TRNC

NEAR EAST UNIVERSITY

INSTITUTE OF HEALTH SCIENCES, DEPARTMENT OF PHARMACOLOGY

Assessment of Awareness and Attitudes of Community Pharmacists in Northwest Nigeria towards Chronotherapy

A THESIS SUBMITTED TO THE GRADUATE INSTITUTE OF HEALTH SCIENCES NEAR EAST UNIVERSITY

BY

UGOCHUKWU CHIDIEBERE CHUKWUNYERE

In Partial Fulfillment of the Requirements for the Degree of Master of Science in Pharmacology

NICOSIA 2017

TURKISH REPUBLIC OF NORTHERN CYPRUS

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Master of Science in Pharmacology

Advisor

Prof. Dr. Nurettin Abacıoğlu

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DEDICATION

I dedicate this work to Almighty God for giving me the knowledge, wisdom and strength. So also, to my father, mother, siblings and my friends for believing in me and giving me the well deserved encouragement.

Approval

Thesis submitted to the Institute of Health Sciences of Near East University in partial fulfillment of the requirements for the degree of **Master of Science in Pharmacology.**

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LIST OF ABBREVIATIONS

S/No.	Abbreviations	Meaning
1.	5FU	5-Fluorouracil
2.	AMI	Acute Myocardial infarction
3.	ACEI	Angiotensin-converting enzyme inhibitor
4.	AUC	Area Under the Curve
5.	BP	Blood Pressure
6.	cAMP	cyclic Adenosine MonoPhosphate
7.	cGMP	cyclic Guanosine MonoPhosphate (cGMP)
8.	DBP	Diastolic Blood Pressure
9.	DHC	Dihydrocodeine
10.	ECG	Electrocardiogram
11.	EC	Ethylcellulose
12.	FDA	Food and Drug Administration
13.	HMG Co-A	Hydroxymethylglutaryl- Coenzyme A
14.	HPMC	Hydroxypropyl Methylcellulose
15.	IFN-β	Interferon-β
16.	IRI	Immuno-reactive insulin
17.	ISDN	Isosorbide dinitrate
18.	ISMN	Isosorbide mononitrate
19.	NO	Nitric Oxide
20.	PK	Pharmacokinetics
21.	PE	Pulmonary Embolism
22.	PSVT	Paroxysmal Supra Ventricular Tachycardia
23.	SBP	Systolic Blood Pressure
24.	T1DM	Type 1 diabetes mellitus
25.	T2DM	Type 2 diabetes mellitus
26.	VPB	Ventricular Premature Beats
27.	WHO	World Health Organization

ABSTRACT

Chronotherapy refers to the time of administration of medications in synchronization with the body's circadian rhythm in order to optimize the therapeutic effects while minimizing its adverse effects. Although good practice and application of chronotherapy can optimize therapeutic effects, its benefit can be compromised by poor knowledge and attitudes towards the application of principles of chronotherapy during patient management and care. The aim of this study was to assess the awareness and attitudes towards chronotherapy among community pharmacists practicing in Northwest region of Nigeria.

Data was collected using a modified questionnaire based on current literature. 77 out of 125 community pharmacists that were included in this study were male (61%). Data analysis showed that most of the participants were between the ages of 26 and 30 years (42.4%), with years of practice below 6 years (56.8%). Results showed that awareness was influenced by age and years of practice. Mean total awareness and attitude scores were 8.49 ± 1.38 and 46.19 ± 5.92 respectively. Spearman's rank-order correlation showed a statistically significant ($p= 0.015$) positive correlation ($r=0.216$) between awareness and attitude.

The findings from this study highlight the need for a comprehensive chronotherapy-related educational program for community pharmacists practicing in this region.

Keywords – Chronotherapy, circadian rhythm, community pharmacists, medications.

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1. Introduction.

1.1 Overview

There have been increased interest in understanding the molecular regulatory systems that connect circadian components and, more importantly, in understanding how the output signals triggered by circadian rhythms maintain the body's physiology in accordance with its evolving environment. These discoveries have yielded broad advances in several aspects of science, health, and medicine, including an important molecular foundation with regards to chronotherapy, in which medications are administered at times most likely to yield the greatest efficacy.

Chronotherapy refers to the administration of medications in synchronization with the body's circadian rhythms in order to optimize the therapeutic effects and/or minimize adverse effects (Kaur et al., 2016). Circadian rhythms are daily cycles of physiological and behavioral processes endogenously generated by an organism that can be externally modulated by cues such as light, temperature, and food intake (Dibner et al., 2010). It has been reported that circadian rhythms is relevant to human diseases and is associated with altered insomnia and sleep syndromes (Huang et al., 2011), jet-lag (Waterhouse et al., 2007), allergies and asthma, cardiovascular diseases and stroke (Portaluppi et al., 2012), hypertension (Shea et al., 2011), metabolic diseases (Masri et al., 2013), neurological and psychiatric disorders (Kronfeld-schor and Einat, 2012), and hormone-dependent cancers (Markt et al., 2015; Monsees et al., 2012).

Several studies have established that circadian rhythms influence both effectiveness and toxicity of many medicines, for example acetaminophen (Kamali et al., 1987) and theophylline (Watanabe et al., 1984) show different pharmacokinetics in the morning compared to evening

and also, therapeutic outcome of drugs maybe improved following medication administration at certain times of the day (Kaur et al., 2013; Hermida et al., 2011; Hassan and Haefeli, 2010; Smolensky et al., 2015; Lemmer, 2005). The concept of homeostasis, where the internal physiological environment of the human body is maintained at a relatively constant state, is one familiar area of study among health professionals in clinical practice. The homeostatic systems are actively regulated by a multifunctional timer called circadian clock which is controlled by the human suprachiasmatic nuclei over a period of 24 h cycle.

For several drugs, a twenty-four hour change in drug bioavailability has therefore been set up and these changes are the outcome of several time-dependent modifications of both physiological and molecular factors that influence drug absorption and distribution (Dallmann et al., 2014). Desired effects or side effects of medications are dependent on several factors including physio-chemical properties of the compound, dosage, its pharmacokinetics, and particularly on its pharmacodynamics (Baydar and Erkekoglu, 2009). Since it has been reported that pharmacodynamics is affected by circadian variability, circadian rhythm should be taken into consideration before drug administration in order to prevent the temporal variations in the mode of action.

Therefore, this study is aimed at assessing the awareness and attitudes of community pharmacists in Northwest Nigeria toward Chronotherapy. This study will identify if community pharmacists in Northwest Nigeria incorporate the principles of Chronotherapy into clinical practice, since a sound knowledge and deeper understanding of pharmacokinetics, pharmacodynamics and therapeutic effects and safety which all amount to the principle of Chronotherapy are invaluable in patient counseling and optimizing patients' pharmacotherapy.

2. Background

Chronotherapy can be described as the therapeutic application of chronopharmacology and it covers several specific principles that relate to the type of disease being treated, the patient's physiology, and the efficacy and tolerability that the patient exhibits to a certain medication considering the biological timing and endogenous periodicities. The goal of chronotherapy is aimed at optimizing the therapeutic outcomes and minimizing the adverse effects by ensuring that a drug is made available particularly at a time that correspond with the rhythms of the disease (Kaur et al., 2013). Several aspects of pharmacology are considered to be time-of-day-dependent, including (1) the numerous activities of molecules which are involved in drug absorption and metabolism (considered under chronokinetics); (2) the physiological system targeted by the therapeutic (considered under chronodynamics); (3) the controlled-release formulations (considered under chronomodulated delivery and chronoformulation); and (4) the effectiveness of a therapeutic (considered under chronoeffectiveness) (Dallmann et al., 2014). Also, since toxicological data is vital to the objectives of chronotherapy, chronotoxicology considers the harmful reactions and unfavorable impacts of active substances and in this manner, characterizes ideal circumstances at which drugs should be administered to improve both effectiveness and tolerance (Kaur et al., 2013).

To expand the remedial advantage and decrease the adverse effects of drugs, checking of markers, for example, clock genes may be valuable to choose the most suitable time of day for administration of drugs. Some chronotherapeutic parameters for clinical studies suggested by Food and Drug Administration (FDA) are as follows:

(1) Drug administration time of the day.

(2) Patient's normal habits and sleep patterns.

(3) Biological factors which are time-related.

The impact of circadian rhythms on pharmacological effects of medications has turned into a major test for drug discovery and drug delivery researchers. In recent years, Pharmaceutical industries have thought of different innovations, for example, time-controlled, pulse, triggered and modified drug delivery gadgets for delivery of synchronized drugs to rhythms in disease states.

2.1 Chronopharmacology

Chronopharmacology is the study of how the effects of drugs vary with biological timing and endogenous periodicities. Chronopharmacologic phenomena can be viewed as resulting from an adaptation of the organism to cyclic environmental changes in 24 hours and the toxicity of xenobiotics is modified by the chronicity of cellular activity of organs (Mazzoccoli, 2011; Kalsbeek et al., 2011). The circadian pharmacokinetics and especially pharmacodynamics that modify drug effectiveness and toxicity are signs of the circadian control of xenobiotic detoxification and could have vital clinical applications since they permit treatment alteration with a specific end goal to increase efficacy and safety and then, minimize side effects. Nevertheless, studies excluding the timing of dosing may be as a result of the reports that discovered several carcinogenic substances (Mazzoccoli, 2011)

2.2 Coordinating drug pharmacokinetics with circadian rhythms

Certain group of organ systems like intestine, hepatobiliary, and renal, have rhythmic physiology that determines the viability of medication absorption, distribution, metabolism, and excretion

and, subsequently, its blood concentration and bioavailability (Dallmann et al., 2014). Rational molecular and biochemical information support the presence of a gut clock that regulates time-dependent gut physiology and, thus, impacts the absorption and distribution of drugs (Dallmann et al., 2014; Musiek and Fitzgerald, 2013). Accordingly, clock mutant mice which carry specific disruptions in circadian genes show a serious deregulation in the absorption of nutrients that is accompanied by disturbances in circadian activity (Pan and Hussain, 2009).

Absorption of orally administered drugs in the gastrointestinal tract is dependent on some key factors, which include (1) the chemical properties of the drug (i.e., hydrophobicity and degree of ionization as pH changes); (2) the rhythmic physiology of the gastrointestinal tract (i.e., circadian changes in gastric pH (Bron and Furness, 2009), gastrointestinal blood flow (Lemmer and Nold, 1991), gastric emptying time and motility (Goo et al., 1987; Kumar et al., 1986) and (3) the expression of unique transporters on epithelial cells of the gut (Ando et al., 2005; Stearns et al., 2008). Several animal models and human clinical studies that evaluated various pharmacokinetic parameters revealed that circadian variation exists in the patterns of absorption for lipophilic drugs and water-soluble compounds (e.g., maximum [peak] drug concentration [C_{max}], time to reach C_{max} following drug administration [t_{max}], and area under the plasma concentration–time curve [AUC] (Musiek and Fitzgerald, 2013; Baraldo, 2008). Therefore, rapid absorption of most lipophilic compounds is observed in the morning than in the evening hours in humans as some factors that aides it's absorption are simultaneously favored, thus, resulting to higher C_{max} and shorter t_{max} values during morning administration (Lemmer and Nold, 1991; Baraldo, 2008). On the contrary, circadian variation is not observed in water-soluble compounds, thus, these compounds show a steady absorption throughout the day (Baraldo, 2008; Sukumaran et al., 2010)].

Studies involving healthy volunteers have revealed the benefits of the method for delivering lipophilic compounds (e.g., immediate vs. sustained release, intravenous) and administration time in the pharmacokinetics of absorption. As the concept of bioavailability, defined as the extent and rate at which a substance becomes available at the site of action, relates to absorption, formulations of lipophilic drugs with immediate release have shown a circadian phase dependency on their action that has not been observed when using sustained-release formulations or after intravenous inoculation. Consequently, absorption of immediate-release preparations of, for example, nifedipine (Lemmer and Nold, 1991), cyclosporine (Baraldo et al., 2003) and non-steroidal anti-inflammatory drugs (Levi and Schibler, 2007) peak in the early morning (e.g., 8 a.m., 22.5 min t_{max} for nifedipine [peroral administration]; 6 a.m. for acetaminophen [peroral administration]; beginning of the active period for cyclosporine (Ritschel and Forusz, 1994) for which drug bioavailability is greater than at night).

Effective drug distribution is to a great extent subject to its lipophilicity, affinity, and abundance of carrier proteins in the blood flow. These factors affect a drug's efficacy and the magnitude of adverse effects since it has been reported that unbound drug does not distribute within the target site but accumulates in unwanted tissues. The circadian time-dependent function of the autonomic nervous system impacts the daily alterations in blood flow- daytime increase and night-time decline (Anderson et al., 1999). Therefore, blood flow plays a role in the variation of drug distribution observed when dosing takes place at different times (Innominato et al., 2010).

The circadian variation in the level of plasma proteins is indicative of the great extent of the fluctuations in metabolic activity in the liver. Examples include albumin, globulins, and α-glycoproteins, whose concentrations are elevated during the daytime, peak at noon, and fall to a minimum at night (Scheving et al., 1968). Therefore, the expected diurnal variation in the drug-

protein binding is dependent on the carrier molecule in the plasma (Ritschel and Forusz, 1994; Lemmer and Bruguerolle, 1994). While this hypothesis has been shown to work in clinical settings for different therapeutic agents- antiepileptic, chemotherapeutics, and anti-inflammatory drugs (Erkekoglu and Baydar, 2012), other drugs with more specific carriers show peak binding at night example, carbamazepine peak at approximately 8 p.m, as evaluated in patients with epilepsy not responding to conventional dosing schedules (Dallmann et al., 2014) or early afternoon (e.g., 5-fluorouracil [optimal timing varies according to sex and genetic background], as evaluated in patients with metastatic colorectal cancer receiving standard dosage (Beaver et al.,2010).

Unlike the case of hydrophobic molecules, distribution of water-soluble drugs in tissues requires the expression of transporters and channels that allow for transit to occur. In this scenario, drug distribution depends on the circadian expression of specific transporters (Goo et al., 1987; Kumar et al., 1986). Beyond distribution, there is a point at which the dose becomes toxic to consider when the therapeutic treatment takes place at a constant rate over a 24-h period.

The liver is primarily the site for xenobiotic detoxification, although there are other extrahepatic systems (e.g kidney, lungs) which are involved in a two-phase detoxification process in which drugs undergo chemical modification (phase 1) and then are conjugated to aid its excretion into bile, feces, sweat, urine (phase 2). Several studies have reported that a circadian oscillatory pattern of expression in most genes encoding members of the microsomal cytochrome P450 family of enzymes involved in phase I, including other enzymes relevant to phase II (e.g epoxide hydrolases, oxidoreductases and sulfo-, glutathione-S-, UDP-glucuronosyl-, methyl-, and acetyl-transferases) (Panda et al., 2002; Storch et al., 2002; Akhtar et al., 2002.).

The excretion of metabolized drugs via bile and urine depends on the ability of the kidney to reabsorb or secrete drugs across the epithelium and the rate of drug elimination and efficiency depends on two important factors: (1) renal blood flow and (2) glomerular filtration rate. The renal blood flow is to a great extent responsible for daily elimination of most ionized drugs into urine with a peak of secretion during the active phase; On the contrary, the glomerular filtration rate happens to be a steady process that prevails even during the resting phase (Koopman et al., 1989). The CRY1/CRY2 knockout animals were used to prove that clock components play a role in regulating the renal rhythmic elimination of metabolites. It has been reported that CRY knockout mice show an abnormal renal blood flow which is most likely to be as a result of a disruption in renin-angiotesin-aldosterone system and to a lesser extent, swaying arterial blood pressure and cardiac output (Doi et al., 2010).

In addition, the expression of the sodium-proton exchanger 3 framework, vital for the daily variations in urine pH (from 4.5 to 8.0), is annulled in the proximal tubule of CRY knockout animals, showing the clock's control of drug elimination by means of urine acidification. In the proximal tubule of the kidney, reabsorption/secretion of water-soluble drugs and small organic ions is accompanied by vigorous diurnal expression of membrane transporters (Zuber et al., 2009).

2.3 Hierarchical Organization of Clocks

The numerous hierarchical levels have to be considered, from molecular and cellular to tissue and organ, at which drugs can act while evaluating their biochemical and physiological effects on the body.

This idea, at first grasped within the fundamental principles of pharmacodynamics, now considers that various cells from various organs have distinctive periodicities in the activities of their biochemical procedures (from transcription/translation to metabolism) and in the transient organization of their signal transduction cascade. However, the concept of chronopharmacodynamics appears to be suited to describe how circadian oscillations in a physiological framework impacts therapeutic outcomes.

Among the earliest biological processes to be pharmacodynamically grouped are the circadian variations in parameters related to ligand receptor interaction and signaling. Findings resolved the presence of diurnal variations in potency (linked with receptor occupancy), efficacy (linked with intracellular signaling and tissue response), sensitivity (linked with receptor binding) (Reinberg, 1992), and the level and action of downstream signaling segments activated by ligand–receptor binding including enzymes, metabolites, and even second messengers (Musiek and Fitzgerald, 2013).

Today, knowledge of the chronopharmacodynamic actions of cardiovascular medications, chemotherapeutic agents, analgesic and non-steroidal anti-inflammatory drugs, antidepressants, anxiolytics, and antipsychotic agents has presented various advantages to patients with different clinical conditions.

2.4 Chronotherapy of cardiovascular diseases

From several reviews, it is established that the numerous cardiovascular conditions including myocardial infarction, stroke and sudden death occur amid the early hours of the day between 6 AM and 12 noon. Blood pressure rises rapidly in the early morning hours, the time when most people wake and start their day. This rise in blood pressure is believed to be associated with

increased secretion of catecholamine's and increased plasma rennin activity (Hofstra et al., 2008). Hence, vascular tone and total peripheral resistance increase in the morning hours, with a corresponding increase in blood pressure.

The distinctions in patterns of illness at different times of the day for cardiovascular diseases, for example, hypertension, angina, heart attack, sudden cardiac death and stroke have been reported. Chronotherapeutic approach gives more precise assurance of the time when patients are most vulnerable and in greatest need of treatment. For instance, it has often been revealed that the blood pressure of hypertensive patient rise rapidly after awakening in the morning, then peaks in middle to late time of the day, decreases at night and is most minimal while the patient sleeps at night. Platelet aggregation increases and fibrinolytic activity declines in the morning, prompting a condition of relative hyper coagulability of the blood

2.4.1 Various cardiovascular diseases

Blood pressure/hypertension

Various internal factors affect the 24 h variation in blood pressure and some of these factors include: the autonomic nervous system, vasoactive intestinal peptide, plasma cortisol, plasma rennin activity, aldosterone, plasma atrial natriuretic peptide. It has also been reported that both sympathetic activity and the rennin-angiotensin–aldosterone activity peak in the early morning hours. Furthermore, blood pressure is influenced by a variety of external factors including physical assertion, emotional/psychological state, and food and sleep/wake schedule. These extrinsic stimuli also influence the autonomic nervous system hence the 24 h variation in the blood pressure is a representative of both internal diurnal rhythms and external factors (Patel et al., 2011). Blood pressure is described by a circadian rhythm, both in hypertensive and in

normotensive subjects; this pattern is linked with lower blood pressure levels during sleeping time and times of minimal activity and higher blood pressure levels amid wakefulness and mental and physical assertion (Lemmer, 2006; Prisant, 2001; Douglas, 2002; Prisant, 2004). Blood pressure fluctuations may be short or long and the most identified and significant blood pressure variations are the diurnal changes related to the sleep-wake cycle. The pattern of blood pressure values obtained during the sleep-wake cycle from characteristic circadian rhythm showed a pattern with minimum values of systolic and diastolic pressure between midnight and 4 am. The pressure rises during waking hours then maintain a plateau for several hours, before reaching a maximum values early in the morning. Certain disease conditions, such as preeclampsia and chronic hypertension alter the diurnal blood pressure fluctuation (Larry et al., 2000).

Hypertension is a typical chronic condition and also an important risk factor for strokes, heart attacks and other vascular and renal disease. The Pharmacological management of high blood pressure reduces the incidence of these complications and prolongs life. The treatment of hypertension not only includes the usual clinical goal of reducing mean blood pressure levels, but also the normalization of the entire blood pressure circadian pattern. The anticipated day-night variation in the symptoms of chronic medical conditions, risk of extreme life threatening cardiovascular conditions and in medical conditions that are inclining to serious disease presents the need for Chronotherapeutic treatment technique that includes the delivery of medications in synchronization with the biological timing which varies according the chronobiology of the targeted tissue. Several antihypertensive medications do not have any effect on the early morning blood pressure when given once daily early in the morning (Patel et al., 2011).

Presently, once daily extended release antihypertensive medication provide safe and viable blood pressure reduction over a 24 hour interval yet their static pattern of drug release may not be designed to suit daily physiologic blood pressure variations. A Chronotherapeutic calcium channel blocker is currently available in the market for the management of certain cardiovascular conditions (Pandit and Suresh, 2009).

Acute myocardial infarction/ pulmonary embolism

It is notable that acute myocardial infarction (AMI) and pulmonary embolism occurs frequently in the early morning. Various physiological functions show diurnal variation including blood pressure (BP), heart rate, coronary blood stream, platelet work, blood coagulability and fibrnolytic activity. In the early morning, the increase in systemic BP and heart rate augment the oxygen demand of the heart and also, the vascular tone of the coronary artery rises and coronary blood flow diminishes in the morning. This rise in oxygen demand and decrease in oxygen supply overstate a imbalance between oxygen demand and supply in the morning. Likewise, platelet functions and blood coagulability increases in the morning. A hypercoagulable state resulting from a reduction in fibrinolytic activity could evoke the morning onset of thromboembolic conditions. Several reports suggest that the autonomic nervous system play an important role in the circadian variation of onset of acute myocardial infarction. Diabetic patients with autonomic nervous system dysfunction do not experience a morning increase in the frequency of ischemic episodes and patients receiving beta-blocker do not show morning increase in the incidence of angina, AMI and sudden death.

A variation in heart rate following a balance in sympathetic/vagal activity is also associated with the onset of ischemic episode in the chronic stable angina. Platelets do not play any role in the

variation of AMI or thromboembolic numbers and their aggregation activity have circadian oscillation. Platelet activation in vivo is instigated by catecholamines secreted from the sympathetic sensory system in a circadian manner. However studies with respect to platelet activation do not present clear circadian expression of any surface marker typical of platelet activation, hence it is uncertain whether the internal clock systems have a direct effect on the circadian functions of the platelets (Patel et al., 2011).

Arrhythmia

Several reports have shown the presence of circadian variation of cardiac arrhythmia. Data suggest that fundamental electrophysiological parameters have circadian variations. Atrial and ventricular refractory periods are strongly influenced by the autonomic nervous system, in which sympathetic activity shortens it and parasympathetic action prolongs the period. Accordingly, fluctuations in the activity of autonomic nervous system within a day can be the main trigger of circadian onset of cardiac arrhythmia. Every parameter of electro-cardiogram (ECG) was studied to determine the presence of diurnal variation and reported that ECG, AV nodal work, QT interim, R&T wave voltage and QT interval, show circadian variation. For the onset of cardiac arrhythmia, paroxysmal atrial fibrillation is classified into two:

(1) Vagatonic platelet activation factor (PAF) which occurs at night time and

(2) Adrenergic PAF which occurs during day time.

There are a few reports indicating distinctive outcomes in term of peak paroxysmal supra ventricular tachycardia (PSVT) from morning to midnight. Nevertheless, they are steady in that it is uncommon for PSVT to happen at night. Nonstop monitoring of ECG disclosed a 24 h variation in the event of ventricular premature beats with a peak between 6 am and 12 noon. The

presence of a circadian onset of ventricular premature beats (VPBs) is dependent on the left ventricular capacity. It has been reported that only patients with a left ventricular ejection fraction above 30 % have a circadian variation of VPBs (Patel et al., 2011).

2.5 Chrono-endocrinology

The endocrine system which is rhythmic at different frequencies, have an exceptional complex time structure and it's able to interact with its distinctive components signaling mechanisms and the environment. The effect and efficacy of a hormone or any messenger at different sites is determined by the rhythmic variations; hence, depending on the hormone-receptor interaction and the rhythmic determinants of the response of the target tissue at any given time, the hormonal stimulus at anytime may exert opposing effects or no effect (Sanchez, 1993). This theoretical viewpoint is basic to the design of drug-delivery systems for the chronotherapy of endocrine and other disorders. It is important to take into consideration the body's biological time structure in the design of agents especially neuropeptides, hormones, cytokines and other agents that modify the oscillating systems. By designating the ideal time to accomplish the desired impact, treatment possibilities may emerge, and unwanted side-effects minimized (Erhard, 2007).

Pulsatile administration of drugs in the high or ultradian frequency range that mimics normal biological oscillatory systems may boost a drug's effectiveness and the rise and fall of drug levels given in a time varying manner may also avoid the side effects encountered with continuous dosing in the therapeutic range (Erhard, 2007).

The choice of drug delivery time for a given clinical condition can affect treatment efficacy and safety and this can be clearly illustrated e.g alternate day and/or morning dosing of

glucocorticosteroids in children and adults requiring anti-inflammatory steroid therapy to avoid adrenal suppression and other adverse effects, the afternoon dosing of steroid medications in severe asthmatics to optimize therapeutic outcome, and the evening/nighttime dosing in patients with adrenogenital syndrome.

2.5.1 Chronotherapy of diabetes mellitus

A decline in insulin impact, either as a result of insufficient secretion or insulin resistance of the target tissues, prompts metabolic changes which are rhythmic in nature. In patients with type 1 diabetes mellitus (T1DM) insulin secretion is significantly reduced or absent and there is no noticeable rhythm of insulin or its binding molecule C-peptide. Nevertheless, other various rhythmic variables that act on carbohydrate metabolism, like growth hormone, catecholamines and cortisol, continue and prompt rhythmic changes in glycemia or glycosuria (Ghata and Reinberg, 1979; Lestradet, 1985). In patients with non-insulin dependent type 2 diabetes mellitus, the pulsatility of insulin secretion is adjusted or enormously reduced (Polonsky et al., 1988; Simon et al., 1991); the circadian rhythm of insulin and the insulin-linking molecule C-peptide, as a gathering wonder, maintained in a few reviews (Nicolau et al., 1984) yet discovered to be modified in others (Boden et al., 1999) probably as a result of the seriousness and stage of the disease.

An investigation of age, sex, activity pattern, and diet-matched groups of elderly clinically healthy and type 2 diabetes mellitus (T2DM) patients examined at 4-hour interval over a 24-hour period, revealed in both groups circadian variation in serum glucose, plasma immuno-reactive insulin (IRI) and C-peptide that were similar in both timing and amplitude. With the exception of the higher serum glucose levels in the diabetics there were no significant differences in the 24-

hour means of the other review variables (Nicolau et al., 1984). A similar timing and amplitude circadian rhythm of IRI and C-peptide was found in T2DM patients treated with oral hypoglycemic agents, however with lower circadian mean levels (Nicolau et al., 1984).

The intricate multifrequency range of rhythms that take part in the regulation of carbohydrate metabolism offers various opportunities to enhance diabetes care by the proper timing of ingestion of food and timing of treatment with insulin, insulin-secretagogues, and insulin sensitizers (Erhard, 2007). The decision to use insulin preparation for therapy has to correspond to the daily changes in insulin requirements. Rhythms of both the regulating and counter-regulating components must be considered.

The endogenous biologic time of a diabetic patient is more imperative than external clock time (Erhard, 2007). The periodic sleep–wake routine of the patient is the most effortless approach to acquire a time reference for his/her circadian time organization. Possible contrasts in the circadian time organization in shiftworkers and transmeridian explorers must be considered and timed treatment should be modified appropriately to avoid poor glycemic control (Erhard, 2007).

The design of the circadian chronotherapy for insulin dependent diabetes requires consideration of various factors (Reinberg, 2003).

- The synchronization of the patient in relation to the habitual sleep/wakefulness pattern.
- The highest insulin requirements are expected during the middle of the day and the lowest ones during late night hours.
- The largest amount of insulin is required around mealtime.
- The blood sugar lowering-effect of insulin is greatest in the morning and minimal in the evening at bedtime.

- Restoration of the circadian rhythm in insulin concentration would involve a peak time around 2 p.m. and a trough time around 2 a.m. However, ultradian rhythms have to be considered.
- Blood glucose controls by sensor or self-measurement should be instituted and uniform Values within the desirable range should be obtained. No circadian rhythm of blood glucose beyond this is necessary.

2.6 Other diseases affected by biological rhythms

Chronotherapy of Arthritis

Osteoarthritis patients have a tendency to have less pain in the morning and more pains at night; while patients with rheumatoid arthritis have pain that often peaks in the morning and declines all through the day. For patients with osteoarthritis, the ideal time for a nonsteroidal anti-inflammatory agent, for example, ibuprofen is around noon or mid-afternoon. A similar agent would be more effective for individuals with rheumatoid arthritis when taken amid the evening hours. The plasma concentration of C-receptive protein and interleukin-6 of patients with rheumatoid arthritis have a circadian rhythm (Martain, 1988).

The new cyclooxygenase-3 inhibitors are useful in easing the osteoarthritis indications when taken in the morning and better outcomes are obtained in rheumatoid arthritis when the dose is taken in the evening (Prasanthi, 2007).

Chronotherapy of Allergic Rhinitis

Early-morning sneezing, nasal congestion and runny nose are common in allergic rhinitis. Study also showed that a morning dose of antihistamine was not as effective as the same dose given in the evening (Ohta, 1995).

Chronotherapy of Peptic Ulcer:

The maximal acid secretion, pain associated with peptic ulcer as well as perforation of gastric and duodenal ulcer are more frequent at night time. The nighttime administration of drugs results in more effective control of disease (Moore-Ede et al., 1983). Nocturnal administration of the peptic ulcer drugs reduces the acid secretion more effectively as well as promotes the ulcer healing and reduces ulcer recurrence (Pocock et al., 1989). In a study that evaluated the time dependency for omeprazole 40 mg, the mean gastric pH during daytime was higher after morning administration than after evening administration (0.72 ± 0.91 pH, $p < 0.0\ 1$); whereas, the mean gastric pH during the nighttime was greater after evening administration than after morning administration (0.64 ± 0.83 pH, $p = 0.02$). This suggests that morning administration of omeprazole is preferable for patients with reflux resulting from physical activity, whereas patient with nocturnal reflux prefer evening administration (Hendel et al., 1995).

Chronotherapy of Hypercholesterolemia

Discovery of the circadian rhythm of cholesterol biosynthesis brought about a new turning point (Khasawneh and Affarah, 1992), where it was stated that elevated rates of cholesterol intake and hepatic cholesterogenesis takes place during the evening hours irrespective of fed/fasting state. An evening administration of an HMG-CoA reductase inhibitor lowered serum cholesterol levels than morning dosing.

Chronotherapy of Pain

Circadian rhythms in acute pain such as dental surgery have been documented, with a morning peak during the first postoperative day. The peak of morphine use occurred at 09.00h and was the least at 15.00h in patients undergoing elective surgery. The peak demand for morphine or hydromorphone occurred in the early morning and was lowest during the night in postoperative gynecologic patients (Peppas and William, 2004).

Chronotherapy of Epileptic seizures

Chronobiology includes some working hypotheses in psychophysiology and allows the development of new hypothetical ideas in the field of neurological sciences. It is likewise outstanding that the brain region with the most elevated focus in noradrenergic nerve terminals and nonadrenaline (NA) has a circadian rhythm in their substance of Nor-adrenalin (Bruguerolle and Labrecque, 2007).

2.7 Impact of timing on pharmacodynamic properties of several drugs

Different chemistries (lipophilic vs. hydrophilic) of β-receptor blocking drugs (e.g., atenolol, propranolol) are used to treat cardiovascular disorders (hypertension, arrhythmias, coronary heart disease) based on their chronopharmacodynamic and kinetic properties. This takes into consideration many years of research that established the rhythm organization of the cardiovascular system and is a reflection of circadian variations in hemodynamic parameters, ventricular stroke volume, heart rate, plasma concentration of hormones (e.g., noradrenaline, renin, angiotensin, aldosterone), and cyclic adenosine monophosphate (cAMP), as well as blood-associated parameters such as viscosity, platelet aggregability, and fibrinolytic activity (Paschos et al., 2010; Lemmer et al., 1991)

2.7.1 Cardiovascular drugs

The onsets of cardiovascular diseases and early symptomatology have strong dependencies. For instance, morbid and mortal events in myocardial infarction and ischemic events peak between 6 a.m. and 12 p.m.; consequently, the utilization of β-blockers, for example, oral doses of propranolol in the morning is recommended. Actually, the therapeutic value of propranolol for the most part originates from its dose–response relationship, which is subject to the circadian phase at which it was administered, and less from its chronopharmacokinetics. In this way, ingestion of oral propranolol hours before wakening blocks β-adrenoreceptors in the sympathetic tissue, changing its tone, hemodynamic conditions, and heart rate at hours of early activities in individuals (Langner and Lemmer. 1988).

α-adrenoceptor antagonist's effectively reduces peripheral resistance in the early morning hours than at other times of the day and night. Indeed, a single night time dose of the α-blocker doxazosin reduces both systolic blood pressure SBP and diastolic blood pressure DBP throughout day and night, but its greatest effect is exerted early in the morning; however, following a night time dosing, the peak effect of doxazosin occurs later than predicted as a result of its pharmacokinetics (PK) (Lemmer and Nold, 1991).

Calcium channel blockers are utilized as a part of the treatment of coronary heart disease, myocardial infarction, cerebrovascular diseases and hypertension for a few years (Stanton, 1998). Verapamil and diltiazem have a more outstanding cardiac effect and dihydropyridine calcium blockers, for example, nifedipine have a more prevailing vasodilator effect (Erkekoglu and Baydar, 2012). Several chronopharmacologic studies were carried out on different calcium channel blockers and it was discovered that generally, their blood pressure lowering effect is

higher in daytime than night time and the circadian clock dependent effects of these drugs show similar pattern as β-blockers (White and LaRocca, 2002; Bakris et al., 2002)

Clinical studies of Angiotensin-converting enzyme inhibitors (ACEI) showed a different effect of the following ACEI: benazepril, enalapril, perindopril, quinapril, ramipril, spirapril, and trandolapril when dosed in the morning vs. the evening. The effects of the long-acting lipophilic ACEI trandolapril when ingested just before bedtime or in the morning was investigated and it was revealed that bedtime administration of the medication was a safe and effective means of controlling morning BP in hypertensive patients without the induction of excessive BP reduction at night (Kuroda et al., 2004). The fixed combination of captopril and hydrochlorothiazide was slightly more effective in reducing nocturnal BP when administered in the evening (Yusuf et al., 2000).

The results from the study of administration-time-dependent efficacy of spirapril, an ACEI recommended for once-daily administration because of its extended duration of action and long half-life of about 40h, showed that morning administration of spirapril, was significantly more effective than bedtime administration in reducing the diurnal BP mean and is significantly less effective in controlling nocturnal BP (Hermida et al., 2006). However, the diurnal/nocturnal BP ratio was significantly reduced with spirapril ingestion on awakening and significantly increased with spirapril ingestion at bedtime (Hermida et al., 2006).

Nitrate derivatives such as nitroglycerin and isosorbide mononitrate/dinitrate (ISMN/ISDN) which act as vasodilators, are used to treat angina pectoris symptoms to relieve chest pain. The therapeutic action of nitrate derivatives result from activation of the vascular nitric oxide (NO)/cyclic guanosine monophosphate (cGMP) pathway, which, in turn, elicits venodilation and

aortic and large elastic artery distensibility, promoting hemodynamic changes (Bode-Boger and Kojda, 2005). Interestingly, though isosorbide dinitrate shows only slight pharmacokinetic changes in relation to the time of drug administration, its therapeutic action is more pronounced when administered orally in the early morning (~2 a.m.), with most angina attacks taking place between 2 and 4 a.m. (Lemmer et al., 1991). These are just a few examples in which time of administration is critical to restoring aspects of vascular physiology that are either directly regulated by the master clock or indirectly modulated through slave oscillators. Actually, the release of glucocorticoids, angiotensin II, and catecholamines, as well as the activity of endothelial nitric oxide synthase is subject to diurnal variations, affecting vasoactive response and blood pressure. Additionally, processes such as vascular angiogenesis and thrombogenesis depend on signaling events that are clock regulated; hence, targeted therapies need to consider the time-of-day activity of target tissues when developing procedures for administration.

In summary, findings from antihypertensives assessed based on their ability to control nocturnal BP revealed that, night-time administration is suggested for perindopril (Morgan et al., 1997) , morning for amlodipine (Qui et al., 2003), evening/bedtime administration for ramipril (Hermida and Ayala, 2009), candesartan (Eguchi et al.,2012), telmisartan(Hermida et al, 2007), amlodipine/hydrochlorothiazide (Zeng et al., 2011), amlodipine/olmesartan (Hoshino et al., 2010) and amlodipine/valsartan (Hermida et al., 2010; Kasiakogias et al, 2015).

2.7.2 Statins

The knowledge of the rhythmic activity of hydroxymethylglutaryl (HMG)- coenzyme A reductase, have led to great improvements in the therapeutic management of hypercholesterolemia using statins. Therefore, FDA approved evening time administration for

statins such as cerivastatin and simvastatin, on grounds of their short half-life (2–3 h) (Plakogiannis and Cohen, 2007). However, fluvastatin benefits from bedtime ingestion, lovastatin should be ingested after meals in the evening, and atorvastatin does not exhibit an optimal time-of-day dosing benefit (Schachter, 2005). Atorvastatin, its active metabolite and rosuvastatin all have long half-lives (14, 20 and 30 h, respectively), and the FDA approved "any time" administration for these medicines (Plakogiannis and Cohen, 2007).

2.7.3 Anti-cancer drugs

The timing for the administration of anti-cancer drug is rarely indicated in study protocols or seldom reported in the reports from clinical trials (Hrushesky et al., 2004). As a consequence inter-individual and intra-individual variations can be observed. For some unclear reasons, most intravenous chemotherapy is being administered during daytime and oral drugs are usually administered once daily, preferably in the morning, without stating the reason for this time of day dosing (Levi et al.,2010; Stupp and Weber, 2005; Di Leo et al., 2008; Motzer and Basch, 2007; Shepherd, 2005; Mok and Ramalingam, 2009). This traditional approach cannot be used for some of the drugs as this lack of consideration towards the timing of therapy assumes that biological parameters are either constant throughout the 24 hours, or that their variations are unpredictable and/or theoretical. Nevertheless, the timing of therapy must be based on information on proper circadian rhythms obtained from rodent and human chronotherapy studies in order to justify the administration time for each drug and optimize its therapeutic index (Innominato, 2010). For anti-cancer drugs used in infusion form, in order to define the drug regimen, the parameters commonly used include: dose administered per unit (usually body surface area or body weight), duration of infusion and frequency of administration (Innominato, 2010). If the cancer drug will be infused for a short time, chronotherapy studies can be

performed with a timed bolus or a short infusion where the timing of administration (start, peak and stop times) are stated (Levi et al.,2010; Hrushesky et al., 2004). However, a constant-rate infusion over 24 hours, or integral multiples of this span, does not consider chronopharmacodynamic properties. Therefore, this procedure should be used as a control administration plan for studies of cancer chronotherapy for drugs whose pharmacologic properties allow long term infusion (Levi et al., 2010).

The differences between the conventional and the chrono-modulated applications are not constrained to the time of administration, but include differences in delivery mode, infusion duration and drug sequence (Garufi et al., 2006). A new anti-cancer delivery system named as "chrono-modulated delivery" presents another infusion parameter, "the time of peak-flow rate". In this type of delivery, the administration pattern does not remain constant. It is somewhat semi-sinusoidal, with an increasing flow rate, a peak administration rate at a time specification and a gradual symmetric decrease in flow rate. The chrono-modulated application profile is particularly suitable for drugs with a short half-life where a relatively long duration of administration is preferred (Innominato, 2010).

Some examples that show the modifications in the pharmacodynamics of some anti-cancer drugs include: DNA synthesis in the main target tissues of 5-fluorouracil (5-FU)-induced toxicity (e.g., bone marrow, skin, and oral and rectal mucosa) is lowest during the night and highest during daytime (Longley et al., 2003; Wood et al., 2006; Lincoln et al., 2000; Bjarnason et al., 2001). Hence, when the whole-body clearance of 5-FU is increased at night, the fraction of healthy cells potentially denatured by 5-FU is decreased. Whole-body pharmacodynamics of 5-FU, thus shows variation along the circadian time scale, with a synchronous phase between different target tissues. The anabolic enzymes (orotatephosphoribosyltransferase, uridinephosphorylase,

and deoxythymidine kinase) that produce cytotoxic forms of 5-FU have their highest activity during the dark span of rats or mice, when 5-FU is most toxic to healthy tissues (Levi, 2006). The action of the "thymidylatesynthetase", the target enzyme of 5-FU has also been studied at the cellular level in the oral mucosa cells of 6 healthy volunteers. The activity of this specific enzyme showed a circadian rhythm with a fall between midnight and 4 a.m (Bjarnason and Jordan, 2002). Therefore, the molecular target of 5-FU is less active at night. This results in a cellular chronopharmacodynamic pattern of this drug consistent with its lower cytotoxicity to the oral mucosa at night.

The circadian profiles of whole-body and cellular chronopharmacokinetics and chronopharmacodynamics in humans would therefore predict a better tolerability of healthy tissues for a nighttime administration of 5-FU. This hypothesis has been approved by several clinical studies. On the other hand, the anti-tumor effects of interferon-β (IFN-β) in mice are more efficient during the early rest phase than during the early active phase. The dosing schedule-dependent effect of IFN-β is also closely related to that of IFN receptors and "interferon-stimulated gene factor (ISGF)" expression in tumor cells or lymphocytes (Takane et al., 2000; Nakagawa et al., 2006).

Tyrosine kinase is an enzyme that transfers a phosphate group from ATP to a protein in the cell. Imatinib mesylate is a molecule that inhibits the function of various receptors with tyrosine kinase activity. In mice, the influence of dosing time on the ability of imatinib mesylate to inhibit tumor growth has been studied and it was observed that the growth of tumor cells implanted in mice was more severely inhibited by the administration of imatinib mesylate during the early rest phase than during the early active phase.

The dosing time-dependency of anti-tumor effects is parallel to that of the imatinib-induced antiangiogenic effect. Hence, optimizing the dosing schedule of the drug could lead to a potent therapeutic efficacy of the drug (Mahdi et al., 2011).

2.7.4 Analgesics and non-steroidal anti-inflammatory drugs

Biological rhythms in pain sensitivity have been studied extensively both in rodents and in humans (Ray et al., 2004; Labrecque and Vanier. 1995; Gallerani et al., 2001). Apart from its potent analgesic properties, morphine shows a broad range of other pharmacological effects. In humans, reduction in gastrointestinal motility, sedation, inhibition of the micturition reflex, and miosis were observed. In rodents, though some of the pharmacologic effects were similar; different reactions were also found, like mydriasis as an opposite reaction (Martin del Campo et al., 2000). Pharmacological tolerance to the analgesic effect of morphine was observed in several studies after chronic administration of morphine in both experimental animals and in humans. However, the clinical tolerance in humans develops more slowly. However, chronopharmacological studies on the development of tolerance to the analgesic effect induced by morphine are very rare. In one of the studies of chronopharmacodynamics of morphine, Yoshida et al., (2003) used hot-plate method to induce pain in mice and the researchers observed the chronopharmacodynamic response towards morphine by using this model (Yoshida et al., 2003). The scientists discovered that there was a significant 24 hours rhythm in the latency of thermal response after morphine injection with a decline at the light phase and a peak at the dark phase. Particularly, at the dark phase, the time spent on the hot-plate after morphine injection was significantly longer compared with non-drugged state. The rhythmic pattern of analgesic effect induced by morphine was similar to that of the sensitivity of mice to painful stimuli in

non-drugged state (Yoshida et al., 2003) and the results of that study were consistent with the previous findings performed by naloxane and morphine (Ray et al., 2004; Li et al., 2004).

Chronopharmacological studies performed on the heat-pain reducing effects of fentanyl in humans revealed that a peak in pain relief occurred late in the afternoon (5.30 p.m) and a decline in the early morning hours (5.30 a.m). However, the analgesic effects of two other opioids (dihydrocodeine and tramadol) were investigated in humans and the results showed a vigorous increase in the painful intensity of the chemical stimuli during evening sessions. Thus, both dihydrocodeine and tramadol exerted stronger analgesic effects when administered in the evening (Hummel et al., 1995).

In a study in which kaolin-induced pain mouse model was used, indomethacin was orally administered to the mice at different hours of the day and the chronopharmacodynamic effect was evaluated. The findings suggest the analgesic effect of indomethacin in mice with the kaolin-induced pain is greater after dosing in the early resting period.

2.8 Chronotherapeutic delivery system

A basic Chronotherapeutic system consists of a drug containing core and a barrier layer of polymer to control drug release from the core. Several drug release techniques have been developed and applied to design chronopharmaceutic delivery systems. The techniques are basically classified into three categories.

(1) Time controlled chronotropic systems.

(2) Stimuli induced pulsatile drug delivery systems.

(3) Externally regulated pulsatile drug delivery systems

2.8.1 Time controlled chronotropic systems

The drug is released as a burst within a short period of time, immediately after a pre-determined off release period.

Time controlled chronotropic systems based on capsules

These systems are composed of an insoluble capsule body, swellable and degradable plugs made of hydrophilic polymers (Like hydroxyl propyl cellulose, poly vinyl acetate, polyethylene-oxide), lipids and bioactive molecule. The lag time is controlled by plug, which is pushed away by swelling or erosion and drug is released as a pulse from the insoluble capsule i.e Pulsincap® a swellable hydrogel seals the drug contents into the capsule body. The hydrogel plug swells when the capsule comes in contact with fluid and after a lag time, the plug pushes itself outside the capsule and rapidly releases the drug (Gohel and Sumitra, 2002).

Time controlled reservoir systems with rupturable polymer coating

The core is coated with a protective polymeric rupturable layer and an outer water insoluble semi permeable rate controlling membrane. Pressure is required to rupture the coating which can be achieved by using swelling agents, gas producing effervescent agents or osmogens (Ueda et al., 1994). Swelling agent includes super-disintergrents like carboxy methylcellulose, sodium starch glycollate, L-hydroxyl propyl cellulose. Polymers like polyacrylic acid, polyethylene glycol etc and a mixture of tartaric acid and sodium bicarbonate is used as effervescent agent. Water ingress to system causes the coating to sell, rupture and release of drug occurs.

Release of drug is independent of pH or solubility of drug and lag-time can be varied by varying thickness of coating or by changing amount of plasticizers in the outermost layer. Rapid release

of drug after lag-time can be observed with increase in the concentration of the osmotic agent (Qureshi et al., 2008).

Time controlled reservoir systems with soluble or eroding polymer coating

Ethylcellulose (EC) of varying particles sizes has been used as an outer coating layer to design a novel dry-coated tablet of sodium diclofenac by direct compression for time-controlled drug release. The drug release from dry-coated tablet exhibited an initial lag period depending on the particle size of the EC powder could modulate the timing of drug release from such a dry coated tablet. The period of lag time for sodium diclofenac released from dry-coated tablets was correlated with the penetration distance of the solvent into dry-coated tablet by in vitro dye penetration study. The results suggest that these dry-coated tablets prepared with different particle sizes of EC powder as an outer coating layer might offer a desirable release profile for drug delivery at the predetermined time and sites (Narisawa et al., 1993).

2.8.2 Pulsatile systems based on changed membrane permeability

Change in permeability of polymeric coating layer is responsible for drug release in presence of certain counter ions of surrounding media (Narisawa et al., 1993).

Stimuli induced pulsatile Drug delivery system

The drug release from these systems is based on the body's physiochemical processes. These systems are meant for the site specific targeted drug delivery by the induction of various physiochemical stimuli at target site. Biological stimuli like release of enzymes, hormones, antibodies, pH of the target site, temperature of the site, concentration of biomolecules (Glucose,

neurotransmitters, inflammatory mediators) etc acts as stimuli to trigger the release of drug from these types of drug delivery systems.

2.8.3 Externally regulated pulsatile drug systems

External stimuli like ultrasound, magnetic field, electrical effect and irradiation are required to control the drug release from these types of systems. When these external factors are applied on the delivery system, conductors present in the delivery system get sensitized and trigger release of drug from the delivery system. Magnetic beads prepared by interfacial polymerization of polyamide microcapsules shows this type of delivery mechanism. Various technologies to develop time controlled per-oral drug delivery systems have been extensively studied in recent decades and some of these systems are discussed below:

Enteric-coated systems

Enteric coatings have been used to prevent the release of a drug in the stomach. Enteric coatings are pH sensitive and drug is released when pH is raised above 5 in the intestinal pH level above about 6, has been successfully used (Shegehiro, 2010). The system contains a core which is fill coated with two polymers, first with hydroxypropyl methylcellulose (HPMC) and then with a gastro-intestinal polymer. In this system, the duration of the lag phase in absorption can be controlled by the thickness of HPMC layer.

Layered systems

These are one or two impermeable or semi permeable polymeric coatings (films or compressed) applied on both sides of the core (Bogin and Ballard, 1992). The two layers both contain a drug dose; the outer drug layer contains the immediately available dose of drug. An intermediate

layer, made of swellable polymers separates the drug layers. A film of an impermeable polymer coats the layer containing the other dose of drug. The first layer may also incorporate a drug-free hydrophilic polymer barrier providing delayed (5 h) drug absorption. A multi-layer tablet system consisting of a hydrophilic matrix core containing the drug dose have been discovered (Conte and Maggi, 1996) and this kind of three layer device have been used in the treatment of Parkinsonian patients using L-dopa/benserazide (Ghika et al., 1997).

2.9 Rational Drug Use

The World Health Organization (WHO) defines the rational and safe use of drugs as a series of steps where appropriate diagnoses and prescribing are followed by appropriate usage of medicines (WHO, 2016). According to an estimate by WHO, over half of all medicines are inappropriately prescribed, dispensed and about half of all patients fail to adhere to instructions. The reasons for this irrationality can be summarized to include: ignorance, incompetence and inadequate resources. As a result, the WHO have stipulated principles which suggest that the patient should be informed about their medication, understand the importance of the prescribed treatment and take the medicines as required and at the right time (WHO, 2012).

Since it's the duty of a physician to diagnose and prescribe drugs, ensuring proper use of a medication is usually the professional task of pharmacists; hence, pharmacy practitioners should take into account that the time of administration may be play an important role in maximizing benefits and eliminating the risk of toxicity of certain medications.

3. Materials and Methods

3.1 Materials:

Questionnaires

SPSS V20.0 Software

Excel 2010

3.2 Study Setting

The study was a cross sectional study conducted between April and May, 2017. Nigeria is a Sub Saharan country in the West African region with a land mass of 923,768 square kilometers [26]. Nigeria comprises thirty-six states and the Federal Capital Territory and these states are grouped into six geopolitical zones. The Northwest geopolitical zone which make up about 20% of the total population of Nigeria, consist of seven states namely: Jigawa, Kaduna, Kano, Kastina, Kebbi, Sokoto and Zamfara. List of registered pharmacists in Nigeria as at December 2016 is 5, 307 out of which 474 community pharmacists are practicing in the Northwest region of Nigeria.

In Nigeria, only two out of the seventeen universities accredited for pharmacy programmes are located in the Northwest zone and a pharmacy-based chronotherapy module is yet to be adopted in the curricula of these institutions but topics on chronobiology are expected to be covered in elective courses from other departments.

The study was approved by the association of community pharmacists' of Nigeria (ACPN).

3.3 Data Collection

Data was collected using a modified questionnaire based on current literature (Kaur et al., 2016). Pilot tests were conducted on five (5) community pharmacists to determine the applicability of the questionnaires. The self administered, pretested and structured questionnaire was designed to consist of twenty-seven (27) close ended questions divided into three (3) sections: demographics, awareness, and attitude.

3.4 Statistical Analysis

Data were analyzed using Statistical Package for Social Sciences (SPSS) version 20.0 software. Descriptive statistics were used to report community pharmacists' demographics. The mean scores of awareness and attitude sections were compared with the participants' demographics using *Mann–Whitney U test* and Kruskal–Wallis test.

Spearman's rank-order correlation was used to determine the relationship between awareness and attitudes. Statistical significance was considered at $p \leq 0.05$.

4. Results

A total of one hundred and thirty one (131) community pharmacists in Northwest region of Nigeria filled the survey instruments. Only one hundred and twenty five (125) survey instruments were included in the final analysis as six (6) were excluded due to some missing data.

4.1 Demographics

4.1.1 Gender distribution of Community Pharmacists.

Table (1) showed a total of (n=125) community pharmacists that participated in this study. From the gender distribution table, most of the participants were male (61.6%).

Table 1: Gender distribution of Community Pharmacists.

Gender	**Number of community pharmacists**	**Percentage (%)**
Female	48	38.4
Male	77	61.6
Total	**125**	**100**

4.1.2 Age distribution of Community Pharmacists.

Table (2) showed the age distribution of community pharmacists that participated in this study. The results showed that most of the participants were between ages of 26 and 30 years (42.4%) and only (26.4%) of the respondents were between the ages of 21 and 25 years.

Table 2: Age distribution of Community Pharmacists.

Age	Number of community pharmacists	Percentage (%)
21 – 25	33	26.4
26 – 30	53	42.4
31 & above	39	31.2
Total	**125**	**100**

4.1.3 Years of Practice.

Table (3) showed the years of practice by community pharmacists that participated in this study. The results showed that only four (4) participants have being practicing for over 15 years (3.2%) while most of the participants have years of practice of below 6 years (56.8%).

Table 3: Years of Practice

Years	Number of community pharmacists	Percentage (%)
1 – 5	71	56.8
6 – 10	37	29.6
11 – 15	13	10.4
16 – 20	4	3.2
Total	**125**	**100**

4.1.4 Level of Education.

Table (4) showed the level of education attained by the community pharmacists who participated in this study. The results showed that most of the participants have only Bachelor degree (76.0%) while only three (3) respondents have Ph.D (2.4%).

Table 4: Level of Education

	Number of community pharmacist	**Percentage (%)**
Bachelor	95	76.0
Master	27	21.6
Ph.D	3	2.4
Total	**125**	**100**

4.2 Awareness Items

The awareness section examined the knowledge of the community pharmacists and data analysis (Table 4) showed that the participants have fair knowledge of circadian rhythms. Only (46.4%) of the participants believed that disruption of the circadian rhythm of the human body can initiate disease conditions. Only (56.0%) of the participants answered correctly the question relating to pattern of peaking of cholesterol biosynthesis (item 5) and their response to that question was reflective in the next question (item 6) as only (68.8%) answered correctly, the time of the day administration of Statins with longer half life. Most of the participants were aware of the optimal time of drug administration and this was shown in their responses to questions on the clinical

case studies (items 9, 10, 11), however, only (44.8%) of the participants answered correctly the clinical case question (item 12) relating to time of the day administration of a once daily dose of Perindopril for a non-dipper patient.

Table 5: Participants' response to awareness items

		Proportion of community pharmacists who answered items correctly (%)
1	Circadian rhythms affect human physiological processes.	85 (68.0%)
2	Disease conditions cannot be initiated by the disruptions of the circadian rhythms of the human body.	58 (46.4%)
3	Circadian rhythms are cycles in the physiological processes of many species, with a period (cycle duration) of roughly 24 hours.	121 (96.8%)
4	With respect to pharmacokinetics of drugs, circadian rhythms can influence drug absorption.	109 (87.2%)
5	In a 24-h time period, the rate of cholesterol biosynthesis follows a pattern of peaking in the morning.	70 (56.0%)
6	Statins with longer half life can be taken at anytime of the day.	86 (68.8%)
7	In a 24-h time period, Gastrointestinal motility follows a pattern of decreasing at night.	76 (60.8%)

8	Asthma symptoms are most likely to worsen at night.	116 (92.8%)
9	NSAIDs (Non steroidal anti-inflammatory drugs) are often administered for pain conditions such as rheumatoid arthritis. The most appropriate time of the day to take this medication is Evening.	103 (82.4%)
10	AJ is a 25 yr old man who is diagnosed with peptic ulcers. His doctor advised him to take Omeprazole 40mg daily. Omeprazole is more effective in raising gastric pH when administered in the morning.	92 (73.6%)
11	Prednisone is a corticosteroid used in wide range of condition for its anti-inflammatory and immunosuppressant effects such as asthma attacks. The most appropriate time to administer the drug in a non emergency situation is morning.	94 (74.4%)
12	Perindopril is angiotensin converting enzyme inhibitor used commonly for the treatment of hypertension. The best time for a non-dipper (blood pressure does not decrease during sleep) patient to take a once daily dose is evening.	54 (43.2%)

4.3 Attitude:

Participants' attitudes toward chronotherapy are shown in table (5). Most of the participants agreed that chronotherapy could increase the efficacy of a drug (91.2%) and reduce the incidence of adverse drug effects (88.0%). Although only (79.2%) of the community pharmacists agreed that counseling patients on chronotherapy will increase the number of patients returning to their pharmacy, data analysis indicated that most of the respondents agreed to statements (items 3, 4, 5) relating to counseling patients about taking their medications at more effective circadian time window . Most participants (97.6%) agreed that chronotherapy should be an area covered in the continuing pharmacy education activities for pharmacy professionals and were willing to dedicate sometime to learn about chronotherapy (95.2%).

Table 6: Attitude scores

	Proportion of community pharmacists who answered either 'Disagree' or 'Strongly disagree' to statements on attitude section (%)	Proportion of community pharmacists who neither 'Agreed' nor 'disagreed' with statements on attitude section (%)	Proportion of community pharmacists who answered either 'Agree' or 'Strongly agree' to statements on attitude section (%)
Chronotherapy could increase the efficacy of a drug.	5 (4.0%)	6 (4.8%)	104 (91.2%)

Chronotherapy could reduce the incidence of adverse drug effects.	10 (8.0%)	5 (4.0%)	110 (88.0%)
At therapy initiation, doctors/general practitioners should counsel their patients about more effective 'circadian-time windows' for drugs where this is applicable.	9 (7.2%)	10 (8.0%)	106 (84.8%)
Where applicable, counseling the patients about taking their medication at more effective 'circadian-time windows' for a particular drug may lead to improved adherence.	8 (6.4%)	11 (8.8%)	106 (84.8%)
Where applicable, counseling the patients about taking their medication at more effective 'circadian-time windows' for a particular drug may lead better cost effectiveness.	15 (12.0%)	10 (8.0%)	100 (80.0%)
Counseling patients on Chronotherapy will increase the number of patients returning to your pharmacy.	13 (10.4%)	13 (10.4%)	98 (79.2%)
Chronotherapeutic information	7 (5.6%)	10 (8.0%)	108 (86.4%)

should be included in drug references.			
Future direction			
Chronotherapy should be an area covered in the continuing pharmacy education activities for pharmacy professionals	3 (2.4%)	0 (0.0%)	122 (97.6%)
As a pharmacist, I am willing to dedicate some time to learn about Chronotherapy.	5 (4.0%)	1 (0.8%)	119 (95.2%)
The principles of Chronotherapy should be taught in the pharmacy course.	14 (11.2)	0 (0%)	111 (88.8%)
Chronotherapeutic studies should be included as part of therapeutic goods administration (TGA) regulatory requirements for registering a drug.	16 (12.8%)	0 (0.0%)	109 (87.2%)

4.4 Groups' relationship with total awareness and total attitude.

With regards to mean total awareness, results showed that there were significant differences between participants aged 21 – 25 years and 31 years & above (p=0.019) and also, participants aged 26 – 30 years and 31 years & above (p = 0.004). Results also showed that there were significant differences between participants with years of practice of 1 – 5 and 11 – 15 (p = 0.041) and also, participants who have being practicing for 6 – 10 years and 11 – 15 years (p = 0.031)

Table 7: Groups' relationship with total awareness and total attitude scores.

	Total Awareness			**Total Attitude**		
	Mean	SD	p-value	Mean	SD	p-value
Gender						
Male	8.62	1.52	0.307	46.25	6.55	0.426
Female	8.29	1.76		46.10	4.82	
Age						
21-25	8.60*	1.57	0.019	45.67	5.18	0.351
26-30	8.90*	1.44	0.004	46.58	6.75	
31 & above	7.84	1.70		46.10	5.38	
Years of Practice						
1-5	8.77#	1.56	0.041	46.09	5.44	0.685
6-10	8.37#	1.60	0.031	45.67	7.52	

11-15	7.23	1.69		47.46	3.95	
16-20	8.75	0.50		48.50	0.57	
Education						
Bachelor	8.63	1.56	0.147	46.51	5.51	0.058
Master	8.22	1.71		46.25	5.91	
Ph.D	6.66	1.52		35.33	10.50	

*. Significant at $p < 0.05$; #. Significant at $p< 0.05$

4.5 Correlation between total awareness and total attitude.

Table 7 shows that the value of correlation (r) between total awareness and total attitude is 0.216. This value of 'r' signifies that the correlation is weak but positive.

Table 8: Correlation between total awareness and total attitude scores.

	Total awareness		**Total attitude**	
	r	p- value	r	p- value
Attitude	1		0.216	0.015*
Awareness	0.216	0.015*	1	

*. Correlation is significant at the level of 0.05 (2-tailed test)

5. Discussion

Community pharmacists are easily accessible and often the first contact health care provider for most Nigerians. To our knowledge, this is the first study assessing community pharmacists' awareness and attitudes towards principles of chronotherapy in this region. Results confirmed our hypothesis that there would be inadequate chronotherapy-related knowledge among community pharmacists practicing in this region. In addition, results indicated remarkable positive attitudes towards chronotherapy and willingness to learn and apply principles of chronotherapy in practice. This finding implies that any misconception about chronotherapy in this region could be addressed if dedicated educational programmes are organized to impact more knowledge about chronotherapy.

The results of our study highlight a lack of awareness of evidence based information on chronotherapy especially in areas involving circadian rhythms in normal physiological functions. These results are in line with previous study conducted on similar cohorts assessing their viewpoints about and experience with application of chronotherapy principles in practice (Kaur et al, 105) and also consistent with another study that assessed the awareness and attitudes of final year pharmacy students towards chronotherapy (Kaur et al., 2016); both studies reported a lack of awareness about current therapeutic evidence on chronotherapy.

This study revealed that only (44.8%) of the community pharmacists provided correct answers to evidence based recommended time for Perindopril (item 12) (Hermida et al, 2011; Smolensky et al., 2015). It could be that community pharmacists in this region mostly apply the FDA approved optimal administration time (morning) for Perindopril and may not be able to help patients maximize benefits from their medications by suggesting appropriate administration times for

such a condition in which blood pressure control is altered by disruptions in circadian BP patterns or sleep disorders. Therefore, with the development and introduction of the new evidence based clinical guidelines for use of chronotherapies in clinical practice, it is important that information about principles of chronotherapy be updated and made available for pharmacy practitioners, as this would help make them knowledgeable enough to provide adequate patient management and care.

It is interesting to note that only (56.0%) of the participants know the circadian pattern of cholesterol biosynthesis as seen on (item 5) of the awareness section. Their response to that question was reflective as only (68.8%) of the respondents answered correctly 'any time of the day' administration of statins with a long half life (item 6) as approved by FDA (Plakogiannis and Cohen, 2007). This observation indicates that almost half of the participants may have forgotten or have a poor understanding of the basic pharmacology and physiology concepts. Our finding is in line with a previous study that reported a low performance by final pharmacy students regarding 'optimal administration time' for statins with a long half life (Kaur et al., 2016) and also supports the findings from a study that was carried out on community pharmacists in an eastern state of Nigeria, where the authors reported that about 53% of the community pharmacists do not attend refresher courses, seminars and workshops frequently (llodigwe and Chima, 2010).

Most of the pharmacists agreed to statements relating to patient counseling and willingness to apply chronotherapy in practice. This indicates that they acknowledge their role in providing patient medication counseling, considering the fact that modern pharmacy practices have evolved from a product oriented practice to pharmaceutical care. This finding supports the result from a

study carried out in Nigeria which reported that community pharmacists participate in patient education on health matters (Olumide and Oladipo, 2015).

This study showed that awareness was influenced by age and years of practice. Pharmacists below the age of 31 years and pharmacists with years of practice below 11 years had a better significant level of awareness. It could probably be because in Nigeria, the young pharmacists are the frontiers in transforming medical care systems and also, they have more access to the internet where you can find a lot of information. Moreover, the teachings in the schools are now advocating evidence based facts other than the crude facts.

This study highlights a positive correlation between awareness and attitude scores. This finding reaffirms adequate awareness can lead to a positive attitude towards chronotherapy, which could eventually result in good practices.

6. Study Limitations.

This study has some limitations and shortcomings. It was difficult to get hold of the community pharmacists in charge as some of the pharmacies were managed by non pharmacists. This study may not be generalized to all parts of Nigeria considering the relative small sample size. Therefore, we suggest that similar study be carried out in other parts of the country.

Conclusion

Majority of the community pharmacists had satisfactory level of awareness and impressive positive attitude towards chronotherapy. However, the findings from this study highlight the need for educational interventions especially in the area of evidence based practice, to improve the knowledge of community pharmacists practicing in this region.

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Tick the boxes appropriately.

1. **Demographic data of respondents**

Gender		Male		Female	
Age		21 – 25		26 -30	31 and above
Years of practice		1 – 5	6 – 10	11 – 15	16 – 20	21 and above
Level of education	Bachelor degree		Master degree		PhD	

2. **Awareness**

	Agree	Disagree
Circadian rhythms affect human physiological processes.		
Disease conditions cannot be initiated by the disruptions of the circadian rhythms of the human body.		
Circadian rhythms are cycles in the physiological processes of many species, with a period (cycle duration) of roughly 24 hours.		

With respect to pharmacokinetics of drugs, circadian rhythms can influence drug absorption.		
In a 24-h time period, the rate of cholesterol biosynthesis follows a pattern of peaking in the morning.		
Statins with longer half life can be taken at anytime of the day.		
In a 24-h time period, Gastrointestinal motility follows a pattern of decreasing at night.		
Asthma symptoms are most likely to worsen at night.		
NSAIDs (Non steroidal anti-inflammatory drugs) are often administered for pain conditions such as rheumatoid arthritis. The most appropriate time of the day to take this medication is Evening.		
AJ is a 25 yr old man who is diagnosed with peptic ulcers. His doctor advised him to take Omeprazole 40mg daily. Omeprazole is more effective in raising gastric pH when administered in the morning.		
Perindopril is an angiotensin converting enzyme inhibitor used commonly for the treatment of hypertension. The best time for a non-dipper (blood pressure does not decrease during sleep) patient to take a once daily dose is evening.		

Prednisone is a corticosteroid used in wide range of condition for its anti-inflammatory and immunosuppressant effects such as asthma attacks. The most appropriate time to administer the drug in a non emergency situation is morning.		

3. General attitude

	Strongly disagree	Disagree	Normal	Agree	Strongly Agree
Chronotherapy could increase the efficacy of a drug.					
Chronotherapy could reduce the incidence of adverse drug effects.					
At therapy initiation, doctors/general practitioners should counsel their patients about more effective 'circadian-time windows' for drugs where this is applicable.					
Where applicable, counseling the patients about taking their medication at more effective 'circadian-time windows' for a particular drug may lead to improved adherence.					
Where applicable, counseling the patients about taking their medication at more effective 'circadian-time					

windows' for a particular drug may lead better cost effectiveness.					
Counseling patients on Chronotherapy will increase the number of patients returning to your pharmacy.					
Chronotherapeutic information should be included in drug references.					
Future direction					
Chronotherapy should be an area covered in the continuing pharmacy education activities for pharmacy professionals.					
As a pharmacist, I am willing to dedicate some time to learn about Chronotherapy.					
The principles of Chronotherapy should be taught in the pharmacy course.					
Chronotherapeutic studies should be included as part of therapeutic goods administration (TGA) regulatory requirements for registering a drug.					