Chronopharmacological Evaluation of Diclofenac in Albino Mice to Determine Effect of Dosing Time on Analgesic Efficacy Using Hot Plate Method.

Miss. Hrucha Gajanan Thubokar[1], Miss. Vaishnavi Prakashrao Joshi[2], Miss. Rupali Ramrao Londhe[3], Miss. Vaishali Vitthal Theng[4]., Miss. Saloni Chandrakant Pingle[5], Prof Dr. K.R. Biyani[6]
[1,2,3,4,5]*B. Pharm VIII Semester, Anuradha college of pharmacy, chikhli*
[6]*Guide, M. Pharm, Ph. D, DPPM, Anuradha college of pharmacy, chikhli*

***Abstract*—This study focuses on the chronopharmacological effects of diclofenac in albino mice using the hot plate method. Chrono pharmacology is the study of how biological rhythms, especially circadian rhythms, can affect the action, effectiveness, and safety of medicines. The purpose of this research was to evaluate whether the time of drug administration influences the analgesic activity of diclofenac, a commonly used non-steroidal anti-inflammatory drug (NSAID). In the experiment, diclofenac was administered orally to albino mice at different time intervals. The analgesic effect was determined by recording the reaction time of mice placed on a hot plate, which is a standard method for assessing pain response. Differences in reaction times at various dosing schedules were compared to understand the role of circadian rhythms in pain sensitivity and drug response. The data obtained were analyzed statistically to identify any significant differences between the treatment timings. The results of this study may help in understanding how the body's internal biological clock can influence the therapeutic effect of diclofenac.**

Overall, the study highlights the importance of chronotherapy, where medicines are given at the most suitable time of the day to improve efficacy and reduce unwanted side effects.

I. INTRODUCTION

Chrono pharmacology will represent a specialized discipline within pharmacology that will investigate the influence of biological timing and circadian rhythms on drug action and efficacy. Both humans and experimental animals will exhibit endogenous rhythmicity in numerous physiological and biochemical processes, including hormone secretion, enzymatic activity, thermoregulation, and nociceptive sensitivity. These temporal variations will exert a profound impact on the pharmacokinetic and pharmacodynamic profiles of therapeutic agents.

Diclofenac, which will be a widely utilized non-steroidal anti-inflammatory drug (NSAID), will be clinically employed for the management of pain and also inflammation. Its primary mechanism of action will involve the inhibition of cyclooxygenase (COX) enzymes, thereby attenuating the synthesis of prostaglandins that mediate pain perception and inflammatory responses. However, emerging evidence will suggest that the analgesic efficacy of diclofenac may fluctuate according to the time of administration, potentially due to circadian modulation of pain threshold and drug metabolism.

The present investigation will be designed to assess the chronopharmacological effects of diclofenac in albino mice by utilizing the hot plate method a well-established experimental paradigm that will evaluation.

II. CONCEPT OF CHRONO PHARMACOLOGY

Chrono pharmacology is the branch of pharmacology that explores the influence of biological rhythms particularly circadian rhythms, which follow an approximately 24-hour cycle on the pharmacological activity of drugs. These rhythmic variations can significantly modify several pharmacological processes, including drug absorption, drug distribution, drug metabolism, and elimination (collectively termed chronokinetics), as well as drug–receptor interactions and tissue responsiveness

(□hromodynamics). Furthermore, circadian oscillations affect various physiological parameters such as pain perception, hormone secretion, and body temperature, which may alter the body's response to pharmacological agents.

In mammals, circadian rhythms are primarily regulated by the hypothalamus, which acts as the central biological clock. The superachiasmatic nucleus (SCN) regulates and synchronizes peripheral clocks across the body and maintains synchronization with the light–dark cycle, ensuring temporal harmony between internal physiological functions and environmental cues. Understanding these temporal patterns provides a scientific basis for chronotherapy, wherein drug administration is aligned with biological rhythms to maximize therapeutic efficacy and minimize adverse effects**.**

III. DESCRIPTION OF DICLOFENAC

Diclofenac is classified as a non-steroidal anti-inflammatory (NSAID) medication belonging to the phenylacetic acid derivative class. It is extensively used for the management of pain, inflammation, and fever associated with various clinical conditions such as osteoarthritis, rheumatoid arthritis, ankylosing spondylitis, and postoperative pain. pharmacologically, Diclofenac exerts its therapeutic action primarily through the inhibition of cyclooxygenase (COX) enzymes, particularly COX-1 and COX-2, that catalyze the conversion of arachidonic acid into autapoid like prostaglandins and thromboxanes.

Diclofenac demonstrates high plasma protein binding and undergoes first-pass metabolism in the liver, primarily via cytochrome P450 enzymes (mainly CYP2C9). The resulting metabolites are subsequently conjugated and excreted through the renal and biliary pathways too. Its elimination half-life is relatively short, typically ranging between one and two hours, although sustained-release formulations have been developed to prolong therapeutic action.

Clinically, Diclofenac is available in multiple pharmaceutical forms, including oral tablets, topical gels, transdermal patches, suppositories, and parenteral preparations, allowing flexibility in administration based on the severity and nature of pain or inflammation. While generally well-tolerated, prolonged or excessive use may lead to gastrointestinal irritation, hepatotoxicity, renal impairment, and cardiovascular risks due to its effect on prostaglandin balance.

Overall, Diclofenac remains a cornerstone among NSAIDs due to its potent anti-inflammatory and analgesic properties, favorable efficacy profile, and wide therapeutic applicability. However, appropriate dosing and consideration of circadian influences may enhance its safety and therapeutic outcomes within the framework of chronopharmacological optimization.

IV. 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.

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.

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.

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.

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.

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.

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.

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.

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.

b. Complete Freund's Adjuvant (CFA) Model

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

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.

b. Spared Nerve Injury (SNI)

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

c. Sciatic Nerve Ligation

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

V. AIM AND OBJECTIVE

aim:

The present study will aim to evaluate the chronopharmacological effect of diclofenac on analgesic efficacy using the hot plate method in albino mice.

Objectives:

1. The study will aim to investigate the influence of dosing time on the analgesic activity of diclofenac.
2. It will compare the morning and evening dosing responses in mice.
3. It will analyze the variation in pain threshold according to the circadian rhythm.
4. It will identify the optimal dosing time for better therapeutic efficacy

Need of Project

This project will be needed to understand the influence of biological timing on pain management. Drug action will not only depend on its dose or type but also on the time of administration. Chronopharmacological studies will help to identify time-dependent variations in drug action, which will lead to improved therapeutic outcomes. The recent study will help to optimize the time of diclofenac administration to achieve better analgesic efficacy and minimize side effects.

VI. 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 "chrono pharmacology," "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.
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.

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.

VII. MATERIALS AND METHODS

a. Animal
1.Species: Albino mice
2.Weight: 20–30 grams
3.Sex: Either sex
4.Number of animals: 12(divided into 4 groups of 3 each)
5. Animals will be maintained under standard laboratory conditions like25 ± 2°C temperature 12 h light/dark cycle).
6. Standard pellet diet and water will be provided.

b. Chemicals
1.Drug: Diclofenac sodium (voveran 100 mg).
2.Vehicle: Normal saline.
3.Dose: Diclofenac will be administered at a dose of 10 mg/kg body weight of mice.

c. Equipments
1.Hot plate apparatus
2.Oral feeding cannula
3.Stopwatch
4.Weighing balance
5.Syringe

Evaluation of Analgesic Activity by using Hot Plate Method

1. Evaluate analgesic activity by using Hot Plate Method.
2. The hot plate temperature maintained at 55 ± 0.5°C.
3. The cut-off time will be fixed at 15 seconds to prevent injury.

VIII. EXPERIMENTAL DESIGN/PROCEDURE:

Chronopharmacological Evaluation of Diclofenac using Hot Plate Method in Albino Mice-
1. Dose Calculation
Standard dose of diclofenac (mice): 10 mg/kg (i.p./oral as per protocol)
Formula: Dose (mg) = Body weight (kg) × Dose (mg/kg)
Example:
Mouse weight = 25 g = 0.025 kg

So, each 25 g mouse requires 0.25 mg diclofenac

2. Preparation of Diclofenac Solution
Accurately weigh required amount of diclofenac sodium.
Dissolve in normal saline (0.9% NaCl) or suitable vehicle.
Prepare a known concentration (e.g., 1 mg/mL).
Example:
Dissolve 10 mg diclofenac in 10 mL saline → 1 mg/mL

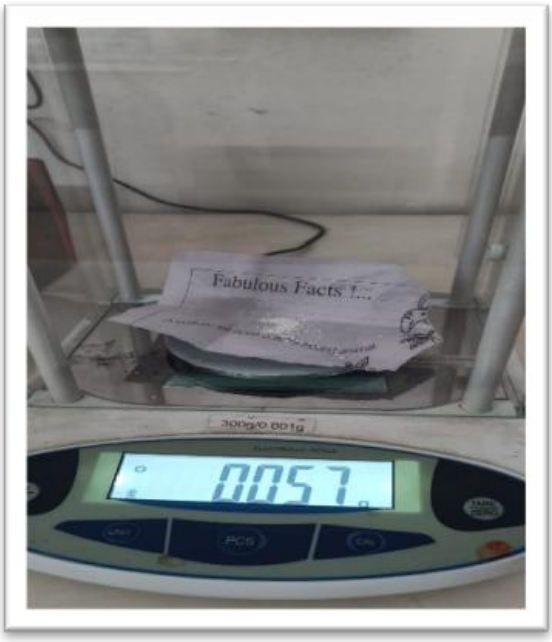


Fig no .1

3. Dose Volume Calculation
Volume to administer= Required dose (mg) /Concentration (mg/mL)
Example:
0.25/1=0.25ml
Each mouse receives 0.25 mL

4. Animal Grouping
Divide mice into groups (n = 3 or more per group):
Group 1 – 6:00 pm dosing
Group 2 – 12:00 am dosing
Group 3 – 6:00 am dosing
Group 4 – 12:00 pm dosing
Each group contains:
Control
Test (Diclofenac)
Vehicle

Fig no.2

5. Hot Plate Setup
Maintain hot plate temperature at 55 ± 1°C
Set cut-off time = 15 seconds (to avoid tissue damage)

Fig no .3 Eddy's hot plate aapratus

6. Baseline Reaction Time
Place each mouse on hot plate.
Record latency time (paw licking/jumping).
This is baseline reaction time (t_0).

Fig no .4

7. Drug Administration
Administer:
Diclofenac → Test group
Saline → Control
Vehicle → Vehicle group
Use appropriate route (i.p./oral)

Fig no .5

8. Observation Time Points
Record reaction time at:
30 minutes
60 minutes
90 minutes
120 minutes
Ensure same procedure each time.

9. Recording Response
Place mouse on hot plate
Record latency (in seconds)
Remove immediately after response or at cut-off time

10. Calculation of Analgesic Activity
Method, you used
a. (Baseline method):
% = (t-baseline time) / baseline time× 100
Where:
t = reaction time after drug
Example:
Baseline = 4.8 sec
After 60 min = 9.2 sec
% = (9.2-4.8)/4.8 x 100 = 91.7%

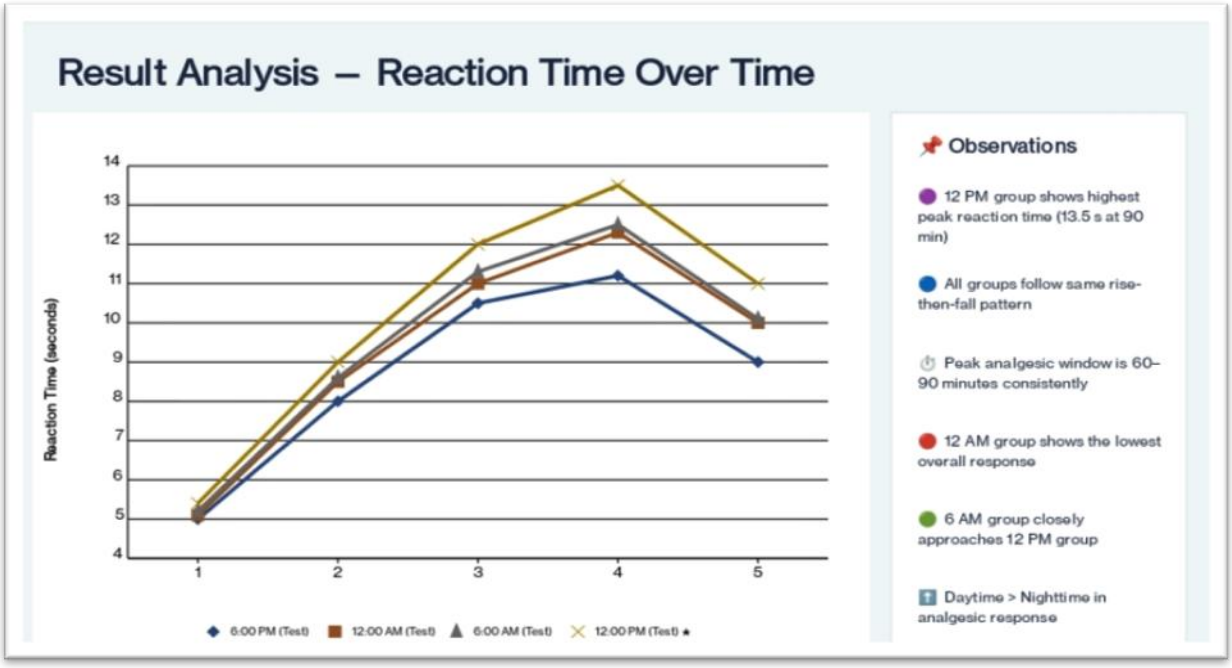
b. (cut-off formula)
% of cut-off time (15 sec) = t / cut-off time × 100
Example:
t=9.2 sec
cut-off time= 15 sec
(9.2/15) × 100 = 61.3%

11. Data Analysis
Compare:
Different time groups (chrono pharmacology)
Test vs control
Identify:
Peak effect time (usually 60–90 min)
Maximum effective dosing time (e.g., 12:00 pm)

12. Result Interpretation
Increased latency = analgesic effect
Higher % = greater efficacy
Compare across dosing times to determine circadian influence

Observation table:

Mice	Weight	Dose	Group	Time of administ-ration	Baseline reaction time	30 min	60 min	90 min	120 min	Cut-off time	30 min %	60 min %	90 min %	120 min %	Response
Group-1															
1	21.4	0.085	Control		4.8	7.5	9.2	10.1	8.4	15	50.0 %	61.3 %	67.3 %	56.0 %	Licking+Jumping
2	28.2	0.11	Test	6:00 pm	5.0	8.0	10.5	11.2	9.0	15	53.3 %	70.0 %	74.6 %	60.0 %	Jumping
3	30.2	0.12	Vehicle		5.2	8.3	10.8	12.0	9.5	15	55.5 %	72.0 %	80.0 %	63.3 %	Licking
Group-2															
1	29.1	0.10	Control		4.9	7.8	9.6	10.4	8.6	15	52.0 %	64%	69.3 %	57.3 %	Licking
2	30.7	0.12	Test	12:00am	5.1	8.5	11.0	12.3	10.0	15	56.6 %	73.3 %	82.0 %	66.6 %	Jumping
3	34.0	0.13	Vehicle		5.3	8.9	11.5	13.0	10.5	15	59.3 %	76.6 %	86.6 %	70.0 %	Licking+jumping
Group-3															
1	29.7	0.11	Control		5.0	8.1	10.2	11.4	9.2	15	54.0 %	68.0 %	76.0 %	61.3 %	Licking
2	31.6	0.12	Test	6:00 am	5.2	8.6	11.3	12.5	10.1	15	57.3 %	75.3 %	83.3 %	67.3 %	Jumping
3	25.0	0.10	Vehicle		4.7	7.6	9.8	10.9	8.8	15	50.6 %	65.3 %	72.6 %	58.6 %	Licking
Group-4															
1	29.9	0.11	Control		5.1	8.2	10.6	11.8	9.4	15	54.6 %	70.6 %	78.6 %	62.6 %	Jumping
2	38.0	0.15	Test	12:00pm	5.4	9.0	12.0	13.5	11.0	15	60.0 %	80.0 %	90.0 %	73.3 %	Licking+jumping
3	36.8	0.14	Vehicle		5.3	8.8	11.6	13.2	10.8	15	58.6 %	77.3 %	88.0 %	72.0 %	Jumping



Result:

The chronopharmacological study of diclofenac in albino mice was performed using the hot plate method at different administration times (6:00 pm, 12:00 am, 6:00 am, and 12:00 pm). The analgesic effect was calculated as the percentage increase in reaction time compared with the baseline value. The diclofenac-treated groups showed a clear increase in reaction time when compared with the control and vehicle groups at all observation periods, indicating significant analgesic activity.

A variation in response was observed depending on the time of administration. At 6:00 pm, diclofenac produced a moderate analgesic effect, with the highest response seen between 60 and 90 minutes after dosing. At 12:00 am, the analgesic effect was lower than other dosing times, although the reaction time increased gradually over the study period.

At 6:00 am, the drug produced a better analgesic response than the midnight dose, suggesting greater efficacy during the early morning hours. The highest analgesic activity was recorded at 12:00 pm, where the percentage increase in reaction time was maximum among all treatment groups.

In all dosing schedules, the peak analgesic effect of diclofenac was generally observed between 60 and 90 minutes after administration, followed by a slight reduction at 120 minutes.

The control and vehicle groups showed only minor changes in reaction time, confirming that the increase in pain threshold was mainly due to diclofenac treatment.

Key Findings:

Diclofenac demonstrated significant analgesic activity in albino mice.

The analgesic response varied with dosing time, showing chronopharmacological influence.

Maximum analgesic effect was observed at 12:00 pm.

Minimum analgesic effect was noted at 12:00 am.

Peak action occurred between 60 and 90 minutes after administration.

IX. CONCLUSION

The present study concludes that diclofenac shows a significant time-dependent variation in analgesic activity in albino mice. The highest analgesic effect was observed when the drug was administered during daytime at 12:00 pm, while the lowest effect was noted during midnight dosing at 12:00 am.

The maximum analgesic response was generally seen between 60 and 90 minutes after administration. These findings indicate that the effectiveness of diclofenac is influenced by the time of dosing.

Overall, the study emphasizes the importance of chronopharmacological principles in optimizing drug therapy. Proper timing of diclofenac administration may improve its analgesic effect and lead to better therapeutic outcomes.

Discussion:

The present study showed that the analgesic effect of diclofenac changes according to the time of administration when evaluated by the hot plate method in albino mice. The percentage increase in reaction time, calculated from the baseline value, indicated that the pain-relieving activity of diclofenac is influenced by biological timing.

Diclofenac is a non-steroidal anti-inflammatory drug (NSAID) that produces analgesic effects mainly by inhibiting cyclooxygenase (COX) enzymes, which leads to reduced prostaglandin synthesis. Since prostaglandins and pain sensitivity are known to vary with circadian rhythms, the differences observed in this study may be related to normal physiological changes occurring throughout the day.

The highest analgesic response was observed at 12:00 pm. This may be due to greater metabolic and physiological activity during daytime hours, which can improve drug absorption, distribution, and response. In addition, sensitivity of pain receptors and central nervous system processing may be higher during this period.

The lowest response was noted at 12:00 am. This reduced effect may be associated with slower metabolic activity during the night, changes in pharmacokinetic and pharmacodynamic processes, and lower responsiveness of pain pathways. Another important finding was that the maximum analgesic activity was generally seen between 60 and 90 minutes after drug administration in all treatment groups. This suggests that diclofenac may reach its optimum effective concentration during this time interval. The control and vehicle groups showed only minor changes in reaction time, confirming that the increase in pain threshold was mainly due to diclofenac treatment rather than random experimental variation.

Overall, the findings support the concept of chrono pharmacology, which states that the effectiveness of drugs can vary depending on the body's internal biological clock. These results may help in planning better dosing schedules to improve therapeutic outcomes.

ACKNOWLEDGEMENT

This is to acknowledge and express our deepest gratitude to Principal Dr. K R Biyani sir our project guide for his most valuable guidance during the course of project work, without which it would have been difficult, rather impossible task for me. He provided valuable motivation, constructive suggestions and precious time during the course of my dissertation work. His guidance and encouragement sustain all a long and helped me greatly in this work. We consider it would be our fortune and honour to have been given opportunity to work under guidance Of Dr. K R Biyani Sir.

REFERENCES

[1] R. H. P. Rang, M. M. Dale, J. M. Ritter, R. J. Flower, and G. Henderson, Rang and Dale's Pharmacology, 9th ed. Elsevier, 2020.

[2] K. D. Tripathi, Essentials of Medical Pharmacology, 9th ed. New Delhi, India: Jaypee Brothers Medical Publishers, 2018.

[3] L. L. Brunton, R. Hilal-Dandan, and B. C. Knollmann, Eds., Goodman & Gilman's The Pharmacological Basis of Therapeutics, 14th ed. New York, NY, USA: McGraw-Hill Education, 2023.

[4] A. Reinberg and M. H. Smolensky, "Chrono pharmacology and chronotherapeutics," in Goodman & Gilman's The Pharmacological Basis of Therapeutics, 14th ed., L. L. Brunton, R. Hilal-Dandan, and B. C. Knollmann, Eds. New York, NY, USA: McGraw-Hill Education, 2023.

[5] M. H. Smolensky and N. A. Peppas, "Chronobiology, drug delivery, and chronotherapeutics," Advanced Drug Delivery Reviews, vol. 59, nos. 9–10, pp. 828–851, 2007, doi: 10.1016/j.addr.2007.07.001.

[6] R. A. Turner, Screening Methods in Pharmacology. New York, NY, USA: Academic Press, 1965.

[7] N. B. Eddy and D. Leimbach, "Synthetic analgesics. II. Dithienylbutenyl- and dithienylbutylamines," Journal of Pharmacology and Experimental Therapeutics, vol. 107, no. 3, pp. 385–393, 1953.

[8] R. Dallmann, S. A. Brown, and F. Gachon, "Chrono pharmacology: New insights and therapeutic implications," Annual Review of Pharmacology and Toxicology, vol. 54, pp. 339–361, 2014, doi: 10.1146/annurev-pharmtox-011613-135923.

[9] B. G. Katzung, T. W. Vanderah, and S. B. Masters, Basic and Clinical Pharmacology, 16th ed. New York, NY, USA: McGraw-Hill Education, 2021.

[10] G. J. Tortora and B. Derrickson, Principles of Anatomy and Physiology, 16th ed. Hoboken, NJ, USA: Wiley, 2021.

[11] R. Altman, B. Bosch, K. Brune, P. Patrignani, and C. Young, "Advances in NSAID development: Evolution of diclofenac products using pharmaceutical technology," Drugs, vol. 75, no. 8, pp. 859–877, 2015, doi: 10.1007/s40265-015-0392-z.

[12] N. M. Davies and K. E. Anderson, "Clinical pharmacokinetics of diclofenac: Therapeutic insights and pitfalls," Clinical Pharmacokinetics, vol. 33, no. 3, pp. 184–213, 1997, doi: 10.2165/00003088-199733030-00003.

[13] M. N. Ghosh, Fundamentals of Experimental Pharmacology, 3rd ed. Kolkata, India: Hilton & Company, 2005, pp. 153–158.

[14] G. Labrecque and P. M. Bélanger, "Biological rhythms in the absorption, distribution, metabolism and excretion of drugs," Pharmacology & Therapeutics, vol. 52, no. 1, pp. 95–107, 1991.

[15] W. A. Ritschel and H. Forusz, "Chrono pharmacology: A review of drugs studied," Methods and Findings in Experimental and Clinical Pharmacology, vol. 16, no. 1, pp. 57–75, 1994.

[16] G. Kaur, D. Singh, G. Singh, and B. Singh, "Chronotherapeutics: A chronopharmaceutical approach to drug delivery in the treatment of asthma," Journal of Controlled Release, vol. 324, pp. 204–224, 2020.

[17] S. C. Sweetman, Ed., Martindale: The Complete Drug Reference, 39th ed. London, U.K.: Pharmaceutical Press, 2017.
[18] H. G. Vogel, Drug Discovery and Evaluation: Pharmacological Assays, 4th ed. Berlin, Germany: Springer, 2008.
[19] X. M. Li, F. Delaunay, S. Dulong, B. Claustrat, S. Zampera, Y. Fujii, et al., "Cancer chronotherapeutics: Experimental, theoretical, and clinical aspects," in Handbook of Experimental Pharmacology, vol. 217, pp. 261–288, 2013.
[20] M. Baraldo, "The influence of circadian rhythms on the pharmacokinetics of drugs in humans," Expert Opinion on Drug Metabolism & Toxicology, vol. 4, no. 2, pp. 175–192, 2008.