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The work embodied in this thesis entitled "***In-vivo* evaluation of anti-depressant activity of *v. oxycoccus* in albino wistar rats"** is original, and has been carried out by the Roll No. 2004650003. Under the guidance and supervision of Dr. Arun Garg, M. Pharm., Ph.D. Department of Pharmacology, School of Medical & Allied Sciences, K. R. Mangalam University, 122103. It has not been submitted in full or part for any other degree of this or any other University.

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Sohna Road, Gurugram, (Haryana)

IN-VIVO EVALUATION OF ANTI-DEPRESSANT ACTIVITY OF *V. OXYCOCCUS* IN ALBINO WISTAR RATS

Thesis Submitted to School of Medical & Allied Sciences,
K. R. Mangalam University, Sohna Road, Gurugram, Delhi- NCR,
In partial fulfillment for the
Award of the Degree of
Masters of Pharmacy (Pharmacology)
2022

Submitted by
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2020 – 2022

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1. INTRODUCTION

1.1 BACKGROUND

Depression is a mental disorder categorized by a sense of despondency, inadequacy, pessimism, decreased activity, sadness, and anhedonia where these symptoms sternly disturb and negatively affectpatient's life, sometimes to the extent of developing suicidal tendencies or committing the act itself.

Depression is the most common mental and physical disorder all over the world. It could be a major reason for disability [1]. Depressive illness comes in diverse forms, just as numerous other illnesses [2]:

Table 1.1: Types of Depression

S.No	TYPES OF DEPRESSION	DEFINITION
1.	Major Depression (Unipolar/ Clinical Depression)	A prolonged feeling of sorrow or a lack of interest in external stimuli characterizes this condition. These disabling episodes of hopelessness can occur several times in a life span.
2.	Dysthymia(Persistent Depressive Disorder)	Persistent depressive disorder is defined as depression that lasts for at least two years. A milder form of depression; involving chronic, long-term symptoms that do not disable but prevent you from feeling good.
3.	Bipolar Disorder (Maniac Depression)	It is a mental illness that produces significant mood swings as well as changes in energy, thought, behavior and sleep.
4.	Seasonal Affective Disorder (SAD)	It is a type of serious depression that occurs frequently during the winter months, when the days are shorter with lesser sunlight.
5.	Psychotic Depression	It is a type of major depression along with psychotic symptoms such as Hallucinations, Delusions and Paranoia.

6.	Peripartum (Postpartum Depression)	Women who conceive and battle with misery, nervousness and/or stress for quite some time or more.
7.	Premenstrual Dysphoric Disorder (PMDD)	It is a cyclic and hormone-based disorder considered as quite a severe form of premenstrual syndrome (PMS).
8.	Situational Depression (Reactive/Adjustment Disorder)	It is described as a short term, stress that can develop when a person encounters a traumatic event or progression of changes to their everyday life.
9.	Atypical Depression	It is termed as a "specified" or subtype of major depression portraying a series of patterns of depression such as oversleeping, overeating, irritability, heaviness in arms and legs.
10.	Treatment Resistant Depression	People diagnosed with depression are given a series of treatments that lead to no success which is referred to as treatment resistant depression.
11.	Disruptive Mood Dysregulation Disorder (DMDD)	It is usually diagnosed in children. They have a difficult time regulating their mood and emotions in an age- appropriate manner. As a result, these children have frequent temper outbursts (verbally or behaviorally) in response to frustration.
12.	Persistent depression	It is the mood that lasts for couple of years maybe two or three. A patient diagnosed with this depression has various symptoms with long term effects on behaviour.
13.	Postnatal depression	After getting a baby some women will get this type of depression. It is also called "Baby Blues". It is comparatively a lite depression and anxiety that clears within two weeks after delivery.

1.2 SIGNPOSTS TO RECOGNIZE DEPRESSION

The specific *DSM-5* criteria for depressive disorder are outlined below. At least 5 of the following symptoms must be present during the same 2-week period. [5, 6]

Some signs to recognize depression are:

- Depressed mood: For adolescents and children, this can be as subtle as an ill-tempered mood
- Loss of pleasure or Less interest in almost all work done (anhedonia)
- Appetite disturbance or considerable weight change
- Disturbance in sleeping (hypersomnia or insomnia)
- Retardation or Psychomotor agitation
- Loss of energy or Fatigue
- Feeling of worthlessness
- Indecisiveness; Decreased ability to concentrate or think
- Recurrent suicidal ideation without a specific plan, recurrent thoughts of death, or a specific plan for committing suicide or a suicide attempt .

It is extremely difficult to design an animal model that can perfectly replicate the symptoms of depression in human patients. In Animals, we cannot expect to find such self-awareness, self-reflection & consideration of others; let alone being conscious of depressed mood, low vanity, and suicidal thoughts. However, some of the manifestations of depression such as altered behaviour in stress, impaired cognitive abilities, and dysfunctional reward-processing behaviour can be easily modelled in animals [7] and these changes in behaviour can be related to physiological, endocrine and anatomical changes [8].

1.3 PREVELANCE

Depression is a widespread ailment that affects 3.8 percent of the world's population, with 5.0 percent of adults and 5.7 percent of adults over 60 years old. Around 280 million people worldwide suffer from depression i.e., 5.0 percent of adults. In any given year, depression roughly affects 1 in 15 adults, and 1 in6 people will experience depression at some point in their lives.[3] Worldwide over 350 million humans of all age group are sickened by depression. It forms a major component in the global load of diseases. In a survey done in 17 countries, it was detected that 1 in 20 people suffered a depressive episode within the previous year [5] . The depressive disorder

peaks at secondto fourth decade of a patient's life. So it reduces the functioning capacity of people grossly and makes it aleading cause of disability adjusted life year (DALY) next to cardiovascular disease. Taking the current trend of disease into consideration it will clearly overcome cardiovascular illness to top the chart. It is morecommon in women.[8]

It currently affects 12.3 percent of the world's population and has increased to 15% by 2020.

According to the World Health Organization's Global Burden of Disease report, depression has become asthe second leading cause of long-term disability in 2020 and will become the primary cause by 2030.

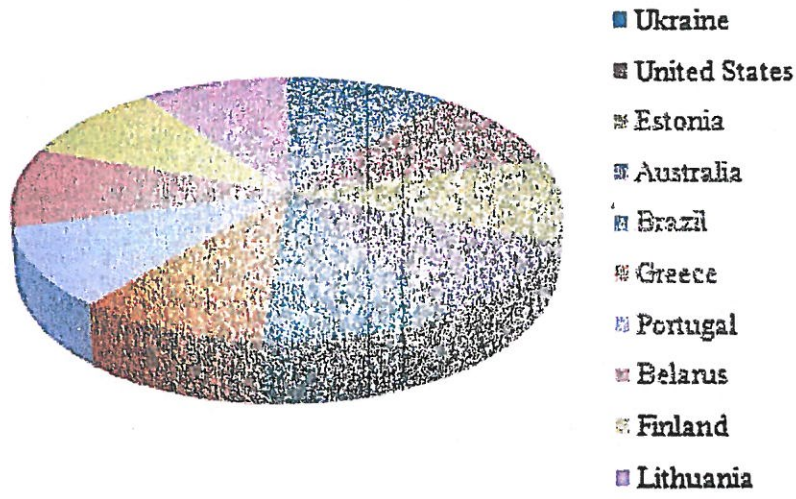

FIG 1.1: Top 10 Countries with the Highest Rates of Depression

1.4 PATHOPHYSIOLOGYOF DEPRESSION

The underlying pathophysiology of major depressive disorder has not been clearly defined. Current evidence points to a complex interaction between neurotransmitter availability and receptor regulation and sensitivity underlying the affective symptoms.

A disturbance in central nervous system serotonin (5-HT) activity as an important factor. Other neurotransmitters implicated include norepinephrine (NE), dopamine (DA), glutamate, and brain-derived neurotrophic factor (BDNF) [9]. However, drugs that produce only an acute rise in neurotransmitter availability, such as cocaine or amphetamines, do not have the efficacy over time that antidepressants do.

The role of CNS 5-HT activity in the pathophysiology of major depressive disorder is suggested by the therapeutic efficacy of selective serotonin reuptake inhibitors (SSRIs). In addition, studies have shown that an acute, transient relapse of depressive symptoms can be produced in research subjects in remission using tryptophan depletion, which causes a temporary reduction in CNS 5-HT levels. However, the effect of SSRIs on 5HT reuptake is immediate, but the antidepressant effect requires exposure of several weeks' duration. Also, some antidepressants have no effect on 5HT (e.g., desipramine), and the antidepressant tianeptine enhances 5HT uptake. All this, together with preclinical research findings, implies a role for neuronal receptor regulation, intracellular signalling, and gene expression over time, in addition to enhanced neurotransmitter availability.

Seasonal affective disorder is a form of major depressive disorder that typically arises during the fall and winter and resolves during the spring and summer. Studies suggest that seasonal affective disorder is also mediated by alterations in CNS levels of 5-HT and appears to be triggered by alterations in circadian rhythm and sunlight exposure.

Vascular lesions may contribute to depression by disrupting the neural networks involved in emotion regulation—in particular, frontostriatal pathways that link the dorsolateral prefrontal cortex, orbitofrontal cortex, anterior cingulate, and dorsal cingulate [10]. Other components of limbic circuitry, in particular the hippocampus and amygdala, have been implicated in depression.

1.4.1 Causes

The causes of depression are not fully understood and may not be down to a single source. Depression is likely to be due to a complex combination of factors that include:

- Genetic
- Biological - changes in neurotransmitter levels
- Environmental
- Psychological and social (psychosocial)

1.5 PATHOPHYSIOLOGY OF DEPRESSION

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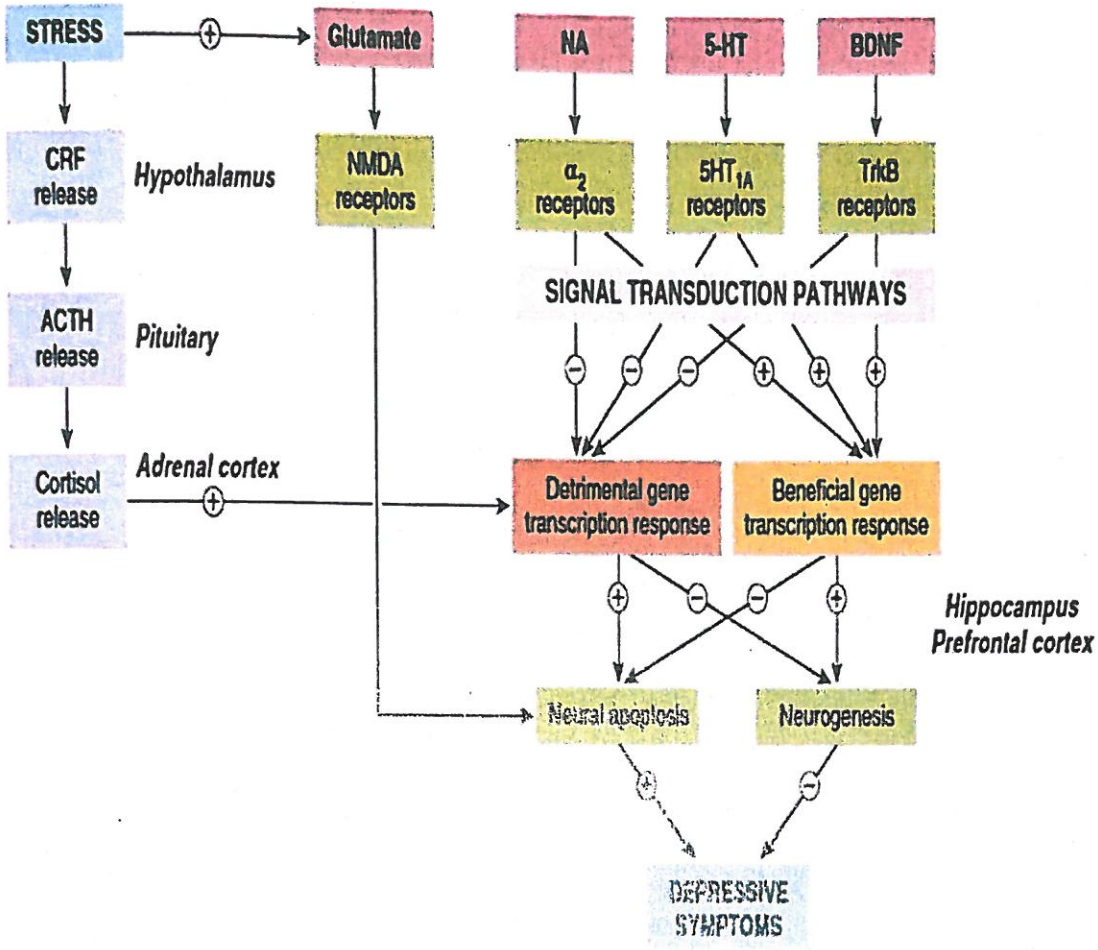

FIG 1.2: Mechanisms leading to and resulting from depressive disorder

1.6 ANTIDEPRESSANTS

An antidepressant, as the name implies, is a type of drug primarily used for the treatment of depression. Depression is a common disorder that affects the chemistry and function of your brain. Antidepressants can help correct the dysfunction by altering the circuits and chemicals that pass signals along nerve routesto the brain. [13]

Antidepressants are grouped into classes based on how they affect the chemistry of the brain. While the antidepressants in a class will tend to have similar side effects and mechanisms of action, there will be differences in their molecular structures which can influence how well the drug is absorbed, disseminated,or tolerated in different people.

There are five major classes of antidepressant and several others that are less commonly used. Each has itsown benefits, risks, and appropriate uses. While some may be considered preferred options, the selection of drug can vary based on your symptoms, history of treatment, and co-existing psychological disorders.

1.6.1 ANTIDEPRESSANTS WORKING

There are three basic molecules, known chemically as monoamines that are believed to be involved in mood regulation. These primarily work as neurotransmitters, which literally transmit nerve signals to theircorresponding receptors in the brain.

Antidepressants work by influencing these neurotransmitters, which include:

Table 1.2: Antidepressants Mechanism of Action

Serotonin	The neurotransmitter whose role it is to regulate mood, appetite, sleep, memory, social behavior, and sexual desire
Norepinephrine	Which influences alertness and motor function and helps regulate blood pressure and heart rate in response to stress
Dopamine	Which plays a central role in decision-making, motivation, arousal, and the signaling of pleasure and reward

1.7 DIFFERENT COMMERCIAL THERAPIES FOR DEPRESSION

Medication, psychotherapy, or both are commonly used to treat depression. If these therapies fail to alleviate symptoms, brain stimulation therapy may be a viable alternative. In milder forms of depression, psychotherapy alone may be used as a first line of defence, with medication added if symptoms persist. Many mental health providers advise starting treatment with a combination of medication and counselling for moderate or severe depression. The best treatment plan should be based on a person's unique needs and medical status while under the care of a professional. Finding the medicine that works best for the person may take some trial and error.

1.7.1 MEDICATIONS

Antidepressants are prescription drugs that are frequently used to treat depression. They take time to work - usually 4 to 8 weeks and symptoms like insomnia, appetite loss, and attention issues frequently improve before mood improves. Before evaluating whether or not a drug works, it is critical to give it a chance. [15]

1. **Selective serotonin reuptake inhibitors (SSRIs):** are drugs that blocks the reuptake of serotonin (SSRIs). SSRIs are frequently prescribed by doctors. These medications are thought to be safer and have fewer negative side effects than other forms of antidepressants. Citalopram (Celexa), escitalopram (Lexapro), fluoxetine (Prozac), paroxetine (Paxil, Pexeva), sertraline (Zoloft), and vilazodone (Viibryd) are examples of

CERTIFICATE

This is to certify that the dissertation entitled **"Evaluation of Anti -Parkinson activity of *Acorus calamus Linn*. Using Haloperidol catalepsy Model"** submitted to K.R. Mangalam University in partial fulfilment of the requirement for the award of Degree of MASTER IN PHARMACY in Pharmacology, embodied the original research work carried out by Sarfaraz Ahmad under our supervision and guidance.

It is further stated that no part of this dissertation has been submitted, either in part or full for any other degree of K.R. Mangalam or any other university/institution.

Supervisor

Dr. Arun Garg

Co-supervisor

Dr. Lakhveer

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Evaluation of Anti -Parkinson activity of *Acorus Calamus Linn.* using Haloperidol catalepsy Model

Thesis Submitted to

K.R.Mangalam University in partial fulfilment of the requirement for the award of Degree of

MASTER OF PHARMACY

IN

PHARMACOLOGY

By

Sarfaraz Ahmad

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2019-21

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1. INTRODUCTION

The most common neurodegenerative movement disorder is Parkinson's disease (PD).It is the most prevalent motion disorders and the second most common central nervous system degenerative disease. It mainly caused by lack of dopamine in the basal ganglia.

It can be characterized by the tremor, stiffness, bradykinesia/akinesia, and posturalinstability apart from the other clinical motor and non-motor symptoms.

It is caused by the degradation of dopaminergic neuron in substantia nigra and decreases neuronal output from basal ganglia to thethalamus. Dopaminergic neurons are damaged by a complex interplay of toxic-synuclein aggregates, mitochondrial dysfunction, oxidative stress, decreased intracellular calcium homeostasis, and neuroinflammation (21).

PD prevalence and incidence rates in Europe are estimated around 108–257/100,000 and 11–19/100,000 per year, respectively. Risk factor of PD is age, male gender and several environmental factors. In various study it is found that it affects 1% of the population that are above 60 years(5). Recent work has reported that global prevalence of PD was estimated 0.3 percent which has risen dramatically with age to >3 percent in patients who are above 80years(20).More recently, researchers have observed that PD incidences are more in males as compared in females. The rise in the risk of Parkinson's disease was thought to be linked to the substantial changes in smoking behavior that occurred in the latter half of the twentieth century. Air pollution caused by traffic has also been linked to an increase in the prevalence of PD.

It has been expected that 0.3% population in developing countries are affected by PD (1).

It is expected that people with PD will get double up to 2030 (20).

The condition was first characterized as paralysis agitans or shaking palsy by James Parkinson in 1817, and the term "Parkinson's disease" was coined later by Jean-Martin Charcot in the 19th century (3).

(a) DA & ACh balance in normal condition (b) DA & ACh balance in Parkinson's disease

Figure 1: Imbalance of Ach and DA in Parkinson Disease

1.1Causes of Parkinson's disease

The specific etiology of the disease is unknown, however oxidative stress, free radical generation, mitochondrial malfunction, apoptosis, neuroinflammation, and hereditary vulnerability are all important pathogenetic variables in PD. Certain endogenous or exogenous toxins such as 6-hydroxydopamine and 1- methyl-4-phenyl-1,2,3,6- tetrahydropyridine rotenone, Parquet, manganese, toluene, N Hexane, carbon monoxide, Mercury, Cyanide, Copper, Lead and Trichloroethylene certain medications, viral infection, Alzheimer's disease, amyotrophic lateral sclerosis (ALS), Creutzfeldt-Jakob disease, Wilson's disease and Huntington's disease. Administration of dopamine directly into brain and cell loss in the dopaminergic nigrostriatal tract of the brain can causes the parkinsonism(4).

Parkinson's disease is a neurodegenerative disorder that affects both motor and nonmotor circuits. It is characterized by two key pathologic processes: (a) premature selective death of dopamine neurons; and (b) the buildup of Lewy bodies, which are made up of misfolded -synuclein and accumulate in numerous systems of Parkinson disease patients (6).It's not clear which step takes place first. According to pathologic research, neurons degenerate in a stepwise manner over many years, with each afflicted place correlating to a distinct pathology. This has been linked to illnesses outside the brain, such as the spinal cord and peripheral autonomic nervous system (4).

Currently, Parkinson disease is diagnosed primarily on the basis of clinical symptoms, gleaned from history and examination of the patient, as well as the responsiveness to dopamine drugs and the emergence of motor variations over time. A resting tremor, a faint voice (hypophonia), veiled facies (first showing as reduced blink rate), small handwriting (micrographia), stiffness (rigidity), slowness of movements (bradykinesia), shuffling steps, and difficulties with balance are all common motor signs of the disease. Resting tremor is a hallmark symptom that often affects one upper limb, however 20% of patients do not have it; 30% of patients may initially show tremor in a lower leg, and there may also show lip, jaw, or tongue tremor at rest. Head and vocal tremors are infrequent, and in such situations, essential tremor should be included in the differential diagnosis. Bradykinesia has the strongest link to the severity of dopamine insufficiency of all the key symptoms. The criteria of the UK Parkinson's Disease Society Brain Bank have been standardized, with diagnosis accuracy of up to 90% (2).

1.2 Diagnosis

Parkinson's disease is mainly diagnosed clinically; the clinical diagnosis includes normal ageing, essential tremor, drug-induced Parkinsonism, the Parkinson-plus syndromes, vascular Parkinsonism, and normal pressure hydrocephalus. Less common entities with Parkinsonism dopa-responsive dystopia juvenile-onset Huntington's disease, pallidopontonigral degeneration. Now a days MRI, EEGs, PET, CT and SPECT techniques have considerably contributed in the diagnosis of PD. Laboratory test like CBC can also performed to diagnose PD (6).

1.3 Treatment

Treatment using synthetic drugs includes Levodopa is the first line treatment for parkinsonism it is a metabolic precursor of dopamine that is decarboxylated to dopamine within the presynaptic terminals of dopaminergic neurons in the stratium, responsible for the therapeutic effectiveness of the drug in Parkinson disease, Peak concentrations of the levodopa in plasma is between 0.5 and 2 hours after an oral dose with the half-life of 1 to 3 hours it is combined with a peripheral dopa decarboxylase inhibitor, either carbidopa or benserazide, which diminishes the peripheral side effects and also combined with plus dopa decarboxylase inhibitor entacapone (inhibitor of COMBT) to inhibit its degradation, About 80% of patients show initial improvement with levodopa , particularly of rigidity, hypokinesia, tremor and bradykinesia, and about 20% are restored virtually to normal motor function (6,9).

Apart from Levodopa dopamine agonist, MAO-B inhibitor and Anti-cholinergic drugs are also employed in the treatment and management if PD. Some of these anti-PD are described below (7).

1.3.1 MAO-B inhibitor

- **Selegiline**

Is a MAO inhibitor that is selective for MAO-B, Inhibition of MAO-B protects dopamine from intraneuronal degradation, thus decreases the metabolism of dopamine and has been found to increase dopamine levels in the brain and was initially used as an adjunct to the levodopa.

1.3.2 Dopamine receptor agonists

Bromocriptine is an ergot derivative, and few newer, non-ergot drugs, ropinirole, pramipexole, rotigotine and Apomorphine.

- **Bromocriptine**

Inhibits the release of prolactin from the anterior pituitary gland, its duration of action is longer (plasma half-life 6-8 hours) than that of levodopa. Newer dopamine receptor agonists include lisuride, pergolide, ropinirole, cabergoline and pramipexole. They are longer acting than levodopa and need to be given only once or twice daily, with fewer tendencies to cause dyskinesias and on-off effects. Apomorphines are available in inject able and transdermal delivery systems respectively, meant to be used for the acute management of the hypomobility phenomenon, alleviate the motor deficits in both levodopa patients.

- **Amantadine**

They have many possible mechanisms for its action includes increased dopamine release, inhibition of amine uptake, or a direct action on dopamine receptors and inhibiting the N-methyl-D-aspartate (NMDA) type of glutamate receptors.

1.3.3 Acetylcholine antagonists

Benztropine, trihexyphenidyl, procyclidine and biperiden interfere with this inhibitory effect on dopaminergic nerve terminals, suppression of which compensates for a lack of dopamine by muscarinic acetylcholine receptors

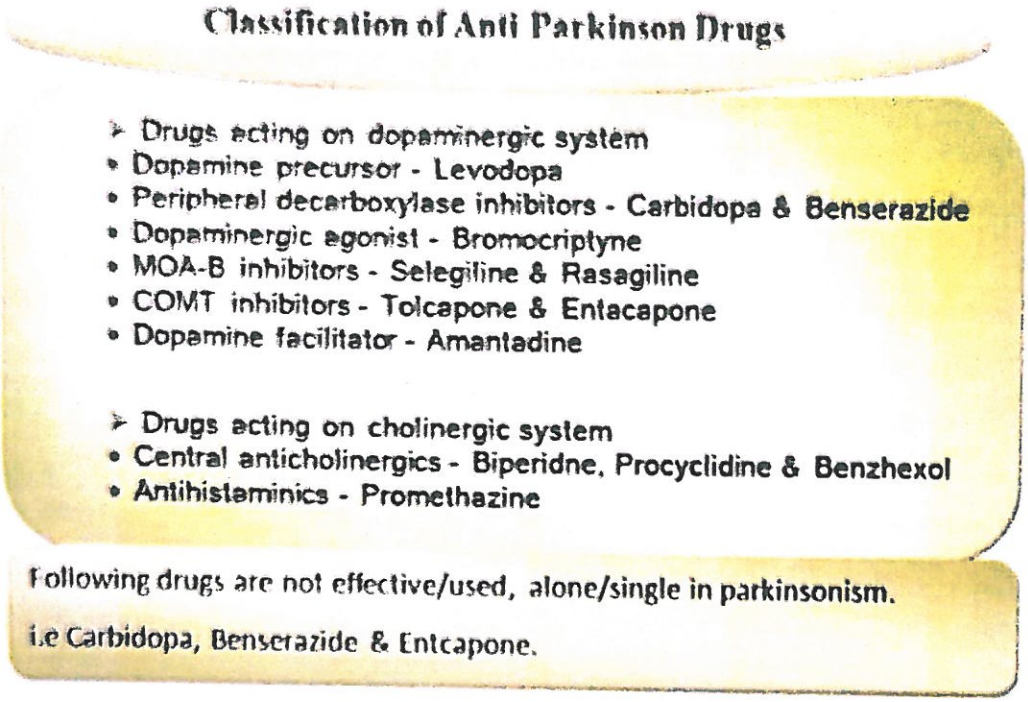

Figure 2: Classification Of Anti-Parkinsonian Drugs

1.4 Adverse effect of currently used Anti-Parkinsonian Drugs. (22, 23)

Many anti-Parkinson drugs are already available in the market but they are associated with some serious side effect (figure No. 3) so, better anti-Parkinson drugs are still needed.

Drugs	Adverse effects
Dopamine agonists	Compulsive behavior (i.e., pathological gambling) Insomnia, Serotonin syndrome
Levodopa	Headache, dizziness, somnolence
COMT inhibitors	Somnolence/insomnia
Anticholinergics	Confusion
MAO B inhibitors	Insomnia, Headache, Serotonin syndrome
Amantadine	Confusion

Figure 3: Adverse effect of currently used Anti-Parkinsonian Drugs

1.5 Use of herbal medicine for treatment of PD

Ethno pharmacology, a knowledge-driven method of drug development, plays a part in drug development centred on natural or conventional awareness of the pharmaceutical or toxicological effects in the human population from animals, plants, and fungi (24). Over 119 commercially accepted medicines are actually extracted from medicinal herbs. Of these, 74 percent have been identified through chemical detection of the components which are necessary for patient therapeutic use. Such 119 herbal medicines are made commercially from more than 90 plant species. With more than 25,000 organisms on the planet, their comprehensive study will contribute to more effective medicines being produced against common viruses (25). Ethno pharmacological method for drug research is found to be highly effective for the development of new drugs ranging from digitalis to vincristine. The most critical step in identifying medicines from plant sources is choosing the most appropriate materials based on ethno medicinal and ethno botanical applications (26).

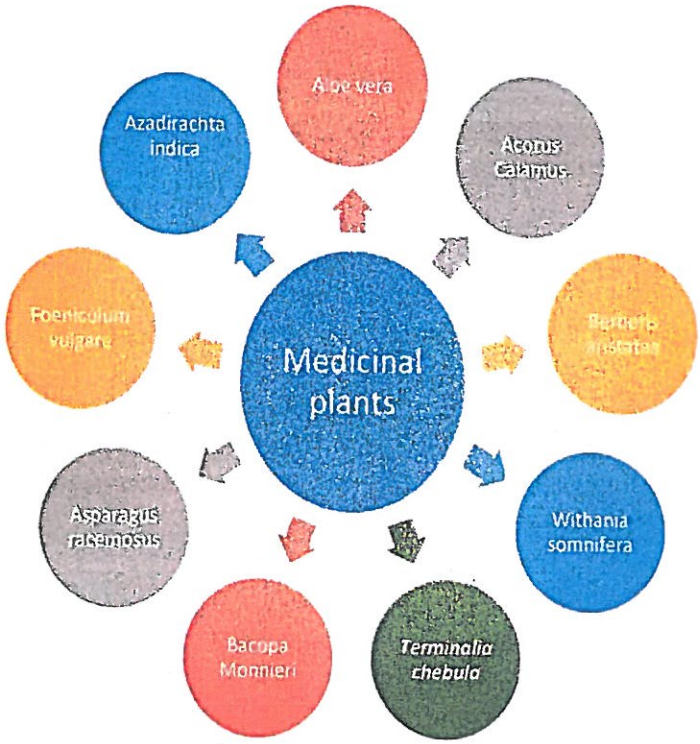

Figure 4: Medicinal plants utilized in the treatment of neurological disorders

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2. LITERATURE REVIEW

(Toncho et al., 2021) investigated the effect of methanol extract of *Acorus calamus at a dose* (64 mg/mL) exhibited the highest antimicrobial activity against eight fungal strains (F. oxysporum, A. flavus, A. niger, F. graminearum, A. ochraceus, Alt. Alternate, A. carbonarius and P. chrysogenum) with an (zones of inhibition ≤ 10.3 mm), and compare the same antifungal activity of Amphotericin B (zones of inhibition ≤ 13.8 mm).

(Rouauda et al., 2021) contemplated that Parkinson's illness (PD) is an intricate, age-related, neurodegenerative sickness whose pathogenesis remains not completely comprehended. They survey the job of mitochondria, natural poisons, alpha-synuclein and neuroinflammation in the advancement of PD. They additionally talk about later information from hereditary qualities, which firmly support the endosomal-lysosomal pathways and mitophagy as being integral to PD. In their audit they examined the arising job of the gut-cerebrum pivot as a modulator of PD movement.

(Chen et al., 2020) clarified that Parkinson's infection (PD) is a neurodegenerative sickness with a long-term long examination history. Autonomic brokenness is a fundamental class of non-engine aggregates that has as of late become a state-of-the-art field that coordinates wilderness research in PD. In their survey, they portray the study of disease transmission of dysautonomic indications in PD. Then, at that point, we play out a careful investigation of the pathophysiology of autonomic brokenness in PD and recommend that the fringe autonomic sensory system might be a critical course for α-synuclein pathology spread from the outskirts to the focal sensory system. In their survey they sum up the techniques presently accessible for the treatment of autonomic brokenness in PD and propose that top notch; better-planned, randomized clinical preliminaries ought to be led later on.

(Sharma et al., 2020) clarified that vacha Acorus calamus Linn (Acoraceae) is a conventional Indian therapeutic spice, which is polished to treat a wide scope of wellbeing illnesses, including neurological, gastrointestinal, respiratory, metabolic, kidney, and liver issues. Until now, 145 constituents have been confined from this spice and recognized, including phenylpropanoids, sesquiterpenoids, and monoterpenes. Convincing proof is reminiscent of the biopotential of its different concentrates and dynamic constituents in a few metabolic and neurological problems, like anticonvulsant, stimulant, antihypertensive, mitigating, immunomodulatory, neuroprotective, cardioprotective, and hostile to stoutness impacts. In their writing study they expected to give

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CERTIFICATE

This is to certify that the dissertation entitled **"*In-vivo* evaluation of wound healing activity of *C. limetta* in Albino wistar rats"** is bonafide research work carried out by Ms. Megha Mittal (2004650004) in partial fulfillment of the requirement for the award of the Degree of "Master of Pharmacy in Pharmacology of School of Medical & Allied Sciences, K. R. Mangalam University, Sohna, Gurugram (Haryana), India. The assignment has been carried out in the laboratories of K. R. Mangalam University and is a bonafide research work carried by her under our supervision and guidance of **Mr. Sanjeev Kumar** at Department of Pharmacology, K. R. Mangalam University, Sohna Road, Gurugram-122103, Haryana during the academic year 2021-22.

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ABSTRACT

Background and Objective: Research project deals with the screening of different extract of *Citrus limetta* peel for wound healing activity. It has been reported that *Citrus limetta* has antibacterial, antifungal, antidiabetic, anticancer and antiviral activities. The wound healing activity of this plant has not been reported and is therefore widely used in folklore medicine. We tried to study this activity. We attempted to investigate this activity. The purpose of the study was to investigate the acceleration *in-vivo* evaluation of the wound healing properties from *Citrus limetta*'s peel extract in Albino wistar rats.

Method: There were 30 Albino wistar rats as an animal trial divided into 6 groups, including control, standard Turmeric, *Citrus limetta* in aqueous, Chloroform & ethanol. The animal model selected is incision wound model.

Result: In incision wound model, *Citrus limetta* peel extract treated rat showed significant wound healing.

Interpretation and Conclusion: It can be concluded that the *Citrus limetta* extract had the potential to accelerate wound healing activity. It can be concluded that *Citrus limetta* peel extract topically have wound healing activity which may be due to its anti-oxidant property and d-limonene.

KEYWORDS

Herbal, Incision model, wound healing, Ethanolic extract, Chloroform extract, aqueous extract, *C. limetta,* Mausambi

1. INTRODUCTION

1.1 BACKGROUND

Skin is made of water, fats, minerals and proteins the largest organ in the body. It protects against infections, thermoregulation, and enables for touch (tactile) sensory stimuli. The layers of skin that is vulnerable to acne, wrinkles, rashes & skin cancer [1].

- The **Epidermis** is a thin, outermost layer of epidermal cells that represents as a water resistant barrier and provides colour to our skin [1, 2].
- The **dermis** is a denser, thicker layer that contains sweat glands, rigid connective tissue and hair follicles. It is majorly consisting of connective tissue [1, 3].
- The **Hypodermis**, or subcutaneous fat layer, connects the overlying dermis to the body constituents. This layer of adipose cells is primarily responsible for body insulation and structural protective measures against physical shock [1, 4].

1.2 CLASSIFICATION OF WOUNDS

The aetiology of a wound, its anatomical position, whether wound is chronic or acute, the method of closure, the presenting symptoms, or the visual appeal of the prevailing different tissues in the wound bed can all be used to describe it [7]. Wounds are classified according to the extent of harm to the various layers of skin.

In broad sense, wounds are classified as follows:

1. Those involving only a superficial loss of epidermis,
2. Those encompassing the epidermis and dermis,
3. Those comprising the dermis, subcutaneous fat, and occasionally the bone [8].

Wounds can indeed be characterized by an acute depending on how long it takes for them to heal. Acute wounds heal without complications in the expected amount of time, with the final outcome of anatomical & functional restoration. Chronic wounds take longer to heal and are more likely to cause complications [9].

Table 1.1: Classification of wounds according to Wound Thickness **[5]**

S.No.	TYPES	DESCRIPTION
1.	Type I	Superficial loss of epidermis; the deeper portions like sweat ducts are not destroyed. Here, wound size is around or equal to 1 cm.
2.	Type II	**Type II A:** The wound encompasses epidermis and dermis, has no significant issue loss. Here, wound size is in between 1 to 10 cm.
		Type II B: The wound encompasses the epidermis, dermis, subcutaneous fat & bone, substantial tissue loss occurs. Here, wound size is above 10 cm.

Table 1.2: Types of Wounds according to the Morphological Characteristics

S.No.	TYPES	DESCRIPTION
1.	Incisional Wounds	This is an injury with no tissue loss and only minor tissue damage. Sharp objects, such as a scalpel or knife, are the primary cause [7].
2.	Excisional Wounds	A tiny portion of whole thickness skin is entirely eliminated from the wound area in these wounds [7].
3.	Burn Wounds	The wounds in which the skin or other organic tissue is burned or injured as a result of heat, radiation, radioactivity, electricity, friction, or chemical contact [9].
4.	Contusion/ bruise	It is usually caused by a blunt blow, the overlying skin is unbroken, but tissues and blood vessels below are damaged. RBCs are trapped in the tissue spaces become deoxygenated and dark colored [10].
5.	Avulsions	This term describes a wound where there is tissue loss, preventing the closure of the wound edges [11].
6.	Sprains	A sprain describes an injury to the fibrous tissues surrounding a joint. Fibrous ligaments around the joint are injured, usually as a result of excessive movement of the joint [12].
7.	Strains	Strains are injuries to muscles, fascia or tendons caused by stretching forces [13].
8.	Penetration Wounds	A knife like object going in or out of the skin is the most common cause of penetration wound [7].
9.	Gunshot wounds	They're usually caused by a bullet or other projectile that pierces or penetrates the body [7].

1.3 PREVALENCE OF WOUNDS

The global burden of injuries can only be estimated, and whatever facts and figures exist is limited to a few countries [13]. Acute and chronic wounds have a significant financial impact on the health-care system, which is measured in both indirect & direct costs. Chronic and acute injuries have a significant financial impact on the health-care system, which is measured in both indirect & direct costs. Since its inception, the NWCSP has sought to establish a decent level of wound healing all over England by reducing defects, improving safety, and better health outcomes, all while reducing the pressure of wound treatment for people, caregivers, and health professionals across a variety of wound types. An efficient comprehensive patient evaluation, accompanied by an organized wound bed assessment, is crucial to enhancing timely wound healing [44].

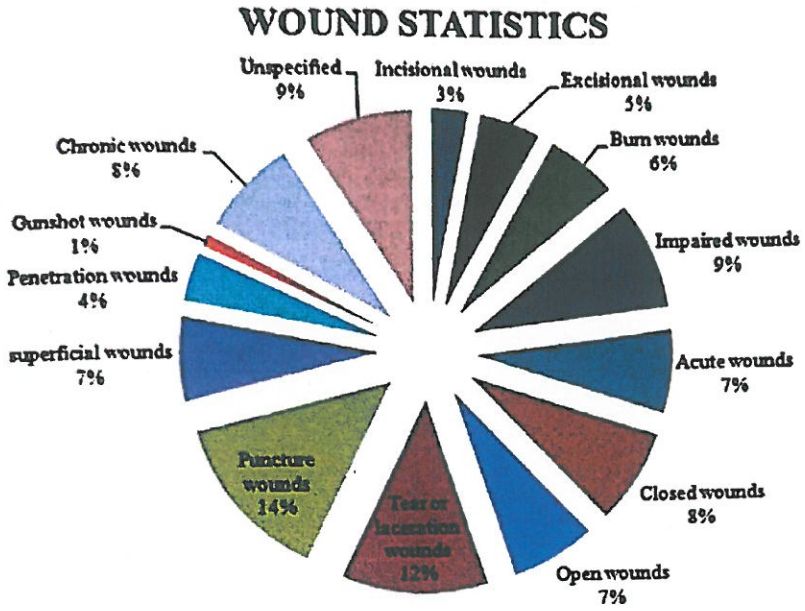

Fig 1.1: Prevalence of Wound Incidence [43]

1.4 PATHOPHYSIOLOGY OF WOUND HEALING

Wounding and wound healing take place in all of the body's tissues and organs, including the skin. The procedure of wound healing begins almost immediately after the injury & all injuries go through an active repair process, though different tissues have different healing processes, as well as different times for complete healing and different pathways for healing [14]. In general, wound healing is the body's natural response to an injury and involves a series of events. The healing process refers to the series of actions taken by the body to heal a wound. Normal wound healing entails a dynamic & complex series of events that culminate in the restoration of injured tissues.

1.4.1 Phases of Wound Healing Process

An injury to the skin or any other tissue triggers a complex repair process aimed at restoring the injured body site. To re-establish the whole organism's integrity and homeostasis, the various events associated in this recovery process should be strictly regulated and synchronized [16]. According to Hutchinson, the wound healing process is divided into four phases [6]:

1.4.1.1 Hemostasis [30-36]

Hemostasis is the immediate reaction to injury by contraction of capillaries to reduce bleeding. It is the process of stabilizing the circulatory system by clot formation and stops the blood leakage at the wound site or it can also be defined as a physiologic (primary and

secondary hemostasis) or surgical procedure, it stops bleeding by keeping blood within a ruptured blood artery.

Chemical Released: Epinephrine [37]

Cells Involved: Platelet cells and cytokines (PGDF & Collagen cells).

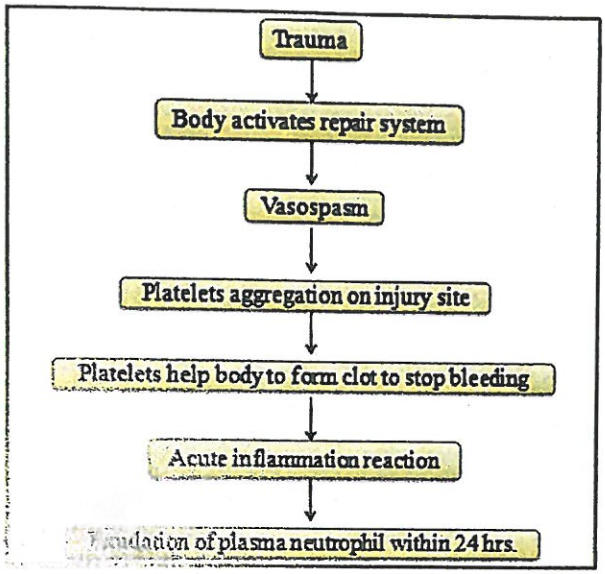

Fig 1.2: Flowchart of Hemostasis Phase

1.4.1.2 Inflammation [30-36]

As tissue repair begins in the injured area, inflammation develops. Fibrin and blood platelets form a loose blood clot to prevent for the blood loss, occurs to help control bleeding, prevent infection at the wound site. Initiated when injury blood vessels begin to leak fluid, continues 2 days up to 2 weeks. Body's protective response to the injury and infection, it begins with increase in vascular permeability in the affected areas. WBCs are the first responders at the medical emergency. Body releases chemicals which dispatch WBCs and platelets to the site of injury, this is called *Chemotaxis*.

Following any kind of injury there are two types of inflammation:

- Acute inflammation – normally results on onset of injury or infection is gone;
- Chronic inflammation – sometimes inflammation is ongoing for months are chronic. Eg: TB and autoimmune disease.

Chemical Released:

- Bradykinin [38]
- Factors C_3a and C_5a [39]
- Histamine (via mast cells) [40]

They all happen all together not in sequence.

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Cells Involved:

- Neutrophils [41]
- Macrophages/ Monocytes [42]

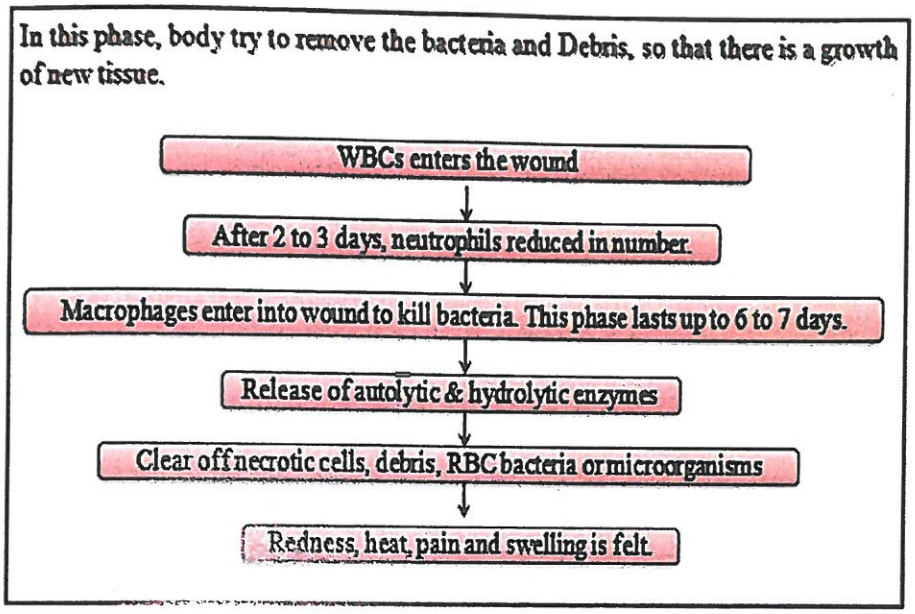

Fig 1.3: Flowchart of Inflammation Phase

1.4.1.3 Proliferation [30-34]

In initial portion, the clot exterior dries forming a scab, fibroblasts infiltrate the wound and secrete collagen to strengthen the clot. Fibroblasts also cause endothelial cells in the wound's surrounding area to proliferate, causing ruptured blood vessels to begin to regenerate. *Granulation tissue* is a fine net developed by these cells. Granulation tissue has a bumpy texture and is distinctive for its colour. They secrete chemicals switch to great the existing clot. The *angiogenesis* (physiological process of formation and assembling of new blood vessels from pre-existing vessels, also known as *neovascularization*) ensures that nutrients are supplied for granulation tissue formation and is essential for wound healing. *Re-epithelialization* begins when granulation tissue is nearly level with the surrounding skin; epithelial cells rearrange shape to aid motility and crawl across the wound bed to cover it.

Cells Involved: The wound cavity is filled with granulation tissue, which is made up of macrophages, immature collagen, fibroblasts, blood vessels and ground particles [21].

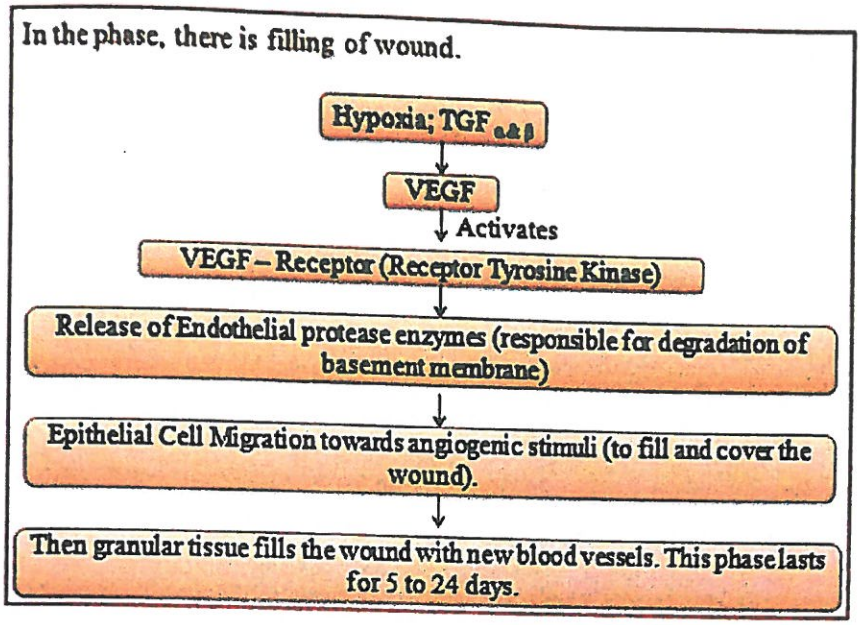

Fig 1.4: Flowchart of Proliferation Phase

1.4.1.4 Remodeling [30-36]

The transition from granulation tissue to scar tissue involves reorganization and maturation of collagen fibers to maximize tensile strength. The fibres are aligned around the lines of tension and cross connected to generate a robust wound during the remodeling phase. The scab sloughs off, collagen fibres and extracellular matrix become more organized and fewer fibroblast at present. A new network blood vessel is restored to normal to supply oxygen blood to rebuild tissue. During this phase, new scar tissue is built to repair the wound & scar tissue formed by a process called *fibrosis*. Scar tissue differs from normal skin in that it has denser arrangement of collagen fibres and a reduced elasticity. Remodeling begins within a week of injury & can take up to two years after wounding. During remodeling, the tissue created by fibroblast matures and regain its normal function.

Cells/Enzymes Involved [23, 43, 44]:

In this phase, for transitioning Type III collagen to Type I collagen - fibroblasts, Matrix metalloproteinases (MMPs), Growth Factors, Collagenase are critically required for regressing capillaries & maturing the scar.

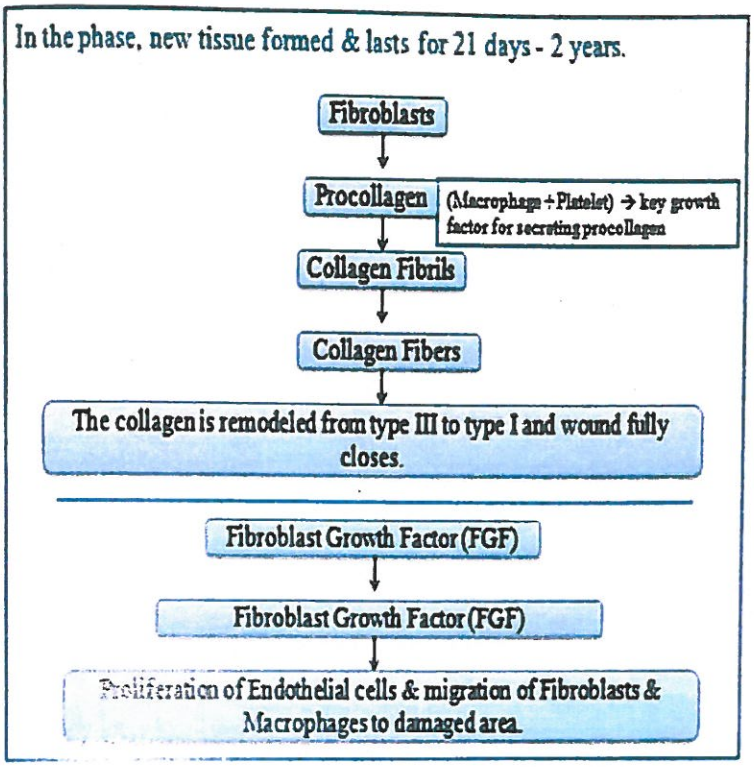

Fig 1.5: Flowchart of Remodelling Phase

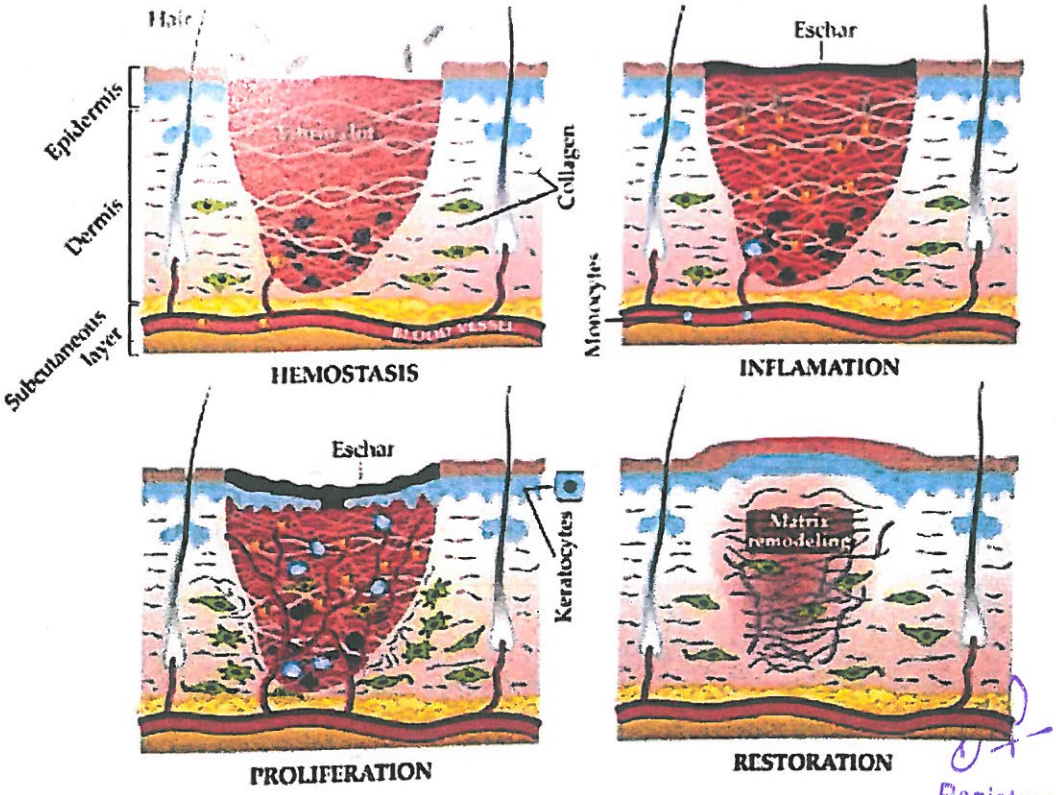

Fig 1.6: Phases of Normal Wound Healing [91]

CERTIFICATE FROM THE GUIDE

This is to certify that the work dissertation **"To evaluate the platelet enhancing activity of Carissa carandus in Sprague Dawley (SD)"** being submitted by Ms. Mahika Jain in partial fulfillment of the requirement for the award of the Degree of Master of Pharmacy in Pharmacology of School of Medical & Allied Sciences, K. R. Mangalam University, Sohna , Gurugram (Haryana), India. The assignment has been carried out in the laboratories of K. R. Mangalam University and is a bonafide research work carried by her under our supervision and guidance of **Dr. Arun Garg** at Department of Pharmacology, K. R. Mangalam University, Sohna Road, Gurugram-122103 Haryana, during the academic year 2021-22. The research work carried out meets the requirement of the present course.

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TO EVALUATE THE PLATELET ENHANCING ACTIVITY OF *CARISSA CARANDUS* IN SPRAGUE DAWLEY (SD)

Thesis Submitted to School of Medical & Allied Sciences,
K. R. Mangalam University, Sohna Road, Gurugram, Delhi- NCR,
In partial fulfillment for the
Award of the Degree of
Masters of Pharmacy (Pharmacology)
2022

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2020 – 2022

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1. INTRODUCTION

THROMBOCYTOPENIA

It is a condition in which one has a low blood platelet count. Thrombocytopenia is due to decreased production, increased destruction, and increased sequestration in spleen. Of this infection is the most common cause. Fever with thrombocytopenia narrows the differential diagnosis of the clinical entity. Infection like malaria, dengue, leptospirosis, typhoid, HIV, and miliary tuberculosis are some of the common causes of fever with thrombocytopenia. Platelets (thrombocytes) are colorless blood cells that help blood clot. Platelets stop bleeding by clumping and forming plugs in blood vessel injuries. Thrombocytopenia might occur as a result of a bone marrow disorder such as leukemia or an immune system problem or it can be a side effect of taking certain medications. It affects both children and adults. Thrombocytopenia can be mild and cause few signs or symptoms. In rare cases, the number of platelets can be so low that dangerous internal bleeding occurs. Thrombocytopenia means you have fewer than 150,000 platelets per micro litre of circulating blood. Because each platelet lives only about 10 days, your body normally renews your platelet supply continually by producing new platelets in your bone marrow [1].

Sometimes, you don't have enough platelets because they get trapped in your spleen, an organ that fights infection. And women may get thrombocytopenia during pregnancy because their bodies get rid of platelets more quickly than usual.

THROMBOCYTOPENIA SYMPTOMS

Sometimes, you don't have any symptoms from thrombocytopenia. When you do, the main problem is bruising and bleeding in your skin that looks like tiny red or purple spots, called *petechiae* [1].

You can bleed outside or inside your body. Sometimes, it can be heavy or hard to stop.

You might also have:

- Bleeding from your gums or nose
- Blood in your urine or poop
- Heavy menstrual periods

The following factors can raise your risk of thrombocytopenia.

- **Environment:** Exposure to toxic chemicals — such as pesticides, arsenic, and benzene — can slow the production of platelets.

- **Lifestyle habits:** Alcohol slows the production of platelets. Drinking too much alcohol can cause your platelet count to drop for a short time. This is more common in people who have low levels of vitamin B12, or folate.
- **Medicines:** Some medicines can slow the production of platelets. Also, a reaction to medicine can confuse your body and cause it to destroy its platelets [2].

1.2.1 Cause

Drugs can cause thrombocytopenia by two main mechanisms:

- Decreased platelet production

  For example cytotoxic drugs induce bone marrow toxicity, which results in decreased blood cell production (red and white blood cells and platelets). Drugs with an influence on folic acid metabolism can also cause thrombocytopenia by this mechanism.

- Increased destruction of platelets (whether immune-mediated or not). [3]

Apart from these two main mechanisms, a phenomenon called Pseudothrombocytopenia has to be taken into account when looking into the cause of low platelet numbers. Pseudothrombocytopenia is caused by the clumping of platelets in vitro, result-ing in low platelet counts. However, the number and function of platelets in vivo is normal. This effect arises from the cold reacting IgM or IgG antibodies that bind to platelets causing clumping at room temperature. It is probably caused by the use of the anticoagulant ethylene diamine tetra-acetic acid (EDTA) instead of citrate [4]. However, pseudothrombocytopenia may also occur in anticoagulants such as heparin and citrate." The same symptoms that are caused by thrombocytopenia may occur in patients with thrombocytopathy. The platelet counts are normal but the function of thrombocytes is impaired. This effect is caused, for example, by aspirin (acetylsalicylic acid), NSAIDs and selective serotonin reuptake inhibitors.

ORIGIN OF THROMBOCYTOPENIA

Platelets are small anucleated cell fragments that originate from megakaryocytes (MKs), which are large cells located in the bone marrow (BM). MKs extends long cytoplasmic protrusions, a process which is called proplatelet formation, into the lumen of the sinusoidal vessels where platelets are sized by the bloodstream. During the process of platelet biogenesis, segments of the MK penetrate the endothelium and, through cytoskeletal remodeling inside the MK, proplatelet fragments are released.

Megakaryocytes

The name megakaryocyte (MK) originates from the greek words mega (big), karyon (core) and kytos (cell) and translates into the definition of 'cell with a big nucleus'. Besides their polyploid nucleus, MKs are with an average size of 50 – 100 µm the largest cells in the BM, but account for less than 0.1% of nucleated cells [5].

Megakaryopoiesis

It is a complex process that involves the commitment of hematopoietic stem cells (HSCs) to the megakaryocyte (MK) lineage, proliferation of the progenitors, MK maturation and terminal differentiation that produces platelets. HSCs are multipotent cells that can either self-renew or differentiate into various blood lineages including the common lymphoid progenitor (CLP) and the common myeloid progenitor (CMP) [6].

Thrombopoiesis

It is the process which is characterized by the extension of long cytoplasmic protrusions (proplatelets) by mature MKs through the endothelium into the vessel lumen where platelets are sized by the bloodstream [7].

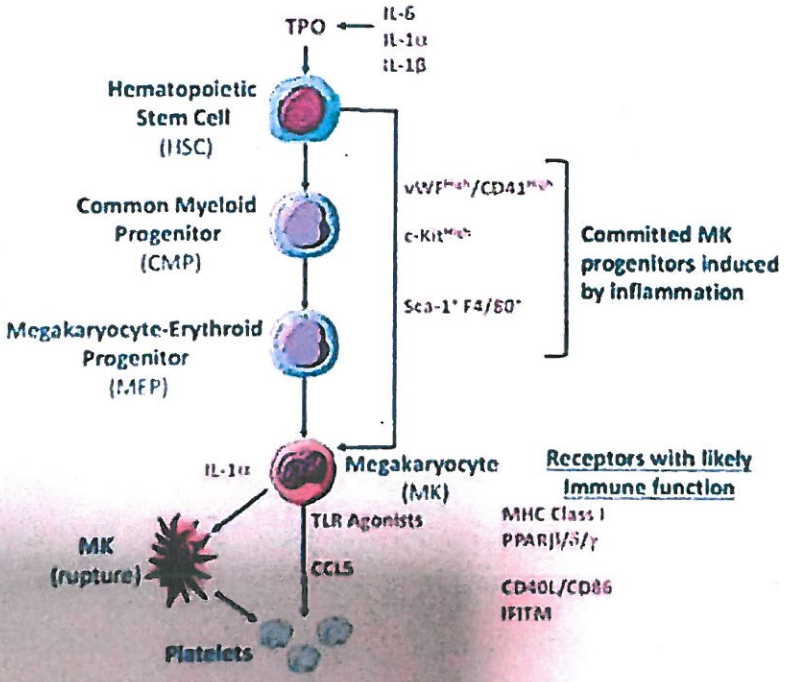

FIG 1.1: Platelet Formation

Before beginning of thrombopoiesis the hematopoietic progenitor cell megakaryoblast undergo DNA replications without cell division (a process called endomitosis), leading to

generation of polyploid megakaryocytes (matured form). The abundant genomic DNA in the polyploid megakaryocytes enhances their ability to synthesize proteins and package them into specific platelet granules [6]. The exact mechanism of platelet release from megakaryocytes is still under debate. In vitro studies demonstrated that platelet formation begins at one pole of megakaryocytes, and then the whole cell is disintegrated, resulting in the generation of numerous proplatelets. However, a recent intravital microscopy study revealed that megakaryocytes extend long protrusions into bone marrow sinusoids and release proplatelets from the tip of the protrusions under shear stress, suggesting that platelet generation in vivo is drastically different from in vitro cell culture conditions [7]. Proplatelets then undergo further division to generate mature platelets in vivo. Interestingly, a recent study suggested that platelets are capable of cell division and progeny generation even without a nucleus, although more evidence is required to confirm this finding. Thrombopoiesis is driven by the interplay of several transcription factors, with a negative feedback role of thrombopoietin in the final stage of platelet production. Thrombopoietin, which is produced in the liver, stimulates the thrombopoietin receptor (cMpl/CD110) on megakaryocytes to induce the formation of proplatelets via a JAK/STAT mechanism that is activated at low platelet counts in blood. In reactive thrombopoiesis, such as that occurring in inflammatory states, IL-6 enhances the process of proplatelet formation by increasing thrombopoietin levels5 [8].

TABLE 1.1: History of Platelets discovery

S.No.	Year	
1.	1957	The Intrinsic Membrane System (IMS) is an extensive complex of cisternae and tubules distributed throughout the MK cytoplasm that is continuous with the plasma membrane and is thought to exist as a membrane reservoir for proplatelet formation.
2.	1968	Megakaryocytes (MKs) increase in size & become full of platelet specific granules, expand their cytoplasmic content of cytoskeletal proteins, and develop a highly tortuous invaginated membrane system.
3.	1976	Endomitosis is a primarily TPO-driven process by which MKs become polyploidy through cycles of DNA replication without cell division Gurney et al., 1994 latter on confirmed this fact.
4.	1982	Megakaryocytic develop from hematopoietic stem cells (HSCs) that reside mainly in the bone marrow but are also present in the yolk sac, fetal liver,

		and spleen during early development.
5.	1994	Role of Thrombopoitin (TPO) were studied which functions as the major regulator that promotes the growth and development of MKs from their Hematopoitic Stem Cell precursors.
6.	1996	Relationship of size of platelets and their activity and efficacy has being studied in many studies
7.	1999	Mature MKs extends long branching processes called proplatelets into the sinusoidal blood vessels of the bone marrow. Functions as the assembly lines of platelet production, are comprised of platelet-sized swellings in that are connected by thin cytoplasmic bridges.
8.	2003	Megakaryocytes release a various sized platelet fragments into the blood, which indicate that terminal platelet formation continues in the blood stream.
9.	2000	Firstly Megakaryocyte proliferate into 2n stage and then begin endomitosis and accumulate a DNA content of 4n, 8n, 16n, 32n, 64n, and even 128n in a single polylobulated nucleus before proceeding with their final maturation and proplatelet formation.
10.	2011	A developed and mature IMS aided by the spectrin membrane skeleton helps establish and maintain proplatelets during platelet biogenesis. All these studies proposed that platelet size correlates with platelet reactivity; larger platelets have greater prothrombotic potential. Elevated platelet size (mean platelet volume) is associated with increased platelet aggregation, increased expression of adhesion molecules, and elevated risk of cardiovascular and peripheral arterial diseases. After they enter the circulation, platelets have a life span of 7 to 10 days.

PLATELETS

Primary hemostasis is dependent on the adhesion, activation and aggregation of platelets, but these processes are also key factors during acute arterial thrombotic occlusion causing fatal disease states such as myocardial infarction or ischemic stroke [9]. Furthermore, platelets have been described as mediators of thrombo-inflammation, atherogenesis and tumor vascular integrity.

The platelet surface contains integrins (e.g., β1, β3, α2, αIIb), glycoproteins (GPs), such as the GPIb-V-IX complex, G-protein coupled receptors (e.g., Gi, Gq, G12/13) and the (hem) immunoreceptor tyrosine-based activation motif (ITAM) receptors GPVI and C-type lectin-like receptor 2 (CLEC-2), among others. Platelets possess a mixture of intracellular granules, with three major subtypes: α-granules, dense granules, and lysosomes. Under physiological conditions, platelets circulate in a discoid shape in close proximity to the vessel wall and are not activated until the endothelial layer covering the vascular wall is disrupted and the subendothelial matrix is exposed [10].

During platelet activation in mice, which is mainly mediated through the receptor GPVI, CLEC-2 and integrins α2β1 and αIIbβ3, the PLC isoform γ2 (PLCγ2) is phosphorylated and thus activated, which induces the release of Ca2+ from intracellular stores. The increase of Ca2+ in the cytoplasm then contributes to further steps of cellular activation, such as degranulation and inside-out activation of integrin αIIbβ3 and cytoskeletal rearrangements pivotal for platelet shape change [11].

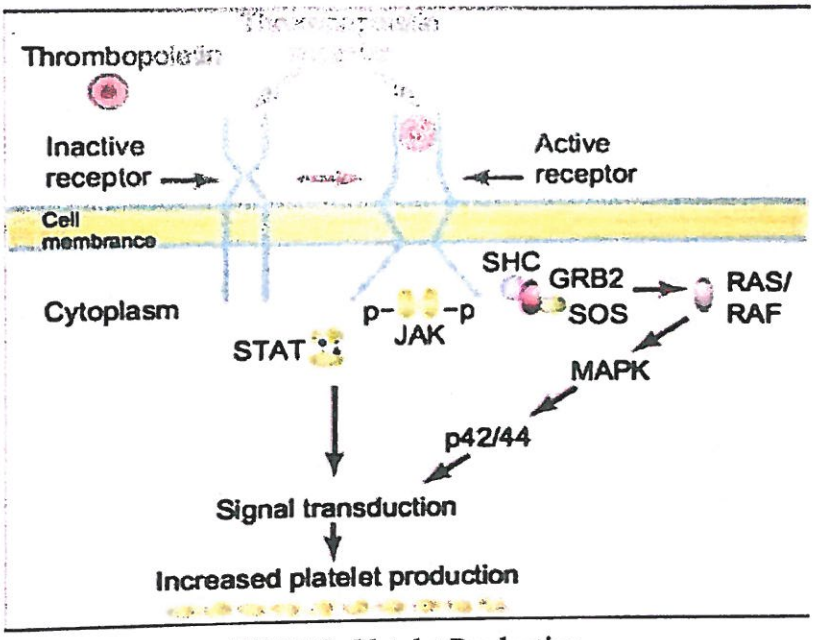

FIG 1.2: Platelet Production

STRUCTURE OF PLATELETS

I] Platelets in Resting Phase

Some of the major receptors found on the surface of resting platelets include the glycoprotein receptor for von-Wille brand factor (VWF); the major serpentine receptors for ADP,

thrombin, epinephrine, and thromboxane A2; the Fc receptor Fcγ RIIA; and the β3 and β1 integrin receptors for fibrinogen and collagen [12].

The surface of the platelet plasma membrane is smooth with the lipid bilayer of the resting platelet contains a large concentration of transmembrane receptors. Platelet plasma membrane composed of phospholipid bilayer, which is the site of expression of various surface receptors and lipid rafts which helps in signaling and intracellular trafficking [12].

III Intracellular Components:

a. ***Granules:*** Dense granules (or dense bodies) are 250 nm in size, with electron-dense cores; they function primarily to recruit additional platelets to sites of vascular injury. These granules contain a plenty of hemostatically active substances which are released upon platelet activation, these includes serotonin, catecholamines, adenosine 5 - diphosphate (ADP), adenosine As mentioned in their study, P. Blair suggested that the main role of α granules are, coagulation, inflammation, atherosclerosis, antimicrobial host defense, angiogenesis, wound repair, and tumorigenesis [13].

b. ***Organelles:*** Paroxisomes are small organelles that contains catalyze enzymes and Lysosome contains β- galactosidase, cathepsin, aryl sulfatase, β-glucuronidase, and acid phosphatases. Platelets is an anuclear structure that contains small numbers of mitotically active mitochondria which are essential to fulfill the energy need of the circulating Platelets for maximum of 7 days [14].

c. ***Open Canalicular system:*** As a reservoir of plasma membrane and membrane receptors since one-third of the thrombin receptors are located in the OCS of the resting platelet which are to be exposed to plasma membrane if needed. The open canalicular system (OCS) has being studied; and found that it is the "tunnel" system present throughout the platelet cell and remains connected with the plasma membrane. After cell activation, specific membrane receptors are also transported in the reverse direction from the plasma membrane to the OCS, this process is called as down regulation [15].

d. ***Dense Tubular System (DTS):*** Platelets contain a dense tubular system (DTS), which is believed to be similar in function to the smooth endoplasmic reticular system in other cells and serves as the predominant calcium storage system in platelets [16].

e. ***The cytoskeleton of the resting platelet:*** The disc shape of the resting platelet is maintained by a well-defined and highly specialized cytoskeleton. The three major cytoskeletal components of the resting platelet are: - Marginal microtubule coil - Actin cytoskeleton - Spectrin membrane skeleton [17].

II] Platelets in Activation Phase

The response of Platelets to vascular damage, and found that Platelets undergo rapid and dramatic changes in cell shape, on activation by agonists thrombin, TXA2, ADP, collagen, and VWF; upregulate the expression and ligand-binding activity of adhesion receptors, and secrete the contents of their storage granules and they change their shape from discs to spheres with pseudopodia.

Platelet receptors are different in different phases as in Resting phase, in activation phase and in stabilization and negative regulation phase. During platelet activation, the actin filament content doubles from a resting platelet concentration of 0.22 μM to 0.44 μM. Filopodia are composed of tight bundles of actin filaments that originate near the center of the platelet.

These shape changes are determined by the remodeling of the platelet cytoskeleton which is dependent upon the new congregation of actin filaments. Conversion into sphere from disc is mainly brought by raising cytoplasmic calcium level of Platelets many folds from the resting concentration of 10 to 20 nM. The formation of platelet lamellipodia and filopodia requires the assembly of actin filaments [19].

FUNCTIONS OF PLATELETS

Platelets play various roles in addition to the main one i.e., Hemostasis and Thrombosis. Various fuctions of the Platelets are listed as follows [19]-

A. Hemostasis and Thrombosis:

- Adhesion
- Activation
- Spreading
- Secretion
- Aggregation
- Procoagulant
- Clot Retraction
- Tissue Repair

CERTIFICATE

This is to certify that the dissertation entitled "**In silico screening and pharmacokinetic studies of anticancer herbal plant products**" submitted to K.R. Mangalam University in partial fulfilment of the requirement for the award of Degree of **MASTER IN PHARMACY** in Pharmacology, embodied the original research work carried out by **Sunil Kumar Jangra** under our supervision and guidance.

It is further stated that no part of this dissertation has been submitted, either in part or full for any other degree of K.R. Mangalam or any other university/institution.

Lakh 27/1/21

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In Silico screening and pharmacokinetic studies of anticancer plant products

Thesis Submitted to

K.R. Mangalam University in partial fulfilment of the requirement for the award of Degree of

MASTER OF PHARMACY

IN

PHARMACOLOGY

By

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Further arrangement of Thesis

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Chapter -1
Introduction

Cancer: After cardiovascular disease, cancer is the second biggest cause of death (1-4). It is a serious public health problem in both developed and undeveloped countries, and it is commonly treated with herbal plants or their phytochemicals (5,6). It can be characterised by growth of cell without any control and a cancer cell can invade in nearby tissue and cells, undergoes metastasis, angiogenesis as well as other metabolic changes. The cancerous cells can spread to other parts of the body by the lymph and blood. Cancer can be classified based on the type of their tissue of origins, for e.g., lung cancer, breast cancer, colorectal cancer, stomach cancer and prostate cancer. In humans, there are number of varieties of cancer; among these, lung cancer is the most common in men and breast cancer in women [7,8].

1.1 Cancer can also be classified as:

1. Carcinoma
2. Sarcoma
3. Lymphoma and leukemia
4. Germ cell tumor
5. Blastoma

1. **Carcinoma:** Carcinoma cancers are arising from the epithelial cells. The carcinoma cancers are the most common cancers and it is including in the lung, prostate, pancreas, colon and breast cancer (9).
2. **Sarcoma:** Sarcoma cancers are derived from the connective tissues (i.e., fat, nerve, cartilage and bone) (9).1
3. **Lymphoma and leukemia:** These two classes of cancer are arising in the hematopoietic (blood forming) cells. Lymphoma arising in the white blood cells and lymphoma affects the lymph nodes of certain sites – intestine, stomach and brain. Leukemia begins in blood forming tissues and it causes the abnormal blood cells (9).
4. **Germ cell tumor:** Germ cell tumor are derived in the pluripotent cells (cells that are able to make cells). These cells are presenting in the ovary and the testicle.
5. **Blastoma:** Blastoma cancers are arising in the embryonic tissue or immature precursor cells.

1.2 Signs and symptoms of cancers:

There are some signs and symptoms of cancer:

- Change in weight, excessive weight loss and gain (10,11).
- Trouble in breathing and chronic cough (11).
- Fatigue (10,11)
- Fever (10,11)
- Chest pain (10,11)
- Dyspnea (11)
- Sputum with blood (11)
- Changing in bladder and bowel habits (10).
- Thickening of lymph area under the skin.
- Skin alterations, such as redness of skin, yellowish, darkening and sores that are not heal.
- Hoarseness (10).
- Formation of breast lump in case of breast cancer. (10).
- Abdominal pain and muscle pain (10).
- Difficulty swallowing (11).
- Diarrheal and constipation.

1.3 Risk factors of cancer:

There are some risk factors of cancer:

- Unhealthy diet (12-16)
- Alcohol use (13,14)
- Tobacco use (12-16)
- Less physical activity (12-16)
- Air pollution
- Excessive body weight (12-16)
- Some chronic infection

In 2018, 13% of cancers credited by carcinogenic infections, like hepatitis B virus, hepatitis C and helicobacter pylori (HP), human papillomavirus (HPV) and Epstein-Barr virus (17). HPV increases the risk of cervical and liver cancer and Human immunodeficiency virus (HIV) significantly increase the risk if cervical cancer.

1.4 You can prevent cancer by:

- Quitting smoking. (18,19)
- Maintain the body weight. (18,19)
- By taking healthy diet. (18,19)
- Doing regular physical activity. (18,19)
- Avoiding intake of alcohol. (18,19)
- Get vaccine against Human papillomavirus (HPV), hepatitis B and hepatitis C if vaccination is recommended. (19,20,21)
- Avoiding from the direct contact of ultraviolet radiation (UV), Xray's and gamma rays (19,22,23).
- Early diagnosis and screening of cancer (19,24,25,26)
- Reducing contact of indoor air pollution and outdoor pollution (19).

1.5 Epidemiology:

Cancer is a main cause of death globally, the projected number of deaths with cancer is 10 million in 2020 (27)

The most common number of new cases in 2020 which are shown in figure 1:

- Breast cancer (2.26 million cases)
- Lung cancer (2.21 million cases)
- Colon rectum (1.93 million cases)
- Prostate cancer (1.41 million cases)
- Skin (non-melanoma) (1.20 million cases)
- Stomach (1.09 million cases)

The most common causes of cancer death in 2020 which are shown in figure 2:

- Lung cancer (1.80 million deaths)
- Colon and rectum (935000 deaths)
- Breast (68500 deaths)
- Liver (830000 deaths)
- Stomach (769000 deaths)

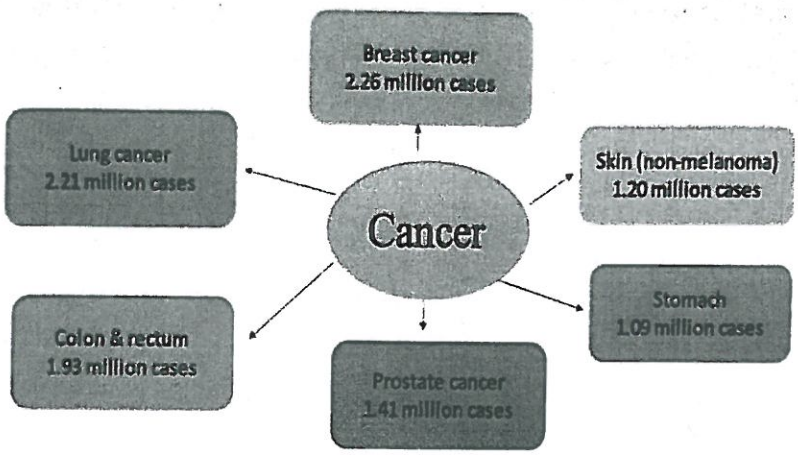

Figure 1. The number of new cases in 2020.

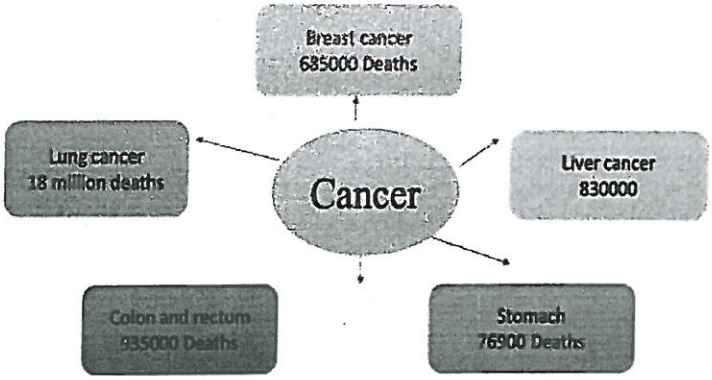

Figure 2. Total number of deaths in a particular cancer type in 2020.

In India 1392179 cancer patients were diagnosed with various type of cancer in 2020 year (28). The number of projected cancer patients are higher in females as compare of males patients. In india 712758 number of cancer patients are projected in females in 2020 year and 679421 number of cancer patients are projected in males. One in 68 males have lung cancer and one in 29 females have breast cancer the five most common cancers in males are prostate, lung, tongh, mouth and stomach. The five most common cancer in females are breast, lung, ovary andcervix uteri. In Asia 58.3% cancer deaths are estimated in 2020 and 59.5% deaths in global population.

Globally new cancer patients cases percentage are- female breast cancer (11.7%), prostate (7.3%), lung (11.4%), stomach (5.6%), corecrtal (10.0%), liver (4.7%), cervix uteri (3.1%), esophagus (3.1%), bladder (3.0%), thyroid (3.0%), and (36.9%) are other cancers.

Worldwide approximately 28.4million new cancer cases are projected in 2040 which are 47% increase from the year 2020 cases (29).

1.6 Pathophisiology of cancer:

Some endogenous and exogenous agents such as smoke, radiation, metal viruses, genotoxins, ROS can damage in the cell DNA. Due to damage in DNA epigenetic alteration is occur which do not repair DNA genes and DNA start mutation in the genes which indirectly synthesis foulty protiens lacking the particular biological activity. So DNA repair proteins are reduced (30,31,32). When DNA repair defiency is occurred damaged DNA increased in cell, which caused mutation. Mutation rate of cell is increased by the defective DNA mismatch (33,34). Which caused the progression of cancer. The foulty proteins has a role in cell division then, cell cycle will not be regulated and as a result in starting dividing in uncontrolled way. At the end uncontrolled cell division leads to formation of mass of cells called tumor.

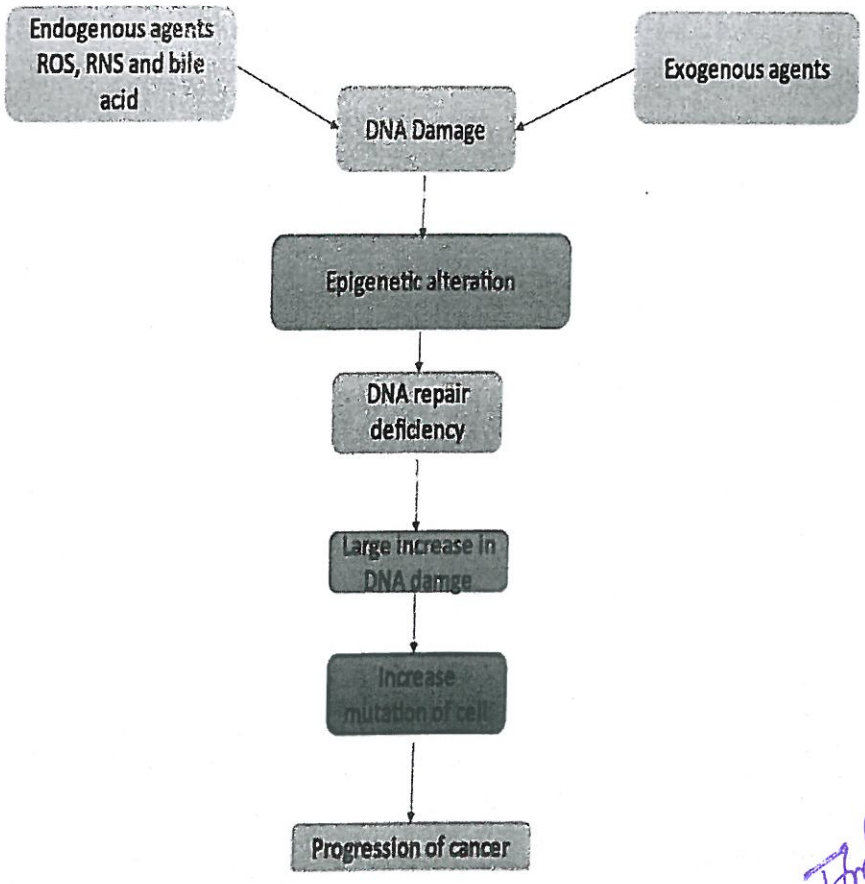

Figure 3. Pathophisiology of cancer.

1.7 Current treatment strategies for cancer:

Currently there is no specific treatment of cancer but there are four different approaches which are used for the treatment of cancer. They are applied either alone and they can be applied in combination with each other. These cancer therapies are radiotherapy, immunotherapy, chemotherapy and surgery.

1. **Surgical approach:** in surgery approach tumor is removed physical from its tissue of origin. The surgery therapy provides a complete cure of single treatment. In this therapy small residual of cancer cells are remains which form a new cancer. This therapy cannot remove secondary tumors, so it is often combined with the radiotherapy or chemotherapy for the increasing rate of the treatment (35). This therapy has several side effects for example in brain tumors it does not increase the survival rate (36,37). On other hand surgical removal of tumor cause unnecessary pain at excision site of patient.
2. **Radiotherapy:** in this therapy cancer cells and tumors tissues are exposed by the gamma rays and X-rays. These rays induce DNA damage in cancer cells, which cannot be repaired and cause arresting of DNA replication and consequently cancer cells are undergo apoptosis. The main side effect radiotherapy is that it effects the surrounding healthy cells. DNA repair is necessary in healthy cells but radiotherapy damage or killed the healthy cells (38,39), another side effect is caused in a long-term treatment, radiotherapy induce mutation of cells and forms a new cancer (40,41).
3. **Chemotherapy:** in this therapy, treatment of cancer is done with the help of drugs which contains the active pharmaceutical ingredients (APIs). These APIs are isolated from plants or synthesized in lab through chemical reaction. The chemotherapy imparts advantages over surgical method in that it can easily reach at the tumor sites. The drugs can kill the primary and secondary tumors even if their specific location is unknown (42). The chemotherapy can be combined with the radiotherapy in order to increase the therapeutic efficacy (43). But problem is that chemotherapeutic agents are acts on the cancer cells as well as on healthy cells or tissues. Therefor chemotherapy also cause serious side effects like hair loss, bone marrow depression and make weak immune system (44,45).
4. **Immunotherapy:** In this therapy our immune system is used as weapon against the cancer. Immunotherapy is the most selective treatment and it has very less side effects (46,47). Immunotherapy can be combined with the chemotherapy (48). In the immunotherapy APIs, proteins and peptides are used. In this therapy vaccines are used for the prevention of cancer, for example vaccines against Human Papilloma Virus

CERTIFICATE

This is to certify that the dissertation entitled "**IDENTIFICATION AND MOLECULAR DOCKING STUDY OF NATURAL COMPOUND FOR TREATMENT OF COVID 19**" submitted to K.R. Mangalam University in partial fulfilment of the requirement for the award of Degree of MASTER OF PHARMACY in Pharmaceutics, embodied the original research work carried out by MOHD KAFEEL under our supervision and guidance.

It is further stated that no part of this dissertation has been submitted, either in part or full for any other degree of K.R. Mangalam or any other university/institution.

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LIST OF ABBREVIATIONS

1. SARS-CoV-2- Severe Acute Respiratory Syndrome Coronavirus 2
2. ACE-2- Angiotensin-converting enzyme-2
3. TNF-α-Tumor Necrosis Factor
4. IL-6 - Interleukin-6
5. CHM- Chinese herbals medicine
6. M protein- Membrane Protein
7. S protein- Spike Protein
8. E protein- Envelope Protein
9. N protein- Nucleocapsid Protein
10. RdRp- RNA-dependent RNA polymerase
11. M pro- Main Protease
12. HCQ- Hydroxychloroquine
13. COVID-19- Coronavirus Disease 2019
14. MERS-CoV- Middle East Respiratory Syndrome Coronavirus

CHAPTER 1: INTRODUCTION

The first case of the newly emerged Human Coronavirus (HCoV) was reported in the month of Dec 2019 in Wuhan city of China (COVID-19) [1]. On 12th January 2020 World Health Organization (WHO) declares COVID-19 is a pandemic and caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2). SARS-CoV-2 is a non-segmented RNA virus and is mainly identified in the broncho-alveolar secretions, sputum, and saliva [2]. The virus belongs to the family Coronaviridae and genus Betacoronavirus which is interrelated to severe acute respiratory syndrome coronavirus (SARS-CoV) and (MERS-CoV) [3,4]. The various symptoms of COVID-19 are chest congestion, fever, respiratory distress, myalgia, fatigue loss of appetite, shortness of breath, cough and headache, the 1st case of COVID-19 in India was reported on 30 Jan 2020 in Kerala and as on December 2020 more the 95 Lakhs peoples were suffered from the diseases with more than 1 lakh of deaths [5,6].

Critics of the specific Coronavirus CSS idea have guaranteed that"cytokine storm has basically no definition" [7] and that there is absolute "no proof that will Coronavirus will provoke cytokine storm" in the patient [8]. Cytokine storm is a clinical aggregate of immune dysregulation recognized by perpetuated service of lymphocytes and macrophages that leads to secretion of large quantities of cytokines which causes systemic swelling and multi-organ failure with high mortality.

1.1 Sign and symptoms

Fever, Dry cough, Tiredness, Sore throat, Diarrhoea, Conjunctivitis, Headache, Loss of taste or smell, A rash on the skin, or discoloration of fingers or toes, [9,10]

Serious symptoms: Difficulty breathing or shortness of breath, Chest pain or pressure, Loss of speech or movement[11]

1.2 Genomic structure of SARS-CoV-2

The novel coronavirus is composed positive single-stranded ribonucleic acid (RNA) structure which acts as a molecular message that allows the assembly of proteins required for other elements of the virion [12,13]. The virus is large in size is about 9 to 12 nm with a diameter of 60 to 140 nm and the particles of this virus are spherical and polymorphic.HCov is divided into 4 different protein which plays a critical role to bind with the human receptor that are namely spike (S), membrane (M), nucleocapsid (N), and envelope (E) proteins and all these protein is used to

protect the genome of this virus in which spike protein (S) is located on the surface of the virus having three-segment which are ectodomain (ED) region while (E) proteins are the smallest (8.4–12 kDa size) protein of this virus which is situated on the endoplasmic reticulum and Golgi complex in the host cells [14-16]. Viral morphogenesis, CoV assembly, and budding formation is the main role of the (E) proteins and act as as a virulence factor [17,18]. (M) protein mainly used for viral assembly which hundred times bigger than (E) proteins [19,20]. The function of (M) protein is to maintain the shape of the viral envelop and also responsible for viral intracellular homeostasis. The N protein plays an important role when the virus is ready for packaging its viral genome into a helical ribonucleocapsid (RNP) [21]. It regulates the replication and transcription of viral RNA and maintains the formation of RNA complexes.[22,23]

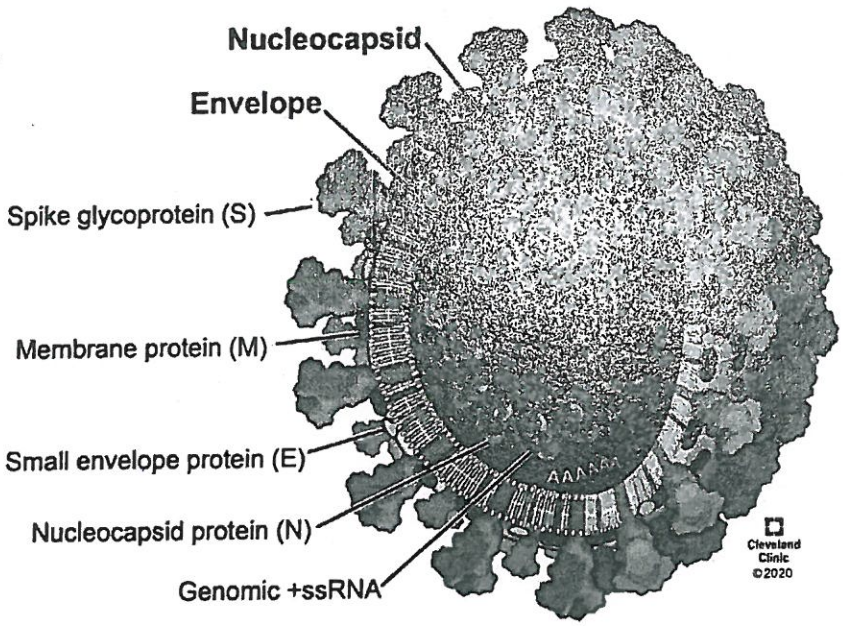

Fig. 1 structure of COVID 19

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1.3 Life cycle of COVID-19

The life cycle of COVID-19 begins in host cells once it reaches to lungs and binds to the type-II pneumocytes of the alveoli using its spike proteins (S protein) to the cellular receptor Angiotensin-converting enzyme-2 (ACE-2) [24-26]. After binding to the receptor conformational changes in the S protein facilitates viral envelope fusion with the cell membrane through the endosomal pathway [27,28]. Following fusion with host cells, the virus injects its positive single-stranded RNA (+ssRNA) into the host cell which then translates into viral replicase polyproteins pp1a and 1ab and results in cleavage to small products by viral proteinases [29]. The polymerase produces a series of sub-genomic mRNAs by discontinuous transcription and is finally translated into relevant viral proteins. Host RNA-dependent RNA polymerase converts the viral RNA into a new virions genome [30,31]. Viral proteins and genome RNA are subsequently assembled into virions in the endoplasmic reticulum and Golgi apparatus and then transported via vesicles and released out of the cell (Fig. 2).

A healthy person gets infected when they come in contact with an infected person [32]. Once at the surface, the virus reaches in alveolar type-2-pneumocytes where it attaches with spike protein with ACE2 receptor of type-2 cells [33]. Following fusion with host cells, the virus injects its positive single-stranded RNA (+ssRNA) into the host cell. Host cells, ribosome's translates the viral into large viral proteins which are further chopped into smaller proteins and used to make the structural proteins of progeny virions [34,35]. Host RNA-dependent RNA polymerase converts the viral RNA into a new virions genome. Progeny virus after completing the life cycle bursts the host cells starts infection into nearby cells. In severely infected patients, the virus also affects vital organs like the kidney and heart [36,37]. To date there is no effective medicine available for the treatment of COVID-19 hence in this review we focused on repurposing drugs used in traditional Chinese and Indian as well as an allopathic system of medicine (Fig. 3) which may be used alone or in combination to get maximum advantage of therapy [38,39].

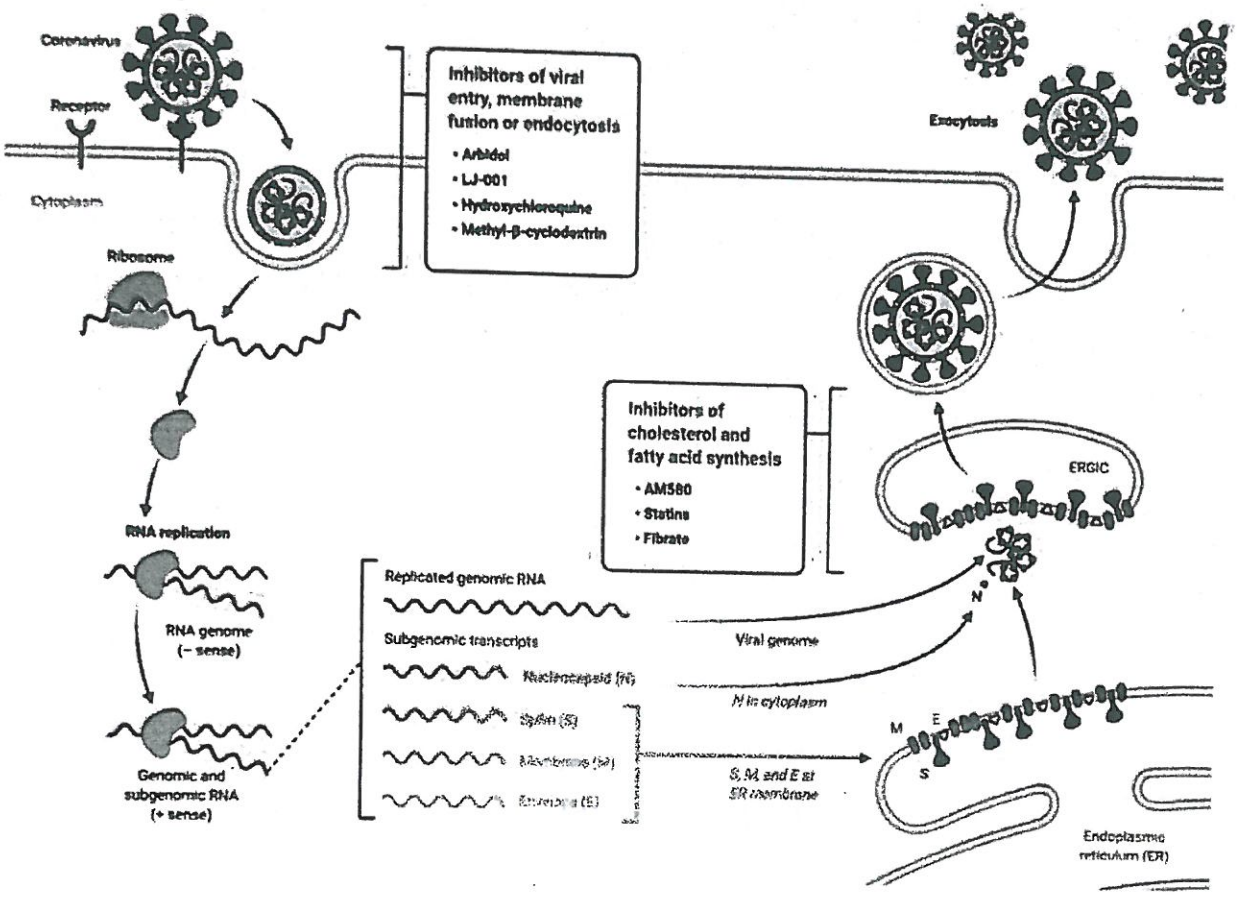

Fig. 2 Life cycle of COVID-19

I.4 Pathophysiology

nterleukin -6 and COVID -Cytokines storm

n early scientific studies, another mediator in COVID-19 is an elevated level of interleukins-6 IL-6) [40-42]. In COVID-cytokines storm numerous inflammatory cytokines like IL-1, IL-10, ınd tumor necrosis factor(TNF)-α are raised approximately 2-100 times above normal levels, vhereas IL-6 shows much higher concentration as compared to other interleukins. Some studies eported a marked elevation in serum IL-6 levels in the 100-10, 000 pg/mL range in patients with he serious disease [43-45]. These markedly elevated IL-6 levels in COVID-CSS are similar in nagnitude to serious CAR T-cell CRS [19] and higher than other hyper-IL-6 syndromes such as netacentric cattleman disease, where IL-6 is elevated but typically <100 pg/mL [46]. HLH is said o encompass a varied spectrum of "hyper ferritinemia hyper-inflammatory syndromes with a :ommon terminal pathway but with different pathogenetic roots"[47].

IL-6 is difficult disks immune dysregulation plus respiratory failing within COVID-CSS will be quickly accumulating. Raised serum IL-6 will be linked with lymphopenia, reduced lymphocyte cytotoxicity, and endothelial service. These types of defense defects may become partially renewed simply by therapy with IL-6 blockade with tocilizumab [48-51]. Study-related to IL-6 pointed out that the concentration of IL-6 > 80 pg/mL and C-reactive proteins concentration > 97 mg/L is very predictive and is associated with respiratory system failure [52-55].

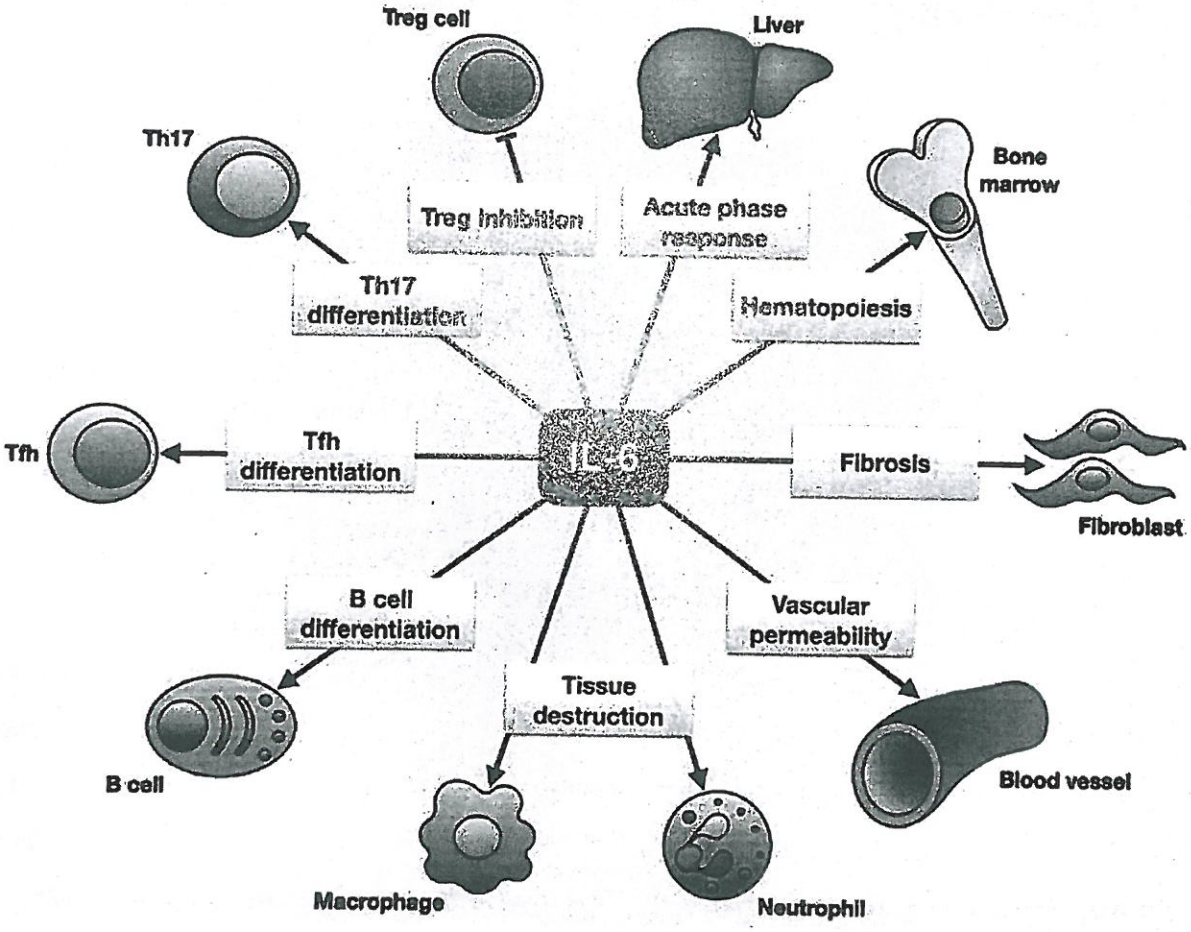

Fig. 3. Interleukin -6

1.5 Treatment strategiesfor COVID-19

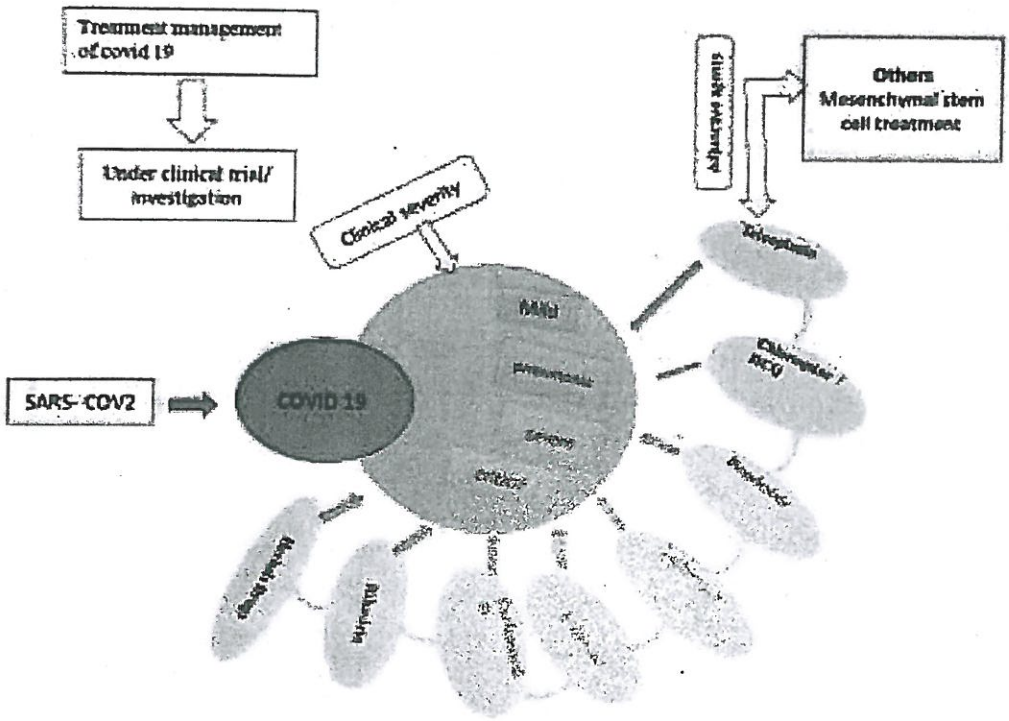

Fig. 4. Treatment strategiesfor COVID-19

Antibacterial drug

Teicoplanin

It is a semisynthetic glycopeptide antibiotic used to treat serious infections caused by gram-positive bacteria. This drug showed prominent effects when taken at a dose of 400mg once a day to treat infection associated with coronavirus [56]. It acts by inhibiting spike viral protein by cathepsin L which is responsible for the release of genomic viral RNA [57].

Antiprotozoal drug

Chloroquine/hydroxyl chloroquine

Chloroquine acts by inhibiting the virus at the entry-level to the host cell while if it already inside the cell then it prevents the replication of the virus by changing the acidic pH of DNA replication and its organelles. Some researchers found that hydroxyl chloroquine is more safer as