

Evaluation of Analgesic and Anti-Inflammatory activity of *Ananas comosus* fruit peel extract by using experimental animals

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ABSTRACT:

The present study was undertaken to evaluate the analgesic and anti-inflammatory activities of the ethanolic extract of *Ananas comosus* (pineapple) fruit peel using experimental animal models. The extract was prepared by maceration of dried pineapple peel powder in 96% ethanol and was subjected to acute toxicity studies according to OECD Guideline 423. The extract was found to be safe up to 2000 mg/kg body weight, and doses of 100, 200, and 400 mg/kg were selected for pharmacological evaluation. Analgesic activity was assessed using the hot plate and tail flick methods, while anti-inflammatory activity was evaluated by the carrageenan-induced paw edema model in Wistar rats. Ibuprofen (20 mg/kg) served as the standard drug. The extract exhibited significant dose-dependent analgesic effects by increasing reaction time in both pain models. It also produced marked anti-inflammatory activity, reducing paw edema and lowering inflammatory biomarkers such as TNF- α and IL-6. The findings indicate that *Ananas comosus* fruit peel possesses promising analgesic and anti-inflammatory properties, supporting its potential as a natural therapeutic agent for pain and inflammatory disorders.

KEY WORDS: Pineapple Peel Extract, Bromelain, Analgesic Activity, Anti-inflammatory Activity.

INTRODUCTION:

Inflammation is a complex biological and protective response of the body against harmful stimuli such as pathogens, damaged cells, toxic compounds, and physical injury. It serves as a defense mechanism intended to eliminate the initial cause of cell injury, remove damaged tissues, and initiate the healing process. The classical signs of inflammation include redness, heat, swelling, pain, and loss of function. Although inflammation is essential for survival and tissue repair, excessive or uncontrolled inflammation can contribute to the development of several acute and chronic diseases. Inflammatory responses are mediated through various chemical substances such as prostaglandins, leukotrienes, histamine, bradykinin, cytokines, and platelet-activating factors. These mediators regulate vascular permeability, leukocyte migration, tissue destruction, and repair processes. (Corrado et al., 2009; Salvemini et al., 1996).

Inflammation may be classified as acute or chronic depending on the duration and nature of the response. Acute inflammation is an immediate and short-term reaction characterized by vasodilation, increased vascular permeability, and infiltration of neutrophils at the site of injury. It generally lasts for a few days and is followed by healing and tissue repair. In contrast, chronic inflammation is a prolonged inflammatory condition associated with tissue destruction, fibrosis, and infiltration of macrophages, lymphocytes, and plasma cells. Persistent inflammation has been implicated in the pathogenesis of numerous diseases such as rheumatoid arthritis, osteoarthritis, asthma, inflammatory bowel disease, cardiovascular disorders, diabetes mellitus, neurodegenerative diseases, and cancer. (Harshmohan, 2010; Karin et al., 2006).

Several inflammatory mediators play a major role in the initiation and progression of inflammation. Cyclooxygenase enzymes (COX-1 and COX-2) are responsible for the synthesis of prostaglandins from arachidonic acid. Prostaglandins contribute significantly to pain, swelling, and fever during inflammation. COX-2 is particularly induced during inflammatory conditions and is considered an important therapeutic target for anti-inflammatory drugs. Histamine released from mast cells contributes to vasodilation and increased vascular permeability, while bradykinin is involved in pain production and edema formation. Cytokines such as tumor necrosis factor-alpha (TNF- α) and interleukins (IL-1 and IL-6) further amplify the inflammatory response by promoting leukocyte recruitment and activation. (Nathan, C. 2002; Holgate, S. T. 1986; Dinarello, C. A. 1984).

Non-steroidal anti-inflammatory drugs (NSAIDs) are among the most widely prescribed medications for the treatment of pain and inflammation. These drugs exert their pharmacological effects mainly through inhibition of cyclooxygenase enzymes and suppression of prostaglandin synthesis. Common NSAIDs such as aspirin, ibuprofen, and diclofenac are effective in reducing pain, fever, and inflammation. However, prolonged use of NSAIDs is often associated with adverse effects including gastric irritation, peptic ulcers, renal toxicity, cardiovascular complications, and hypersensitivity reactions. Opioid analgesics, though highly effective for severe pain management, may also produce adverse effects such as respiratory depression, tolerance, dependence, and addiction. Therefore, the development of safer and more effective anti-inflammatory and analgesic agents remains an important area of pharmaceutical research. (Vane, J. R. 1971; Insel, P.A. 1994).

Pain is defined by the International Association for the Study of Pain (IASP) as an unpleasant sensory and emotional experience associated with actual or potential tissue damage. It acts as a protective mechanism that warns the body against harmful stimuli and helps prevent further injury. Pain may be classified as acute or chronic, nociceptive or neuropathic, depending on its origin and duration. Nociceptive pain arises from tissue injury and activation of nociceptors, whereas neuropathic pain results from injury or dysfunction of the nervous system. Pain perception involves four major processes namely transduction, transmission, modulation, and perception. These processes involve specialized nociceptors, afferent nerve fibers, spinal pathways, and higher brain centers. (Ma & Turner, 2012; Willis, W.D. et al., 1997).

The search for safer therapeutic agents has led researchers to investigate medicinal plants extensively. Medicinal plants have been used since ancient times in traditional systems of medicine for the treatment of pain, inflammation, fever, and various other diseases. Plants contain several bioactive phytoconstituents such as alkaloids, flavonoids, tannins, glycosides, saponins, terpenoids, and phenolic compounds that possess important pharmacological activities. Compared to synthetic drugs, herbal medicines are often considered safer and are associated with fewer side effects. Numerous medicinal plants have shown promising anti-inflammatory and analgesic activities in experimental studies, supporting their traditional use in the management of inflammatory disorders and painful conditions. (Halliwell et al., 1992; Latha et al., 2021; Alagesaboopathi et al., 2011).

Natural products continue to play a significant role in modern drug discovery and development. Many clinically important drugs including morphine, aspirin, and several anti-inflammatory agents have originated from plant sources. Scientific validation of medicinal plants through phytochemical, pharmacological, and toxicological investigations is essential for the development of novel therapeutic agents. Therefore, exploration of medicinal plants for anti-inflammatory and analgesic activities remains a valuable and promising approach for discovering safer, cost-effective, and more effective medicines for the treatment of inflammation and pain-related disorders.

MATERIAL AND METHODS:

Drug Profile of *Ananas comosus*



Fig: 1. *Ananas comosus*

Name of the drug: *Ananas comosus*

Family: Bromeliaceae

Synonyms: Pineapple, Ananas, *Ananas sativus*, *Bromelia comosa*

Taxonomical Classification (*Ankit Sharma et al; 2024*)

- Kingdom: Plantae
- Phylum: Tracheophyta
- Class: Lilopsida
- Order: Bromeliales
- Family: Bromeliaceae
- Genus: *Ananas*
- Species: *A. comosus*

Cultivation: Pineapple is mainly cultivated in humid tropical and subtropical regions with high rainfall. It grows best at temperatures between 22°C and 32°C and prefers well-drained sandy loam soil with a pH range of 5.0–6.0. Propagation is mainly carried out vegetatively through crowns and suckers. Commercial cultivation is possible in plains and hilly regions up to 1,100 meters altitude, provided frost conditions are absent. (Rana S. et al; 2023).

Phytochemical Constituents: *Ananas comosus* contains several important phytochemicals with medicinal properties. Studies have reported the presence of bromelain, tacorin, flavonoids, alkaloids, saponins, phytosterols, carbohydrates, proteins, and cardiac glycosides in the stem. The peel is rich in antioxidants such as phenolics, terpenoids, carotenoids, coumarins, tannins, lignin, plant sterols, and ascorbic acid. Major polyphenols identified include catechin, epicatechin, gallic acid, ferulic acid, tannic acid, and salicylic acid. Bromelain is considered one of the major bioactive constituents responsible for many pharmacological activities. (Meena R, et al 2021; Soni S, et al; 2022; Ketnawa S, et al; 2012; Nadzirah KZ, et al; 2012).

Preparation of Pineapple Peel Extract: The pineapple peels were washed, dried, and powdered before extraction. About 10 g of peel powder was soaked in 96% ethanol for 48 hours and filtered. The extract was concentrated using a rotary evaporator at 40°C, sterilized under UV radiation, and stored at 4°C until further use. (Renu N.J. et al 2025).

Dose determination: Acute toxicity studies of the extract of *Ananas comosus* fruit peel were performed according to OECD Guideline 423. The animals were fasted for 12 hours prior to the experiment, and the extract was administered orally at a single dose of 2000 mg/kg body weight. The rats were observed continuously for the first 4 hours and further monitored daily for 14 days for any signs of toxicity or mortality. Parameters such as respiration, eye movement, vomiting, diarrhea, writhing, body movements, and changes in fur were evaluated. No mortality or toxic symptoms were observed during the study period, indicating that the extract was safe at the tested dose. (Samira Kargutkar, et al 2016).

Based on the acute toxicity study, the experimental doses selected were 100, 200, and 400 mg/kg (p.o.), which correspond to 1/20th, 1/10th, and 1/5th of the calculated safe dose, respectively. Based on the literature review, Standard drug Ibuprofen was selected at dose 20 mg/kg.

Selection of animals: Albino Wistar rats weighing 150–250 g were used for the study. The experimental protocol was approved by the Institutional Animal Ethics Committee (IAEC) and conducted according to the guidelines of the Committee for Control and Supervision of Experiments on Animals (CCSEA). The animals were housed in polypropylene cages containing rice husk bedding and maintained under standard laboratory conditions with a temperature of $24 \pm 2^{\circ}\text{C}$, relative humidity of 30–70%, and a 12:12 hour light-dark cycle. Animals had free access to standard pellet diet and water throughout the study.

The rats were randomly divided into six groups: Vehicle Control, Negative Control, Standard, Test Group I (100 mg/kg), Test Group II (200 mg/kg), and Test Group III (400 mg/kg).

Method of Analgesic:

1) Hot plate Method:

Wistar rat weighing between (150-200g) were divided into 5 groups of 6 animals in each group (n=6). The animals were pretreated with drugs 60 minutes before tail flick. Group I served as control (vehicle treated) orally, group II served as positive control (Ibuprofen treated) 20 mg/kg orally as a standard drug, groups III, IV, V received 100, 200 and 400 mg/kg of extracts of *Ananas comosus*. The latency to flick the hind paw or lick or jump from the hot plate was the reaction time. The reaction time of animals was noted down on hot plate at 0, 30, 60, 120 and 180 minutes after the treatment. The basal reaction was time taken by observing hind paw licking or jump response (whichever appear first) in animals while placed on hot plate, which was maintained at constant temperature 55°C . A cut off period of 10 seconds was observed to avoid damage to the paws. (A. O. Sijuade et al 2016).

2) Tail Flick Method:

Wistar rat weighing between (150-200g) were divided into 5 groups of 6 animals in each group (n=6). The animals were pretreated with drugs 60 minutes before tail flick. Group I served as control (vehicle treated) orally, group II served as positive control (Ibuprofen treated) 20 mg/kg orally as a standard drug, groups III, IV, V received 100, 200 and 400 mg/kg of extracts of *Ananas comosus*.

The reaction time was recorded using tail flick analgesiometer at 0, 30, 60, 120 and 180 minutes time interval after the drug administration. The temperature was maintained at $50-55^{\circ}\text{C}$. (A. O. Sijuade et al 2016).

Method for Antiinflammation:**1) Carrageenan induced paws edema:**

The method of Winter et al. was used to study anti-inflammatory activity using plethysmograph to measure the paw volume. Wistar rat weighing between (150-200g) were divided into 6 groups of 6 animals in each group (n=6). Group I served as control (vehicle treated) orally, group II served as negative control Carrageenan 0.1ml by sub-plantar route on 1st day, group III served as positive control (Ibuprofen treated) 20 mg/kg orally as a standard drug, groups IV, V, VI received 100, 200 and 400 mg/kg of extracts of *Ananas comosus*.

A mark was made on both the hind paws just below the tibio-torsal junction. After Thirty minutes of treatment, an inflammatory edema was induced in the left hind paw by injection of 0.1 ml of 1% of Carrageenan solution in the plantar tissue of the paw of all the animals. The right paw served as a reference. The initial paw volume was measured plethysmographically within 30 seconds after injection. The relative increase in paw volume was measured in all groups at 30 minutes, 1, 2 and 3 hrs after Carrageenan injection. The percentage increase in the paw volume over the initial reading was calculated. This increase in the paw volume were compared with group I. (A. O. Sijuade et al 2016).

The percentage inhibition of edema volume was calculated by using the formula,

$$\text{Percentage of inhibition} = 100 (1 - V_t / V_c)$$

Where,

V t is increase in paw volume in treated groups

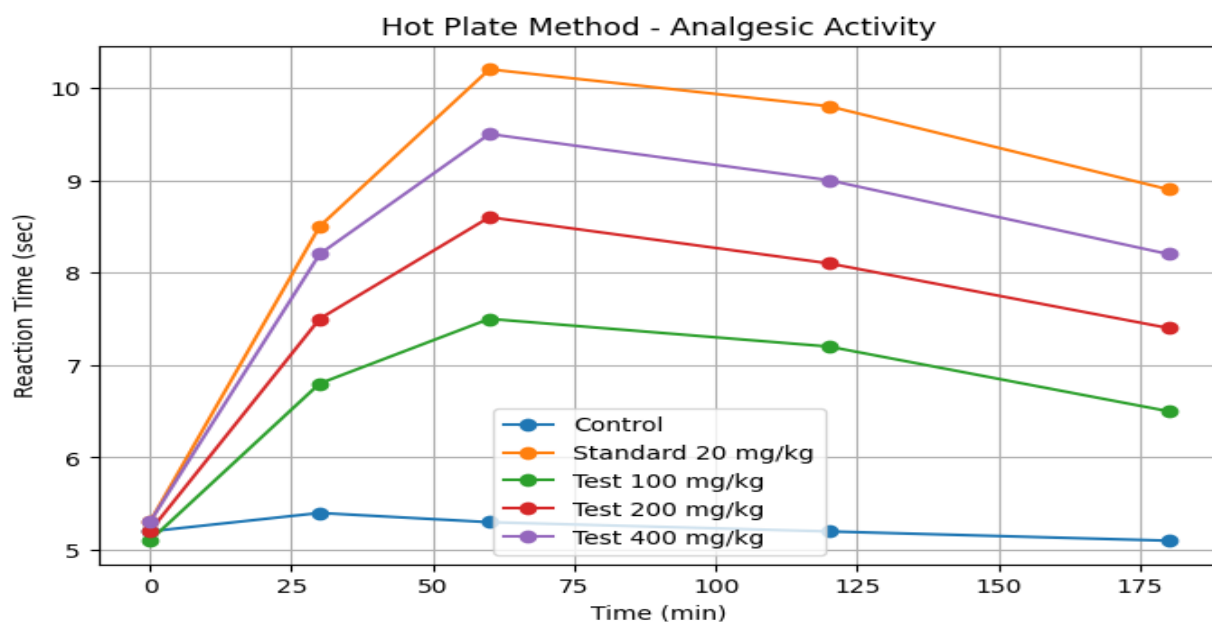
V c is increase in paw volume in control groups

RESULT:**Hot Plate Method (Reaction time in sec)**

Table No.1: Hot Plate Method

Group	Dose	0 min	30min	60min	120min	180min
Control	-	5.2 ± 0.3	5.4 ± 0.3	5.3 ± 0.3	5.2 ± 0.2	5.1 ± 0.3
Standard	20mg/kg	5.3 ± 0.2	8.5 ± 0.3 ***	10.2 ± 0.4 ***	9.8 ± 0.3 ***	8.9 ± 0.2 ***
Test	100mg/kg	5.1 ± 0.3	6.8 ± 0.2 *	7.5 ± 0.3 *	7.2 ± 0.2 *	6.5 ± 0.3 *
Test	200mg/kg	5.2 ± 0.2	7.5 ± 0.3 **	8.6 ± 0.3 **	8.1 ± 0.2 **	7.4 ± 0.3 **
Test	400mg/kg	5.3 ± 0.3	8.2 ± 0.2 ***	9.5 ± 0.4 ***	9.0 ± 0.3***	8.2 ± 0.2 ***

All values are presented as mean ± SEM, n=6. One way ANOVA, followed by Dunnett's test was performed as the test of significance *p<0.05, **p<0.01, ***p<0.001

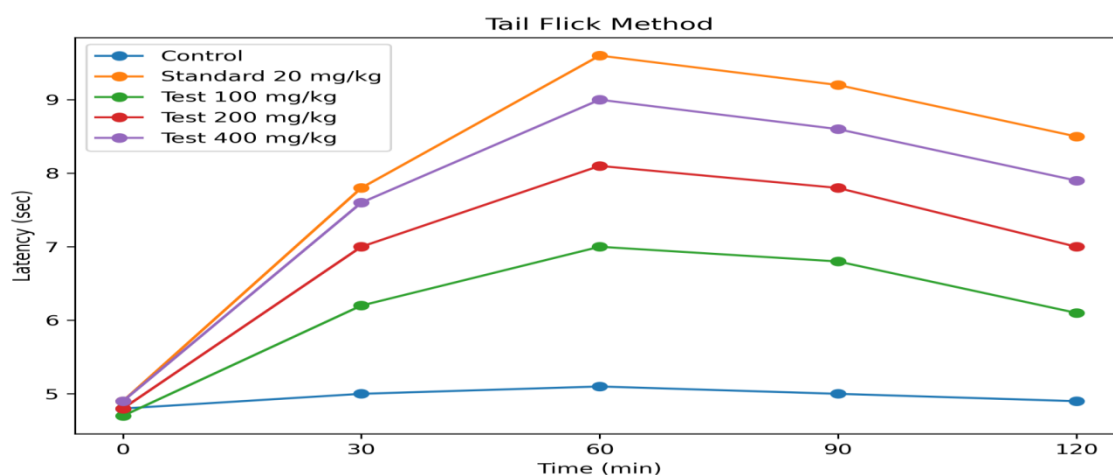
**Fig.No 2: Hot Plate Method**

Tail Flick Method (Latency in sec)

Table No. 2: Tail Flick Method

Group	Dose	0 min	30min	60min	90min	120min
Control	-	4.8 ± 0.2	5.0 ± 0.3	5.1 ± 0.2	5.0 ± 0.3	4.9 ± 0.2
Standard	20mg/kg	4.9 ± 0.2	7.8 ± 0.3 ***	9.6 ± 0.4* **	9.2 ± 0.3 ***	8.5 ± 0.2* **
Test	100mg/kg	4.7± 0.3	6.2 ± 0.2 *	7.0 ± 0.3*	6.8 ± 0.2 *	6.1 ± 0.3 *
Test	200mg/kg	4.8 ± 0.2	7.0 ± 0.3 **	8.1 ± 0.32**	7.8 ± 0.3 **	7.0± 0.2 **
Test	400mg/kg	4.9 ± 0.3	7.6 ± 0.2 ***	9.0± 0.3***	8.6 ± 0.2 ***	7.9 ± 0.2* **

All values are presented as mean ± SEM, n=6. One way ANOVA, followed by Dunnett's test



was performed as the test of significance *p<0.05, **p<0.01, ***p<0.001

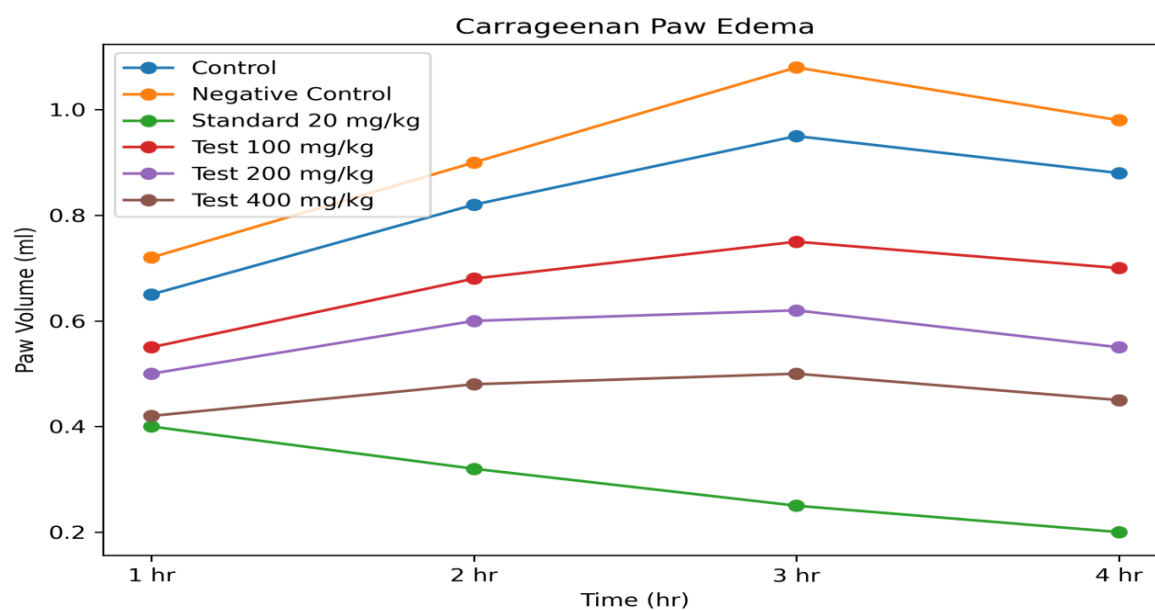
Fig.No.3:Tail Flick Method

Carrageenan Induced Paw Edema (Paw Volume in ml)

Table No. 3: Carrageenan Induced Paw Edema

Group	Dose	1hr	2hr	3hr	4hr	% Inhibition
Control	-	0.65 ± 0.02	0.82 ± 0.03	0.95 ± 0.02	0.88 ± 0.03	-
Negative Control	0.1 ml	0.72 ± 0.03	0.90 ± 0.03	1.08 ± 0.04	0.98 ± 0.03	-
Standard	20mg/kg	0.40 ± 0.02 ***	0.32 ± 0.02* **	0.25 ± 0.01* **	0.20 ± 0.02* **	77%
Test	100mg/kg	0.55 ± 0.03*	0.68 ± 0.02 *	0.75 ± 0.03 *	0.70 ± 0.02 *	20%
Test	200mg/kg	0.50 ± 0.02 **	0.60 ± 0.03 **	0.62 ± 0.02 **	0.55 ± 0.03 **	38%
Test	400mg/kg	0.42 ± 0.02 ***	0.48 ± 0.02 ***	0.50 ± 0.02 ***	0.45 ± 0.02 ***	52%

All values are presented as mean ± SEM, n=6. One way ANOVA, followed by Dunnett's test was performed as the test of significance *p<0.05, **p<0.01, ***p<0.001

**Fig. No.4: Carrageenan induced Paw Edema**

DISCUSSION:

The present study was conducted to evaluate the analgesic and anti-inflammatory activities of the ethanolic extract of *Ananas comosus* fruit peel in experimental animal models. Preliminary phytochemical screening revealed the presence of flavonoids, phenolic compounds, tannins, proteins, amino acids, carbohydrates, and triterpenoids. These phytoconstituents are known to possess significant pharmacological activities, particularly antioxidant, analgesic, and anti-inflammatory properties.

The analgesic activity of the extract was assessed using the hot plate and tail flick methods. In both models, the extract produced a significant and dose-dependent increase in reaction time compared with the control group. The highest dose (400 mg/kg) showed maximum analgesic activity and produced effects comparable to the standard drug Ibuprofen. The increase in latency period observed in the hot plate and tail flick tests suggests that the extract possesses central analgesic activity.

The anti-inflammatory activity was evaluated using the carrageenan-induced paw edema model, a widely accepted model of acute inflammation. Treatment with the extract significantly reduced paw edema at all tested doses. The highest dose (400 mg/kg) exhibited 52% inhibition of edema, while the standard drug Ibuprofen produced 77% inhibition.

CONCLUSION:

In conclusion, the ethanolic extract of *Ananas comosus* fruit peel possesses significant analgesic and anti-inflammatory activities. These effects may be attributed to the presence of flavonoids, phenolic compounds, tannins, and bromelain-like constituents. The findings support the traditional use of pineapple peel in the management of pain and inflammation and suggest its potential as a natural source for developing safer and effective therapeutic agents. Further studies are required to isolate the active constituents and establish their clinical efficacy.

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