

**Research Article****Assessment of Anti-inflammatory, Analgesic, and Antipyretic Properties of Exclzyme EN™: An in-vivo Approach**

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**Abstract**

**Objective:** The current study investigated the anti-inflammatory, anti-arthritis, analgesic, and antipyretic properties of the Exclzyme EN™ using in-vivo models.

**Method:** The anti-inflammatory activity of Exclzyme EN™ was evaluated using the carrageenan-induced paw edema model, in which carrageenan solution was injected subcutaneously into the right hind paw of rats. Paw volume was measured with a highly sensitive Basile Plethysmometer, and changes from baseline were recorded for both control and test groups. For assessing the anti-arthritis potential, arthritis was induced by injecting Freund's complete adjuvant. Analgesic activity was examined using the acetic acid-induced writhing assay, while antipyretic properties were tested through the Brewer's yeast-induced pyrexia model. In all experiments, animals in the test groups were pretreated with Exclzyme EN™. Reductions in paw volume, improvements in the arthritic index, decreases in the number of writhings, and reductions in rectal temperature were observed and compared with baseline and control groups to determine the therapeutic potential of Exclzyme EN™.

**Results:** Exclzyme EN™ demonstrated significant efficacy in reducing paw volume in rats subjected to carrageenan injection compared with the control group. The greatest improvement was observed at a dose of 200 mg/kg Exclzyme EN™ after 24 h. At this concentration, Exclzyme EN™ also improved the arthritic index by 77.78% from baseline, confirming its anti-arthritis potential. Analgesic activity was evident in the acetic acid-induced writhing assay, where a marked reduction in writhing by 82.73% and 93.01% was observed at both 40 mg/kg and 80 mg/kg doses, respectively. Furthermore, Exclzyme EN™ produced a sustained antipyretic effect lasting up to 5 h at an effective dose of 100 and 200 mg/kg, with a significant decrease in rectal temperature compared to controls.

**Conclusion:** The findings in the current investigation highlights prominent anti-inflammatory, anti-arthritis, analgesic, and antipyretic properties of Exclzyme EN™ in mice at the studied doses. Further, pre-clinical and clinical studies are required to confirm and warrant the findings in the study and also elucidate the associated mechanisms.

**Key words:** Exclzyme EN™; Anti-inflammatory; Anti-arthritis; Analgesic; Antipyretic

## 1. Introduction

Inflammation is the body's protective response to injury, infection, or invading pathogens, clinically characterized by swelling, pain, redness, and heat. Acute inflammation involves excessive free radical generation and the release of multiple inflammatory mediators [1]. Inflammation typically manifests as pain and edema at the site of injury, accompanied by fluid leakage from vascular tissues due to increased vessel wall permeability, migration of inflammatory cells, and subsequent tissue damage [2]. A wide range of non-steroidal anti-inflammatory drugs (NSAIDs) and other pharmacological agents are available to manage inflammation and its associated symptoms such as pain and swelling. However, prolonged use of NSAIDs is frequently associated with adverse effects, including gastrointestinal toxicity, renal impairment, and increased cardiovascular risk [3]. This underscores the need to explore alternative therapeutic strategies for managing inflammation.

Enzymes play a key role in digestion, metabolism, immune modulation, and inflammation control with clinical evidences showing their systemic effectiveness [4]. Exclzyme EN™ is a multi-enzyme blend that combines various systemic enzymes with plant nutraceuticals like amla and rutin [5]. Digestive enzymes enhance macromolecules breakdown, release of nutrients, and absorption [6,7]. Systemic enzymes and flavonoids contribute to immune modulation, inflammation control, and metabolic regulation [8]. Proteases degrade inflammatory mediators, fibrin, and immune complex resulting in anti-inflammatory and immunomodulatory activity. Multi-enzyme cascades improve metabolic regulation by reducing toxic intermediates like hydrogen peroxide and oxidases and helps in improving substrate clearance [4,9]. The plant components amla (*Emblia officinalis*) and rutin in the blend are rich in polyphenolic compounds. Amla contains vitamin C, gallic acid, ellagic acid, phyllemblic acid, tannins, flavonoids, and pectin, while rutin is a flavonoid glycoside of quercetin, contributing antioxidant, anti-

inflammatory, and antipyretic properties [10]. These enzymes and flavonoids have demonstrated potential to alleviate inflammation, pain, and pyrexia. The proteolytic enzymes are well-documented for their anti-inflammatory, analgesic, and antipyretic activities, while the digestive enzymes enhance nutrient breakdown and absorption, contributing to improved metabolic efficiency [11]. The phytochemicals present in amla and rutin provide antioxidant and immunomodulatory effects, further supporting therapeutic outcomes [12,13]. Importantly, systemic enzymes such as bromelain and serratiopeptidase may aid in injury recovery by modulating fibrosis, influencing inflammatory mediator levels, and contributing to immune regulation [14].

The carrageenan-induced paw edema, acetic acid-induced arthritis, and Brewer's yeast-induced pyrexia models are well-established experimental systems widely employed to evaluate novel pharmacological agents. These assays respectively serve as standards for acute inflammation, analgesic efficacy, and rectal temperature reduction, providing reliable measures of anti-inflammatory, analgesic, and antipyretic activity in-vivo. Subplantar carrageenan injection produces a biphasic edema; the early phase (~1 h) is mediated by histamine, serotonin, bradykinin, and prostaglandins generated via cyclooxygenase (COX) enzymes, while the delayed phase (>1 h) reflects neutrophil infiltration and sustained prostaglandin synthesis [15,16]. Evidence suggests that agents targeting COX enzymes, free radical formation, and pro-inflammatory protein expression may provide superior control of inflammatory states compared to currently available therapies [2].

This study investigated the anti-inflammatory, anti-arthritis, analgesic, and antipyretic properties of Exclzyme EN™ using established in-vivo mouse models. The carrageenan-induced paw edema assay was employed to evaluate anti-inflammatory efficacy, while the Freund's complete adjuvant

(FCA) model was used to assess anti-arthritis potential. Analgesic activity was determined through the acetic acid-induced writhing test, and antipyretic efficacy was measured using the Brewer's yeast-induced pyrexia model. Across these models, Exclzyme EN™ demonstrated significant reductions in edema, pain responses, and rectal temperature, along with improvements in the arthritic index, highlighting its wide pharmacological potential.

## 2. Materials and Methods

### 2.1. Animals

Male adult Wistar rats and female Swiss albino mice were used to evaluate the anti-inflammatory, analgesic, and antipyretic activity of Exclzyme EN™. All animals were housed under a 12-h light/dark cycle and provided free access to food and water throughout the experiment. The Institutional Animal Care and Ethical Committee (IAEC), formed under Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA) approved the pharmacological protocols.

### 2.2. Chemicals and Reagents

The investigational product (IP), Exclzyme EN™ was generously provided by Specialty Enzymes and Probiotics, Chino, USA. All the reagents and solvents, including carrageenan and acetic acid (99.5%), were of laboratory grade and procured from reliable suppliers. Freund's Complete Adjuvant (FCA) was obtained from Sigma USA (Product No. F5881, Lot No. 080K8926, CAS No. 9007-81-2). Each milliliter of FCA contained 1 mg of *Mycobacterium tuberculosis* (H37RA, ATCC 25177), heat-killed and dried, suspended in 0.85 mL mineral oil and 0.15 mL Mannide monooleate.

### 2.3. Evaluation of Anti-inflammatory activity

Wistar rats weighing 90–120 g, with paw volume variation restricted to  $\pm 0.5$ , were randomly allocated into four groups of eight animals each. Paw edema was induced in the right hind paw by subcutaneous injection of 0.1 mL of a 1% carrageenan solution. The test groups received Exclzyme EN™ at doses of 50,

100, and 200 mg/kg, while the control group was administered an equal volume of distilled water (vehicle) following the same procedure. Paw volume was measured using the UGO Basile Plethysmometer, a highly sensitive instrument, both prior to and after carrageenan injection at defined time intervals (1, 2, 3, and 24 h). Changes in paw volume were calculated for each animal at each interval, and drug-treated groups were compared with the control group to evaluate the anti-inflammatory effect of the intervention.

### 2.4. Evaluation of Anti-Arthritic activity

The FCA model was employed to evaluate the anti-arthritis activity of the intervention, Exclzyme EN™. Male Wistar rats weighing 130–200 g were selected and divided into three groups, each consisting of eight animals. On day 1, 0.1 mL of FCA was injected subcutaneously into the sub-plantar region of the left hind paw to induce arthritis. The FCA administration produced inflammation as a primary lesion, which reached its peak within 3–5 days. Secondary lesions appeared after approximately 11–12 days, characterized by inflammation in non-injected sites including hind paws, forepaws, ears, nose, and tail. In Lewis strain rats, FCA also caused a reduction in body weight and immune responses. Following FCA injection, animals were treated for 13 days (once daily) with either the test compound (Exclzyme EN™ at 100 or 200 mg/kg) or distilled water (control group). Paw volumes of both hind paws, along with body weight, were recorded on days 0, 1, 5, 13, and 21 using the UGO Basile Plethysmometer (Model 7140). On day 5, the volume of the injected paw was measured to assess the primary lesion and the effect of therapeutic agents during this phase. The severity of adjuvant-induced disease was further evaluated by measuring the non-injected paw (secondary lesions) with the same instrument. From Day 13 to day 21, animals were intentionally left untreated to observe the progression of the disease. On day 21, body weight was recorded again, and the severity of secondary lesions was visually assessed and graded according to the

secondary lesion-scoring scheme described in the Table 1.

For primary lesions, the percentage inhibition of paw volume in the injected left paw relative to the control group was determined on day 5. For secondary lesions, the percentage inhibition of paw volume in the non-injected right paw relative to the control group was measured on day 21. The arthritic index was calculated as the sum of the lesion scores assigned to each animal. The mean values obtained for the treated groups were compared with those of the control group to assess the efficacy of the intervention.

**Table 1:** Lesion scoring scheme

Secondary Lesions	Symptoms	Score
Ears	Absence of nodules and redness	0
	Presence of nodules and redness	1
Nose	No swelling of connective tissue	0
	Intensive swelling of connective tissue	1
Tail	Absence of nodules	0
	Presence of nodules	1
	Forepaws: absence of inflammation	0
	Inflammation of at least one joint	1
Hind paws	Absence of inflammation	0
	Slight inflammation	1
	Moderate inflammation	2
	Marked inflammation	3

### 2.5. Evaluation of Analgesic activity

The analgesic activity of Exclzyme EN™ was assessed using the acetic acid-induced writhing test in Swiss albino mice (20–25 g, either sex). The animals were randomly divided into five groups, each comprising eight mice. Test groups received Exclzyme EN™ at different pretreatment intervals prior to acetic acid administration, while the control group was given distilled water. After 3-h, all mice were injected intra-peritoneal with 0.1 mL of 0.6% acetic acid. Each mouse was then placed individually in an observation chamber, and the

number of writhes was recorded over a five-minute period. The writhing counts of the test groups were compared with those of the control group to evaluate the effect of the intervention. For scoring purposes, a writhe was defined as abdominal stretching accompanied by the simultaneous extension of at least one hind limb. The percent inhibition of writhing was calculated using below Equation. The time interval showing the highest percentage of inhibition was designated as the peak effect. Concentrations demonstrating more than 70% inhibition of writhing were considered to possess strong analgesic activity, while concentrations producing less than 70% inhibition were classified as having poor analgesic activity.

$$\% \text{ Inhibition} = \frac{A_c - A_t}{A_c} \times 100$$

Where,  $A_c$  and  $A_t$  are Average writhes in control and test

### 2.6. Evaluation of Antipyretic activity

The antipyretic effect of Exclzyme EN™ was evaluated using a Brewer's yeast-induced pyrexia model in female Wistar rats (150 g). Rectal temperatures were measured with a lubricated thermocouple inserted 2 cm into the rectum to establish baseline values. Fever was induced by subcutaneous injection of 10 mL/kg of a 15% Brewer's yeast suspension in 0.9% saline, administered at the nape of the neck. The injection site was massaged to ensure even distribution beneath the skin. Rats were housed at 22–24 °C, and food was withdrawn immediately after yeast administration. Animals with rectal temperatures  $\geq 38^\circ\text{C}$  were included in the study. The control group received distilled water, while the standard group was treated with paracetamol at doses of 150 and 300 mg/kg. Test groups received Exclzyme EN™ at doses of 100, 200, and 400 mg/kg body weight. Rectal temperatures were recorded at 60, 120, and 180 min post-dosing. For each interval, the change from baseline was calculated, and the maximum reduction in rectal temperature relative to the control group was determined. Results were compared with the standard drug (paracetamol).

## 2.7. Statistical Analysis

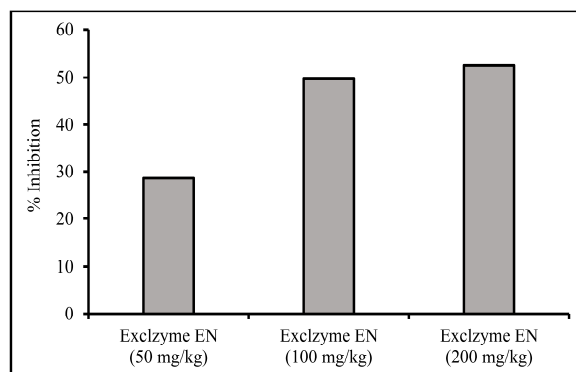
The results are expressed as Mean  $\pm$  S.D. Statistical analysis was performed using one-way ANOVA, followed by Dunnett's post hoc test to compare treated groups with the control. A probability value of  $p < 0.05$  was considered statistically significant.

## 3. Results

### 3.1. Anti-inflammatory activity

Anti-inflammatory activity refers to ability to reduce redness, swelling, fever, and pain caused by the body's immune response to injury or disease. The administration of carrageenan produced a marked increase in paw volume (inflammation) in the control group. The onset of edema was evident after one hour ( $0.27 \pm 0.15$  mL), with the maximum increase observed at the third hour ( $0.47 \pm 0.16$  mL). A reduction in paw swelling was noted after 24 h. Rats pretreated with Exclzyme EN™ (50 mg/kg), one hour prior to carrageenan injection, resulted in a reduction of paw edema at the first hour (19.25%), with the maximum reduction observed at the third hour (28.61%) compared to the control group. However, this reduction was not statistically significant. At a dose of 100 mg/kg, Exclzyme EN™ produced its greatest effect at the third hour, showing a more pronounced reduction in carrageenan-induced paw edema. Similarly, the 200 mg/kg dose demonstrated maximum inhibition at the third hour with mean difference of  $-0.73 \pm 0.19$  at the end of 24 h, indicating a dose-dependent anti-inflammatory activity of Exclzyme EN™.

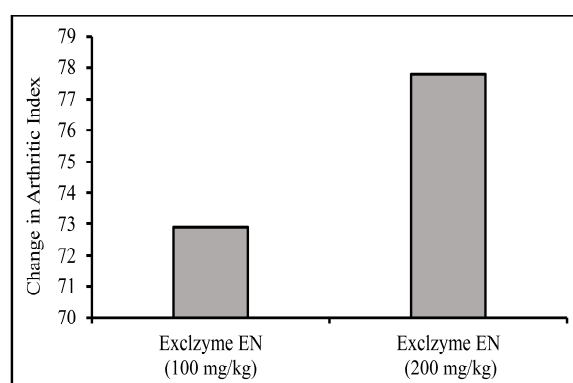
A dose-dependent inhibition of paw edema was observed following administration of Exclzyme EN™. Notably, the 100 mg/kg and 200 mg/kg doses demonstrated a prolonged duration of action, with significant inhibition persisting even at 24 h. The percent inhibition of edema was found to be 23.68% at the dose of 100 mg/kg and 37% at 200 mg/kg. These results indicate that Exclzyme EN™ possesses strong anti-inflammatory activity. The detailed outcomes are summarized in the accompanying Table 2 and Figure 1.



**Figure 1:** Effect of Exclzyme EN™ on inflammation after 3 h of carrageenan injection

### 3.2. Anti-arthritic activity

Anti-arthritic activity refers to the ability to alleviate joint pain, and slow or prevent the progression of arthritis. The control group animals, demonstrated evident inflammation in the left paw post administration of FCA. No secondary lesions were observed on the ears, nose, tail and right hind paw. However, left paw showed evident edema with the arthritic index of three indicating the onset of inflammation. Exclzyme EN™ was administered orally at a dose of 100 or 200 mg/kg after the injection FCA in test group animals. The inflammation due to FCA injection was noticeable after one hour of administration in all the animals. The test group treated with Exclzyme EN™ demonstrated less inflammation compared to the animals in the control group. The arthritic index reduced by 72.89% at the dose of 100 mg/kg and 77.78% at the dose of 200 mg/kg. The change in paw volume and arthritic index are reported in the Table 3 and Figure 2.



**Figure 2:** Effect of Exclzyme EN™ on Change in Arthritic Index in FCA induced Arthritic rats

**Table 2:** Dose-dependent effect of Exclzyme En<sup>TM</sup> on paw volume in carrageenan-induced rat edema

Group	Change in paw volume				% Inhibition			
	1 h	2 h	3 h	24 h	1 h	2 h	3 h	24 h
Control (DW)	0.27 ± 0.15	0.37 ± 0.16	0.47 ± 0.16	-0.53 ± 0.16	-	-	-	-
IP: 50 mg/kg	0.22 ± 0.18	0.30 ± 0.13	0.45 ± 0.33	-0.48 ± 0.12	19.25	20.2	28.61	11.01
IP: 100 mg/kg	0.18 ± 0.19	0.24 ± 0.15	0.24 ± 0.05	-0.59 ± 0.22	33.8	36.03	49.73	-23.68
IP: 200 mg/kg	0.20 ± 0.14	0.24 ± 0.08	0.22 ± 0.02*	-0.73 ± 0.19	26.29	36.7	52.41	-37

**Table 3:** Effect of Exclzyme EN<sup>TM</sup> on change in paw volume and arthritic index in FCA induced arthritic rats

Group	Change in paw volume				Change in Arthritic Index
	1 h	2 h	3 h	24 h	
Control (DW)	0.42 ± 0.22	1.42 ± 0.28	1.15 ± 0.37	1.38 ± 0.45	
IP: 100 mg/kg	0.21 ± 0.28	1.24 ± 0.31	1.10 ± 0.38	1.16 ± 0.35	72.89
IP: 200 mg/kg	0.19 ± 0.26	1.05 ± 0.31	0.88 ± 0.46	0.87 ± 0.43	77.78

### 3.3. Analgesic activity

Analgesic activity refers to the effectiveness in resolution of pain without causing a loss of consciousness. The injection of 0.1 mL acetic acid (0.6%) produced  $34 \pm 9.97$  writhes in the control group mice. In contrast, the mice pretreated with Exclzyme EN<sup>TM</sup> exhibited a marked reduction in writhing behavior, with  $19.9 \pm 6.7$  writhes at 20 mg/kg,  $16.8 \pm 6.1$  writhes at 30 mg/kg,  $5.9 \pm 3.5$  writhes at 40 mg/kg dose, and  $2.4 \pm 2.6$  writhes at 80 mg/kg respectively. Notably, pretreatment with the higher dose of Exclzyme EN<sup>TM</sup> significantly attenuated acetic acid-induced writhing responses ( $p < 0.05$ ), indicating a dose-dependent analgesic effect. The findings for changes in writhing in mice are presented in Table 4 and Figure 3.

**Table 4:** Effect of Exclzyme EN<sup>TM</sup> on Acetic acid writhing in mice

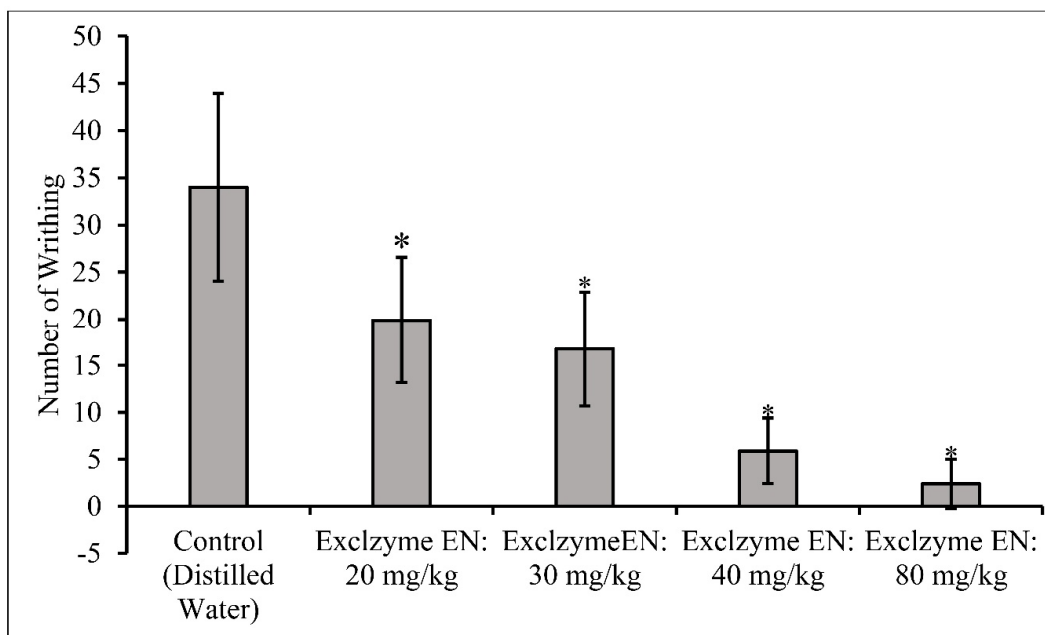
Group	Number of Writhing	% Inhibition
Control (DW)	$34 \pm 9.97$	-
IP: 20 mg/kg	$19.9 \pm 6.7$	41.55
IP: 30 mg/kg	$16.8 \pm 6.1$	54.41
IP: 40 mg/kg	$5.9 \pm 3.5$	82.73
IP: 80 mg/kg	$2.4 \pm 2.6$	93.01

### 3.4. Antipyretic activity

The antipyretic activity of Exclzyme EN<sup>TM</sup> was evaluated in a Brewer's yeast-induced fever model. Subcutaneous injection of an aqueous yeast suspension significantly elevated rectal temperature in the control group by  $0.43 \pm 0.12$  °C, rising from 38.6 °C to 39.03 °C. Mice treated with Exclzyme EN<sup>TM</sup> or paracetamol showed a significant reduction in rectal temperature compared with controls.

Among the test groups, the 200 mg/kg dose of Exclzyme EN<sup>TM</sup> produced the greatest decrease ( $0.63 \pm 0.12$  °C), comparable to both paracetamol doses in the standard group. The antipyretic effect of Exclzyme EN<sup>TM</sup> was evident from the first hour, reached its peak at the third hour, and persisted for up to five hours across all doses. The 100 mg/kg and 200 mg/kg doses were the most effective. Paracetamol also induced a marked reduction, with a peak decrease of  $0.97 \pm 0.50$  °C at 300 mg/kg.

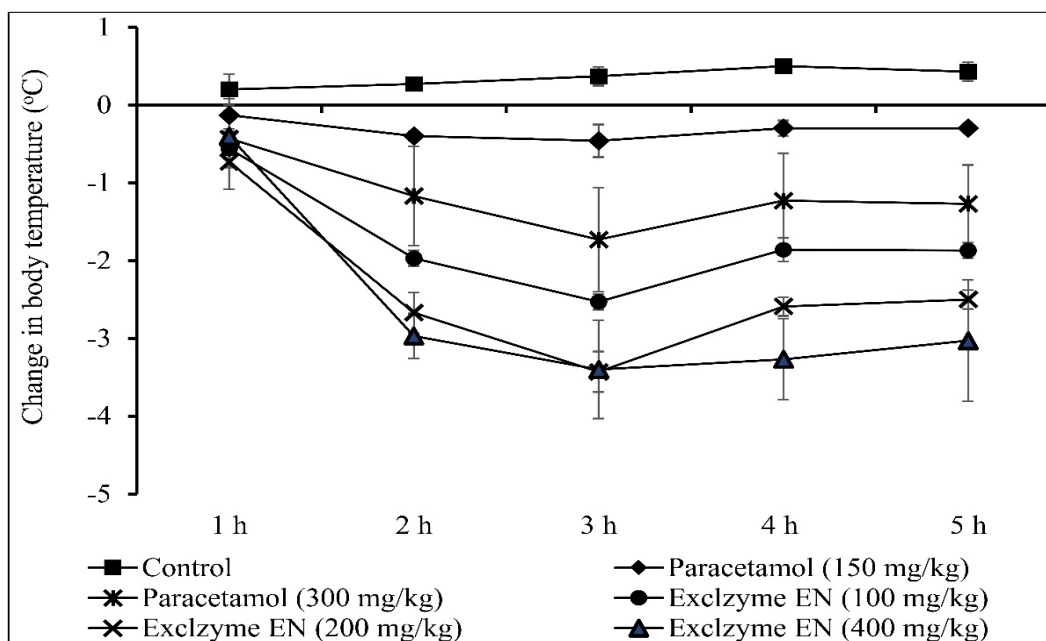
Overall, the reduction in rectal temperature achieved with Exclzyme EN<sup>TM</sup> was comparable to paracetamol across all tested doses, demonstrating its potential as an effective antipyretic agent. The mean changes in rectal temperature of mice from baseline (0<sup>th</sup> day) are presented in Table 5 and Figure 4.



**Figure 3:** Effect of Exclzyme EN™ on writhing behaviour in mice

**Table 5:** Mean change in rectal temperature post administration of yeast at 0<sup>th</sup> h

Group	Temperature at 0 h	Change in temperature from 0 <sup>th</sup> h (°C)				
		1 h	2 h	3 h	4 h	5 h
Control	38.6±0.36	0.2±.2	0.27±.06	0.37±0.12	0.5±0	0.4 ±0.12
Standard: 150 mg/kg	38.6±0.46	-0.33±0.21	-0.67±0.06	-0.83±0.21	-0.8±0.10	-0.73±0.06
Standard: 300 mg/kg	38.7±0.15	-0.3±0.36	-0.77±0.64	-1.27±0.67	-0.93±0.61	-0.97±0.50
IP: 100 mg/kg	38.9±0.21	-0.13±0.25	-0.8±0.1	-0.80±0.10	-0.63±0.15	-0.60±0.10
IP: 200 mg/kg	38.4±.06	-0.17±0.35	-0.7±0.26	-0.90±0.26	-0.73±0.12	-0.63±0.12
IP: 400 mg/kg	38.15±.61	0.33±0.29	-0.3±0.29	-0.03±0.63	-0.68±0.52	-0.53±0.78



**Figure 4:** Effect of Exclzyme EN™ on rectal temperature changes in mice

#### 4. Discussion

Non-steroidal anti-inflammatory drugs (NSAIDs) are widely prescribed for pain and inflammation, but long-term use these drugs often results in gastrointestinal ulcers, bleeding, and renal complications [17]. This highlights the need for safer alternatives that provide symptom relief without such adverse effects. The present study investigates the anti-inflammatory, anti-arthritis, analgesic, and antipyretic potential of Exclzyme EN™; a multi-enzyme blend enriched with herbal components.

Proteolytic enzymes such as bromelain, papain, and serratiopeptidase are well-documented for their anti-inflammatory activity. Bromelain, a cysteine proteinase containing peroxidase, acid phosphatase, protease inhibitors, and calcium, remains stable across a wide pH range. It has a well-established analgesic and anti-inflammatory effects, alongside additional benefits including immune modulation, wound healing, and anti-tumour activity [18,19]. This study demonstrates the analgesic effect of Exclzyme EN™, observed via the reduction in the number of writhes in acetic acid induced writhing model, these results aligns well with the prior findings [20,21].

Papain, derived from papaya, also exhibits significant anti-inflammatory action. It effectively reduced carrageenan-induced paw edema, cotton pellet granuloma, and arthritic inflammation in rat models [22]. In a clinical study, it has effectively reduced pain ( $p=0.001$ ) in lumber spine of osteoarthritic patients and influenced the biochemical markers such as ALP ( $p=0.054$ ) and serum creatinine ( $p=0.035$ ), suggesting systemic therapeutic potential beyond symptom control and improved quality of life [23]. These properties account for the observed outcomes of Exclzyme EN™, specifically the reduction of carrageenan-induced paw edema in rats and the improvement in the arthritic index noted in this study.

Fever is a complex physiological response to disease, driven by cytokine-mediated elevation of body temperature, acute-phase reactant

production, and activation of multiple endocrine and immune pathways [24]. Brewer's yeast, a fungal source of lipopolysaccharides, binds to macrophages and triggers the release of cytokines such as interleukin-1 into circulation. This cascade reduces blood-brain barrier integrity, liberates arachidonic acid, and ultimately stimulates prostaglandin E2 (PGE2) synthesis in the anterior hypothalamus, producing pyrexia [25]. Proteases including serratiopeptidase and bromelain have demonstrated antipyretic effects in-vivo by suppressing inflammatory cytokines and systemic responses [26]. Serratiopeptidase, in particular, exerts anti-inflammatory and antipyretic activity through proteolytic and mucolytic mechanisms, hydrolysing pyrogenic mediators and modulating the cyclooxygenase (COX) pathway to reduce fever [27,28]. Amla has validated antipyretic efficacy in rat models, showing significant reduction of yeast-induced pyrexia, further supporting its role as a natural fever-modulating agent [25]. Amla, rich in vitamin C, polyphenols, and potent antioxidants, provides strong antioxidant and anti-inflammatory benefits, including notable anti-collagenase activity [29,33]. Rutin, a therapeutic flavonoid, has demonstrated effectiveness in alleviating inflammation and fever in both arthritis and pyrexia in-vivo models. Its anti-arthritis activity is mediated through antinociceptive, antioxidant, and anti-inflammatory mechanisms, positioning it as a promising therapeutic agent for inflammatory pain and arthritis [30]. Moreover, rutin exhibits synergistic interactions with NSAIDs and paracetamol, enhancing their anti-inflammatory, analgesic, and antipyretic effects in-vitro and in-vivo [31]. As a pain-relieving flavonoid, rutin provides consistent evidence of anti-inflammatory and analgesic efficacy, supporting its role in the management of chronic inflammatory conditions [32].

The antinociceptive and antipyretic properties of Exclzyme EN™ were evaluated using established mouse models. The study demonstrated that Exclzyme EN™ significantly suppressed carrageenan-induced paw edema in

rats, confirming marked acute anti-inflammatory efficacy. In addition, the formulation effectively improved the arthritic index in acetic acid-induced arthritis models. These outcomes reflect the multi-enzyme composition of the intervention, which engages several mechanisms underlying analgesic activity. Furthermore, Exclzyme EN™ produced a significant reduction in rectal temperature in the Brewer's yeast-induced pyrexia model, validating its antipyretic potential. Collectively, the consistent results across inflammation, arthritis, and pyrexia models highlight the broad therapeutic impact of Exclzyme EN™ under in-vivo conditions.

### 5. Conclusion

In summary, this study demonstrates in-vivo therapeutic potential of Exclzyme EN™ in alleviation of inflammation, arthritis, and pyrexia in experimental models. Exclzyme EN™ reduced carrageenan-induced paw edema, confirming its anti-inflammatory efficacy; improved the arthritic index in acetic acid-induced arthritis, validating its anti-arthritic potential; demonstrated its analgesic properties by reducing the writhes in affected mice, and significantly lowered rectal temperature in Brewer's yeast-induced pyrexia, establishing its antipyretic activity. Collectively, these findings highlight Exclzyme EN™ as a promising multi-enzyme intervention with broad therapeutic relevance. However, further studies are warranted to elucidate the precise molecular mechanisms underlying its pharmacological effects.

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**Conflict of Interest:** The authors declare that there are no conflicts of interest

### Ethical statement

All procedures performed in studies involving animals were in accordance with the ethical standards of the institution or practice at which the studies were conducted.

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**Declaration of Non-Use of AI:** The authors confirm that no artificial intelligence tools were used in this study.

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