ASSESSMENT OF THE PROTECTIVE ROLE OF GREEN TEA EXTRACT AGAINST THE CIPROFLOXACIN INDUCED TOXICITY ON MANDIBULAR CONDYLAR CARTILAGE OF WISTAR RATS

AMEERA KAMAL KHALEEL ALSOCOTY

UNIVERSITI SAINS MALAYSIA

2022

ASSESSMENT OF THE PROTECTIVE ROLE OF GREEN TEA EXTRACT AGAINST THE CIPROFLOXACIN INDUCED TOXICITY ON MANDIBULAR CONDYLAR CARTILAGE OF WISTAR RATS

by

AMEERA KAMAL KHALEEL ALSOCOTY

Thesis submitted in fulfilment of the requirements
for the degree of
Doctor of Philosophy

July 2022

-

ACKNOWLEDGMENTS

First of all, thanks to ALLAH, for blessing me with this education and allowed me fulfil this hard work, Alhamdulillah.

I would like to thank the Dean of School of Dental Sciences, Health Campus, Universiti Sains Malaysia, and all the stuff in the college for their support.

I would like to express my thanks and gratitude to my main supervisor A/prof Dr Ramizu Bin Shaari and my co-advisors Dr Mohamad Arif Awang Nawi and Dr Ali Mihsen Hussein Al-Yassiri for their generous, excellent supervision, patience, guidance, friendly supervision and advices which had been extremely valuable in completing this thesis.

Great amount of gratitude for Al-Daqah and Al-Jawadin Medical laboratories in Karbala for thoughtful co-operation and help in the biochemical part of the study.

Great amount of gratitude for Al-Kafeel Hospital in Karbala and Ugene laboratory in Kufa for their help in the histopathological and immunohistochemical part of the study.

Sincere thanks to all the staff in the Laboratory Animal Research Center, College of Pharmacy, University of Karbala, Iraq for all their helps and support.

Great thanks to my husband, Prof Dr Ali Sultan Al-Rifai, Dean of Collage of Dentistry, Ahl Al Bayt University, who offered me his time, care, support, and endless encouragement and made my graduate study a very enjoyable time.

Thanks to the wonderful friends and colleagues specially Sharifah Mastura Syed Mustafa and Marina Binti Che Mat Nawi, and all the staff in the IPS/ USM for their continuous help.

Last but not least I would like to thank everyone who provided any assistance even with a single word.

.

TABLE OF CONTENTS

LIST OF TABLES

Page

LIST OF FIGURES

LIST OF SYMBOLS

A	Absorption
α	Alpha
β	Beta
C	Concentration
^{0}C	Centigrade
%	Percent

LIST OF ABBREVIATIONS

ADAM	A disintegrin and metalloproteinases
ADAMTS	A disintegrin and metalloproteinase with thrombospondin motifs
ANOVA	Analysis of Variance
AP-1	Activator protein-1
Bax	Bcl2 associated x protein
Bcl2	B-cell lymphoma 2
b.w.	Body weight
Ca	Calcium
CIP	Ciprofloxacin
COX-2	Cyclooxygenase-2
CRP	C-Reactive Protein
DAB	Diaminobenzidine
Dl	Deciliter
DNA	Deoxyribonucleic acid
EC	(-)-epicatechin
ECG	(-)-epicatechin gallate
EDTA	Ethylene diamine tetra acetic acid
EGC	(-)-epigallocatechin
EGCG	(-)-epigallocatechin gallate
ELISA	Enzyme-Linked Immunosorbent Assay
G	Gram
GT	Green tea
H&E	Hematoxylin and eosin
H_2O_2	Hydrogen peroxide
HRP	Horseradish peroxidase
IHC	Immunohistochemistry
IL	Interleukin
i.m.	Intra-muscular
iNOS	inducible nitric oxide synthase
Kg	Kilogram
L	Liter

MCC	Mandibular condylar cartilage
MDA	Malondialdehyde
Mg	Milligram
Mg	Magnesium
Ml	Milliliter
Mmol	Millimole
MMP	Matrix metalloproteinase
NaCl	Sodium chloride
NADP	Nicotinamide Adenine Dinucleotide Phosphate
NADPH	Nicotinamide Adenine Dinucleotide Phosphate Hydrogen
NK-κB	Nuclear factor kappa-light-chain-enhancer of activated B cells
ng	Nanogram
Nitro-PAPS	2-(5-nitro-2-pyridylazo)-5-(N-propyl-N-sulfopropylamine) phenol disodium salt
nm	nanometers
P53	Tumor suppressor protein
PBS	Phosphate buffered saline
PCNA	Proliferating Cell Nuclear Antigen
PGE_2	prostaglandin E_2
Ppm	part per million
RCF	Relative Centrifugal Force
ROS	Reactive Oxygen Species
S	Saline
s.c.	Subcutaneous
SPSS	Statistical Package for the Social Science
TIMPs	Tissue Inhibitors of Metalloproteinases
TNF	Tumor Necrosis Factor
U	Unit
µg	Microgram
µm	Micrometer
VEGF	Vascular Endothelial Growth Factor
W	Water
Zn	Zinc

PENILAIAN PERANAN PERLINDUNGAN EKSTRAK TEH HIJAU TERHADAP TOKSISFIKASI CIPROFLOXACIN TERHADAP CARTILASI CONDYLAR MANDIBULAR TIKUS WISTER

Abstrak

Kepala kondilus mandibula ditutupi dengan rawan kondilus mandibula yang berfungsi sebagai pusat pertumbuhan dalam kompleks kraniofasial. Ciprofloxacin ialah fluoroquinolone yang penting untuk merawat jangkitan bakteria yang mengancam nyawa. Ia mempunyai kesan kondrotoksik dalam pertumbuhan rawan kondilar. Teh hijau telah menarik perhatian yang signifikan untuk pelbagai manfaat kesihatannya. Penyelidikan terdahulu mendedahkan kepentingan pilihan diet untuk pencegahan kesan kondrotoksik ciprofloxacin. Objektif kajian ini adalah untuk menyiasat potensi ekstrak teh hijau untuk pencegahan kondrotoksisiti mandibula yang disebabkan oleh ciprofloxacin dalam tikus Wistar Albino jantan juvana. Dalam kajian ini, dua puluh lima ekor tikus telah digunakan. Lima ekor tikus digunakan untuk kajian rintis dan dua puluh ekor lagi dibahagikan kepada empat kumpulan. Pada hari ke-32 hari, semua tikus dalam kumpulan Ciprofloxacin/Air dan Ciprofloxacin/Teh hijau (kumpulan yang dirawat) diberi dua suntikan subkutan ciprofloxacin sebanyak 600 mg/kg berat badan selang lapan jam, manakala Saline/Air dan Saline/teh hijau disuntik dengan garam secara subkutan. Kumpulan Salin/Teh Hijau dan Ciprofloxacin/Teh hijau telah disuntikan secara intragastik dengan ekstrak teh hijau dalam dos oral 300 mg/kg/hari selama lapan hari sebelum suntikan salin atau ciprofloxacin secara subkutan. Pada hari ke-34, semua tikus telah dibius, seterusnya pengumpulan darah melalui tusukan jantung dan sampel kondilus mandibular diambil serta-merta dan diproses. Keputusan menunjukkan bahawa gavage intragastrik ekstrak teh hijau tidak signifikan terhadap paras serum magnesium, kalsium, dan vitamin E dalam semua kumpulan yang dikaji ($p>0.05$), tetapi paras serum zink dalam Saline/Teh hija dan Ciprofloxacin/Teh hijau menunjukkan peningkatan yang signifikan berbanding dengan dua kumpulan yang lain ($p<0.05$). Kumpulan Ciprofloxacin/Air menunjukkan penurunan signifikan dalam ketebalan rawan kondilus bilangan sel tulang rawan, dan kandungan proteoglikan berbanding dengan tiga kumpulan lain ($p<0.05$). Analisis statistik juga menunjukkan penurunan yang signifikan

terhadap skor Mankin dalam kumpulan Ciprofloxacin/Teh hijau dengan kumpulan Ciprofloxacin/Air ($p<0.05$). Kumpulan Ciprofloxacin/teh hijau menunjukkan peningkatan yang signifikan terhadap ekspresi imuno Bcl-2 ($p<0.05$) dan tiada perbezaan yang signifikan ($p>0.05$) dalam ekspresi imun kolagen II terhadap kumpulan Ciprofloxacin/Air. Kajian ini memberikan bukti pertama bahawa ekstrak teh hijau boleh mengurangkan kesan kondrotoksik ciprofloxacin dalam model tikus.

ASSESSMENT OF THE PROTECTIVE ROLE OF GREEN TEA EXTRACT AGAINST THE CIPROFLOXACIN INDUCED TOXICITY ON MANDIBULAR CONDYLAR CARTILAGE OF WISTAR RATS

Abstract

The head of the mandibular condyle is covered with mandibular condylar cartilage which acts as the center of growth in the craniofacial complex. Ciprofloxacin is a fluoroquinolone which is important for treating a life-threatening bacterial infection. It has a chondrotoxic effect in growing condylar cartilage. Green tea has attracted a significant attention for its multiple health benefits, and the previous researches uncover the importance of dietary choices for prevention of the chondrotoxic effect of ciprofloxacin. The objective of the present study was to investigate the preventive potential of green tea extract on the mandibular chondrotoxicity induced by ciprofloxacin in juvenile male Wistar Albino rats. In the present study, twenty-five rats were used. Five rats were used for the pilot study, and the other twenty were divided into four equal groups. On day 32 of age, all the animals in Ciprofloxacin/Water and Ciprofloxacin / Green tea treated groups were subcutaneously injected by ciprofloxacin as two subcutaneous injections of 600 mg/kg of body weight, eight hours apart, while the Saline/Water and Saline/Green tea groups were subcutaneously injected by saline. The Saline/Green tea and Ciprofloxacin / Green tea groups were intragastically gavaged by green tea extract in an oral dose of 300 mg/kg/day, eight days before the subcutaneously injection of saline or ciprofloxacin. On day 34, all the animals were anaesthetized, blood collection by cardiac puncture was taken, and the mandibular condyle samples were taken immediately and processed. The results showed that the intragastric gavage of green tea extract can cause a non-significant change in the magnesium, calcium, and vitamin E serum levels in all the groups studied ($p>0.05$), but the zinc serum level in the Saline/Green tea and Ciprofloxacin / Green tea groups showed a significant increase in comparison with the others two groups ($p<0.05$). The Ciprofloxacin/Water group showed a significant decrease in the mandibular condylar

cartilage thickness, cartilage cells number, and proteoglycans content in comparison with the other three groups ($p<0.05$). Statistical analysis also showed a significant decrease in Mankin score in the Ciprofloxacin / Green tea group in comparison with the Ciprofloxacin/Water group ($p<0.05$). The Ciprofloxacin / Green tea group showed a significant increase in the Bcl-2 immune expression ($p<0.05$) and a non-significant increase ($p>0.05$) in collagen II immune expression in comparison with the Ciprofloxacin/Water group. This study provides the first evidence that green tea extract can decrease the chondrotoxic effects of ciprofloxacin in a rat model.

CHAPTER ONE

INTRODUCTION

1.1 BACKGROUND

1.1.1 Temporomandibular joint

The temporomandibular joint is a synovial and hinge-type joint, consist from a group of anatomic structures like the mandibular condyle, mandibular fossa, articular eminence, articular disc, muscles, and ligaments. The head of the condyle is formed by expanding of the upper projection from the posterosuperior part of the ramus. The mandibular condyle has ovoid condylar process on a tapered mandibular neck. The head of the condyle is articulating with temporal bone and is covered with fibrocartilage. The temporomandibular joint in rat and human are nearly close and a few anatomical differences have been found. For this reason, the rat appears as an alternative to temporomandibular joint model (Orset *et al*., 2013).

1.1.2 Cartilaginous joint types

In general, different types of cartilages at specific anatomic locations are present. The hyaline type includes the articular cartilage of the long bones, costal cartilage, nasal cartilage, laryngeal and tracheal cartilages (Amerman, 2016). The elastic cartilage is present in the body's parts in which the stretchability is important like epiglottis, external ear, and at the attachment site of the larynx to the vocal cords (Amerman, 2016). The fibrocartilage is tough and forms the intervertebral discs, the articular cartilage which

cover the mandibular condyle, at bone attachment areas of ligaments and tendons, and the joint between sternum and clavicle (Nicke *et al*., 2018).

The cartilaginous joint classified into primary and secondary cartilages. Primary cartilages are hyaline, immovable and regarded as a part of primary skeletal cartilage and present in growing end of bone in which the interstitial cell proliferation occurs in chondrocytes, and later on, this primary cartilage is calcified. The secondary cartilages are independent of the primary cartilage, it shows appositional proliferation in chondrocytes, and appear later in embryonic development (Mizoguchi *et al.*, 2013), and need low functional loading and oxygen tension to sustain all the cartilaginous properties (Hinton *et al*., 2015).

1.1.3 Mandibular condylar cartilage

This fibrocartilage is covering the head of the mandibular condyle and is often classified as secondary cartilage, and is considered as the center of the highest growth in the craniofacial area and is important in the temporomandibular joint function and the morphogenesis of craniofacial skeleton (Felsenthal and Zelzer, 2017; Nicke *et al.*, 2018). This fibrocartilage is avascular, and depend on the intra-articular synovial fluid for nourishment. The fibrocartilaginous structure of the mandibular condylar cartilage (MCC) along with the lubrication action of the synovial fluid ensure that all the masticatory loads are spread over larger contact areas and then absorbed (Tanaka *et al*., 2006). The MCC is differ from the primary cartilages in the histological organization, proliferation mode, differentiation, calcification; in addition to their response to different environmental stimuli (Mizoguchi *et al*., 2013).

Even though the MCC is only a few millimeters thickness, it has a lasting durability and extraordinary mechanical properties. This unique composition provides the joints

surface with a low friction, high lubrication, wear resistance, and shock absorption even it is a continuous load bearing area throughout a person's life (Khojastepour *et al.*, 2017; Bellinghen *et al.*,2018).

Microscopically, MCC is made up of chondrocyte and the extracellular matrix which surrounding it (Chen *et al*., 2012). The inorganic dissolved salts like magnesium (Mg), calcium (Ca), sodium, and potassium are present in the extracellular tissue fluid. The transport of nutrients to the chondrocytes occurs through the flow of water in the cartilage. The interaction of this water with the proteoglycan, they provides the MCC with the tremendous compressive strength (Bellinghen *et al*., 2018).

A variety of collagen types are present, which is mainly collagen type II; they are synthesized by the chondrocytes cells and considered as the major structural macromolecules of the extracellular matrix and distributed in the various zones of the MCC with a variable orientation (Oinas *et al*., 2018).

Proteoglycans are macromolecules interspersed in between the collagen fibers, and produced by chondrocytes. The proteoglycan macromolecules, especially the aggregates provide the osmotic resistance necessary and contribute to the gel-like property as a shock absorber.These proteoglycans contain a high number of carboxyl and sulfate groups which are fixed along their glycosaminoglycan chains, which in a physiological environment, they become negatively charged. The negatively charged glycosaminoglycans can bind to cationic dyes such as Safranin O (Wang *et al*., 2009).

1.1.4 Green tea

Second to the water, tea is the widely consumed beverage in the world. It is cultivated in approximately more than thirty countries in the world and is globally consumed. Green tea (GT) is prepared by initial heating or steaming process of Camellia sinensis

leaves. The chemical composition is proteins, carbohydrates, vitamins (like B, C, E), caffeine and theophylline, and minerals like Mg, Ca and zinc (Zn). The polyphenolic flavonoids in GT is the most important constituents of GT leaf, which are attributed to the most beneficial effect of GT (Cabrera *et al*, 2006).

The preparation methods of GT can influence the catechins content both quantitatively and qualitatively; and this amount can also vary according to the origin of tea leaves due to the varieties of growing conditions. Because of the presence of different other antioxidant constituents in the extract of GT, it was reported that the GT extracts are more stable than the pure epigallocatechin gallate alone (Khokhar and Magnusdottir, 2002).

GT has attracted a significant attention for it's a wide range benefits for different disorders, ranging from weight loss to cancer therapy (Nesran *et al*., 2020). GT was evaluated in a number of diseases because it is the best known for its antioxidant activity and anti-apoptosis properties (Rains *et al.,* 2011). It was also found that GT can also reduce the kidney oxidative damage caused by gentamicin in rats (Abdel-Raheem *et al*., 2010).

1.2 PROBLEM STATEMENT

Nalidixic acid (Quinolone) was discovered as a byproduct of the chloroquine synthesis in 1960 (Hall *et al*., 2011). In the 1980, various fluorinated derivatives were synthesized (Stahlmann, 2002). Quinolone-induced arthropathy has been described in different juvenile animals such as rats (Forster *et al*., 1996), dogs (Stahlmann *et al*., 2000), rabbits (Machida *et al*., 1990), guinea pigs (Bendele *et al*., 1990), and in juvenile

horses (Davenport *et al*., 2001). In human, they can induce tendon rapture and tendinopathy (Kim, 2010).

Ciprofloxacin (CIP) is a fluoroquinolone antibiotic which shows a broad-spectrum activity against gram negative and gram positive bacteria and are important for treating a serious life threatening bacterial infections (Blessed *et al*., 2018). It has a chondrotoxic effect in growing condylar cartilage. Different histological changes are usually detected like necrosis of chondrocytes, collagen and proteoglycan degeneration, and cleft formation in the articular cartilage which may lead to the formation of erosions (Kato, 2008). The chelation of Mg ions with CIP has been reported to induce electrolyte imbalance and matrix degeneration in the cartilage (Adikwu and Brambaifa, 2012).

1.3 JUSTIFICATION OF THE STUDY

Unlike the other tissues in the body, the damaged MCC has less ability for repair it self, because of the limited ability of the chondrocytes to proliferate and regenerate a new cartilaginous tissue and the absence of the blood vessels . This regeneration and repair of the MCC represent the important challenges for most investigators worldwide due to the avascular structure of the MCC, in addition to its cellular arrangement, and dense extracellular structure (Eftekhari *et al*., 2020).

Because of the chondrotoxic effect of CIP in growing condyle, it is contraindicated during pregnancy and lactation periods, in children and adolescents, according to the toxicological results in animals' studies during the postnatal growth period (Sendzik *et al*., 2005). In Egerbacher *et al* (2001), Pfister *et al* (2007), and Halawa (2010) studies, they revealed that the CIP induced chondrotoxicity can be diminished by Mg or vitamin E supplementation. Adikwu and Brambaifa (2012) found that the administration of Mg,

Zn chloride or vitamin E can prevent or reverse the CIP induced chondrotoxicity by excess collagen formation, increase bone growth and osteoblastic activity, and inhibit the free oxidation radical's formation by preventing oxidative stress and the DNA oxidation. Channa (2014) study revealed that CIP induced chondrotoxicity effects could be reduced by simultaneous administration of Zn chloride in albino rats pre and postnatally. The oral administration of Ca gluconate can also ameliorate the articular cartilage damage by inhibiting the over expression of cyclooxygenase-2 (COX-2), which is important enzyme in prostaglandin synthesis and associated chondrocyte apoptosis (Kang *et al*., 2014).

Since GT contain different vitamins, like vitamin E, and different minerals like Mg, Ca and Zn, and associated with different activities like the antioxidant and anti-apoptosis activity, so its uses may ameliorate the articular cartilage damage and facilitate the use of CIP during the nursing period, pregnancy period, in children and adolescents and can publish a new database for further future studies. The previous scientific researches uncover the importance of dietary choices. The dietary therapy, if effective, it might be a less expensive and safer method for prevention of the chondrotoxic effect of CIP.

RESEARCH OBJECTIVES

1.3.1 General objective: To investigate the preventive potential of green tea extract on the mandibular chondrotoxicity induced by CIP in juvenile Wistar Albino rats.

1.4.2 Specific objectives: To compare the rats which were subcutaneously injected by CIP with or without the gavage by green tea extract for:

1. The biochemical levels of serum Mg, Ca, and Zn.
2. The biochemical level of serum vitamin E.
3. The histomorphometrical analysis for the MCC sections stained with:
    a) Haematoxylin and eosin (H&E) as a measure for the MCC thickness and chondrocytes cells numbers.
    b) Safranin-O stain, as a measure of the glycosaminoglycans loss and distribution.
    c) Toluidine blue stain, for detection of proteoglycans.
4. Bcl-2 immunohistochemical expression level as antiapoptotic marker in the MCC.
5. Collagen II staining intensity level, since it is considered as the main collagen type in the MCC and responsible for the stability and healthy condylar cartilage.

1.5 RESEARCH HYPOTHESIS

Since the GT contains different minerals like Mg, Ca, and Zn, and different vitamins like vitamin E, and contains the highest concentrations of different antioxidants than any other types of tea, in addition to its intrinsic free radical scavenging activity which likely contributes to its antiapoptotic activities, so this study was to assess the hypothesis that GT can prevent or decrease the chondrotoxic effect of CIP.

CHAPTER TWO
LITERTURES REVIEW

2.1 TEMPOROMANDIBULAR JOINT

2.1.1 Human temporomandibular joint

Human temporomandibular joint is a synovial and hinge-type joint which is covered by a fibrocartilaginous cartilage and consist of anatomic bony structures which has a unique function and character in comparison with the other diarthrodial joints. The components of this joint are mandibular condyle, mandibular fossa, articular tubercle, articular disc, and articular capsule (Khojastepour *et al.*, 2017; Stocum and Roberts, 2018; Runci Anastasi *et al.*, 2021). Because, both the MCC and the temporomandibular joint disc are capable to adapt their shape to the shape of the bony articular surfaces, they are capable to prevent any loads by this deformation (Nokar *et al*., 2018; Bordoni and Varacallo, 2021).

The muscles of mastication are masseter, temporalis, medial and lateral pterygoid, they are in direct action with the temporomandibular joint (Bender *et al.*, 2018; Sushant *et al.*, 2019). The accessory muscles of mastication are consisting from the suprahyoid, and infrahyoid muscles which are located bilaterally on the side of the neck. The buccinator is a facial expression muscle that helps in mastication by keeping food pushed back within the oral cavity (Morton *et al*.,2011).

The temporomandibular ligament is important ligament which can prevent any excessive retraction movement of the mandible, which might cause a problem with the joint. The sphenomandibular and stylomandibular ligaments are two minor accessory ligaments and are not directly attached to the joint. They become taut when the mandible is protruded (Sushant *et al*., 2019; Bordoni and Varacallo, 2021).

2.1.2 Rat's temporomandibular joint

The temporomandibular joint is unique to mammals, but the animal models will not be the closest mimics of the human condition, but the most used species in researches are rats, rabbits and pigs (Herring, 2003). The rats are mostly considered in the medical research, they are easy to handle, economic, and the temporomandibular joint of the rat presents certain similarities with that of the human (Figure 2-1). This allows the obtained results from the rat experimental model to reproduce again in the patients (Porto et al., 2010), Vimal Adithyan and Karthik Ganesh (2017) study found that morphologically the articular structure of rats, as a whole, is nearly similar to that of humans, but the angles between the mandible corpus and condyle were found to be different (150° vs. 125° in human) as seen in Figure 2-2. Orset et al (2013) study also considered the rats as a good alternative to model temporomandibular joint.

The temporomandibular joint of rat is consisted of the mandibular head and fossa, and the fibrocartilaginous disc, and surrounded by a thin capsule. The mandibular capsule extends from the border of the mandibular fossa to the neck of the mandibular condyle. The biconcave disc divided the articular joint space into upper discotemporal and lower discomandibular spaces. The glenoid fossa appears flat without articular eminences (Porto *et al.,* 2010).

Figure 2.1. Rat's photographs show: (A) Lateral view of temporomandibular joint. (B) Superior view of the mandible. (C) Inferior view of the glenoid fossa (Porto *et al*., 2010).

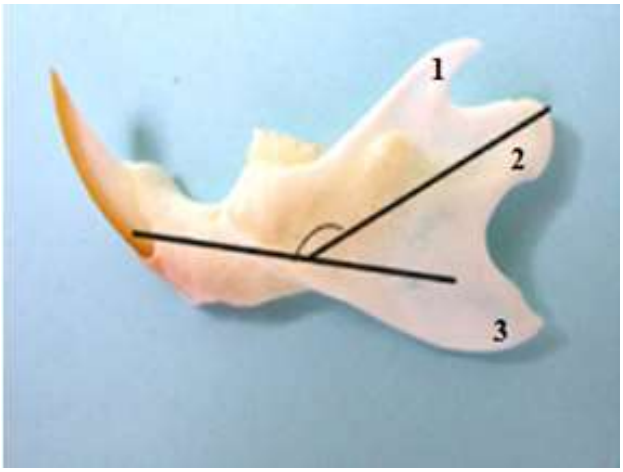

Figure 2.2. The angle between the mandible corpus and condylar axis in a sagittal plane of rat's mandible.1. Coronoid process. 2. Condyloid process. 3. Angular process (Orset *et al*., 2013).

About the histological features, an obvious similarity between the human and rat temporomandibular joints was seen. The rat condyle is covered by a layer of fibrocartilage, but with less layers of chondrocytes than that of the articular condyle of human. These layers of chondrocytes are maturing gradually until reaching the area near the bone tissue and then becomes hypertrophic. A human-like synovial membrane is also seen in the temporomandibular joint of the rat, and the disc is also consisting from a fibrocartilaginous tissue, as in the human (Fuentes *et al*.,2017).

2.2 MANDIBULAR CONDYLAR CARTILAGE

2.2.1 Mandibular condylar cartilage cellular constituents

The MCC is a tissue which contains cartilage progenitor cells, fibrocartilage stem cells, and chondrocytes cells at different stages of maturation (Chen *et al*., 2012; Mizoguchi *et al.,* 2013).

A. Cartilage progenitor cells: The chondroprogenitor cells are present predominantly in the upper layer of the proliferative zone of the MCC. The cells in this zone are actively proliferating. These cells are of great importance because they are the origin of nearly all cell divisions of the MCC cells. After the cessation of the growth, the MCC becomes phenotypically similar to other articular cartilages by entering into a post-mitotic state. However, it was found that the progenitor cell population are still capable of reactivating even in response to changes in mechanical loading (Robinson *et al.*,2015).

B. Fibrocartilage stem cells: Condylar cartilage has lacked the vascular supply and has limited regenerative properties. In 2016, a novel stem cell niche containing a reservoir of fibrocartilage stem cells was discovered in the condylar cartilage of rats, and later were

identified in mouse, rabbit, and human condylar cartilage. It plays important role in the development and regeneration of fibrocartilage. When tissue injury occurs, this specific stem cells can proliferate and differentiate to regenerate the damaged tissue. But, the number of these cells becomes decreases gradually with age due to the lower ability for the proliferation and differentiation with increasing age (Bi *et al*., 2020). More importantly, engraftment treatment of fibrocartilage stem cells has been successfully applied in animal models with temporomandibular joint disorders (Fan *et al*.,2021).

C. Chondrocyte cells: These cells are varying in numbers, shapes, from flat to round or oval, and sizes according to the tissue layer they are located in (Hinton *et al*., 2015).

2.2.2 Mandibular condylar cartilage layers

Microscopically, the MCC is composed of several layers (Rogers *et al.,* 2018), they are (Figure 2-3):

A. Fibrous layer (Articular): The condylar cartilage's most superficial layer is made up of dense fibrous connective tissue with scattered flat-shaped fibroblast-like cells. It serves as a a protective layer for the underlying cartilaginous layers (Hinton *et al*., 2015).

B. Proliferative cells layer: This layer act as a separating zone between the fibrous and the mature layers and plays a significant role as a cell reservoir. In this layer, the collagen synthesis activity was low, and the cells can differentiate into chondrocytes and have multilineage potential (Bellinghen *et al*., 2018).

This zone is divided into two layers based on their cellular morphology: (a)The upper polymorphic cell layer in which a densely packed polygonal cells with a thin cell processes and round nuclei are present. The proliferative activities are mainly limited to

this cell layer (b) The lower layer in which the flattened cells are arranged with their long axes parallel to the condylar articular surface (Chen *et al*., 2012).

C. Chondrocytic cells layer (Mature zone): The cellular shape varies from flattened to spherical in this layer, which comprises chondrocytes at various stages of maturity. The extracellular matrix in this mature zone has an increased area with hematoxylinphilic and metachromatic staining with toluidine blue, which indicate the cartilage matrices active deposition. The collagens, proteoglycans, and glycosaminoglycans synthetic activity is high in this layer (Bellinghen *et al*., 2018).

D. Hypertrophic cells layer: It is the chondrogenic lineage's last stage of differentiation which is important in order to replace the cartilage by the bone through endochondral ossification. The hypertrophied chondrocytes cells are increase in size, and bone marrow vasculature infiltrates the region, along with chondroclastic and osteogenic precursor cells. The bone matrix which is newly secreted is then deposited and the hypertrophied chondrocytes undergo apoptosis, or survive to be transform to bone formative cells (Bellinghen *et al*., 2018).

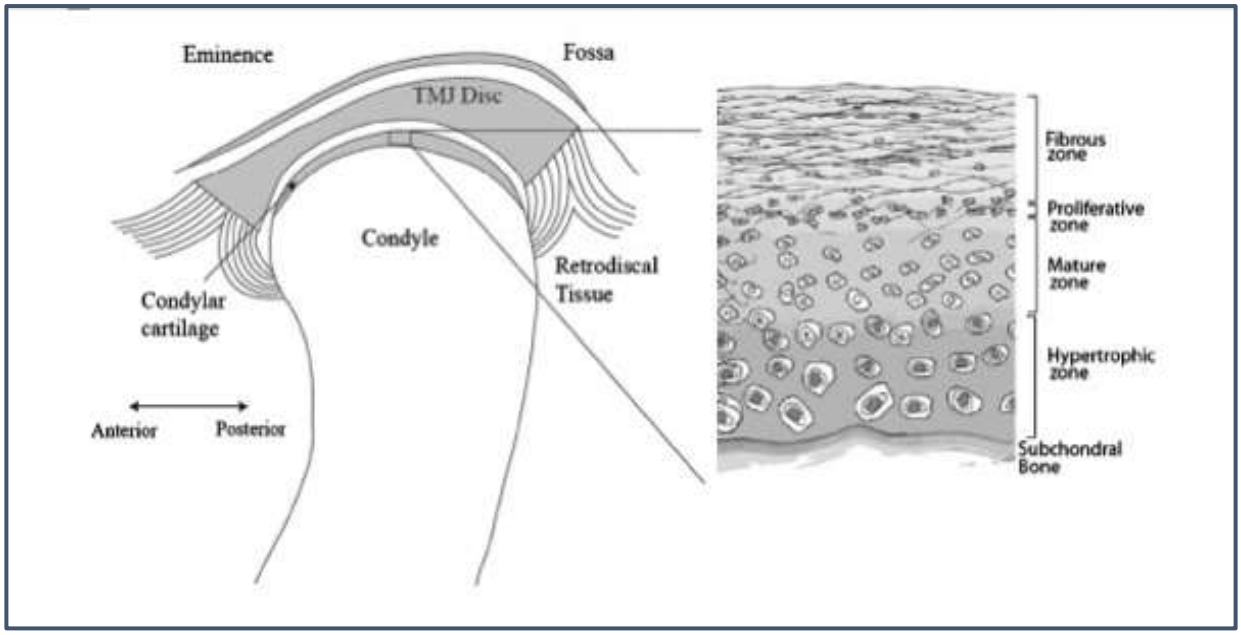

Figure 2.3. Diagram of the articular layers in the central section of the rat's mandibular condyle cartilage (Singh and Detamore, 2008).

2.2.3 Articular cartilage extracellular matrix

Between the young and the adult articular cartilage, the chemical composition of the extracellular matrix was seen differs, it consists of:

2.2.3(a) Water: The articular cartilages in general is highly hydrated and the water content forms about 65–75% of the cartilage wet weight. This water content is different from nearly 80% of the wet weight in the surface zone to about 65% in the deeper zone. The extracellular matrix water plays a role in the determination of the physiological and physical characteristic of the articular cartilage, like the elasticity, the ability to withstand the mechanical load, and the transport of different nutrients like the inorganic sults and metabolites through the cartilaginous tissue (Roughley and Mort, 2014). Proteoglycans are the most important element in cartilage hydration. The polyanionic structure of proteoglycans,

along with their hydrophilicity, allows them to bind a considerable amount of water. Proteoglycans can osmotically draw water into cartilage and establish a high osmotic pressure in the tissue, allowing it to retain a significant amount of water even under the presence of mechanical compression (Xia *et al.*, 2016).

2.2.3(b) Proteoglycans and glycosaminoglycans: The interfibrillar space of the cartilage matrix is occupy by aggrecans. Aggrecan deposition is a hallmark of the chondrogenesis process and is considered as an important marker in different medical studies to elucidate the different molecular mechanisms of chondrogenesis process. The proteoglycan aggrecans bind to hyaluronan and form the proteoglycan aggregate in the presence of a link protein (Roughley and Mort , 2014), as seen in Figure (2-4).

Aggrecan is synthesized by chondrocytes and secreted into the extracellular matrix, accounting for 10–15 percent of cartilage's wet weight. In the extracellular matrix, aggrecan is considered as the second largest group of macromolecules. They contain a core protein and covalently attached sulfated glycosaminoglycans. The core protein can have more than 100 glycosaminoglycan molecules which are laterally bounded to it in a bottle brush like arrangement. To resist the compressive loads, aggrecan serves as the primary and the direct role in providing osmotic resistance important for the condylar cartilage. The significant abundance of this aggrecan in the extracellular matrix of the articular cartilage below the superficial zone is linked to its capacity to resist compressive loads (Xia *et al.*, 2016).

Glycosaminoglycans are a polysaccharide with linear chain structure. Glycosaminoglycans have a significant function in determining the biomechanical properties of cartilage with the presence of collagen fiber. The important glycosaminoglycans present in the condylar cartilage are chondroitin 4-sulphate, dermatan sulphate, chondroitin 6-sulphate, and keratan

sulphate. It carries a negative charge, which are a major source of the repulsive electrostatic interactions in extracellular matrix of cartilage (Neves *et al.,* 2020).

Proteoglycans function in cartilage will improve our understanding of the cartilage degeneration and chondrogenesis. Proteoglycans can be broken down by fragmenting the protein core with various proteolytic enzymes or free radicals with the subsequent hydrolysis of glycosaminoglycan, proteoglycans can be broken down (Neves *et al*., 2020).

Other proteoglycans that are also present and expressed during chondrogenesis in normal cartilage include:

- Small leucine-rich proteoglycans family, which include decorin, biglycan, fibromodulin, asporin, lumican, and epiphycan, has been identified as important components in the extracellular matrix and are involved in several biological and pathological processes. It play significant roles in regulating the extracellular matrix arrangement, matrix mineralization, as well as in immunity and tumor growth (Randilini, *et al.*,2020).
- Cell surface syndecans and glypican are attached to the cell membrane. It regulates molecular interactions that mediate cell adhesion, migration, proliferation, and differentiation. Through these activities, surface proteoglycans modulate critical biological processes of development, inflammation, infection, tissue repair, and cancer metastasis (Park, 2020).
- Basement membrane proteoglycan, perlecan which shows a well-documented role in the basement membrane and considered as an important component of the cartilage pericellular matrix (Melrose, 2020).

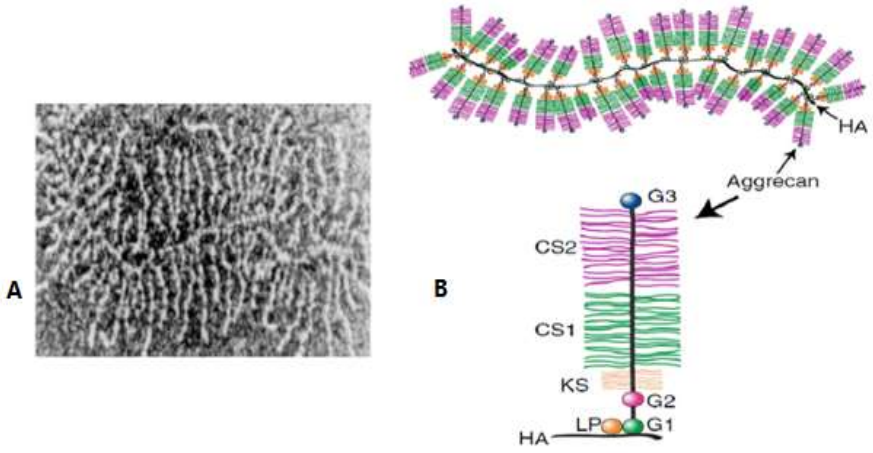

Figure 2.4. Photographs shows: (A) An electron micrograph of a proteoglycan aggregate of articular cartilage (DeLee and Drez, 2009). (B) Diagram of proteoglycan aggregate macromolecule consisted of central hyaluronic acid (HA), and aggrecan with link protein (LP) attached to it. Glycosaminoglycan side chains with the associated sulfated molecules, the chondroitin sulfate (CS1, CS2), the keratan sulfate (KS), and the aggrecan core protein (G1, G2, G3) are also seen (Roughley and Mort, 2014).

2.2.3(c) Collagen: It is a structural protein and a key solid component of the condylar cartilage extracellular matrix, acting as a structural framework for the articular cartilage's extracellular matrix. Nearly 15−20% of wet weight of the adult human articular cartilage is consisted of collagen. Collagen and collagen-like proteins are present in almost 50 different types in vertebrates, and almost 28 varieties are in humans. In adult condylar articular cartilage, the type of principal importance is type II collagen (Shibata, *et al.,* 2020).

In general, during the skeletal growth, the collagen of the articular cartilages is synthesized by the chondrocytes, which ceases in adult articular cartilage. Collagen synthesis can increase after an injury, but it isn't enough to fully repair the injured articular cartilage. The types III, VI, IX, X, XI, XII, and XIV of collagen are also found in the extracellular matrix of articular cartilage, but in lesser amounts and with a specialized structural role. Types IX and XI are

more common in early articular cartilage during skeletal development than in mature cartilage. Collagen type I is only important in ligaments and tendons (Hulmes, 2008).

2.2.3(d) Other components: The extracellular matrix in the cartilage contains several minor components, like the integrin, chondronectin and anchorin; they assist in the attachment of chondrocytes to the extracellular matrix. The phospholipids in the articular cartilage are surface-active molecules, they help in the lubrication of the articular cartilage surface (Xia *et al*., 2016).

2.2.4 Regions in the extracellular matrix of articular cartilage

The extracellular matrix, which is synthesize by chondrocytes, provides the structural environment for the chondrocytes with a remarkable biomechanical property of the condylar articular cartilage. This interactions between the extracellular matrix and the chondrocytes cells are important to both matrix biosynthesis and degradation in addition to cell anchorage. The extracellular matrix can be divided into (Khojastepour *et al*., 2017; Doweidar, 2019):

A. Pericellular matrix: A thin layer that surrounds the chondrocyte cells. It mainly consists from proteoglycans and glycoproteins with different non-collagenous proteins.

B. Territorial matrix: It surrounds the pericellular matrix and mainly consists from fine collagen fibrils that form a network.

C. Interterritorial matrix: This largest region is important and contributes mostly for the biomechanical properties of the condylar cartilage. The proteoglycans are abundant

in this zone. Any pathological processes in the condyle may result in a degradation or inadequate repair of the extracellular matrix components.

2.2.5 Breakdown mechanism

The extracellular matrix must be degraded in a timely manner for tissue formation, repair, and remodeling to occur. It is regulated in a normal physiological state, but when it is disrupted, it can lead to a variety of disorders . The collagen fibers and the aggrecan are important structural components of the condylar cartilage extracellular matrix, and their degradation is correlated with the progression of diseases (Melching, *et al*.,2006).

There are 23 Matrix metalloproteinases (MMPs) in humans, their expression is controlled by many factors like the growth factors, the inflammatory cytokines, and different hormones interaction. MMP can digest extracellular matrix macromolecule, and cleave non- extracellular matrix molecules like the growth factors and its receptors. Collagenases like MMP-1, MMP-8 and MMP-13 are the causes of the degradation of the major collagens at physiological pH. MMP-2 can also digest type IV, V, VII, X and XI collagens, elastin, laminin, and fibronectin (Wang *et al.*,2013).

A disintegrin and metalloproteinases (ADAM) are also expressed in cartilage. It was also shown that among the 34 known ADAM, several types of them was seen in areas with the greatest damage and proteoglycan loss, for this reason, this enzyme can contribute to condylar cartilage damage (Troeberg and Nagase, 2012).

It was found that ADAM9, ADAM10, and ADAM12 can affect chondrocyte cells differentiation and proliferation. ADAM-10 has also different other functions like the extracellular matrix degradation with a localized shedding of different cell surface proteins (Yang *et al*., 2017; Khaleel *et al*.,2020).

The ADAM with thrombospondin motifs (ADAMTS) is a group of 19 members which involved in different development and several types are expressed in cartilage. Proteoglycans degradation is a process catalyzed by various enzymes for glycosaminoglycan chains and core proteins, and ADAMTS are usually involved (Troeberg and Nagase, 2012). The two important ones are the 'aggrecanases', namely ADAMTS-4 and -5, which can degrade aggrecan. The core protein of aggrecan is sensitive to proteolysis along its length at numerous sites. At these sites, cleavage results in removal of a part of chondroitin sulfate chains and affecting the negative charge (Troeberg and Nagase, 2012). The extracellular proteolytic enzymes are present in a latent form in healthy cartilage. The four members of tissue inhibitors of metalloproteinases (TIMPs) family can inhibit MMPs, ADAM and ADAMTS families (Nagase and Murphy,2008). TIMPs have also different biological activities like anti-angiogenic, promoting cell proliferation, with proapoptotic and antiapoptotic activities which are independent of MMP inhibition (Melching, *et al.*,2006).

The Vascular Endothelial Growth Factor (VEGF) is formed by the chondrocytes and during development, it is considered as a chondrocyte survival factor. In addition, it is essential for skeletal growth and the postnatal homeostasis. Through vascular invasion, it can induce destruction of cartilage which is considered as an early mechanism to change the cartilage into the bone. Furthermore, the VEGF can regulate the production of TIMPs and MMPs and this play a role in extracellular matrix remodeling (Nishimura *et al.*, 2004). It is also associated with MCC diseases and its increase in expression is correlated with the increase in disease severity or mechanical overload (Pufe *et al.*, 2004).

2.3 FLUOROQUINOLONE

2.3.1 Background

The old quinolone (nalidixic acid) was discovered as a byproduct of the chloroquine production. The spectrum of activity against the gram-negative bacteria of this non-fluorinated substance is very narrow. Later in the 1980s fluorinated derivatives were synthesized (Figure 2-5). They possess a broad antibacterial spectrum that includes the gram negative and the gram positive aerobic with the anaerobic species (Elbashir *et al*., 2013). The active substances of fluroquinolones are CIP, sparfloxacin, enoxacin, cinoxacin, levofloxacin, flumequin moxifloxacin, lomefloxacin, ofloxacin, norfloxacin, pefloxacin, prulifloxacin, and rufloxacin (Brar *et al.,* 2020). They are antibiotics with a broad spectrum, which are prescribed widely and are important for treating life threatening bacterial serious infections (Blessed *et al.,* 2018). Since 1986, the use of fluoroquinolones for the treatment of different types of infectious diseases have become so widely increased, and CIP is the most widely and successfully used fluoroquinolones (de Almeida *et al*., 2007). Fluoroquinolones classification on the basis of their generation and characteristic features is seen in Table 2-1(Sharma *et al.,* 2009).

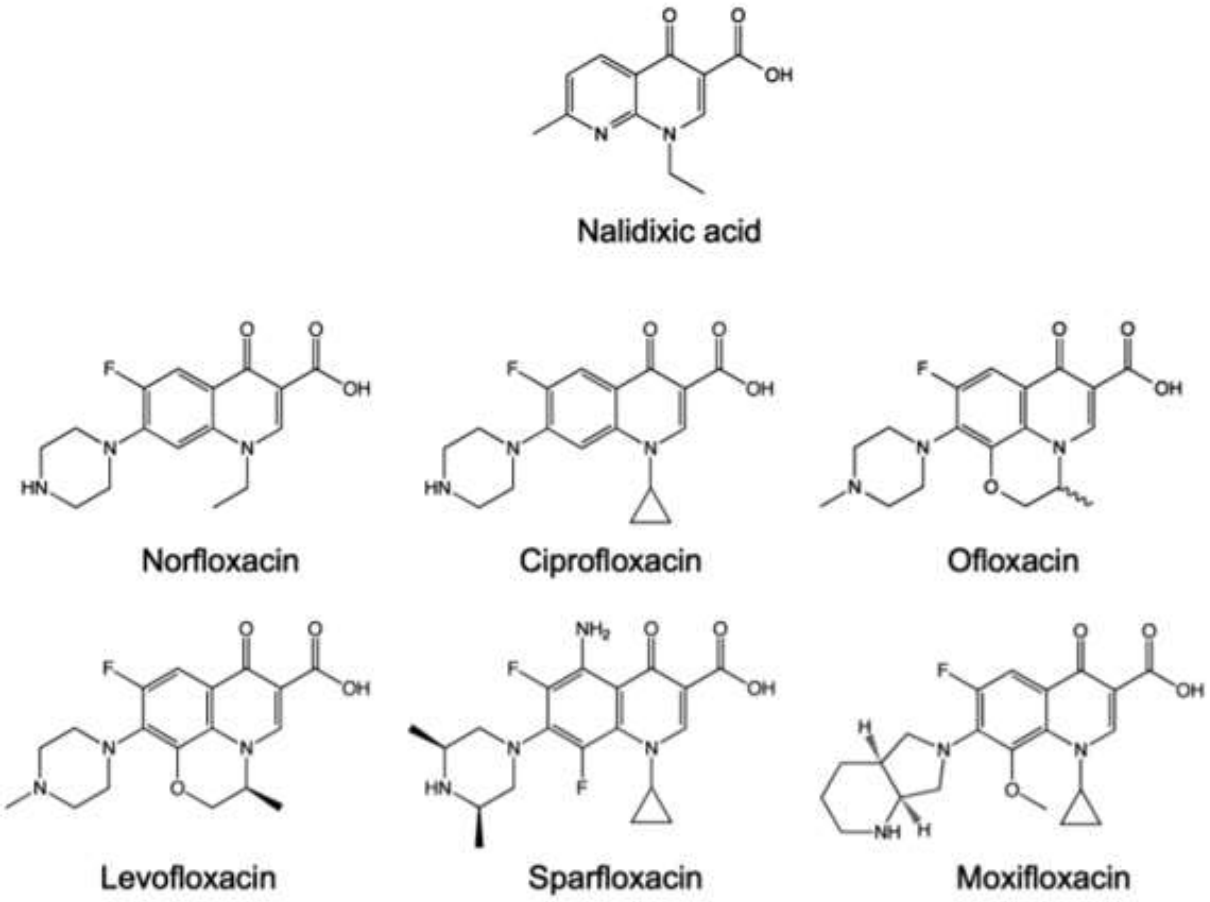

Figure 2.5. Nalidixic acid and some of the fluorinated derivatives structures (Aldred *et al*., 2014).

Table 2.1. Fluoroquinolones classification according to their generation and characteristic features.

Type of drug	Type of generation	Character
Naldixic A Pipemidic A Oxolinic A	First	Activity against gram-negative bacteria. High protein binding about 90%. Associated with little half-life. Bacteria can develop a rapid resistance.
Ciprofloxacin Norfloxacin Enoxacin Ofloxacin Lomefloxacin	Second	Broad spectrum activity against gram positive bacteria. Broad spectrum activity against gram negative bacteria. Protein binding about 50% with improved tissue distribution. Associated with longer half-life.
Temafloxacin Sparafloxacin Grepafloxacin	Third	Active against gram positive bacteria. Active against gram negative bacteria.
Clinafloxacin Gatifloxacin Trovafloxacin Moxifloxacin	Fourth	Extended activity against both strains of bacteria.

2.3.2 Mechanism of action

Inside bacterial cells, it causes inhibition to topoisomerase IV and topoisomerase II (DNA gyrase) enzymes which are involved in the uncoiling and supercoiling of DNA during the replication period. The inhibition can weaken the DNA strands, causing DNA damage and bacterial death. Bacterial gyrase differs from mammalian topoisomerase, and fluoroquinolones have a selectivity for bacteria cells that is roughly 1000 times greater than the comparable enzyme in humans (Blessed *et al*., 2018; Hangas, *et al*., 2018; Fief *et al*., 2019; Brar *et al*., 2020).

2.3.3 Antimicrobial properties

Fluoroquinolones have a good activity against a wide range of gram positive and gram-negative bacteria, and this broad-spectrum of activity against both types was seen associated with some types of them. Excellent activity was seen against gram negative bacteria such as *Enterobacteriaceae, Pseudomonas aeruginosa, Haemophilus influenzae, Moraxella catarrhalis, Neisseria gonorrhoeae,* and *Neisseria meningitides.* Most of gram-negative bacteria which is responsible for the urinary tract infections showed high sensitivity to fluoroquinolones (Appelbaum and Hunter, 2000).

CIP and ofloxacin have a good activity against the *staphylococcus aureus* isolates, like the penicillin-sensitive and the methicillin-sensitive strains. Several other bacteria are inhibited by the fluoroquinolones use like the species of *Chlamydia, Legionella, Mycoplasma, Mycobacterium, and Brucella.* Levofloxacin, grepafloxacin, sparfloxacin, and trovafloxacin shows a significant activity against *Mycoplasma pneumonia, Legionella pneumophila,* and *Chlamydia pneumoniae.* But CIP shows less activity against the *Mycobacterium tuberculosis* (Hooper,2000).

The various types of quinolone derivatives have been studied for their antifungal activities. It was found that some of them was seen exhibited a significant activity against the drug susceptible and the drug resistant fungi (Zhang, 2019). Fluoroquinolone antibiotics exhibit a low antiviral activity against Severe Acute Respiratory Syndrome Coronavirus 2 and Middle East Respiratory Syndrome Coronavirus and its ability to suppress their replication in cultured cells is limited (Scroggs *et al*., 2021). While other propose the use of fluoroquinolones as adjuncts therapy in the treatment of Severe Acute Respiratory Syndrome Coronavirus 2 associated with pneumonia (Karampelaa and Dalamagab, 2020).