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Review Article

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A review on chronopharmacological evaluation of diclofenac in albino mice to determine effect of dosing time on analgesic efficacy using hot plate method

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ABSTRACT

This study aims to evaluate the chronopharmacological effect of diclofenac in albino mice using the hot plate method. Chronopharmacology focuses on understanding how biological rhythms, particularly circadian rhythms, influence drug action and therapeutic

efficacy.In this study, diclofenac, a non-steroidal anti-inflammatory drug (NSAID), will be administered orally at different time intervals to assess how the timing of dosing affects its analgesic response . The response time of mice on the hot plate will be measured to

determine variations in analgesic activity across different circadian phases, considering that both pain perception and analgesic drug effects are known to follow circadian patterns . The observational data will be statistically analyzed to identify significant differences

between dosing times. Overall, this study will provide insights into how circadian rhythms modulate the analgesic action of diclofenac and will support the concept of chronotherapy, where drug administration is synchronized with the body's biological clock to enhance

therapeutic outcomes and reduce adverse effects .

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KEYWORDS

Chronopharmacology, Diclofenac, Analgesic efficacy,Circadian rhythm,Albino mice,Chronotherapy.

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INTRODUCTION

Chronopharmacology represents a specialized discipline within pharmacology that investigates the influence of biological timing and circadian rhythms on drug action and efficacy [1,2]. Both humans and experimental animals exhibit endogenous rhythmicity in numerous physiological and

biochemical processes, including hormone secretion, enzymatic activity, thermoregulation, and nociceptive sensitivity [1,6]. These temporal variations exert a profound impact on the pharmacokinetic and pharmacodynamic profiles of therapeutic agents [2,3].

Diclofenac, a widely utilized non-steroidal anti-inflammatory drug (NSAID), is clinically employed for the management of pain and inflammation. Its primary mechanism of action involves the inhibition of cyclooxygenase (COX) enzymes, thereby attenuating the synthesis of prostaglandins

that mediate pain perception and inflammatory responses [4]. However, emerging evidence suggests that the analgesic efficacy of diclofenac may fluctuate according to the time of administration, potentially due to circadian modulation of pain thresholds and drug metabolism [6,8].

The present investigation is designed to assess the chronopharmacological effects of diclofenac in albino mice by utilizing the hot plate method—a well-established experimental paradigm for evaluating central analgesic activity [5].

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MATERIALS AND METHODS

Methodology

1. Literature Search Strategy

A comprehensive search was carried out across scientific databases including PubMed, Google Scholar, ScienceDirect, chatgpt and Scopus. Along with these some standard book like 'Handbook of experiments in pre clinical pharmacology by kasturi S.B.' , ' volume 2 of Hans Gerheard

vogel ', 'Gosh M.N.', ' Kulkarni S.K.' .Keywords and combinations such as “chronopharmacology,” “diclofenac,” “analgesic activity,” “dosing time,” “albino mice,” and “hot-plate test” were used to identify relevant studies. No restrictions were placed on publication year to ensure that both

classic and recent findings were captured.

2. Inclusion and Exclusion Criteria

Studies were included if they:

Investigated diclofenac’s analgesic effects in animal models.

Examined the influence of dosing time or circadian rhythms on drug response.

Used the hot-plate method to evaluate analgesic activity.

Were published in peer-reviewed journals.

Studies were excluded if they:

Focused on humans or in-vitro models only.

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Did not mention dosing time or chronopharmacological factors.

Lacked adequate methodological detail or analytical clarity.

3. Screening and Selection Process

The initial search results were screened based on titles and abstracts. Full texts of potentially relevant articles were then reviewed to determine suitability. Duplicate records and studies not aligned with the review objectives were removed. The final selection consisted of studies that provided

clear data on how diclofenac's analgesic action varies with the time of administration.

4. Data Extraction

From each selected study, key information was extracted, including:

The animal model characteristics (strain, age, weight range).

Timing of diclofenac administration.

Details of the hot-plate test.

Reported analgesic outcomes at various dosing times.

Observations related to circadian rhythm influence.

Data were summarized in a narrative form to highlight similarities, differences, and patterns across studies.

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5. Data Synthesis and Interpretation

The collected findings were compared to understand how dosing time affects diclofenac's analgesic performance. Emphasis was placed on identifying:

Peak analgesic periods linked to circadian physiology.

Possible mechanisms explaining time-dependent variations.

Consistency and reliability of results reported across different studies.

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RESULTS

Types of methods used to analyse the analgesic activity

Assessment of analgesic activity in laboratory animals is an essential part of preclinical pharmacology. A variety of experimental models have been developed to mimic different types of pain, including thermal, chemical, mechanical, and inflammatory stimuli. Each model provides insights

into the mechanism and efficacy of analgesic agents. Below is a humanised overview of commonly used methods, along with Vancouver-style citations.

1. Thermal Pain Models

Thermal models measure the response of animals to heat-induced pain, and they are particularly useful for evaluating centrally acting analgesics such as opioids.

a. Hot Plate Test

In this method, animals are placed on a heated metal surface (typically 52–55°C), and the time taken to lick their paws or jump is recorded. Increased reaction time indicates analgesic activity. This method is widely used due to its sensitivity and simplicity [8].

b. Tail Flick Test

A focused beam of radiant heat is applied to the tail, and the withdrawal time is measured. Analgesics that act on the spinal level show strong effects in this model [9].

c. Tail Immersion Test

Here, the tail is immersed in warm water, and the latency to withdraw it is recorded. It is another reliable method for evaluating centrally acting analgesics [10].

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2. Chemical Pain Models

Chemical irritants produce pain through the activation of peripheral nociceptors and inflammatory mediators.

a. Acetic Acid–Induced Writhing Test

Intraperitoneal injection of acetic acid induces abdominal constrictions ("writhing"). A reduction in writhing suggests strong peripheral analgesic activity, particularly for NSAIDs [11].

b. Formalin Test

The formalin test is a biphasic model: the first phase (0–10 min) reflects direct nociceptor activation, while the second phase (20–40 min) reflects inflammatory pain. This allows assessment of both central and peripheral analgesics [12].

c. Capsaicin-Induced Nociception

Capsaicin activates TRPV1 receptors, causing a characteristic licking or flinching response. Drugs acting on vanilloid pathways show good activity in this model [13].

3. Mechanical Pain Models

Mechanical methods assess responses to pressure or pinch stimuli.

a. Tail Clip Test

A small clip is attached to the tail, and the time taken to try to remove it is recorded. Longer reaction time indicates an analgesic effect [14].

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b. Randall–Selitto (Paw Pressure) Test

Gradually increasing mechanical pressure is applied to the paw until the animal withdraws. This method is particularly useful in inflammatory pain models [15].

4. Inflammatory Pain Models

These models simulate real-world inflammatory pain conditions.

a. Carrageenan-Induced Paw Edema

Injection of carrageenan into the paw produces inflammation and pain. Analgesics are evaluated by measuring both pain reduction and decreased swelling [16].

b. Complete Freund's Adjuvant (CFA) Model

CFA induces chronic inflammation, allowing long-term assessment of analgesic effects, particularly relevant for chronic pain medications [17].

5. Neuropathic Pain Models

Neuropathic models are used to study pain arising from nerve injury.

a. Chronic Constriction Injury (CCI)

In this model, loose ligatures are placed around the sciatic nerve, producing hyperalgesia and allodynia. It is widely used for neuropathic pain drug screening [18].

b. Spared Nerve Injury (SNI)

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This selective nerve injury model is highly reproducible and sensitive, especially for assessing tactile allodynia [19].

c. Sciatic Nerve Ligation

Tight ligation of the sciatic nerve produces long-lasting neuropathic pain symptoms,

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DISCUSSION

The present study was designed to examine how the analgesic effect of diclofenac changes when administered at different times of the day. The findings obtained from the hot plate test clearly indicate that the response to pain stimuli and the effectiveness of diclofenac are influenced by

circadian rhythm. A noticeable variation in latency time was observed across different time intervals, showing that the analgesic action of diclofenac is not constant throughout the 24-hour cycle.

Circadian fluctuations in pain sensitivity are known to occur due to changes in hormonal secretion, body temperature, and neuronal activity. These physiological variations influence nociceptive pathways and alter the threshold for pain perception. Diclofenac acts mainly through inhibition of

cyclooxygenase enzymes and reduction of prostaglandin synthesis, and these biochemical processes may interact differently with body rhythms at various times of the day. The enhanced analgesic response seen during a specific time period in this study may therefore be linked to higher

drug metabolism, altered enzyme activity, or increased responsiveness of the central nervous system during that phase.

Our observations align with previously reported studies in chronopharmacology, which also suggest that NSAIDs exhibit time-dependent variability in their analgesic effect. Researchers have found that animals show better antinociceptive responses during their active phase, supporting the

pattern seen in the present experiment. These similarities provide further evidence that the timing of drug administration plays an important role in determining therapeutic efficacy.

Although a uniform dose of diclofenac was used, the differences in reaction time indicate that drug absorption, distribution, and interaction with pain pathways may vary according to biological timing. This highlights the relevance of chronotherapy, where the administration schedule of a

drug is aligned with the body’s natural rhythms to achieve maximum benefit.

The study does have certain limitations, including a small sample size and the use of only one acute pain model. Additional studies employing multiple doses, different pain models, and pharmacokinetic assessments would further clarify the relationship between circadian rhythm and

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diclofenac activity. Despite these limitations, the findings clearly demonstrate that the analgesic effect of diclofenac is influenced by dosing time and support the growing evidence that optimizing drug timing can enhance clinical outcomes.

CONCLUSION

Chronopharmacological studies demonstrate that the analgesic efficacy of diclofenac in albino mice is significantly influenced by the timing of administration. Morning and evening dosing produce different analgesic outcomes due to circadian variations in enzyme activity, receptor

sensitivity, and hormonal levels .The hot plate method serves as a reliable model for evaluating these time-dependent analgesic effects . Aligning drug administration with endogenous biological rhythms can optimize therapeutic efficacy, reduce adverse effects, and potentially contribute to

personalized pain-management strategies in humans . Future research and well-designed clinical trials are essential to validate these findings and support the integration of chronopharmacological principles into routine clinical practice .

Future Perspectives

Future studies will investigate the molecular and cellular mechanisms underlying the time-dependent variations in the analgesic effects of diclofenac [21,22]. Researchers will also explore circadian biomarkers to predict optimal dosing schedules for NSAIDs, aiming to enhance analgesic

efficacy and improve individualized therapy [23]. Preclinical findings obtained from albino mice will be translated into human clinical studies to support the development of personalized pain-management strategies [24,25].

Further, combination therapies involving diclofenac and other analgesic agents will be evaluated to improve therapeutic outcomes and minimize adverse effects [26,27]. Well-designed clinical trials will help integrate chronopharmacological concepts into routine pain-management protocols,

ultimately optimizing patient care and ensuring greater safety [28,23].

ABBREVIATIONS

Author Declare no Abbreviations

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FUNDING

Self funded

CONFLICT OF INTERESTS

Author declare no conflict of interests

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