard Group



PLANT EXTRACT (100 mg/kg)

- Mild to moderate infiltration of PMN (Poly Morpho Nuclear Cells I Inflammatory cells)
- Mild to moderate edema and mild to moderate inflammation

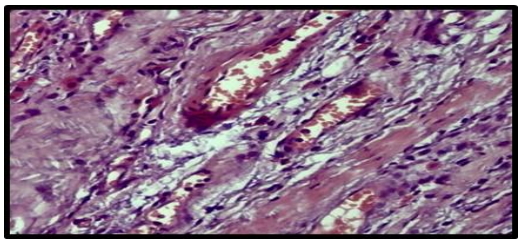
Fig. 7. *Sonchus* Extract (100 mg/kg)



COMBINATION 1

- Mild infiltration of PMN (Poly Morpho Nuclear Cells I Inflammatory cells)
- Mild epithelial hyperplasia (EHP), Mild inflammation (MI) and Mild Sub

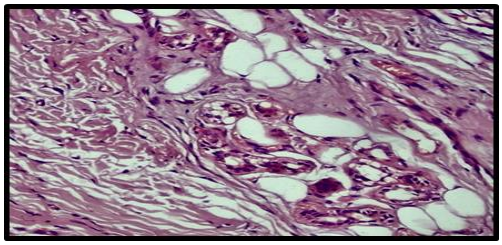
Fig. 8. Combination 1 *Sonchus* EXTRACT (100 mg/kg) + Indomethacin (10 mg/kg)



PLANT EXTRACT (300 mg/kg)

- Mild to moderate infiltration of PMN (Poly Morpho Nuclear Cells I Inflammatory cells)
- Mild to moderate edema and mild to moderate inflammation

Fig. 9. *Sonchus* Extract (300 mg/kg)



COMBINATION 2

- Mild infiltration of PMN (Poly Morpho Nuclear Cells I Inflammatory cells)
- Mild epithelial hyperplasia (EHP), Mild inflammation (MI) and Mild Sub epidermal edema(MSEE)

Fig. 10. Combination 2 *Sonchus* Extract (300 mg/kg) + Indomethacin (10 mg/kg)

Histopathological alterations in rat paw tissues stained with H&E and oil red O staining (4-10). Representative images of paw tissues from animals treated with (4) Normal Control, (5) Positive Control, (6) Standard group, (7) *Sonchus* extract (100 mg/kg), (8) Combination 1 - *Sonchus* extract (100 mg/kg) + Indomethacin (10 mg/kg), (9) *Sonchus* extract (300 mg/kg), (10) Combination 2 - *Sonchus* extract (300 mg/kg) + Indomethacin (10 mg/kg). Under 100X (H&E) magnification, pathological analysis of the tissue portions was carried out using light microscopy.

4. DISCUSSION

There is an urge for better, more secure medications considering the therapy strategies presently in use for the control of antipyretic and anti-inflammatory activity having numerous limitations. Many people have noticed that the majority of developed nations utilize conventional therapies and herbal remedies as a normative foundation for maintaining optimal wellness [29]. Herbal remedies provide healing qualities that are advantageous to both people and animals [30]. Despite the fact that little is known about how herbal medications made from plant compounds work, they are being used extensively for treating a wide range of medical conditions [31]. Numerous animal models have provided us with knowledge regarding potential pathways that contribute to the etiology of inflammation and pyrexia. In the current investigation, we used male Albino Wistar rats that were provoked to pyrexia by Brewer's yeast and edema by Carrageenan to assess the antipyretic and anti-inflammatory properties of SWME as well as its synergistic action when combined with Paracetamol and Indomethacin. The investigation found that in the Brewer's yeast-induced pyrexia model, SWME alone as well as in combination with Paracetamol reduced rectal temperature. Additionally, the same plant alone and in combination with Indomethacin prevented the rise in paw volume and cytokines associated with inflammation (IL-6, IL-1 β, and TNF-α) in the Carrageenan-induced paw edema

model. The plant extract confirmed its antipyretic and anti-inflammatory properties by exhibiting this response.

A complicated physiological response brought on by diseases or aseptic stimulation is termed as fever [31]. Numerous endogenous pyrogens, such as prostaglandin, macrophage protein-1, tumor necrosis factor-α, interleukin-1β, interleukin-6, and interleukin-8, can induce fever [32]. When prostaglandin E2 (PGE2) level rises in certain areas of the brain, the body temperature rises [31]. By regulating heat generation and dissipation, the hypothalamus regulates body temperature. Many diseases, which include infections, damage to tissues, inflammation, and additional medical conditions, can cause fever. Heat must be produced and retained in order to elevate the body's temperature above the desired level. Vasoconstriction reduces the loss of heat through the skin, allowing the blood-brain temperature to align towards the hypothalamic-created modified set-point. When a person suffers from fever, cytokines such as interleukins, interferons (α, β), and tumor necrosis factor-alpha move towards the circumventricular organs of the brain and trigger the arachidonic acid (AA) pathway, which elevates the production of PGE2. This pathway produces and releases PGE2, the last regulator of the febrile response. It is made up of the enzyme phospholipase A2, COX-2, and PGE2 synthase. When PGE2 exists in the hypothalamus, heat synthesis is initiated and loss of heat via the cyclic adenosine monophosphate pathway is minimized, maintaining an elevated body temperature set-point [33]. When applied subcutaneously, Brewer's yeast causes fever in rats by attaching to lipopolysaccharide-binding protein (LBP), that in turn promotes the production of endogenous pyrogens and prostaglandin, the brain's last mediator of pyrexia [3]. This research is useful for determining the antipyretic properties of synthetic medications and botanical components. According to the results of the research, rats given 300 milligrams per kilogram SWME extract & 100 milligrams per kilogram SWME extract + 150 milligrams per kilogram Paracetamol as well as 300 milligrams per kilogram SWME extract + 150 milligrams per kilogram Paracetamol illustrated a noteworthy decrease in the raised body temperature in comparison to Brewer's yeast-induced rats. Through these outcomes, SWME demonstrated both its antipyretic action and synergistic effects with Paracetamol respectively.

An essential biological reaction produced by the vascular tissues to damaging stimulus such as infections, injured cells, or irritating substances, is termed as inflammation [32]. Inflammation is a major indicator of health problems and is marked by redness, discomfort, swelling, and fever. Bradykinin, histamine, serotonin, and prostaglandins are examples of pharmacological cytokines that are important in the processes underlying inflammation, albeit the exact mechanisms are not well known. Paw edema caused by Carrageenan is frequently utilized as a model for creating novel anti-inflammatory medications. Plethysmometer, for measuring mercury displacement, is among the most often used instrument for this purpose [34]. Edema, hyperalgesia, erythema, and inflammation are the cardinal symptoms of the biphasic inflammatory reaction thought to be triggered by Carrageenan [35]. Higher PG synthesis is seen in injured tissues during the first inflammatory phase, which can last as long as two hours and is brought on by histamine and serotonin. This three to five-hour delayed phase is regulated by leukotrienes, phagocytic cells, NO, tissue macrophages and oxygen free radicals. Bradykinin and similar kinin-like compounds are the primary agents in the maintenance phase [36]. According to the research findings, Indomethacin and SWME at varying doses constitute significant defensive agents against acute inflammation caused by Carrageenan. In the current investigation higher doses of SWME (300 milligrams per kilogram) & combination of SWME (100 milligrams per kilogram) + Indomethacin (10 milligrams per kilogram) & SWME (300 milligrams per kilogram) + Indomethacin (10 milligrams per kilogram) revealed substantial decrease of edema in both phases of inflammation although the greater decrease being seen in the second phase of inflammation. The highest rate of reduction was achieved when Indomethacin 10 milligrams per kilogram & SWME 300 milligrams per kilogram were combined.

TNF-α, IL-1β, and IL-6 are among the significant pro-inflammatory cytokines that are released in response to carrageenan administration [37]. The raised production of all these pro-inflammatory cytokines is strongly associated with inflammation [38]. We thus tried to determine if the suppression of TNF-α, IL-1β, and IL-6 production at inflammatory region could

potentially be connected with the anti-inflammatory property of SWME. IL-6 concentrations in plasma constitute essential immunological mediators that show variations in inflammation. One of the key mediators of inflammation is TNF-α and pleotropic inflammatory cytokine, that inhibits the growth of cells in tumours. The acute phase response, is mediated by interleukin-1 also known as Endogenous pyrogen (EP), which is generated primarily by blood monocytes. Though TNF-α and IL-1β paradoxically contribute to inflammatory damage to tissues, they are strong pro-inflammatory cytokines that can trigger various pathways of signalling and assist in defending the host [39]. The research results demonstrated that, in comparison to the Carrageenan-induced control group, the increased levels of TNF-α, IL-1β, and IL-6 were reduced by SWME 300 milligrams per kilogram, SWME 100 milligrams per kilogram + Indomethacin (10 milligrams per kilogram) & SWME 300 milligrams per kilogram + Indomethacin (10 milligrams per kilogram).

There have been multiple reports of pharmacological activities corresponding to the genus Sonchus. Ethnobotanical information suggests that various portions of Sonchus genus are utilized in jaundice, deafness, gout, cough, bronchitis, asthma, eye problems, appendicitis, tonsillitis and in sore throat. [12] Indian village tribes make earaches from the leaves of *S. wightianus* DC [14]. To treat diarrhoea, one teaspoon of the root decoction of this plant twice a day is utilized. Crushed leaves of this plants can be applied immediately to small wounds to stop bleeding. Boils and abscesses are treated with these leaves [12]. According to a previous research investigation and our initial phytochemical assessment, SWME includes a variety of plant-based compounds, such as quinones, glycosides, emodins, fatty acids, triterpenes, sterols, polyphenols, and anthracenosides [16]. Moreover, a number of research investigations have documented the anti-inflammatory and antipyretic properties of fatty acid methyl esters, sterol and triterpenoids molecules [40,41]. According to reports, this substance acts by blocking inflammatory markers like TNF-α, IL1β, and IL6, which can lead to fever and inflammation [42]. It has also been discovered that phytosterol and triterpenoids significantly inhibits the COX-2 enzyme [43,44]. The inhibition of the COX-2 enzyme lowers the PGE2 synthesis, resulting in lowering fever temperature in the Brewer's yeast pyrexia model and reduces paw volume in the Carrageenan-induced paw edema model.

The synergistic effect of *Sonchus wightianus* DC with Paracetamol and Indomethacin indicates a promising improvement in the treatment of fever and inflammation. By potentially reducing the doses of synthetic medications, boosting efficacy, and minimizing adverse effects, this combination could open the way for safer, more effective therapy for a wide spectrum of inflammatory and febrile illnesses. The study focused on cytokine levels (IL-1β, IL-6, TNF-α) but might lack deeper mechanistic insights into how the extract interacts with these pathways. Further molecular or cellular studies would be needed to understand the precise mechanisms of action. Future clinical applications may include improved treatments for inflammatory diseases, infectious fevers, and post-surgical care, with a focus on enhancing patient outcomes while reducing the risk of adverse effects.

5. CONCLUSION

Herbal remedies have been employed to treat a wide range of ailments for numerous ages. They possess significant potential because to their improved accessibility, cost-effectiveness, and reduced incidence of side effects. The S.W plant is among the numerous species that remain unexplored, despite our knowledge of the diverse medicinal properties these plants possess. As a result, the goal of the current investigation was to assess the S.W. plants therapeutic qualities. It was discovered that the different elements in S.W. leaves exhibit varying pharmacologic actions. The main constituents of the S.W. leaves include fatty acids, triterpenes, sterols, polyphenols, emodins, quinones, glycosides, polyose and anthracenosides The aforementioned data indicates that the leaves of SWME exhibit antipyretic and anti-inflammatory properties. They also displayed synergistic effect when combined with Indomethacin and Paracetamol, respectively. There was no attempt made to figure out the mechanism underlying the antipyretic action that was identified in this investigation. Nevertheless, it might be possible that it is operating through one of the two central or peripheral mechanisms mentioned above. Additionally, it is conceivable that the two mechanisms might be involved. The existence of fatty acid methyl esters might be the cause of the antipyretic effect. Furthermore, the paw edema resulting from Carrageenan was significantly reduced in volume and cytokines

associated with inflammation were inhibited by the methanolic extract of leaves. The outcomes of this research suggest that SWME may be useful in treating acute inflammatory conditions. Triterpenes and phytosterol could be the possible cause for the anti-inflammatory effect. Overall, this investigation revealed that the SWME leaf possessed considerable antipyretic and anti-inflammatory properties, as well as synergistic effects in combination with standard medications. The current investigation provided scientific support for the traditional usage of SWME as an anti-inflammatory and antipyretic. We believe this initial data provides a solid foundation for future investigations. In future work, we plan to conduct additional studies to explore the mechanisms of action, including cytokine profiling and enzyme inhibition assays. These follow-up studies will allow us to identify specific molecular pathways involved in the observed synergistic effects.

DISCLAIMER (ARTIFICIAL INTELLIGENCE)

Author(s) hereby declare that NO generative AI technologies such as Large Language Models (ChatGPT, COPILOT, etc.) and text-to-image generators have been used during the writing or editing of this manuscript.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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