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Novel Natural Based Chewable Lozenges for the Treatment of Erectile Dysfunction

By

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DEDICATION

This work is dedicated to my family, Elias, Aida, and Christophe. It is because of their continuous and unwavering support that I can present this work. I am eternally indebted to all of you.

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Novel Natural Based Chewable Lozenges for the Treatment of Erectile Dysfunction

Jean-Pierre Frem

ABSTRACT

Chewable lozenges are easy to use, and highly flavored oral dosage forms used to deliver active ingredients, while providing high patient compliance rates. This work targets the development of chewable lozenges containing multiple natural active ingredients for the treatment of erectile dysfunction. Five batches were developed and analyzed consecutively by adjusting specific processes and material parameters such as the compounding procedure, and excipient quantities based on the physical appearance, stickiness, texture, chewiness, and taste of the lozenges. The most optimal batch obtained is batch "5" and includes adjustments to the compounding procedure to prepare the gelatin base separately while maintaining a heating temperature of 65-70˚C and a mixing speed of 750 rpm and maintaining a lower temperature of 50-60˚C with a mixing speed of 500 rpm while adding the active ingredients. Adjustments of the total liquid content was made to obtain a volume of 1.55 ml/lozenge and a water content of 0.30 ml/lozenge. In addition, the rotary evaporation technique was implemented to remove 6.7 ml (55.83%) of ethanol from the Black Maca liquid extract. Among other adjustments, these were the most significant in impacting the physical parameters and more specifically the stickiness of the lozenges. This project illustrates the successful development of chewable lozenges as a dosage form for the delivery of multiple active natural ingredients with distinct mechanisms of action. Water and solvent contents like ethanol, and process parameters can impact the physical characteristics of the chewable lozenges.

Keywords: Chewable Lozenges, Natural Ingredients, Drug Delivery, Process Parameters, Physical Characteristics, Batch, Rotary Evaporation.

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LIST OF ABBREVIATIONS

ACEIs .....Angiotensin Converting Enzyme Inhibitors
ALP .....Alkaline Phosphatase
API .....Active Pharmaceutical Ingredient
ARBs .....Angiotensin II Receptor Blockers
ATP .....Adenosine Triphosphate
AUC .....Area Under The Curve
BSP .....Bone Sialoprotein
cAMP .....Cyclic Adenosine Monophosphate
CCR2 .....C-C Chemokine Receptor Type 2
cGMP .....Cyclic Guanosine Monophosphate
Cm3 .....Cubic Centimeter
Cmax .....Maximum Serum Concentration
CNE-1.....Human Nasopharyngeal Carcinoma Cells
CNS.....Central Nervous System
COX-2.....Cyclooxygenase-2
CTR .....Calcitonin Receptor
CTSK .....Cathepsin K
CYP2C9 .....Cytochrome P450 Family 2 Subfamily C Member 9
CYP2D6 .....Cytochrome P450 Family 2 Subfamily D Member 6
CYP3A4 .....Cytochrome P450 Family 3 Subfamily A Member 4
DDR2 .....Discoidin Domain Receptor 2
eNOS .....Endothelial Nitric Oxide Synthase
ER .....Estrogen Receptor
ERα .....Estrogen Receptor Alpha
ERβ .....Estrogen Receptor Beta
FSH .....Follicle-Stimulating Hormone
G .....Gram
GI .....Gastrointestinal
GPCR .....G Protein-Coupled Receptor
GSH .....Glutathione

GTP .....Guanosine Triphosphate
IC50 ..... The Half Maximal Inhibitory Concentration
IIEF ..... International Index Of Erectile Function
IL-1β .....Interleukin-1 Beta
ITG B3.....Beta-3 Integrins
L-Arginine AKG .....L-Arginine Alpha-Ketoglutarate
L-Arginine HCL .....L-Arginine Hydrochloride
LD50..... Median Lethal Dose
LH .....Luteinizing Hormone
MEK/ERK ..... ERK Kinase
Mg .....Milligram
Ml ..... Milliliter
mmol .....Millimole
MMP9..... Matrix Metallopeptidase 9
mRNA .....Messenger Ribonucleic Acid
NADPH .....Adenine Dinucleotide Phosphate Hydrogen
NF-Kb ..... Nuclear Factor Kappa-Light-Chain-Enhancer Of Activated B Cells
nM .....Nanomolar
NO ..... Nitric Oxide
NOS..... Nitric Oxide Synthase
ODS..... Orally Dissolving Strips
PDE ..... Phosphodiesterase Enzyme
PDE-5.....Phosphodiesterase Type 5
PDE-5Is ..... Phosphodiesterase-5 Inhibitors
Pg..... Picogram
pH..... Potential Of Hydrogen
PKG..... Protein Kinase G
PTSD .....Prost-Traumatic Stress Disorder
RANK-RANKL ..... Receptor Activator of Nuclear Factor-Kb Ligand
RBC ..... Red Blood Cell
ROCK-II.....Rho-Kinase 2
RPM ..... Rotations Per Minute
SOD..... Superoxide Dismutase
T1/2 .....Half-Life

Tmax.....Time to Reach Maximum Concentration

TNF-A.....Tumour Necrosis Factor Alpha

TRAP.....Tartrate-Resistant Acid Phosphatase

UV-Filters .....Ultraviolet Filters

µg .....Microgram

µmol .....Micromole

˚C ..... Degree Celsius

5'GMP ..... 5' Guanosine Monophosphate

CHAPTER ONE

INTRODUCTION

1.1 Sexual Impotence

Sexual impotence is defined as the inability, difficulty, or insufficient power to initiate, sustain, and accomplish the act of sexual intercourse or copulation (Hammond WA, 1887). Whereas sexual potency is the ability to start, maintain and achieve the copulation act successfully. It is also defined as a symptom that happens due to changes in the neuroendocrine, vascular, and neurologic mechanisms (Giorgi PM, et. al. 1992). Sexual impotence is described to occur in both males and females. Moreover, it is to be differentiated from sexual sterility where there is an inability to produce offspring even when being able to perform the act of copulation successfully. Sexual impotence can be primary in nature or early-onset where sexual impotence develops since the first attempt at copulation, whereas secondary or late-onset sexual impotence develops from the previous competency. (Cooper AJ. 1972)

Sexual impotence includes a mixture of problems that happen in the domains of ejaculation, sexual drive, orgasm, and more importantly erection. (Geboes, et. al. 1975)

1.1.1 Causes

Multiple causes and etiologies have been described in the literature the most important being; psychogenic, psycho-organic, and drug-induced.

1.1.1.1 Psychogenic Sexual Impotence

Psychogenic sexual impotence includes two categories: primary and secondary. Primary psychogenic impotence comprises patients who have a deficit in ejaculation and sexual intercourse knowledge due to taboos and rigid familial education systems resulting in their lack of knowledge. Another example includes patients who never experienced ejaculation. Patients can also lack knowledge of other sexual passions. Secondary psychogenic impotence on the other hand includes patients who suffered previous physical injuries or traumas like patients with a history of orchitis, paraphimosis, sexually transmitted diseases, testicular torsion, or any other testicular injury. (Geboes, et. al. 1975) Other psychogenic factors can be as simple as anxiety and

fear of causing injury to the partner, hostility or feeling or revenge towards the partner (Stekel W. 1927), disgust related to the female reproductive organs, sexual inhibition where some sexual fantasies are shunned down or not properly communicated to the partner in fear of accusations of being perverted leading to the inhibition these sexual desires. This can also be due to cultural taboos that are imbued during early life, which classify some sexual desires as sinful or unnatural. For instance, nocturnal emissions are considered pollution in some societies and cultures. (Masters, W.H. & Johnson, V.E. 1970) Personality factors can also lead to psychogenic sexual impotence, for instance, patients with personality disorders, psychopaths, and egocentric people cannot have an affectionate relationship with their partners leading to symptoms such as ejaculation without orgasm. (Gutheil E.H. 1959) And last but not least, impotence can be caused by aging. It was observed that older patients who suffered from sexual impotence have been performing the act of copulation with the same partner for years and with the same sort of activities, this leads to these patients rapidly habituating and having a lower sexual motivation response over the years to the previously exciting stimulations. (Cooper A.J. 1967)

1.1.1.2 Psycho-Organic Sexual Impotence

Psycho-organic impotence includes a group of patients who suffer from both psychological and organic disturbances that result in impotency. Organic disturbances include a wide range of dysfunctions. For instance, patients suffering from diabetes have neurologic disturbances or vascular troubles that lead to impotency. Atherosclerosis, which is another organic cause alters proper blood flow decreasing blood pooling and trapping in the corpus cavernosum leading to a decrease in erectile function and consequently potency. (Geboes, et. al. 1975) Other factors are classified as anatomical; like congenital deformations, hydrocele, and testicular fibrosis. Cardiorespiratory which includes angina pectoris, Myocardial infarction, and coronary insufficiency. Endocrine factors include diseases like acromegaly, Diabetes mellitus, and Cushing's syndrome. Genitourinary problems like prostatitis, urethritis, and priapism. And last but not least, neurological factors can include diseases like Parkinsonism, Multiple Sclerosis, and peripheral neuropathies. (Cooper AJ. 1972)

1.1.1.3 Drug-Induced Sexual Impotence

Drug-induced causes are defined by medications that have the potential of causing potency disturbances, like chlorpropamide, some anti-hypertensive

medications, and monoamine oxidase inhibitors. (Geboes, et. al. 1975). Other medications include amphetamines, imipramine, atropine, reserpine, nicotine, alcohol, and thioridazine. (Cooper AJ. 1972) Some studies assessing the effect of some drugs on sexual potency have been developed, for instance, a study evaluated sexual function in patients taking atenolol (beta-blocker). It was observed that atenolol affected negatively sexual function; it decreased the number of sexual intercourses from 7.8 to 4.2 ($p<0.01$). (Doumas, et. al. 2006)

1.1.1.4 Constitutional Sexual Impotence

Another cause of sexual impotence is classified as constitutional impotence; it is usually associated with the low presence of sexual motivation or drive that is persistent and continuous, with low responsiveness to external stimuli. This classification is usually diagnosed by assessing sexual motivation and responsiveness in patients; for instance, patients with impotence that is present since the first attempt at copulation if ever made can be diagnosed with constitutional impotence. Other factors include persistent or worsening impotence, waning of sexual motivation that can happen in the early 30s and is continuous, waning of sexual activity, reduced or absent morning and spontaneous erections, and reduced erotic desire in certain sexual situations. (Cooper AJ, et. al. 1970) Among all the factors that could lead to constitutional impotence, the latter does not have any organic or external pathology. (Cooper AJ. 1972)

1.1.2 Resulting Effects

Sexual impotency is the common effect from which many categories have been established. Patients can suffer from disturbances in ejaculation, disturbances of libido, and disturbances in ejaculation.

Ejaculation occurs when the sperm passes from the vas deferens tubes to the urethra where the seminal fluid is released by the seminal vesicles. In a pulsing motion the musculus bulbocavernosus and ischiocavernosus contract to force the semen out. Disturbances in ejaculation can occur as premature ejaculation (ejaculatio praecox), late ejaculation (Ejaculation Retardata), retrograde ejaculation where the semen enters the bladder, and anejaculation also known as true impotence where the patients are unable to ejaculate. (Geboes, et. al. 1975)

Sexual drive is a state of motivation or stimulus that causes individuals to engage in sexual activities. This stimulus comes from a mix of cognitive processes (thoughts and fantasies), neurologic and physiologic mechanisms (arousability), and emotional states (mood). Disturbances of libido are the lack of sexual drive also known as hypoactive sexual desire. According to the Diagnostic and Statistical Manual of Mental Disorders, hypoactive sexual desire cannot be accounted for exclusively due to psychological disorders or physiologic causes like hyperprolactinemia or hypogonadism. However, it is linked to the presence of other sexual dysfunctions like erectile dysfunction or delayed ejaculation. (Corona G, et. al. 2013)

1.2 Anatomy of the Male Genital Organs

The male genital organs have specific functions in the human body; production of sperm, transport of semen, and synthesis of hormones like testosterone. Important elements of the male genital organs that will be discussed are the penis and scrotum.

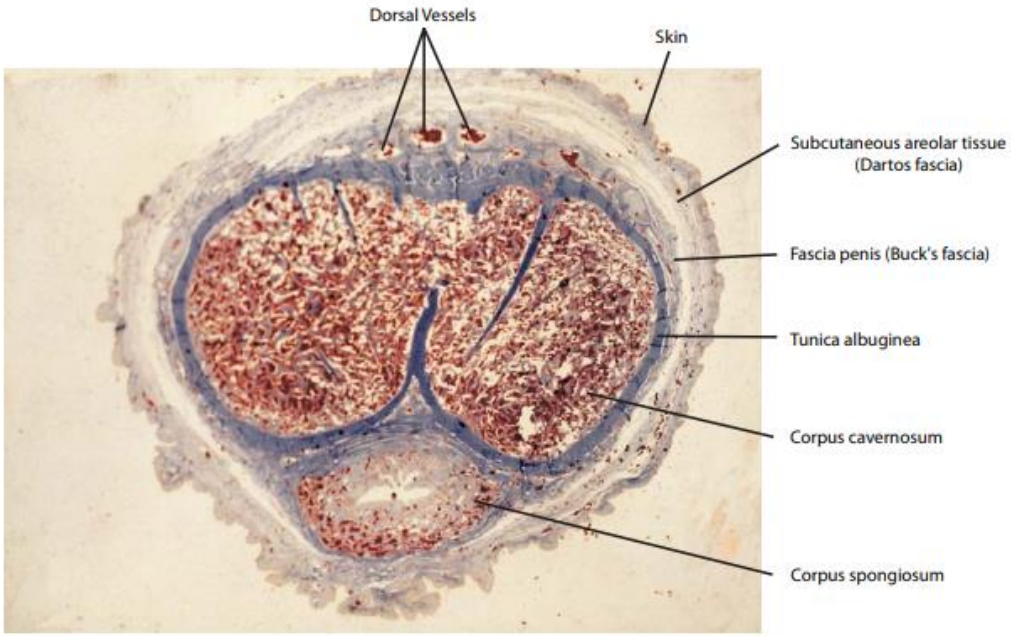
1.2.1 Anatomy of the Penis

The penis is made up of multiple structures, each with specific functions and roles that complement each other. The first is the corpus cavernosa, which is a spongy tissue that is located in the dorsolateral position and split into two lateral masses which in turn are connected by fibrous tissue. The two corpus cavernosa masses are enveloped by a fibrous sheath. The corpus cavernosum muscle relaxes during the erection to increase blood trapping. The corpus spongiosum is the third mass of tissue that is situated in the ventral position between the two muscle masses of the corpus cavernosa. Its engorgement results in the constriction of the urethra to allow for proper ejaculation. (Panchatsharam PK, et. al. 2020). The glans, also known as the head of the penis, vary in appearance between uncircumcised and circumcised men. In uncircumcised men, it is covered by moist tissue (mucosa) and is pink in color. It is also covered by the prepuce. In circumcised men, the moist tissue covering the glans becomes dry. The urethra is a tube that runs through the corpus spongiosum from the bladder reaching the head of the penis. Its role is to conduct urine and receive seminal fluid through the ejaculatory duct and conduct semen. Finally, the meatus is an opening situated at the tip

of the head of the penis and allows the exit of urine and semen. (Clement P, Giuliano F. 2015)

Figure 1 - Cross-section of the penis

Cross section of the penis indicating different parts and structures including the dorsal vessels, subcutaneous areolar tissue, the skin, and the corpus cavernosum and spongiosum muscles.



(Quartey JKM, 2006)

As seen in figure 1, the way these elements work together is tightly linked; the penis is made of three main structures with cylindrical shapes; two masses of corpus cavernosa, and one mass of corpus spongiosum. The two masses of corpus cavernosa are fused by fibrous tissue, with an incomplete septum that separates them. The corpus spongiosum is situated under the incomplete septum, ventrally between the two masses of corpus cavernosa, where the urethra passes centrally through it. The distal end of the corpus cavernosa is covered by the glans, the glans is structured and folded in a way that covers the ends of the corpus cavernosa, with a distinctive shape called the corona. The corpus cavernosa is made of multiple blood spaces which are covered by the tunica albuginea, which is a fibroelastic tough structure. Similarly, the corpus spongiosum contains blood spaces but is covered by a thinner tunica albuginea. (Hsu G, et. al. 2004)

The tunica albuginea itself is directly covered by another tissue, which is the deep fascia (Buck's). The latter binds the two corpus cavernosum masses together and splits ventrally to cover the corpus spongiosum separately.

The dartos fascia is a loose subcutaneous tissue that does not contain fat. It covers the deep fascia penis (Buck's) and contains nerves, lymphatics, and superficial blood vessels.

The dartos fascia itself is covered by the skin, the latter is thin and covers the dartos fascia, the penis, and the scrotum. It also contains the dorsal vessels. (Quartey JKM, 2006)

1.2.2 Anatomy of the Scrotum

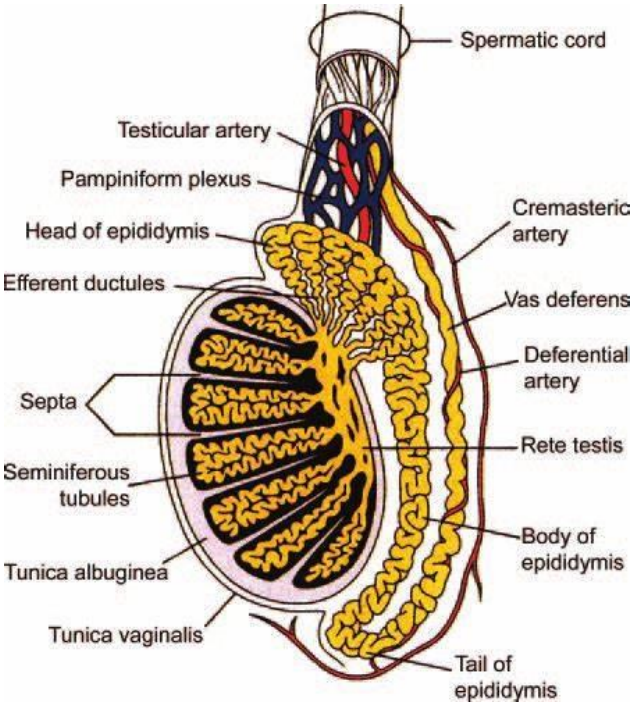
The scrotum is made of skin and subcutaneous tissue that is divided into two pouches, each containing serous membranes that produce the serous fluid which in turn contains the testes. The role of the scrotum is to regulate the temperature of the testes and protect them from physical damage. Inside the scrotum, multiple structures are available each with different roles and functions; vas deferens, epididymis, and testes.

1.2.2.1 Anatomy of the Testicles

The testicles or testes are male reproductive organs that have both endocrine functions (testosterone production) and exocrine functions (production of sperm). They are oval-shaped and are situated in the scrotum, attached to it by the scrotal ligament. The function of the testes is to produce sperm/spermatogenesis from spermatocytes and to make hormones namely androgens.

Figure 2 - Intrascrotal anatomy

Figure representing the different anatomical elements of the scrotum; the arteries, vas deferens, epididymis, and the protective layers: Tunica albuginea and vaginalis



(Deurdulian C, et. al 2007)

As represented in figure 2, the testes are attached and suspended in the scrotum by spermatic cords. Although they might vary slightly in their physical shape, generally the testes have a volume of 30 ml, and they are on average 3.8 cm long, 2.5 cm deep, and 3 cm wide, with a weight range of 10.5-14 g. (Leung M, et. al. 1984) Each testis is surrounded by the tunica albuginea which is a fibrous sac lined internally with a group of blood vessels also known as the tunica vasculosa. Inside the scrotum, the testes receive blood supply from the testicular arteries, they also receive innervations from sympathetic and parasympathetic fibers. The testes are composed of cone-shaped lobules and contain convoluted seminiferous tubules. The latter are supported by a connective tissue that contains the interstitial Leydig cells, these cells are responsible for the production of testosterone. (Hafez ESE. 2016) In addition, the tubules contain the supporting Sertoli cells and spermatocytes which are responsible for spermatozoa

and sperm development. The development of sperm begins with the differentiation of the nuclear part of the spermatid (Gray H. 1918) The tubules become less convoluted at the apex of the lobules, they enter then form a linked group of tubes also known as rete testes (Thomas RD, Dewbury KC. 1993) Then the rete testes enter the tunica albuginea forming at the end of their course a conical mass, also known as the coni vasculosi, constituting the head of the epididymis (Liguori G, et. al. 2011)

1.2.2.2 Tunica Albuginea

The Tunica Albuginea is a fibrous layer that surrounds the testes and contains collagenous and smooth muscle structures. The Tunica Albuginea itself is covered by the Tunica Vaginalis. The latter is a serous membrane pouch that contains fluid and is covered by an endothelial cell layer on the inner surface. It is specifically divided into two parts; the visceral and parietal lamina. The visceral part covers a bigger part of the testes compared to the parietal lamina, whereas the parietal lamina is larger extending from the medial side of the cord to the testes. Both parts create a cavity in the Tunica Vaginalis where, among many pathological processes, mainly fluid accumulation. When excessive serous fluid accumulates, hydroceles occur. (Liguori G, et. al. 2011)

1.2.2.3 Epididymides

The epididymis or epididymides is a structure that resides on top of the testes, it is formed by coiled tubes that are tightly fit, and it is connected to ducts in the testes. If spermatogenesis starts in the testes, sperm cell maturation occurs in the epididymis, the sperm cells enter their non-motile stage, and then they mature and/or are stored and become able to be motile. The epididymis has three parts: the head, body, and tail. The sperm cells mature in the head and body of the epididymis and are stored in the tail. (Bostwick DG. 1997)

1.2.2.4 Ductus Deferentia

The ductus deferentia (vas deferens) is a multitude of muscular tubes, each passing along the medial line of the testes, entering the pelvic cavity, and reaching up to behind the urinary bladder. It unites with the duct of a seminal vesicle forming an ejaculatory duct that empties in the urethra. The role of this structure is to store and transport sperm cells into the urethra. (Tiwana MS, Leslie SW. 2017)

1.3 Erectile Function Mechanism

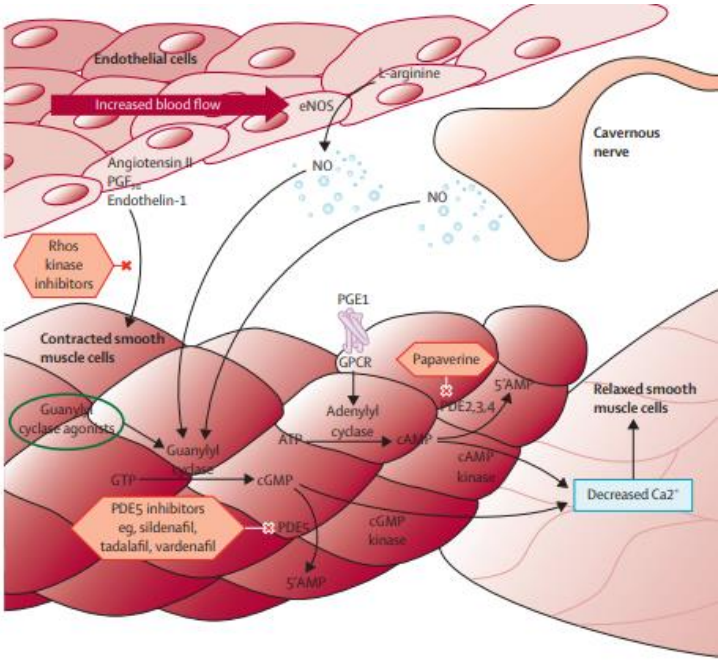
Proper erectile function is one of the most important conditions for proper sexual function, in the following sections normal erection physiology and erectile dysfunction will be described.

1.3.1 Erection Physiology

Erectile function physiology is a web of intricate mechanisms that lead eventually to an erection. Erection physiology starts first with stimulation of the brain by multiple cognitive factors and stimuli like tactile, visual, and olfactory signals. This stimulation, facilitated by the combination of testosterone leads to an erection. Moreover, there is a usual balance between erectogenic (a stimulus that causes erection) and erectolytic (an unpleasant stimulus that reduces erection). A change in this balance can result in a pro-erection signal that passes through the central nervous system, specifically the spinal cord, reaching the cavernous nerves. Contrary to popular beliefs, erection can also happen through subconscious stimulus due to electrical activity changes in the brain, and that usually happens during sleep leading to what is called "reflex erections". (Carson C, Dean J. 2007)

Figure 3 - Erection physiology pathway

Figure indicating the dual release of endogenous nitric oxide; produced from L-arginine by nitric oxide synthase in the endothelium, and the release of nitric oxide from the cavernous fibers. The eventual binding of nitric oxide to the guanylyl cyclase increases levels of cyclic guanosine monophosphate and decreases calcium levels.



(Shamloul R, Ghanem H. 2013)

Nitric oxide (NO) is released from multiple fibers, including the cholinergic, non-noradrenergic, and non-cholinergic fibers. More specifically, as indicated in figure 3, nitric oxide is released from the cavernous nerve. It is also released by the endothelium, in fact, it is produced from L-arginine by an enzyme called endothelial nitric oxide synthase. (Andersson KE, Wagner G. 1995) Consequently, the concentration of nitric oxide increases, and the latter enters the guanosine triphosphate/cyclic guanosine monophosphate (GTP/cGMP) pathway, in fact, NO binds to guanylyl cyclase transforming GTP to cGMP, as such increasing the cGMP levels, in turn leading to the decrease of the intracellular calcium concentration in the cavernosal smooth muscle cells causing them to relax. The relaxation of the smooth muscle causes the compression of the subtunical small veins blocking the venous return of blood. Interestingly, this can cause an eightfold increase in the blood volume accumulated. This succession of events leads to the phenomenon of erection. Conversely, the phosphodiesterase enzyme (PDE) regulates the smooth muscle cells' relaxation activity by hydrolyzing cGMP to its inactive form 5'GMP, consequently reducing muscle relaxation, and as such erection, moreover, the contraction of the smooth muscles is also caused by sympathetic constrictors leading to to the decompression of the subtunical

veins allowing for the return of blood decreasing pressure and blood trapping. (Shamloul R, Ghanem H. 2013)

1.3.2 Erectile Dysfunction

Erectile dysfunction also known as inadequate penile erection, is the inability to start an erection or maintain it sufficiently to perform the act of copulation. It affects mainly but is not limited to men aged 40 years and above, with recent studies showing a 1-10% prevalence of the disease in men younger than 40 years and reaching a prevalence of 50-100% in men older than 70 years old. (Nicolosi A, et. al. 2003) Some findings and studies have attributed the development of erectile dysfunction to a multitude of diseases like; Diabetes Mellitus, hypertension, hyperlipidemia, depression, and metabolic syndromes. (Clark NG, et. al. 2007) Moreover, some other studies have even used erectile dysfunction as a biological marker of cardiovascular diseases. (Ponholzer A, et. al. 2010). Meanwhile, some studies and meta-analyses were able to find a link between lifestyle factors and erectile dysfunction; they found that patients leading an inadequate lifestyle with factors such as smoking, obesity, and low physical activity had an increase in the risk of developing erectile dysfunction. (Dong J, et. al. 2011).

Similarly, to sexual impotency, erectile dysfunction can be classified into three categories: (Shamloul R, Ghanem H. 2013)

- Psychogenic (psychological factors)
- Organic (drug-induced, hormonal, neurogenic, or arterial factors)
- Combination of psychogenic and organic factors

1.3.2.1 Psychogenic Erectile Dysfunction

Psychogenic erectile dysfunction is a combination of psychological factors mainly interpersonal, cognitive, and developmental. Since in most cases, a pro-erection stimulation is needed to initiate the erection pathway, any unpleasant stimulus can lead to erectile dysfunction like a memory of a poor sexual experience. (Carson C, Dean J. 2007) One of the most common psychological factors is performance anxiety it was observed that patients fearful of having a failure during the act of copulation lead to

them developing erectile dysfunction. Nevertheless, psychological factors are currently classified as predisposing factors which include factors that are already present or have already happened like past traumatic experiences or Post-Traumatic Stress Disorders (PTSD), inadequate sexual education linked to strict education, and mental health problems. 85% of patients suffering from PTSD suffered from erectile dysfunction compared to 22% of men who did not suffer from PTSD. (Cosgrove DJ, 2002) Precipitating factors include acute or current relationship and marital problems, emotional pressures that come from family or specific societies, loss of a job, pregnancy, or other major life events. For instance, depression is one of the factors that can reduce erectile function by decreasing arousal and sexual motivation. A statistical study reported that depressed men have 2.5 times more erectile and sexual dysfunction problems compared to men who are not suffering from depression. (Dunn KM, et. al. 1999) Lastly, maintaining factors which include long-term relationship and marital problems, mental problems, and a lack of knowledge about available medications and medical devices that treat erectile dysfunction. (Shamloul R, Ghanem H. 2013) Some psychometric tools and instruments have been used to identify and assess psychogenic erectile dysfunction. For instance, the Beck Depression Inventory is a questionnaire that evaluates the depressive symptoms that lead to erectile or sexual dysfunction. The Minnesota Multiphasic Personality Inventory is another tool that is used to identify and differentiate between the psychogenic or organic factors that lead to erectile dysfunction. A third test that is used is the Psychological Impact of Erectile Dysfunction instrument which evaluates how the quality of life of the patients can affect erectile dysfunction and also how erectile dysfunction affects patients' emotional life and sexual experience. (Bodie JA, et. al. 2003)

1.3.2.2 Organic Erectile Dysfunction

Organic erectile dysfunction includes factors that are physical in nature or outside factors causing changes in the normal physiology of erection, they include:

- Neurogenic factors
- Endocrinological factors
- Vasculogenic factors or local cavernous problems
- Drug-induced

Neurological Factors

Neurogenic or neurological factors are disorders that affect the Central Nervous System (CNS) like the spinal cord, or the autonomic fibers of the penis. Any damage to these nerves can lead to erectile dysfunction. For instance, damage to the peripheral efferent autonomic fibers of the penis can decrease the relaxation of the smooth muscles inducing erectile dysfunction. Another example includes damage to the sensory fibers of the penis, disrupting stimuli transmission and resulting in erectile dysfunction. Diseases that affect the CNS; like multiple sclerosis, Alzheimer's, Parkinson's, and spinal cord injury all have to potential to cause erectile dysfunction. For instance, a study showed that patients with Multiple sclerosis suffered from erectile dysfunction 2.2 times more than patients who did not suffer from Multiple sclerosis (Keller JJ, et. al. 2012) Even patients that have radical pelvic surgeries can suffer from erectile dysfunction. A study showed that 34% of patients under 50 years old who had lumbar spine surgical decompression had post-procedural erectile dysfunction. (Siddiqui MA, et. al. 2012) Peripheral nervous system injuries or neuropathies that occur in patients suffering from Diabetes Mellitus can also lead to erectile dysfunction. (Carson C, Dean J. 2007) A study showed that when lifestyle factors and dietary modifications were offered to patients, their erectile function improved due to lower low-density lipoproteins and cholesterol levels. (Corona G et. al. 2011) One of the tools used to assess neurogenic erectile dysfunction is penile glans biothesiometry; it is an instrument where an electromagnetic device is placed on the shaft and glans of the penis. This electromagnetic device measures electric signals from multiple vibratory stimuli of multiple amplitudes. (Bemelmans B, et. al. 1995) The bulbocavernosus reflex latency test is a tool that measures the reflexogenic mechanism of erections, one electrode is placed on the corona and the second proximally to the corona. These electrodes allow for the delivery of electrical impulses and the measurement of muscle response. (Burnett A. 2011)

Endocrinological Factors

Androgens have an important role in normal sexual development, enhancement of sexual desire, erections, and consequently sexual performance. Any disease affecting androgens levels in the body has the potential to cause erectile dysfunctions. For instance, androgens and more specifically testosterone regulate the expression of NO synthase, smooth muscle relaxation and PDE-5 activity, low levels of testosterone can

consequently induce erectile dysfunction. (Traish AM, et. al. 2003) Hyperprolactinemia is a disease where there is an increase in the levels of prolactin, leading to the inhibition of gonadotropin-releasing hormones, decreasing the release of luteinizing hormone, and as such decreasing the synthesis and secretion of testosterone, reducing testosterone levels. Other diseases like Diabetes Mellitus and hypogonadism can also cause erectile dysfunction. (Carson C, Dean J. 2007) The incidence of erectile dysfunction in patients suffering from Diabetes was 68 cases in 1000 patients compared to 25.9 in 1000 patients who do not suffer from Diabetes. (Bortolotti F, et. al. 2000) The normal testosterone level range is between 280-1000 ng/dl, for patients suffering from hypogonadism testosterone levels are observed to be lower than the normal range. (Morgentaler A, et. al. 2014) Primary hypogonadism, also known as hypergonadotropic hypogonadism, is caused by direct damage to the testes like orchitis, Klinefelter's syndrome, chemotherapy, and undescended testes. In primary hypogonadism, the luteinizing hormone and follicle-stimulating hormone levels are normal and even elevated as a response to low androgen levels. Secondary hypogonadism, also known as hypogonadotropic hypogonadism, is due to damage to the hypothalamic-pituitary axis resulting in low Luteinizing Hormone (LH) and Follicle-Stimulating Hormone (FSH) levels. For instance, hyperprolactinemia is a disease where prolactin levels are elevated, leading to suppression of the secretion of gonadotropin-releasing hormone from the hypothalamus, decreasing, in turn, the secretion of LH and subsequently causing the decrease in testosterone synthesis. (Papagiannopoulos D, et. al. 2015) Hyperthyroidism is another disease that can lead to erectile dysfunction it increases the aromatization of testosterone to estrogen, increases the levels of sex hormone-binding globulin, and decreases consequently the bioavailability of testosterone. (Maggi M, et. al. 2012)

Vascular Factors

Vasculogenic or vascular factors are among the most common causes of erectile dysfunction, they lead to disturbances and changes in the smooth muscle cells' response. Any damage to the blood vessels, whether arteries or veins, can cause erectile dysfunction. For instance, atherosclerosis results in decreased blood perfusion and can even lead to ischemia, as such leading to erectile dysfunction. (Carson C, Dean J. 2007) Other factors or comorbidities like diabetes, hyperlipidemia, hypertension, and other risk factors can cause penile arterial insufficiency, decreasing blood flow coming to the area. (Yao F, et. al. 2012) Endothelial damage or dysfunctions can also lead to arterial

insufficiency causing erectile dysfunction. For instance, Peyronie's disease is defined as a local inflammation of the bilaminar tunica albuginea layers, and it leads to penile fibrosis, deformity, and pain that leads to sexual or erectile dysfunction. 21% of patients with Peyronie's disease younger than 40 years old suffer from erectile dysfunction. (Chung E, et. al. 2011) Reduced relaxation of the smooth muscle cells can lead to reduced compression of the subtunical veins, resulting in an increased blood "outflow" and as such decreasing blood trapping and pressure. Inadequate venous occlusion caused by physical damage or functional/anatomical changes in the tunica albuginea can also lead to erectile dysfunction, such as in the case of patients with Peyronie's disease. (Shamloul R, Ghanem H. 2013)

Drug-Induced Factors

Other than physiologic and metabolic alterations, some drugs can cause erectile dysfunction. The most common class of medications causing erectile dysfunction are psychotropics; they include tricyclic antidepressants, selective serotonin reuptake inhibitors like fluoxetine and sertraline, and selective serotonin-norepinephrine reuptake inhibitors like venlafaxine, anti-psychotics like olanzapine, phenothiazines, and butyrophenones like haloperidol and droperidol. (Serretti A, Chiesa A. 2011) Anti-hypertensives like beta-blockers and thiazide diuretics are the most common blood pressure-lowering agents that can induce erectile dysfunction. Calcium channel blockers were also observed to induce erectile dysfunction, whereas patients taking Angiotensin Converting Enzyme Inhibitors (ACEIs), Angiotensin II Receptor Blockers (ARBs), and alpha-blockers were observed to have lower erectile dysfunction frequencies. (Thomas A, et. al. 2003) Other medications like statins, recreational substances; marijuana, opiates, cocaine, nicotine, and alcohol, antiarrhythmics; digoxin, amiodarone, disopyramide, and anti-androgens; like gonadotropin-releasing hormone agonists were observed to induce erectile dysfunction. Other drugs like chemotherapy agents, ketoconazole, spironolactone, and H_2-blockers also have the potential to cause erectile dysfunction. (Shamloul R, Ghanem H. 2013)

1.4 Erectile Dysfunction Therapies

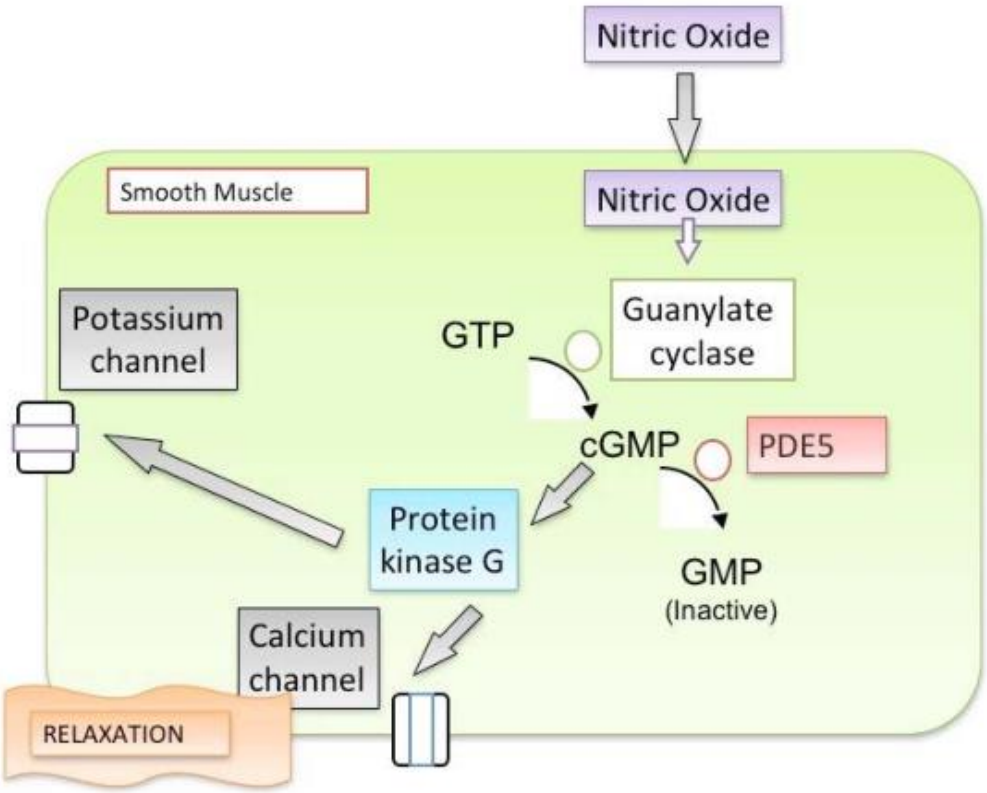
Many therapeutic remedies have been developed and described in the literature for the treatment of erectile dysfunction. They are mainly divided into therapeutic medications, medical devices, and surgical procedures.

1.4.1 Phosphodiesterase-5 Physiology

Phosphodiesterase-5 (PDE5-) is an enzyme purified and identified in 1980 by Dr. Francis Sharron (Francis SH, et. al. 1980) and cloned by Dr. McAllister-Lucas in 1993. (Mcallister-Lucas LM, et. al. 1993)

Figure 4 - PDE-5 mechanism of action

Figure describing the role of Phosphodiesterase-5 in inactivating cyclic guanosine monophosphate



(Gur S, et. al. 2012)

As seen in figure 4, PDE-5 is an endogenous enzyme that is specific to cGMP and eliminates it by hydrolysis rendering it inactive. It is available in vascular beds and corpus cavernosum muscle. It is also available to a much lower extent in the heart and cardiovascular system. cGMP is a messenger that induces muscle relaxation by activating Protein Kinase G (PKG) which in turn phosphorylates other proteins leading to the reduction of calcium levels in the corpus cavernosum smooth muscle cells, hydrolyzing it to its inactive form reducing muscle relaxation and consequently reducing erection. (Gur S, et. al. 2012) PDE-5 enzyme has two allosteric sites for the binding of cGMP, and one site for PKG phosphorylation. (Corbin JD. 2004)

1.4.2 Phosphodiesterase-5 Inhibitors

Phosphodiesterase-5 inhibitors are currently recognized as the first-line therapy for the treatment of erectile dysfunction. As seen in figure 4, they act by inhibiting the

phosphodiesterase-5 enzymes, leading to reduced degradation and hydrolysis of cGMP. Consequently, this leads to increased levels of cGMP and as such increases corpus cavernosum smooth muscle cells relaxation and maintaining this effect, leading to an increased duration of the erection phenomenon. (Huang SA, Lie JD. 2013) This mechanism of action is not limited to the corpus cavernosum, as PDE-5 inhibitors were shown to have systemic effects (Carson C, Dean J. 2007)

1.4.2.1 Phosphodiesterase-5 Inhibitors Dosage Pharmacokinetics

Phosphodiesterase-5 inhibitors are currently available in oral dosage forms such as sildenafil, vardenafil, tadalafil, udenafil, avanafil, and mirodenafil. The most common PDE-5 inhibitors used and studied are sildenafil, vardenafil, and tadalafil. The main advantages of the PDE-5 inhibitors are improvement in sexual performance, ejaculation, and erection. Most of the PDE-5 inhibitors have an onset of action that happens as soon as 30 minutes except for tadalafil which has an onset of action at 45 minutes. Each has a different dosage regimen with udenafil reaching 200 mg/day, sildenafil, mirodenafil reaching 100 mg/day, vardenafil, and tadalafil reaching 20 mg/day. Moreover, tadalafil's efficacy can reach up to 36 hours, udenafil and mirodenafil reach up to 12 hours, and sildenafil and vardenafil reach up to 8 hours. (Shamloul R, Ghanem H. 2013)

1.4.2.2 Phosphodiesterase-5 Inhibitors Drug-Drug Interactions

Phosphodiesterase-5 inhibitors have drug-drug interactions with other medications like anti-hypertensives; ACEIs, beta-blockers, and thiazide diuretics mostly, because their concomitant administration causes a synergistic decrease in blood pressure. Since PDE-5 inhibitors are metabolized by the cytochrome CYP3A4, inhibitors of this cytochrome can prolong the duration of action of PDE-5 inhibitors. For instance, azole group antifungals (ketoconazole), antiretrovirals (ritonavir, indinavir, and saquinavir), and macrolides (erythromycin, and clarithromycin) all have CYP3A4 inhibitory activity, increasing plasma concentrations of PDE-5 inhibitors. Grapefruit juice with PDE-5 inhibitors administration is considered a drug-food interaction since grapefruit juice also inhibits CYP3A4. (Schwartz BG, Kloner RA. 2010)

1.4.2.3 Phosphodiesterase-5 Inhibitors Contraindications

Phosphodiesterase-5 inhibitors are also contraindicated with patients taking nitrates, since the latter are NO-donors, as such inducing the accumulation of cGMP and causing severe hypotension. PDE-5 inhibitors should be also taken with caution for patients on alpha-blockers since both PDE-5 inhibitors and alpha-blockers cause synergistic vasodilation, and consequently synergistic hypotension. (Schwartz BG, Kloner RA. 2010) Only when patients are stable on alpha-blockers can they be administered PDE-5 inhibitors starting with the lowest dose. (Shamloul R, Ghanem H. 2013)

1.4.2.4 Phosphodiesterase-5 Inhibitors Adverse Events

Common adverse events of phosphodiesterase-5 inhibitors include headache, dizziness, flushing, and nasal congestion all of which are due to systemic vasodilation. (Rosen RC, Kostis JB. 2003) Other common side effects observed include back pain, dyspepsia, and myalgia. Sildenafil can also cause visual disturbances also known as "blue vision", and sildenafil, vardenafil, and tadalafil can cause sudden hearing loss. (Carson C, Dean J. 2007)

1.4.3 Subsequent Therapies

Other therapies and remedies described in the literature include other therapeutic medications, medical devices, and surgical procedures.

1.4.3.1 Intraurethral Alprostadil

One of these many treatments is intraurethral alprostadil. It is regarded as one of the second-line treatments for patients where oral PDE-5 inhibitors failed to treat erectile dysfunction. This type of injection can be easily learned by the patients and injected when needed, and the onset of erection is usually very rapid reaching up to 10 minutes after injection. Alprostadil, also known as 11,15-dihydroxy-9-oxoprost-13-en-1-oic acid, is a synthetic form of the naturally occurring prostaglandin E1. PGE1 is found systemically in the human body and the seminal fluid. (Hanchanale V, Eardley I. 2013) Alprostadil is available and approved to be used in two dosage forms: intracavernosal and intraurethral injections. The onset of action of Alprostadil is very fast with a range of 10-15 minutes with its effects lasting 30 minutes to 12 hours. 20% of Alprostadil enters systemic circulation with 80% metabolized in the lungs, with 90% of the

metabolites excreted renally. (Hanchanale V, Eardley I. 2013) The mechanism of Alprostadil follows a specific pathway; it binds to smooth muscle cells receptors (GPCR) activating intracellular adenylate cyclase. This enzyme is responsible for the conversion of ATP to cAMP, which in turn increases intracellular calcium sequestration increasing smooth muscle cell relaxation and vasodilation. (Costa P, Potempa A. 2012). A randomized controlled trial using intracavernosal alprostadil injection was evaluated, and it was shown that this type of injection offered a 70-87% response rate in men with erectile dysfunction. (Godschalk MF, et. al. 1994) Although intraurethral alprostadil injections are more approved by patients, intracavernosal injections have higher efficacy. A trial showed that among 60 men suffering from erectile dysfunction, 90% had improved erectile function when taking intracavernosal injections compared to 60% when taking intraurethral injections. (Shokeir A, et. al. 2001)

1.4.3.2 Intraurethral Papaverine

Another intraurethral injection treatment includes papaverine, which is an alkaloid and a non-specific phosphodiesterase inhibitor, it mainly acts by inhibiting the degradation of cyclic Adenosine Monophosphate (cAMP) to 5'AMP, leading to increased levels of cAMP and subsequent relaxation of the smooth muscle cells and vasodilation. Papaverine also blocks the voltage-calcium channels reducing calcium levels and inducing smooth muscle relaxation and blocking alpha-adrenergic receptors inducing vasodilation. (Steers WD. 2002). Alprostadil injections can reach an efficacy of up to 70% whereas the combination of alprostadil, papaverine, and phentolamine can reach an efficacy of 90% in the treatment of erectile dysfunction. Common side effects observed with these types of injections are priapism and penile fibrosis, with penile pain, hypotension and flushing observed more frequently in patients using intraurethral alprostadil injections. (Shamloul R, Ghanem H. 2013)

1.4.3.3 Testosterone Injections

Testosterone injections are another type of treatment for erectile dysfunction, they also improve sexual libido and ejaculation. They are limited, however, to patients with low bioavailability or levels of testosterone like patients suffering from hypogonadism. Some studies showed the efficacy of testosterone administration in patients with hypogonadism, where their erection performance improved significantly compared to patients given a placebo (57% vs 16.7%). (Jain P, et. al. 2000) Moreover, testosterone administration can be combined with phosphodiesterase-5 inhibitors. This

combination has been studied since androgens and more specifically testosterone is important in the expression of NOS and PDE-5 gene expression. It is also stated that the presence of androgens is essential for the proper function of PDE-5 inhibitors. (Morales A, et. al. 2004) A study showed that the combination of testosterone with PDE-5 inhibitors improved the latter's efficacy; in 70 men, patients administered testosterone 1% gel had 70% improvement in response to sildenafil compared to 48% in patients taking placebo. (Shabsigh R, et. al. 2006)

1.4.3.4 Vacuum-Assisted Erection Devices

Vacuum-assisted erection devices are medical devices used to treat erectile dysfunction. Although they are considered first-line therapy, they are usually limited to patients where phosphodiesterase-5 inhibitors failed to provide efficacy and for patients who are uncomfortable using invasive therapies such as intraurethral injection treatments. They function by applying negative pressure to the shaft of the penis, forcing blood flow inside the corpus cavernosum. An elastic band located at the base of the cylinder and as such at the base of the penis traps the blood in the corpus cavernosum. The penis maintains its erect state for as long as the device is in place, however, the ring must be removed within 30 minutes after it is placed, and that is to avoid the risk of ischemia and tissue necrosis if remained for several hours. This type of medical device offers high efficacy and is a good alternative for patients who want to avoid medical treatments. (Carson C, Dean J. 2007)

1.4.3.5 Surgical Procedures

Surgical procedures and treatment are considered in patients where existing medical therapies did not provide any efficacy. There are two types of surgical procedures: penile prosthesis and penile revascularization. A penile prosthesis or penile implant acts by inserting a semi-rigid prosthesis to replace the activity of the corpus cavernosum. The prosthesis replaces the corpus cavernosum which is surgically removed. It offers a rigidity that is suitable to perform the act of copulation. Types of prostheses include; hydraulic (most popular), semi-rigid, and mechanical. The advantage of this surgical procedure is that it offers a more natural erection offering a more normal sexual life. (Gurtner K, et. al. 2017) Other surgical procedures include improving arterial blood inflow and reducing venous blood outflow. More commonly,

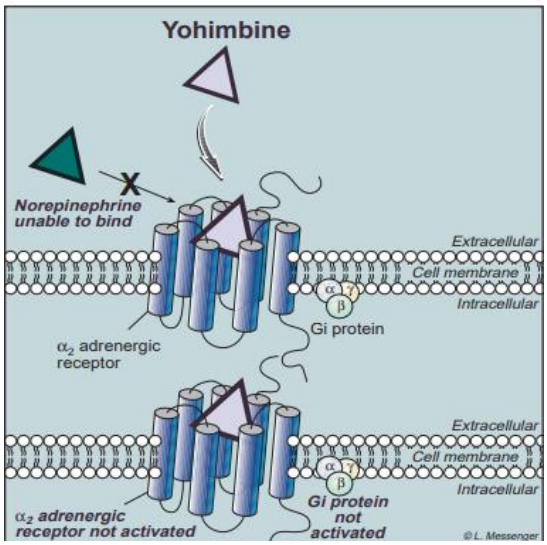
penile arterial revascularization surgeries proved their efficacy in restoring blood inflow in patients with arterial lesions. (Babaei AR, et. al. 2009)

1.4.3.6 Yohimbine

Yohimbine, a natural remedy, is classified as an alkaloid and is extracted from the bark of the Coryanthe Yohimbe tree.

Figure 5 - Mechanism of action of Yohimbine

Figure indicating the competitive inhibition of Yohimbine on the alpha-2 adrenergic receptors



(Morales A. 2000)

As described in figure 5, it acts as an alpha 2-adrenergic receptor blocker in both pre and postsynaptic receptors. (Ernst E, Pittler MH. 1998) Post-synergistic alpha 2-adrenergic receptors that are located in a distal position to the adrenergic nerve terminal are usually activated by epinephrine and other catecholamines inducing contraction of the corpus cavernosum smooth muscle. Yohimbine acts by binding to these receptors instead of epinephrine reducing the contraction of the corpus cavernosum. Although yohimbine proved good efficacy in treating erectile dysfunction, it was observed that its

efficacy decreased with chronic administration. Moreover, its t1/2 is short-lasting up to 35 minutes. (Morales A. 2000)

1.4.3.7 Stem Cell Therapy

Stem cell therapy is another form of treatment and available therapy that includes the use of stem cells. These cells are undifferentiated with properties that include pro-angiogenic, anti-fibrotic, and anti-apoptotic characteristics. Stem cells are divided into four classes; Adult stem cells, embryonic stem cells, induced pluripotent stem cells, and amniotic fluid stem cells. Out of these four classes, adult stem cells are the main focus of studies because of their ability to repair and restore proper function to different tissues. More specifically, mesenchymal stem cells are identified and isolated from other stem cells. They are derived from many tissues and are used in many studies for their activity in treating erectile dysfunction. (He M, Von Schwarz ER. 2020) Some studies showed that mesenchymal stem cells derived from adipose tissue treated erectile dysfunction by restoring and regenerating corpus cavernosum tissue function which sustained damage. (Castiglione F, et. al. 2012)

1.4.3.8 Low-Intensity and Hyperbaric Oxygen Therapies

Low-intensity shock therapy is a non-invasive novel technique that uses shockwave of low intensity to induce structural changes microscopically and as such induce neovascularization, this concept is applied to the penis where it was theorized that the stimulation causes the release of vascular endothelial growth factors and stromal cell-derived factor 1 restoring proper hemodynamics and pathological changes. (Lei H, et. al. 2013) Although the mechanism is not fully understood in the literature, low-intensity shock therapy proved its efficacy to treat erectile dysfunction in patients where phosphodiesterase-5 inhibitors did not prove effective. (Clavijo RI, et. al. 2017) Hyperbaric oxygen therapy is another novel technique where the patient breathes 100% oxygen at pressures above normal atmospheric pressure. This increases oxygen delivery to the tissues. (Sahin M, et. al. 2018) Its mechanism of action is similar to low-intensity shock therapy where it induces angiogenesis by increasing the release of VEGF and hypoxia-inducible factor-1alpha resulting in increased blood flow and improved erectile function. A study showed that hyperbaric oxygen therapy improved erectile function by 88% (Hadanny A, et. al. 2018)

1.5 Natural Ingredients Literature Review

1.5.1 *Ferula Hermonis*

Ferula Hermonis, also known as Zallouh, is a plant from the family of Apiaceae and the genus Ferula. This family includes more than 150 species. Ferula Hermonis is native to Lebanon and Syria (Boghrati Z, Iranshahi M. 2019), and its used parts include primarily the roots, then flowers and seeds to a lower extent. (Chauhan NS, et. al. 2014) Its active constituents include Daucane-type sesquiterpenes like feruhermonin B, feruhermonin A, lancerdiol p-hydroxybenzoate, lancerdiol vanillate, lancerotriol benzoate, lancerotriol p-hydroxybenzoate, lancerotriol vanillate, jaeschkeanin, vaginatin, teferidin, ferutinin, and teferin. (Mazaro-Costa R, et. al. 2010) Volatile constituents such as α-pinene, α-bisabolol, 3,5-nonadiyne, Carvacrol, linalyl acetate, 1,8- cineole, and camphene. Triterpenoid saponins such as sandrosaponin X, sandrosaponin IX, and sandrosaponin XI. Vitamins E and A, and Fatty acids namely Myristic acid, Palmitic acid, Palmitoleic acid, Stearic, Oleic, Linoleic, α-Linolenic, Arachidic, and Arachidonic acid. Its major compounds responsible for its desired activity are ferutinin, teferdin, and teferin. (Sattar Z, Iranshabi M. 2017) Among other benefits, Ferula Hermonis is used to treat erectile dysfunction in men, increase sexual performance in both males and females, and improve bone mineralization in females. Acutely, ferutinin binds to the estrogen receptors alpha and beta that are located in the hypothalamus, consequently activating the hypothalamus-pituitary-gonadal axis, inducing the release of gonadotropins and more importantly testosterone. (Zanoli P, et. al. 2005) As such, testosterone levels are increased, increasing sexual performance and erectile function. It is theorized that testosterone increases NO synthesis in the medial preoptic area, increasing dopamine release and consequently sexual motivation. (Hull et al. 1997) More specifically, ferutinin offers an agonistic or estrogenic activity to the Estrogen Receptor alpha (ERα) and agonistic/antagonistic or anti-estrogenic activity to the Estrogen Receptor beta (ERβ). In addition, ferutinin has a higher binding affinity to the ERα (IC50=33.1 nM) compared to ERβ (IC50=180.5 nM) (Zavatti M, et. al. 2005) which might explain the estrogenic effects when used for short durations of time compared to prolonged use. Teferdin and teferin act in a similar mechanism, however, teferdin has a lower binding affinity to the estrogen receptors compared to ferutinin, and teferin has a negligible affinity (0.01% of 17β-estradiol) to the estrogen receptors

compared to ferutinin. However, when administered sub-chronically or chronically, ferutinin alone caused negative effects; it caused a negative feedback loop of the hypothalamus-pituitary-gonadal axis, consequently decreasing testosterone levels. (Zanoli P, et. al. 2005) Moreover, ferutinin caused an increase in intromission and mount latencies and decreased the ejaculating percentage in rats compared to rats taking a placebo. Although teferdin and teferin were observed to reduce intromission latencies, when compared to rats taking a placebo, this reduction was not statistically significant. (Zanoli P, et. al. 2005) In females, ferutinin was observed to bind to the ERα, consequently increasing the levels of estrogen and progesterone, the effects observed were increased sexual motivation and proceptive behaviors. (Zare Mirakabad H, et. al. 2019) This effect was the opposite, however, when female rats were hormone-primed with estradiol benzoate. In addition, this receptor binding increases estrogen levels and improves bone mineral density by stimulating the Phosphatidylinositol-3-kinase/Akt and MEK/ERK signaling pathways in undifferentiated amniotic fluid human stem cells, as such increasing bone reconstruction. (Ferretti M, et. al. 2010) Moreover, Ferula Hermonis had considerable LH and FSH-like activity; it was observed to increase the number of corpora lutea consequently increasing progesterone synthesis, in addition, multifocal hyperplasia and mild endometrial folding were observed due to the LH-like activity of *Ferula Hermonis*. This leads to increased female fertility. (Hammam A mohsen M, et. al. 2022) *Ferula Hermonis* extracts were shown to have variable effects depending on the method of extraction used; hydrophilic or lipophilic extracts. Hydrophilic extracts were observed to stimulate and enhance sexual performance, and that is due to the high polarity of the extracts. Especially methanolic extracts increased the mount rates, and enhanced sexual motivation levels, whereas, water extracts increased the duration of intromission latency which is a negative effect while keeping mount rate levels unchanged. Moreover, lipophilic extracts, which contain low polarity extracts were observed to suppress and reduce sexual performance. Specifically, petroleum ether and ethyl acetate extracts provided negative effects such as decreased intromission rate and increased intromission latency, and decreased mount rates. (Hadidi KA, et. al. 2003) Some observed side effects of *Ferula Hermonis* were observed especially during chronic administration; for instance, hepatomegaly, atrophy of the testes, decreased body weight, and decreased Red Blood Cell (RBC) count and hemoglobin levels. *Ferula Hermonis* should be administered with caution for patients on vasodilators or patients who suffer from cardiovascular problems, and for patients

taking hormones or hormone replacement therapies like estradiol. (Naguib YMA, Lilling HJ. 2003)

1.5.2 *Lepidium Meyenii*

Lepidium Meyenii Walp. Also known as Maca is a plant from the family of Brassicaceae, and the genus Lepidium L. Maca is native to Central Peru, and its used parts include mainly the roots and areal parts. (He Y, et. al. 2020) Its active constituents include Polysaccharides; ribose, rhamnose, arabinose, xylose, mannose, glucose, and galactose. Essential amino acids such as Threonine, valine, methionine phenylalanine, isoleucine, leucine, and lysine. Non-essential amino acids like aspartate, glutamate, serine, histidine, glycine, arginine, alanine, tyrosine, cysteine, and proline. Macamides which are also known as N-benzylamides of long-chain fatty acids, macamides are the major constituents that offer the desired benefits. Enzymes: amylase, pectin esterase, and polygalacturonase Fatty acids like linoleic acid (major), palmitic, oleic, and stearic acid. Phytosterols like sitosteryl, campesteryl acetate, ergosteryl acetate, brassicasteryl acetate, and ergostadienyl acetate. And other constituents from different classes like organic acids, glucosinolates, amide alkaloids, β–carboline alkaloids, imidazole alkaloids (lepidine A, B, C, and D), pyrrole alkaloids (macapyrrolins A, B, and C), and polyphenols (flavonolignans and phenolic acids). (Singh N, et. al. 2020) There are three phenotypes of Maca; Black, yellow, and red Maca. Black and Yellow Maca were the two phenotypes that had efficacy towards spermatogenesis and increased sexual performance. Black maca showed the highest efficacy on spermatogenesis mainly by affecting the length of spermination stage VIII in the seminiferous tubules; increasing the length by 2.4 times. (Gonzales GF, et. al. 2014) This effect was observed as soon as 7 days and up to 42 days. (Gonzales C, et. al. 2006) Black Maca specifically increases spermination stages VII-VIII and increases germinal cell mitosis stages IX-XI. This leads to an increase in sperm production and epididymal sperm count without changing the levels of Luteinizing, Follicle-stimulating hormones, and testosterone. In some studies, this effect was shown to happen as soon as three days after Maca treatment. (Gonzales GF, et. al. 2013) In addition, another mechanism where Black Maca increases sperm quantity and volume is due to the anti-oxidant and free radical scavenging activity of the macamides, phenolic, and fatty acid compounds. The latter protects sperm from oxidative damage and leads to increased daily sperm production and epididymal sperm

count. (Yucra S, et. al. 2008) Moreover, Maca was shown to increase the expression levels of 3β-hydroxysteroid dehydrogenase messenger Ribonucleic Acid (mRNA). This leads to an increase in the production and activity of this enzyme, increasing androstenedione production. This compound is a precursor of testosterone, increasing as such testosterone levels. (Ohta Y, et. al. 2017) Yellow Maca on the other hand showed intermediate effects compared to Black Maca on spermatogenesis. In addition, black Maca was observed to increase daily sperm production levels, and sperm quality (volume and motility), this effect is theorized to be due to a possible mechanism where Maca enhances the response of Sertoli cells to the follicle-stimulating hormone. (Gonzales GF, et. al. 2001) Maca was also observed to protect spermatogenesis from the degenerative effects of Malathion and organophosphates on the spermination stages. (Bustos-Obregón E, et. al. 2005) In females, some trials showed that Maca was able to increase levels of Luteinizing and follicle-stimulating hormones during the pro-oestrus stage, increasing consequently ovulation by promoting the Hypophysis Pituitary Gonad axis. (Uchiyama F, et. al. 2014) Moreover, maca was observed to increase bone mineral density in females, and that is because of its activity on the hypothalamus axis which caused an increase in testosterone and progesterone levels, and also because has easily absorbable calcium, magnesium, and silica amounts, which increases calcium levels. (Zhang Y, et. al. 2005) In addition, Maca can reduce anxiety and depression symptoms which are commonly seen in menopausal women (Lee MS, et. al. 2011). In addition, Maca was shown to reduce menopausal symptoms by increasing estrogen production at levels above 30 pg/ml, reducing menopausal discomfort. (Serrano ZA. 2017) It even improved sexual function in menopausal women by increasing progesterone and testosterone levels. (Najaf Najafi M, Ghazanfarpour M. 2018) Main extraction techniques include the use of hydro-alcoholic solvents like ethanol and water to extract the active constituents, and petroleum ether extracts can also be used. (Gonzales GF, et. al. 2001) Some side effects observed are mainly gastrointestinal discomfort and headaches. Moreover, Maca should not be administered to patients with hormone-sensitive conditions like patients suffering from breast or ovarian cancer, endometriosis, and uterine fibroids. (Bethesda MD. 2012)

1.5.3 L-Arginine

L-arginine is a conditionally essential amino acid, it is available from the turnover of proteins, protein diet, and through endogenous synthesis. (Morris SM. 2006)

L-arginine is available in two other forms: L-arginine HCl and L-arginine alpha-ketoglutarate (AKG). L-arginine HCL offers increased water solubility compared to L-arginine, in addition, it can mask the taste of L-arginine due to its effect on stabilizing the pH. L-arginine AKG on the other hand is a form that is used mostly for athletes and physical activities due to the presence of the alpha-ketoglutarate molecule which is responsible for energy production in the body. L-arginine is also able to treat erectile dysfunction by increasing NO synthesis by the eNOS, (Chen J, et. al. 1999) consequently inducing vasodilation and increasing cGMP production inducing corpus cavernosum smooth muscle cell relaxation and subsequent erection. (Moody JA, et. al. 1997) Another form of L-arginine is also used to treat erectile dysfunction; L-citrulline is a precursor of L-arginine, through synthesis pathways it is transformed into L-arginine increasing its levels, and as such increasing NO levels. This effect is seen acutely and can provide sustained levels of L-arginine and NO. (Morita M, et. al. 2014). In addition, the combination of L-arginine with L-citrulline enhances levels of L-arginine, cGMP, and NO more significantly than administering L-arginine alone. (Schwedhelm E, et. al. 2008) A study showed a difference in L-arginine levels of approximately 350 vs 270 μmol/l in rabbits and approximately 1000 vs 700 μmol/l in rats, and 130 μmol/l vs 80 μmol/l of NO in rabbits. (Morita M, et. al. 2014) Moreover, L-citrulline inhibits the enzyme arginase, which is responsible for L-arginine metabolism in the hepatic and gastrointestinal tissues, increasing consequently the bioavailability of L-arginine. (Khalaf D, et. al. 2019) Some observed side effects of L-arginine include gastrointestinal discomfort, nausea, and diarrhea. L-arginine should also be used with caution for patients with low blood pressure.

1.5.4 *Punica Granatum*

Punica Granatum, also known as pomegranate, is a plant from the family of Lythraceae, and the genus Punica L. The used parts are mainly fruits and seeds. (Maina C, et. al. 2019) Pomegranate is native to modern-day Iran. Its active constituents include Punicalagin, ellagic acid, punicalin, and ellagic acid derivatives as its major compounds. 3,3´-di-O-methylellagic acid, 3,3´-tri-O-methylellagic acid, 3´-O-methyl-3,4-methylene, Flavonoids (Luteolin), Quercetin, Kampherol, Anthocyanins (delphinidin, cyanidin, and pelargonidin), Phenolics (pedunculagin, punicacortein A-D, granatin A and B, punicafolin, punigluconin, corilagin, and gallocatechins), Fatty acids (Mainly

Linolenic acid, octadecanoic acid, palmitic acid, stearic acid, and arachidic acid), Sterols, Triterpenes, Tannins, and Carbohydrates. (Mena P, et. al. 2011) It was shown to treat erectile dysfunction in males; in fact, it was observed that it increased sexual motivation and libido, intromission frequency, and decreased intromission latency, (Forest CP, et. al. 2007) it was also observed to increase testosterone levels with prolonged use (Maina C, et. al. 2019). It has two distinct mechanisms of action by which it treats erectile dysfunction; the first being NO-dependent. Pomegranate enhances and increases the bioavailability of NO by protecting it from oxidative damage due to its antioxidant properties, increasing such levels of NO and subsequent corpus cavernosum smooth muscle relaxation. (Ignarro LJ, et. al. 2006) The second mechanism of action is specific to ellagic acid. It is a NO-independent mechanism, where ellagic acid activates the Ca-activated K+ channels, and increases the suppression of the L-type Ca channels (Oztekin CV, et. al. 2013), increasing as such the relaxation of the corpus cavernosum smooth muscle cells. In females, pomegranate was seen to improve female post-menopausal symptoms (Adel-Mehraban MS, et. al. 2022) like hot flashes, insomnia, fatigue, vertigo, nervousness, and melancholia. (Ahn KH, et. al. 2010) Pomegranate was also studied and used to increase bone mineral density and prevent osteoporosis; it was seen that pomegranate is transformed to the CLA 9c isoform in the intestines, which in turn induces the inhibition of RANKL osteoclast differentiation, as such it down-regulates a reduces the expression of osteoclast differentiation markers such as CTR, CTSK, CCR2, MMP9, ITG β3, and TRAP markers. (Spilmont M, et. al. 2013) Consequently leads to a decreased Receptor Activator of Nuclear factor-κB Ligand (RANK-RANKL) downstream signaling. On the other hand, pomegranate was shown to increase bone mineralization, Alkaline Phosphatase (ALP) activity, and matrix mineralization by up-regulating transcriptional factors such as Bone Sialoprotein (BSP), and Discoidin Domain Receptor 2 (DDR2). Moreover, due to its anti-inflammatory activity, it offers bone-sparing activity by decreasing cytokines responsible for inflammation such as Tumour necrosis Factor alpha (TNF-α) and Nuclear Factor Kappa-light-chain-enhancer of activated B cells (NF-κB). (Spilmont M, et. al. 2013) Pomegranate is considered safe to use, however, since it inhibits CYP2C9, CYP2D6, and CYP3A4 it should be taken with caution for patients on medications that are metabolized by these cytochromes like carbamazepine. Moreover, medications like ACEIs and PDE-5 can cause synergistic hypotension and a decrease in blood pressure.

1.5.5 Zinc

Zinc is a mineral, and one of the essential trace elements. It is available in the diet and form of supplements. (Hambidge M. 2000) It was shown to improve erectile function, and sexual performance by different mechanisms; it stimulates and increases the human chorionic gonadotropin-induced synthesis and production of cAMP (Prasad AS, et. al. 1996), as such increasing testosterone levels and inducing corpus cavernosum smooth muscle relaxation through the activity of cAMP kinase. Moreover, it increases androstenedione conversion to testosterone by increasing the enzyme 17 β-hydroxysteroid dehydrogenase, as such increasing testosterone levels, and increasing sexual performance and erectile function. (Paniagua R, et. al. 1982) In addition, it decreases the activity and inhibits the enzyme hepatic 5-alpha reductase and decreases the formation of Nicotinamide Adenine Dinucleotide Phosphate Hydrogen (NADPH) cofactor, the hepatic 5-alpha reductase enzyme is responsible for the metabolism and breakdown of testosterone, and as such its inhibition leads to increased testosterone levels. (Leake A, et. al. 1984) In females Zinc was observed to improve fertility and sexual performance; it was shown to facilitate zona pellucida hardening which is the outer shell of the oocytes, increasing as such fertility, it was also shown to prevent polyspermy by decreasing sperm motility. (Garner TB, et. al. 2021) Moreover, in the androgen receptors, namely estrogen and progesterone receptors, the binding domain is a cysteine-rich zinc finger protein, consequently increased levels of zinc allow for more formation of these zinc finger proteins and offer the better function of these receptors, increasing the binding and action of estrogen and progesterone (Prasad AS. 1995). Zinc is considered safe as a supplement, however, side effects like nausea, vomiting, headache, stomach cramps, low appetite, and diarrhea can still appear.

1.5.6 Honey and Vitamin C

Although honey will be used as a sweetener in the formulation, it offers itself benefits in increasing sexual performance, and multiple health benefits, such as anti-oxidant activity, anti-inflammatory, wound healing activity, anti-microbial activity, and vasodilation activity. It is made of multiple active constituents such as Sugars; Sucrose, fructose, glucose, Maltose, isomaltose, maltulose, turanose, panose, centose with maltose being the major disaccharide, and Isomaltotetraose major oligosaccharide. Acids like Gluconic (major), acetic, butyric, lactic, and pyroglutamic acids. Phenolic

acids and flavonoids are responsible for most of their benefits such as; Ellagic acid, Gallic acid, Syringic acid, Cinnamic acid, cafeic acid, coumaric acid, Kaempferol, luteolin, Quercetin, Chrysin, Myricetin, and galangin. Amino acids like Proline (major), Glucosaminic acid, methionine, aspartic acid, alanine, cysteine, valine, glycine, leucine, isoleucine, tyrosine, histidine, arginine, tryptophan. Enzymes such as a-Glucosidase (invertase and sucrose), Glucose Oxidase, Diastase, catalase, and phosphatase. And some important vitamins like Riboflavin, Pantothenic acid, Niacin, thiamine, ascorbic acid, and pyridoxine. (Ahmad RS, et. al. 2017) Honey acts by different mechanisms of action mostly attributed to and caused by the flavonoids and phenolic acids like a free radical scavenging activity (Van den Berg AJJ, et. al. 2008) and antioxidant activity (Chua LS, et. al. 2013), inhibition of the inflammatory cytokines and interleukins IL-8, IL-1β TNF-alpha, and Cyclooxygenase-2 (COX-2). (Hadagali MD, et. al. 2014) In addition, honey helps in wound healing due to its anti-microbial activity and debriding activity; it has activity against *S.aureus*, *S.pneumoniae*, Bacillus species, *E.Coli*, *Klebsiella Pneumoniae*, *H.influenzae*, Salmonella species, Shigella Species, (Molan PC. 1992) and anti-fungal activity against Aspergillus sp, Candida sp, Penicillium sp, and Aspergillus sp. (Irish J, et. al. 2006) Moreover, it has proteolytic enzymes that allow the peeling of necrotic tissue and cells. (Hadagali MD, et. al. 2014) In addition, an interesting mechanism that can also benefit the addition of honey to the other active ingredients is its ability to increase the production of NO; quercetin a constituent available in honey can increase NO synthesis by stimulating eNOS and consequently increasing NO levels. (Khoo NKH, et. al. 2010) Quercetin is also behind the effect of honey in increasing testosterone levels as observed in some studies, moreover honey can increase luteinizing hormone levels, and as such increase the stimulation of 17β-hydroxysteroid dehydrogenase to convert androstenedione to testosterone even at low doses of 100 mg/Kg in rats. Moreover, doses of 120 mg/d in rats provided testicular tissue-protective effects by protecting against pentylenetetrazole-induced damage to the tissue. (Banhihani SA. 2019) Although honey is considered safe to use, it can increase the activity of cytochrome CYP3A4, decreasing the activity of some drugs metabolized by this cytochrome such as anti-platelets. (Ahmed S, et. al. 2018) Since it increases NO production, it can also interact with hypotensive medications, causing a synergistic decrease in blood pressure.

Vitamin C on the other hand, also known as, ascorbic acid is mainly used due to its beneficial effects when combined with Ferula Hermonis. A study showed that the administration of 500 mg/Kg of Vitamin C with 6 mg/Kg of the aqueous extract of *Ferula Hermonis* for 6 weeks partially reversed the negative chronic effects of *Ferula hermonis* and more specifically ferutinin. Vitamin C was able to reduce fibrosis and tissue damage and preserved the ERβ expression to a certain extent. Moreover, it maintained higher testosterone levels compared to using *Ferula hermonis* alone, however, testosterone levels still decreased by the end of the study. (Ayuob NN, et. al. 2014)

1.5.7 *Vitis Vinifera* Seed Oil

Vitis Vinifera is also known as grape seed. It is used mainly as extracts or oils. Grape seed extract contains many phenolic and polyphenolic compounds such as procyanidins which are condensed tannins from the flavonoid class. Specifically, the grape seed contains more than 70% of polyphenolic compounds. It was shown to activate the phosphoinositide 3-kinase/Akt signaling pathway, consequently phosphorylating and stimulating eNOS (Edirisinghe I, et. al. 1979). This increases eNOS activity and increased the production of NO and subsequent corpus cavernosum smooth muscle cell relaxation. It also inhibits aromatase, the enzyme responsible for converting androgens and testosterone to estrogen. (Kijima I, et. al. 2006) Through a non-NO-dependent pathway, it inhibits Rho-kinase 2 (ROCK-II) (Goswami SK, et. al. 2012), causing direct human corpus cavernosum muscle relaxation. Through a NO-dependent pathway, grape seed can protect NO from oxidative stress and environmental damage, due to its antioxidant properties, (Fitzpatrick DF, et. al. 2002) that usually cause the decrease of NO levels, in another study, it protected NO and sperm quality from arsenic-induced damage by increasing levels of glutathione (GSH) and superoxide dismutase (SOD). (Li SG, et. al. 2015) The main extraction techniques used are the methanolic and water extracts. (Clifton PM. 2004)

1.6 Dosage Form Selection

The literature was reviewed for multiple dosage forms that could be used for the development of the product. Four dosage forms were selected while keeping in mind

patient compliance and ease of administration: orally dissolving strips, hard lozenges, syrups, and chewable lozenges.

1.6.1 Orally Dissolving Strips

Orally dissolving strips (ODS) are a type of hydrophilic polymer that is developed with the intent to be placed on the tongue. Some distinctive characteristics of orally dissolving strips include being thin with a large surface area, they should also be non-obstructive, and they do not need the intake of water to disintegrate. ODS also have local and systemic absorption. (Wanjari D. 2014) Oral dissolving strips are divided into three classes; flash release, mucoadhesive melt-away wafers, and mucoadhesive sustained-release wafers. (Bala R, et. al. 2013) Flash-release dissolving strips offer the fastest dissolution reaching up to 60 seconds while being the thinnest with 20-70 μm and having the largest surface area with 2-8 cm2 out of the three classes. Moreover, they are formed by a single layer only with systemic and local activity. Mucoadhesive melt-away wafers have the benefit of including either one layer or multiple layers of different medications, and they can be applied to the gingival or buccal areas. In addition, they have a surface area ranging from 2-7 cm2, they are the thickest of the three classes reaching a thickness of 50-500 μm and taking a few minutes for complete dissolution. Mucoadhesive sustained-release wafers compared to the other two classes have the smallest surface area ranging from 2-4 cm2, their application is mainly to the gingival area, and they also have a thickness of 50-250 μm, and offer a longer duration of action due to longer dissolution time ranging from 8-10 hours. Some advantages of ODS include their fast release of API due to the rapid disintegration of the strips and their large surface area, they are convenient to use offer high compliance rates among patients, they can mask the bad taste of active ingredients, they offer good physical stability, and since they can be applied in the buccal area the active ingredients enter the systemic circulation directly by avoiding the first-pass effect, increasing as such the bioavailability of the active ingredients. (Kumar Vishwakarma P. 2015) The main disadvantages of ODS include low dose limitation; a maximum dose limit of 40 μm can be used. Another limitation is the difficulty to achieve dose uniformity, and difficulty to package due to their high moisture sensitivity. (Kumar P, et. al. 2015)

1.6.2 Hard Lozenges

Hard lozenges are another form of solid dosage form that could be used for the development of the product. They are also known as solid candies, they are made by a mixture of sugar and other carbohydrates, and they have a solid amorphous texture; they are not crystalline and not glass-like either. Hard lozenges are most commonly used for the treatment and relief of sore throat pain where active ingredients such as lidocaine are incorporated. They can be also used for irritation relief due to their long duration to dissolve, and consequently, they provide local activity. They have a dissolution time ranging from 5 to 10 minutes. (Rao M, et. al. 2014) Hard lozenges can also be molded into various shapes and structures to improve patient compliance. In addition, there are conflicting arguments for the use of preservatives; some articles argue that there is no need to include preservatives, whereas other articles explain that due to the moisture content of hard lozenges, which has a usual range of 0.5-1.5%, there is a need to include preservatives to avoid bacterial and microbial growth. Consequently, preservatives that have low aroma and taste have been described as effective preservatives such as 0.125% butylparaben, and 0.015% propylparaben. (Dahiya S, Dahiya R. 2021) Some advantages of hard lozenges include the ease of administration and offering high compliance rates, due to the use of sugars hard lozenges can be used to mask the bad taste of active ingredients, hard lozenges primarily have GI and subsequent systemic absorption, however, they have a slight local absorption and activity due to their long duration of dissolution, as such some of the active ingredients are absorbed through the mucosa avoiding the first-pass effect. (Choursiya S, Indurkhya A. 2020) Some limitations or disadvantages of hard lozenges include the presence of the first-pass effect due to the GI absorption of the active ingredients which reduces the bioavailability of the drugs. (Umashankar M, et. al. 2016) They are more difficult to compound since they need high heating temperatures reaching above 100˚C, and as such heat-labile active ingredients cannot be used. (Majekodunmi S. 2015)

1.6.3 Liquid Dosage Forms

Liquid dosage forms have also been evaluated for the development of the product. There are two classes of liquid dosage forms: monophasic and biphasic. Monophasic liquids include syrups, linctuses, and elixirs. Syrups are concentrated solutions of sucrose; they are viscous in texture and sweet in taste. Similarly, linctuses

are viscous solutions that can be sweet, they are, however, not necessarily concentrated solutions of sucrose, and are most commonly used to relieve cough. Elixirs are hydro-alcoholic solutions that are composed mainly of high volumes of ethanol and sugars like sucrose. They are aromatic and sharp in taste. Biphasic liquid dosage forms include suspensions and emulsions. Suspensions are dispersed solid particles in a liquid medium, they offer fast action in the body, however, they need to be shaken before use. Emulsions are a combination of two immiscible liquids, either oil in water or water in oil phase compositions. Oil-in-water emulsions are commonly used for internal treatments, whereas water-in-oil emulsions are used for topical applications, as such, they need emulsifying agents to stabilize compared to suspensions, and similarly to the latter emulsions need to be shaken before use. (Kalyan PG, et. al. 2017)

1.6.4 Syrup Dosage Form

The dosage form that could provide adequate texture and taste when combined with the active ingredients is syrup. Syrups include the use of sucrose which is compatible with honey, one of the ingredients used in the product, moreover some studies developed herbal syrup formulations using honey as the major ingredient and base of the syrup. (Patil AG, et. al. 2020) Moreover, syrups are sweet in taste and viscous in texture, and their concentration of sucrose is 66.7% W/W or 85% W/V USP. Syrups include two types; medicated and non-medicated, the only difference being the active ingredient that offers therapeutic effects. (Awad A, et. al. 2021) Syrups can contain a low amount of preservatives in the form of alcohol. Syrups have different preparation techniques; with heat, without heat, percolation, and the addition of sucrose to liquid. Preparations with heat are the easiest to prepare, however, the use of heat can render some active ingredients unstable and can cause the inversion of sucrose leading to increased microbial growth susceptibility. Preparations without heat include the use of agitation, this method is used for heat-labile ingredients, however, it is harder to compound. The addition of sucrose to liquid includes simply the addition of sucrose to a liquid mixture of the medication and solvent while stirring. And finally, percolation includes the addition of a prepared syrup base to the percolated extracts of the active ingredients. (Klie G. 1881) Syrups have the advantage of masking the taste of some bitter drugs, they have high osmotic pressures due to the high concentration of sucrose consequently acting as a self-preservative and inhibiting microbial growth, and compared to the other dosage forms they have a direct

absorption to the GI since there is no need for the syrup to disintegrate or dissolve or be chewed. Some limitations include the risk of crystallization during storage, and syrups need to be well sealed or closed to eliminate moisture formation and possible bacterial growth. (Gaikwad A, et. al. 2014)

1.6.5 Chewable Lozenge Dosage Form

Lastly, chewable lozenges dosage forms similar to hard lozenges are evaluated for the development of the product. Chewable lozenges also known as gummies can be highly flavored and slightly acidic in taste, they offer a soft and chewable consistency and texture compared to other dosage forms. They are used commonly to administer medications to special populations like pediatrics. (Umashankar M, et. al. 2016) The gelatin base is usually made of 70% glycerin, 20% gelatin, and 10% purified water. (Rao M, et. al. 2014) However, these percentages can vary depending on how much chewability and hardness are desired in the texture. (Sulaiman TS, et. al. 2015) Similar to hard lozenges, low quantities of effective preservatives can be used to inhibit microbial growth while having a low aromatic profile like 0.125% butylparaben or 0.015% propylparaben. Some advantages include the ease of administration, especially for special populations like pediatrics, they can be also used to mask the bad taste of some active ingredients by using flavoring agents and sweeteners. (Dahiya S, Dahiya R. 2021) As such, this combination can be captivating and appealing to patients offering high compliance rates. They are administered in the buccal area and have good GI absorption leading to systemic absorption and activity, they are also easy to compound compared to other dosage forms. Some limitations include the first pass effect decreasing the bioavailability of the active ingredients, and heat-labile ingredients cannot be used. (Majekodunmi S. 2015)

1.6.6 Selection of the Chewable Lozenge Dosage Form

Comparing these four dosage forms, chewable lozenges are the most adequate choice; they do not have the dose limitation of orally dissolving strips, and they are easier to compound compared to hard lozenges while using lower heating temperatures (Dille MJ, et. Al. 2017), and they do not have the risk of crystallization compared to syrups. Moreover, they are an appealing dosage form in both shape and taste while being easy to use.

CHAPTER TWO

OBJECTIVE AND AIMS

The thesis was designed to develop a suitable dosage form that comprises multiple natural-based active ingredients with the intent to cover multiple mechanisms of action and targeted multiple populations and age groups while being aimed primarily at the treatment of erectile dysfunction.

Objective: Development and optimization of a suitable chewable lozenge

- To prepare and develop an adequate formulation with the specific concentrations of the active ingredients and excipients.
- To optimize the compounding procedure to obtain a pleasant chewable lozenge with acceptable characteristics.
- To optimize the glycerin base percentages and the ingredients to obtain adequate lozenges with the desired physical properties.
- To characterize the physical parameters desired to obtain adequate lozenges.

CHAPTER THREE

METHODOLOGY

3.1 General Pharmacokinetics

3.1.1 L-Arginine Pharmacokinetics

L-arginine is a conditionally essential amino acid, its main pharmacokinetic properties include: It is hydrophilic with a water solubility of 100mg/ml with a melting point range of 217-227 ˚C. According to some pharmacokinetic studies, when 10g of L-arginine was administered orally, the Cmax was 50 µg/ml +/- 13.4 µg/ml after 1 hour of the administration. In addition, there was no significant renal excretion, with the average non-renal excretion reaching 360 ml/min. The mean bioavailability was 21% +/- 4% with a range of 5-50%. The AUC was 6.7 mmol/L.min (Tangphao O, et. al. 1999)

3.1.2 *Ferula Hermonis* Pharmacokinetics

Ferula Hermonis has limited pharmacokinetic studies, as such ferutinin, which is one of the major compounds and active constituents, can be used to obtain some pharmacokinetic data. Ferutinin is a daucane-type phytoestrogen, it is also considered a natural terpenoid. Moreover, isoflavones phytoestrogens have a similar structure to ferutinin. When administered 50 mg, the T1/2 obtained was 4.6-9.3 h with a Cmax reaching 0.76-1.55 nmol/ml. The AUC was 11.6-18.3 nmol/ml per h. (Rowland I, et. al. 2003) In addition, higher urine excretion was observed compared to hepatic excretion. The bioavailability of isoflavones has a range of 13-35% (Viggiani MT, et. al. 2019), and they are also poorly water-soluble. (Kim IS. 2021) *Ferula asafetida* which is another genus was found to tolerate heat up to a temperature of 160˚C. (Niazmand R, et. al. 2020)

3.1.3 Zinc Pharmacokinetics

Zinc is a mineral, there are many salt forms of zinc like zinc oxide, zinc sulfate, zinc gluconate, and zinc bis-glycinate. When administered 45 mg of zinc sulfate orally,

the mean Cmax was 8.2 µmol/l, with a mean AUC of 42.1 µmol/l.h. When administered 0.15-0.23 mmol/day, the calculated bioavailability was around 30%. Zinc also has good renal elimination and is water-soluble. (Neve J, et. al. 1991) Zinc sulfate can also remain stable at heating temperatures reaching 640˚C. (Jones F, et. al. 2013)

3.1.4 *Lepidium Meyenii* Pharmacokinetics

Lepidium meyenii also known as Maca, contains many constituents, more importantly, one of its major compounds that exert its desired effects are macamides also known as N-benzylamides of long-chain fatty acids. (Zhu H, et. al. 2020) When administered 100 mg/kg of macamides, the Cmax obtained was 54-519 nM +/- 149 nM. The Tmax ranged from 3-6 h with a T1/2 of 9-14.6 h. The AUC obtained was 723-3690 nM*h +/- 664 nM*h. (Singh N, et. al. 2020) Macamides are also lipophilic with a polarity K: 4:1:2:2 in respectively petroleum ether: ethyl acetate: methanol: water. The thermal stability of maca reached a temperature of 106˚C. (Zhong J, et. al. 2019)

3.1.5 *Punica Granatum* Pharmacokinetics

Punica Granatum is also known as pomegranate and contains many constituents, the major compounds behind the desired benefits are ellagic acid and ellagitannins. (Luca SV, et. al. 2019) They have a low bioavailability of 10% (Long J, et. al. 2019), and they also contain both lipophilic and hydrophilic moieties. When administered 400 mg of pomegranate extract (330 mg ellagitannins and 22 mg ellagic acid), the Cmax of ellagic acid was found to be 0.11 mmol/L. (Kang I, et. al. 2016) Interestingly, higher doses of ellagic acid did not improve the bioavailability of the compound, the reason behind this effect is theorized to be due to an absorption saturation in the small intestines. (Seeram NP, et. al. 2004) The thermal stability of anthocyanins was observed to be 70-90˚C. (Fisher UA, et. al. 2013). After administration of 0.8 g/kg of ellagic acid in rats, the Cmax obtained was 213 ng/ml, the AUC was 0.838 µg.h/ml, and the T1/2 was 0.77 h. (Lei F, et. al. 2003) Ellagic acid was also shown to have poor absorption (Wang R, Du L. 2010) and rapid renal elimination. (Seeram NP, et. al. 2008) After administration of 180ml pomegranate juice in humans, the Cmax obtained was 18.64 +/- 1.69 ng/ml, with an AUC of 50.07 +/- 5.8 ng/h/ml. The elimination T1/2 was 0.75 +/- 0.078 h and the Tmax was 0.98 +/- 0.059 h. (Seeram NP, et. al. 2006)

3.1.6 *Vitis Vinifera* Seed Oil Pharmacokinetics

Vitis vinifera seed oil, also known as grapeseed oil, is mainly composed of procyanidins. When administered 21 mg/kg orally, the Cmax was observed to be 2.6 +/- 0.93 µ/ml with a T1/2 of 7.3 h and an AUC of 17 +/- 2.7 µg.h/min Procyanidins are also hydrophilic and are renally excreted and have a bioavailability of 10.6%. They also have thermal stability ranging from 100-125˚C. (Stoupi S, et. al. 2010)

Table 1- General pharmacokinetics of the active ingredients

API	L-arginine	*Ferula Hermonis*	Zinc	*lepidium meyenii*	*Punica Granatum*	*Vitis vinifera* seed oil
Description	Amino Acid	Ferutinin	Mineral	Macamides	Ellagic acid	Procyanidins
Cmax	50 µg/ml	0.76-1.55 nmol/ml	8.2 µmol/l	54-519 nM	213 ng/m	2.60 µg/ml
T1/2	10.1 mins	4.6-9.3 h	-	9-14.6 h	0.77 h	7.3 h
AUC	6.7 mmol/L.min	11.6-18.3 nmol/ml per h	42.1µmol/l h	723-3690 nM*h	0.838 µg.h/ml	17 µg.h/min
Excretion	Non-renal	Renal	Renal	-	Renal	Renal
Bioavailability	5-50%	13-35%	30%	-	10%	10.6%
Thermal stability	217-227˚C	160˚C	640˚C	106˚C	70-90˚C	100-125˚C
Lipophilicity	Poor	Good	Poor	Good	Good	Poor

According to the pharmacokinetics data, some problems could be resolved during the preparation of the product. For instance, as seen in table 1, the thermal stability of the ingredients ranges from 70-640˚C, as such it makes sense to opt for the preparation of a chewable lozenge instead of hard lozenges; since hard lozenges need heating temperatures above 100˚C. Heating temperatures above 100˚C will increase the risk of procyanidins and ellagic acid loss. Another problem is the low bioavailability of L-arginine, which is a critical ingredient needed to obtain the desired effects. As such, L-Citrulline is added to the formulation. It inhibits arginase, which is the enzyme responsible for the breakdown and metabolism of L-arginine, increasing the bioavailability of L-arginine. Grapeseed oil also increases the efficacy of L-arginine. Procyanidins are hydrophilic, as such the oil form of grapeseed is used to enhance lipophilicity.

3.2 Dose Selection and Justification

3.2.1 Dose Selection of L-Arginine, L-Citrulline, and Grapeseed Oil

The dose selections will be based on one lozenge, noting that for each patient three lozenges will be administered per day. According to studies evaluating the efficacy of L-arginine for the treatment of erectile dysfunction, effective doses ranged from 6-9 g/day. (Chen J, et. al. 1999). For one lozenge, a dose of 1g will be selected. Some studies assessed the efficacy of combining L-arginine with L-citrulline; they found that a mixture of 2.85 mmol/Kg of L-arginine and L-Citrulline was more effective than 2.85 mmol/Kg of L-arginine alone. The levels of L-arginine and NO were higher with the combination than with L-arginine alone, as such lower individual doses of L-arginine and L-citrulline can be combined while still obtaining higher efficacy than using L-arginine alone. A dose of 500 mg for L-citrulline per lozenge will be chosen. (Morita M, et. al. 2014) Moving on to grapeseed oil, it was observed that a dose of 400 mg/Kg of grapeseed procyanidin extract in mice was effective, an equivalent dose that could be used in the product is 528 mg (Li SG, et. al. 2015), as such a dose of 500 mg per lozenge is selected. Procyanidins are used in combination with L-arginine to enhance its activity and efficacy by stimulating eNOS. The combination of L-arginine 1 g, L-Citrulline 500 mg, and Grapeseed oil 500 mg will give a total dose of 2 g per lozenge.

3.2.2 Frequency of Chewable Lozenges Administration

The recommended frequency of administration of the lozenges that is followed exceptionally for this work will be three times daily, and that is based on the requirement and basis that stability testing, compatibility studies, synergistic studies, and toxicological studies will be done to confirm the dose frequency and selection for the ingredients. Consequently, the total dose will reach 6 g/day, reaching as such the effective range of L-arginine while offering more benefits and higher efficacy compared to administering L-arginine alone.

3.2.3 Dose Selection of the Remaining Active Ingredients

As for teferdin, a study assessed the efficacy of Ferula Hermonis root extract in rats at doses ranging from 1-6 mg/Kg for 10 days, as such an equivalent dose of teferdin equal to 100 mg per lozenge will be selected. (Zanoli P, et. al. 2003). As for Vitamin C, a study showed that the chronic administration of the combination of Vitamin C at 500 mg/Kg with Ferula Hermonis at 6 mg/Kg in male mice partially reversed the negative chronic effects of Ferula hermonis, by maintaining to certain extent testosterone levels. (Ayuob NN, et. al. 2014) Moreover, the upper tolerable limit of Vitamin C is 2 g/day (Bsoul SA, Terezhalmy GT. et. al. 2004) As such, a dose of 500 mg per lozenge will be selected. Moving on to Maca, an effective human dose was observed to be 1500-3000 mg/day. As such, a dose of 500 mg per lozenge will be selected. (Gonzales GF, et. al. 2002) Two studies assessed the efficacy of pomegranate juice and pomegranate fruit extract respectively, the first study assessed the effects of pomegranate juice on human corpus cavernosum tissue by evaluating the relaxation responses; the dose administered was 25.6-415.5 mg. (Gur S, et. al. 2016) As for the second study, pomegranate fruit extract was administered to rats with a dose range of 500-1500 mg/Kg. As such, a dose of 500 mg per lozenge will be selected. (Maina C, et. al. 2019) Lastly, zinc was observed to have doses that reached 300 mg/day with zinc sulfate doses specifically proving efficacy at 250 mg/day in humans (Jalali GR, et. al. 2010) as such a dose of 100 mg can be selected per lozenge.

3.2.4 LD 50 of the Natural Ingredients

The LD50 of the ingredients can also be used to select the doses, for instance, L-arginine has an LD50 of 5110 mg/Kg according to the European Chemicals Agency, Ferula Hermonis has an LD50 of 10.602 g/Kg (El-Thaher TS, et. al. 2001), according to the European Chemicals agency the LD50 for zinc sulfate was 574 mg/Kg. Maca had an LD50 of 7.5 g/Kg. (Meissner HO, et. al. 2019) And lastly, pomegranate fruit extract had an LD50 of 5 g/Kg (Patel C, et. al. 2008)

3.2.5 Possible Synergistic Effects

The dose selection of the ingredients is based on the synergistic effects shown in the literature of some combinations of the ingredients. On a broader scope, possible synergistic effects could exist when all these active natural ingredients are combined. For instance, throughout the literature *Ferula Hermonis*, Zinc, and Vitamin C increase levels of testosterone, indicating a possible synergistic effect between these three ingredients. The effect of the combination of Vitamin C and *Ferula Hermonis* already showed benefits towards testosterone levels by maintaining them on chronic use. (Ayuob NN, et. al. 2014) L-arginine, L-citrulline, *Punica Granatum*, Honey, and *Vitis Vinifera* all increase nitric oxide levels. For instance, the beneficial effect of the combination of L-arginine, and L-citrulline was studied, and it was shown that this combination increases NO levels significantly compared to L-arginine alone. (Schwedhelm E, et. al. 2008)

3.4 Formulation Development

The solubility of Arginine and citrulline is assessed to determine the percent of the water that is going to be used in the formulation. According to the European Chemicals Agency, L-arginine HCL has a water solubility of 730 mg/ml, as such for 1 g of L-arginine HCL 1.37 ml of water is needed, the water solubility of L-arginine AKG is 56.5 mg/ml, and as such 8.84 ml of water is needed to dissolve 500 mg of L-arginine AKG. According to the safety data sheet of L-citrulline by Bioworld, it has a water solubility of 200 mg/ml, as such 2.5 ml of water is needed to dissolve 500 mg of L-citrulline. In total, 12.71 ml of water is needed to dissolve the three ingredients with a range of 1.37 – 8.84 ml. The volume of water per lozenge would be too large following

these calculations, as such a 25% water percent is selected to provide a 1.17 ml volume of water per lozenge which is close to the lower limit, more than 25% water content would increase the risk of obtaining very sticky lozenges with a loose shape and gel-like texture. As such, the three ingredients will be dissolved overheated while compounding the lozenges to increase the water solubility.

The doses of the glycerin base and honey are calculated in a way to have a total volume of 8 cm^3 per lozenge.

Table 2 - Formulation of the chewable lozenge

Ingredients	Uses	Dose for 1 lozenge
Glycerin base (60% glycerin, 15% gelatin, 25% distilled water)	Base and chewable texture	2.80 ml glycerin 0.701 g gelatin 1.17 ml water
Propylparaben (0.015%)	Preservative	0.132 mg
Peppermint oil	Flavoring	Drops
Red food coloring	Color	Drops
L-arginine HCL 1g + L-citrulline 500mg + 500mg Grape seed oil	Erectile dysfunction treatment	2000 mg
Pomegranate extract	Improves potency	500 mg
Black Maca hydroalcoholic extract	Spermatogenesis promoter	500 mg
Teferdin	Erectile dysfunction treatment	100 mg
Elemental Zinc	Improves testosterone production and sexual performance	100 mg

Honey	Sweetener and offers multiple health benefits	100 mg
Vitamin C	Protective effects	500 mg

Table 3 - Volume calculations

Ingredient	Density	Volume for 1 lozenge
Glycerin	1.2613 g/cm^3	2.805 cm^3
Gelatin	0.42 g/cm^3	0.701 cm^3
Water	1 g/cm^3	1.17 cm^3
Propylparaben	1.1 g/cm^3	0.0012 cm^3
L-arginine HCL	1.42 g/cm^3	0.704 cm^3
L-arginine AKG	1.42 g/cm^3	0.352 cm^3
L-citrulline	1.3 g/cm^3	0.384 cm^3
Pomegranate juice (ellagic acid)	1.052 g/cm^3	0.475 cm^3
Grape seed oil	0.95 g/cm^3	0.52 cm^3
Black Maca	1.01 g/cm^3	0.495 cm^3
Zinc	3.2 g/cm^3	0.0312 cm^3
Honey	1.490 g/cm^3	0.0671 cm^3
Vitamin C	1.694 g/cm^3	0.295 cm^3

This volume is chosen since it provides an adequate shape for the chewable lozenges that are not too bulky. As seen in tables 2 and 3, the volume of each ingredient was calculated based on the doses selected and the density respectively. Propylparaben was specifically selected with a percent dose of 0.015%, for a volume of 8 cm^3, the volume of propylparaben equals 0.0012 cm^3. The total active ingredients volume is 3.324 cm^3, as such a volume of 4.676 cm^3 remains. The glycerin base is divided into 25% water, 15% gelatin, and 60% glycerin, calculating the amount results in 1.17 cm^3 water, 0.701 cm^3 gelatin, and 2.805 cm^3 glycerin.

3.5 Compounding Procedure

The compounding steps are done for a batch containing 5 lozenges. This number was chosen to utilize efficiently the available resources. The doses are calculated based on preparing 5.5 lozenges and that is to account for losses during the compounding. The compounding steps are also based on available protocols for gelatin-based preparations.

Some ingredients were not procured, and as such, the compounding procedure and the following demo products were made without them; Teferdin and grape seed oil are missing. As such, we recalculated the quantities of Glycerin, gelatin, and water based on the total volume per lozenge of 8 cm^3. The volume of the glycerin base recalculated was equal to 5.197 cm^3 with the API volume equal to 2.803 cm^3. As such, the distilled water volume becomes 1.3 ml per lozenge, glycerin 3.118 ml per lozenge, and gelatin 0.78 g per lozenge. We also used Black Maca hydroalcoholic extract, vitamin C tablets each of 750 mg instead of vitamin C powder, zinc sulfate powder instead of elemental zinc, and an L-arginine/L-citrulline 50/50 powder mixture. As such, we fixed the doses accordingly to obtain; 1 g of L-arginine HCL and 1 g of L-arginine AKG/L-citrulline.

3.4.1 Initial Compounding Procedure

We weighed and measured accurately each ingredient using a calibrated balance. Then crushed 4 tablets of Vitamin C using a mortar and pestle, and sieved the resulting powder using a mesh number 40 with a mesh opening size of 0.420 microns. We prepared two beakers:

Beaker A contained the hydrophilic phase which included 7.15 ml distilled water, 5.5 g L-arginine HCL, 5.5 g L-arginine AKG/L-citrulline, and 0.00726 g Propylparaben. We mixed the ingredients in the order specified above over heat at 70˚C while stirring at 750 rpm using a magnetic stirrer to obtain a homogenous solution.

We prepared in beaker B the lipophilic phase which includes 2.86 ml Black Maca liquid extract.

In a separate beaker, we put in 17.14 ml of glycerin, and while stirring at 750 rpm and heating at 70˚C, we added the mixture of beaker A (hydrophilic phase) over 5 minutes while making sure that the ingredients are well incorporated. Over a period of 3 minutes, we slowly put in 4.3 g of gelatin while stirring at 750 rpm over heat at 70˚C. Then we added the mixture of beaker B.

To the resulting mixture, we put in the remaining ingredients; 0.55 g Zinc, 2.61 ml Pomegranate juice, 0.6 g Honey, 2.75 g Vitamin C, drops of red liquid food coloring, and drops of peppermint oil.

We continued heating for 30-45 minutes at 70˚C while stirring at 750 rpm. Then removed the heat and cooled down the mixture. While cooling down, we filled the mixture in 5-6 empty molds, and refrigerated overnight or until used.

3.4.2 Adjusted Compounding Procedure

We weighed and measured accurately each ingredient using a calibrated balance. Then we crushed 4 tablets of Vitamin C using a mortar and pestle and sieved the resulting powder using a mesh number 50 with a mesh opening size of 0.297 microns.

We added to a beaker 7.15 ml distilled water and 17.14 ml glycerin. We stirred the mixture slowly at 500 rpm over heat at 50-60˚C for 5 minutes to obtain a colorless solution. Then we left the mixture to rest overheat (no mixing) to remove any bubbles formed.

Over 3 minutes, we put in 4.3 g gelatin and stirred over heat at 65-70˚C and 750rpm, and we kept stirring for 10-20 minutes.

We added the hydrophilic phase ingredients, starting by adding slowly 5.5 g L-arginine HCL, and 5.5g L-arginine AKG/L-citrulline (50/50) while stirring at 500 rpm, and heating at 50-60˚C. Then we put in 0.00726 g Propylparaben, 2.61 ml Pomegranate juice, and 2.75 g Vitamin C while stirring at 500 rpm and heating at 50-60˚C.

We added the lipophilic phase ingredient which is 2.86 ml Black Maca liquid extract while stirring at 500 rpm and heating at 50-60˚C.

Then we put in slowly the remaining ingredients, starting with 0.55 g Zinc, 0.6 g Honey, and drops of liquid red food coloring.

We continued heating for 30-45 minutes or until the mixture started solidifying while stirring slowly at 50-60˚C and 500 rpm

We added 10 drops of peppermint oil, then removed the heat and stopped stirring. We left the mixture to settle and cool down for 5 minutes. While cooling down, we lubricated lightly the empty molds with glycerin and filled the mixture in 5-6 empty molds. We refrigerated in the fridge overnight at 4-8 ˚C or until used.

3.4.3 Optimizing the Glycerin Base and Procedure

In batch "4", the total liquid content volume was taken into account instead of the volume of water alone.

The total liquid volume for 5.5 lozenges included; distilled water, strawberry essence, pomegranate juice, Black Maca liquid extract, and honey. The total liquid volume is equal to 14.014 ml. Consequently, the percentages of glycerin, gelatin, and liquid content are shifted to liquid content (39.52%), glycerin (48.34%), and gelatin (12.13%).

Adjustment: We reduced the volume of distilled water to 1.68 ml per 5.5 lozenges or 0.305 ml per lozenge. The total liquid content became equal to 8.544 ml. We adjusted the amounts of glycerin and gelatin to 20.7 ml, and 5.3 g per 5.5 lozenges respectively. Consequently, the new percentages were as follows: total liquid content (24.73%), glycerin (59.93%), and gelatin (15.34%).

In addition, we used a mesh number 60 which has a mesh opening size of 0.250 microns instead of a mesh number 50. We used a red food coloring powder instead of red liquid food coloring. Moreover, we switched to a strawberry essence flavor instead of peppermint oil.

3.6 Rotary Evaporation

3.6.1 Rational and Concept For the Use of Rotary Evaporation

Throughout compounding the different batches, two major characteristics remained constant; the bitter taste and stickiness of the compounded lozenges.

While tasting each ingredient separately, Black Maca liquid extract had a significant bitter aftertaste. The liquid extract procured is a hydro-alcoholic extract; water and ethanol. The ethanol content is suspected of causing this bitter aftertaste.

As such, two solutions arise: either the removal of ethanol from the liquid extract or procuring non-alcoholic Maca liquid extract. Since ethanol can be easily removed using heat, the first solution was used. The issue, however, is using heating temperatures

below the thermal stability point of Maca to eliminate the risk of losing macamides and other active constituents.

Consequently, using the concept of heating under reduced pressure was considered. Rotary evaporation, also known as rotavap, is a technique that includes a closed system under reduced pressure, while rotating a flask over a heating bath, to allow easier removal of the solvent by reducing the solvent's boiling point.

The Black Maca hydro-alcoholic extract procured consists of water and organic cane alcohol or ethanol as solvents and the Black Maca root extract. The ethanol content is noted by the manufacturer Herb Pharm to be 52-62%.

3.6.2 Rotary Evaporation Protocol

Our protocol was based on the BUCHI r100 protocol and operation manual for the separation of ethanol from Maca liquid hydro-alcoholic extract, and based on existing protocols for the use of rotary evaporation: (Braun Written PV, et. al. 2017) (Sepos E. 2012)

We added 12 ml of Black Maca liquid extract to a 25 ml round bottom flask. Then we attached the latter to the condenser and secured it tightly using a keck clamp. We attached the collection flask using a metal clamp to collect the extracted alcohol. And we made sure that all the tubes were tightly connected to the chiller and condenser.

We turned on the chiller, set it at 6°C, and made sure that the condenser was filled with water. The vacuum pump should be connected to the condenser, and the release valves on the top of the condenser should be closed.

According to existing rotary evaporation techniques for maca (Inoue N, et. al. 2016), we chose the heating bath temperature to be 40°C. (Zhang Y, et. al. 2006)

We filled the heating bath with purified water and lowered the round bottom flask into the hot bath. We selected the vacuum pressure (mbar) for a 40°C boiling point for ethanol to be 175 mbar according to the BUCHI r100 manual solvent list. Then we started the rotary evaporator and set the spin speed of the condenser at 5.5

After two hours we did not observe any ethanol to be extracted, as such we raised the heating bath temperature to 50°C. After the separation was completed, we stopped

the rotary evaporator. We turned off the vacuum pump and waited for the pressure to rise back to 981 mbar, which is the ambient pressure. We turned off the chiller and opened the two release valves at the top of the condenser to release the pressure. We turned off the heating bath, stopped the flask rotation, and raised the bottom flask using the lever.

When cooled down, we removed the round bottom flask and filled the Black Maca liquid extract in a graduated cylinder.

We removed the collection flask and discarded the ethanol, then cleaned the flasks and equipment used.

CHAPTER FOUR

RESULTS

4.1 Batch Development

4.1.1 Batch "1"

Batch "1" was compounded using appropriate amounts for 5.5 lozenges, and the number of lozenges obtained after preparation was 5.5 lozenges.

Figure 6 - Batch "1" including 5 chewable lozenges

Figure indicating the first batch of lozenges prepared using glycerin, gelatin, and distilled water.

One-half of a lozenge is missing since it was used for the assessment of the chewiness of the lozenges. As seen in figure 6, Batch "1" includes chewable lozenges made using the glycerin base only, without the addition of the active ingredients. It is made of 15% gelatin, 25% distilled water, and 60% glycerin, and these percentages are used as such since they are the percentages that are going to be followed for the preparation of the next batches. The lozenges obtained are opaque to white in color, they have an adequate shape that is not too bulky and not too compact, they are non-sticky when held in hand, and they have adequate firmness. They are also chewable when the lozenges were tasted. In addition, they have a shiny exterior with no physical damage or deformations, and they do not have any bubbles formation.

4.1.2 Assessment of Batch "1" for the Preparation of Batch "2"

This batch was compounded to evaluate the glycerin base and to assess if the percentages selected would give adequate lozenges shape and physical characteristics. Since the physical properties are adequate, the compounding procedure followed to prepare these lozenges can be considered adequate. As such, no adjustments are made for this batch, and batch "2" will be compounded following the glycerin base percentages selected.

4.1.3 Batch "2"

Batch "2" was compounded using the glycerin base percentages from Batch "1" with the addition of the active ingredients. Batch "2" was compounded using appropriate amounts for 5.5 lozenges, and the number of lozenges obtained after preparation was 5.5 lozenges.

Figure 7 - Batch "2" including 4 chewable lozenges

Figure representing the second batch of lozenges prepared using the initial compounding procedure steps

As observed in figure 7, the lozenges obtained were very sticky and soft when held in hand. Although no physical damage or deformations are visible in this figure, the lozenges were also very sticky to the molds which caused an increased risk of having deformed lozenges when removed, with one lozenge being damaged when removed

from the mold (not shown in the figure). Although the lozenges were sticky and soft, they still held their shape when holding them in hand. Moreover, as seen in figure 7, the lozenges lack a smooth appearance due to the presence of bubbles. In addition, the color of the lozenges is pink instead of red, and the lozenges lack the shiny appearance that was seen in Batch "1". Moreover, vitamin C coating material residues are still apparent in the lozenges, which is suspected to be due to the large mesh opening size that did not filter out all the vitamin C coating material. When tasted, the lozenges had adequate chewiness and sweetness, however, they had a strong bitter aftertaste that is accentuated by the peppermint oil flavor.

4.1.4 Adjustments Made to the Initial Compounding Procedure

The compounding procedure and steps will be adjusted in a way to prepare the glycerin base first and separately, then the other ingredients are added to the glycerin base. In addition, the mixing speed and heating temperatures will be adjusted in each step to avoid foam and bubble formation during compounding, and that is to adjust the appearance of the chewable lozenges. Moreover, a sieve with a smaller mesh-size opening will be used to reduce the presence of the vitamin C coating material.

4.1.5 Batch "3"

Batch "3" was compounded using the adjustments previously mentioned. The lozenges obtained were slightly less sticky and firmer when held in hand compared to the lozenges obtained in batch "2", however, their firmness was still not ideal.

Figure 8 - Batch "3" including 5 chewable lozenges

Figure showing the lozenges obtained after the adjustments of the initial compounding procedure

As seen in figure 8, similarly to batch "2", no physical damage or deformations are visible, however, the lozenges were still sticky to the molds which caused an increased risk of having deformed lozenges when removed. Although the lozenges were still sticky and soft, they still held their shape when holding them in hand. In addition, the lozenges had a shinier and smoother appearance compared to the lozenges from batch "2". Moreover, as observed in figure 8, the vitamin C coating materials are still apparent in the lozenges, although with a lower amount compared to batch "2". The lozenges also had a pink coloration instead of the red coloration targeted, and they are slightly sweet in taste with a strong peppermint flavor and a noticeable bitter after taste.

4.1.6 Recalculation of the Glycerin Base and Total Liquid Content

The stickiness and softness of the chewable lozenges decreased when the compounding steps were adjusted. However, to reduce the stickiness of the lozenges more effectively, the total amount of liquid per lozenge will be calculated and taken into account instead of the volume of distilled water alone. In addition, instead of using red liquid food coloring, red powder food coloring will be used. To adjust for the firmness of the lozenges, the gelatin percentage will be adjusted and recalculated when the total

liquid content is taken into account. Moreover, a sieve with a smaller mesh opening size will be chosen to eliminate the vitamin C coating residue. To adjust for the bitter after taste of the lozenges, strawberry essence will be chosen instead of peppermint oil as flavor.

4.1.7 Batch "4"

The lozenges obtained were significantly less sticky and much firmer when held in hand compared to batches "2" and "3".

As seen in figure 10, the vitamin C coating residues were eliminated using a mesh number 60 which has a mesh opening size of 0.250 microns.

Figure 9 - Batch "4" including 5 chewable lozenges

Figure showing the lozenges obtained after the adjustments of the total liquid content and glycerin base volume

The lozenges were also shiny and smooth in appearance due to the decrease in foam formation during compounding and had a much better red coloration compared to the previous batches supporting the use of red powder food coloring instead of red liquid food coloring. When tasting the lozenges, they had a sweet taste with an adequate

strawberry flavor, however, the lozenges still had some bitter after taste. However, the strawberry essence flavor was able to cover up some of the bitterness.

4.1.8 Adjustments Made to Remove the Bitter After Taste

The only remaining concern to fix is the bitter aftertaste of the lozenges. After tasting the active ingredients used, the Black Maca liquid extract had a bitter taste. It is suspected that the ethanol solvent used in the liquid extract is the causative agent. As such, it can be removed simply by evaporation.

4.1.9 Batch "5"

The lozenges obtained were nearly non-sticky, and firm when held in hand.

Figure 10 - Batch "5" including 5 chewable lozenges

Figure showing the lozenges obtained after the implementation of rotary evaporation to eliminate the ethanol from the Black Maca liquid extract

As observed in figure 10, the lozenges do not have any vitamin C coating residue, they are smooth and shiny in appearance and their color is adequate. When tasting the

lozenges, they have a sweet taste with an adequate strawberry flavor and a much less noticeable bitter after taste.

The liquid extract of Black Maca obtained after evaporation of the ethanol was observed to be slightly more viscous, darker, and more opaque compared to the liquid extract before ethanol evaporation.

Figure 11- 12 ml Black Maca liquid extract before rotary evaporation

Figure showing the initial volume of Black Maca liquid extract equals to 12 ml

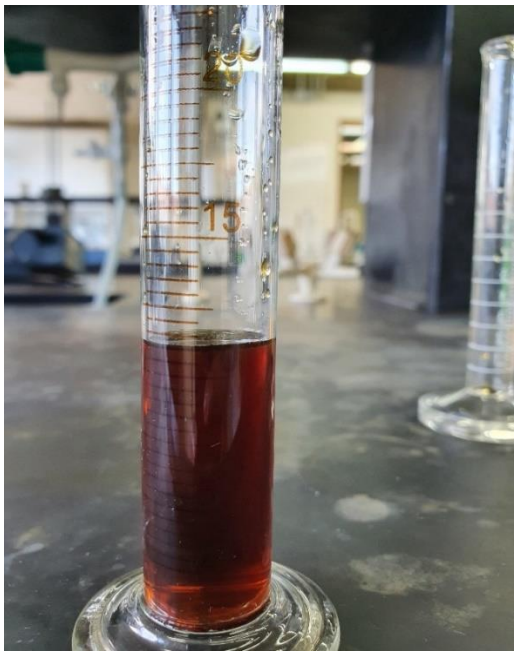
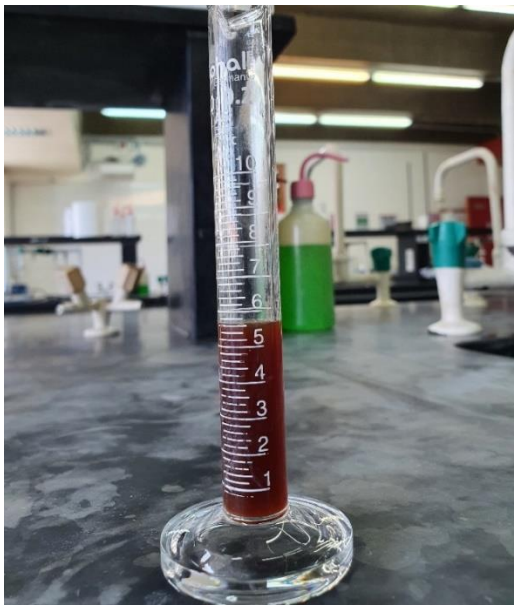


Figure 12 - 5.3 ml Black Maca liquid extract after rotary evaporation

Figure showing the volume of Black Maca liquid extract after extraction of ethanol which equals to a volume of 5.3 ml



As observed in figures 11 and 12, the rotary evaporation process efficiency can be calculated:

- Black Maca liquid extract initial volume: 12 ml
- Black Maca liquid extract final volume: 5.3 ml
- Alcohol extracted: 12-5.3 ml = 6.7 ml (55.83%)
- Efficiency percentage: 90% using the 62% upper limit. Noting that the ethanol content range is 52-62%

CHAPTER FIVE

DISCUSSION

Starting with Batch "1", the lozenges were compounded using glycerin, gelatin, and distilled water only. The rationale behind compounding this batch is to have a reference batch to be used for comparison for all the subsequent batches. More specifically, batch "1", as seen in figure 6, showed that the use of the 60% glycerin, 25% water, and 15% gelatin percentages gave a glycerin base that is suitable for the formation of the non-sticky, chewy, firm, and shiny chewable lozenges. In addition, these lozenges were observed to lack any deformations, and bubbles formed at their surface. Consequently, the glycerin base percentages used for batch "1" were used for the subsequent batches, and adjustments were made in each batch to target the physical results obtained in batch "1".

Moving on to batch "2", as seen in figure 7, the lozenges compounded lacked the smooth appearance described in batch "1", and that is suspected to be due to the formation of foam during the preparation of the lozenges. (Hartel RW, et. al. 2017) In addition, the lozenges obtained were sticky and softer compared to the lozenges obtained in batch "1", and that can be due to the high moisture content of the lozenges (Gunes R. et. al. 2022), it can also be due to a compounding procedure that is not optimized increasing the risk of breaking the glycerin base during compounding. Moreover, the lozenges held their shape even with their high stickiness level, consequently, it can be assumed that the gelatin content is adequate and provided the minimum firmness required for the lozenges to hold their shape. As such, the compounding procedure steps were adjusted to improve the physical appearance of the lozenges. As observed in batch "3", the adjustments made significantly improved the shininess and smoothness of the lozenges by reducing the formation of bubbles on both the interior and exterior areas of the lozenges. And that was due to the reduction of foam formation during compounding by adjusting the heating temperatures and mixing speed parameters at different steps of the compounding procedure. For example, during the gelatin base preparation, the heating temperatures were reduced to a range of 50-60˚C since it was observed that using higher temperatures increased the risk of inducing foam formation, similarly reducing the mixing speed of the magnetic stirrer to 500 rpm also

reduces the risk of bubbles, air entrapment, and foam formation. Consequently, the lozenges obtained using the optimized compounding procedure were observed to have a significant increase in shininess and reduction in the number of pores in the lozenges, increasing the smoothness of the lozenges and decreasing the risk of physical damage or tearing. In addition, leaving the mixture to settle without mixing also reduces the risk of foam formation, and reduces the number of bubbles formed if any were formed during compounding. Moreover, one of the main problems related to the appearance of the lozenges in batch "2" is the presence of the vitamin c tablet coating residues which are light-yellow in color. According to the product's ingredient list, the ingredient used for the coating of vitamin c tablets is hydroxypropyl methyl cellulose. It is also known as hypromellose in the pharmaceutical industry and is specifically the soluble methylcellulose ether. They are mainly used as coating agents by forming water-soluble matrix material. Moreover, it is used to control the release of active ingredients for both hydrophilic and hydrophilic medications. (Kaur G, et. al. 2018) Although hydroxypropyl methyl cellulose is water soluble (Li CL, et. al. 2005), with a solubility of 100 mg/ml at 25˚C according to the Selleckchem hypromellose data sheet, it was observed to remain visible in the lozenges with an incomplete dissolution. To remedy this issue, instead of using a sieve with mesh number 40, mesh number 50 was used for the preparation of the lozenges of batch "3".

The lozenges obtained in batch "3" had a shinier and smoother appearance compared to the lozenges of batch "2" with a considerable decrease in the stickiness and softness of the lozenges. However, compared to the lozenges obtained in batch "1", these results are far from optimal. It was assumed after batch "2"'s observation that adjusting the compounding procedures would reduce the risk of breaking the glycerin base by preparing the latter separately, while reducing the risk of foam formation as much as possible, then adding to it the active ingredients. However, this alone proved to be insufficient as observed in the lozenges from batch "3" in figure 8. Consequently, the moisture content of the lozenges was next assumed to be the major source of the lozenges' stickiness. (Allen LV. 2020) In fact, after calculating the liquid content of the lozenges in batch "3", it was observed to be 39.52% compared to the initially assumed 25% water content. The percentage calculated is much higher compared to the initially assumed percentage and was theorized to be the major reason why the lozenges had significant stickiness. The liquids taken into consideration were Black Maca liquid

extract, pomegranate juice, strawberry essence, and honey. According to the manufacturer of Black Maca liquid extract, it contains 38-48% water as a solvent and 52-62% ethanol as a second solvent, fresh pomegranate juice contains a moisture level of around 85.4% (El-Nemr SE, et. al. 1990), honey contains a moisture level of 18-24% (Singh I, Singh S. 2018). These levels are not precise due to the lack of information from the manufacturers on moisture content levels, nonetheless, these percentages indicate that all these ingredients can add to the moisture content of the chewable lozenges, and need to be taken into consideration during compounding. Although the strawberry essence (Ethyl methylphenylglycidate) should have a high purity level, it might contain water or other solvents mixed with it, or suffered from moisture entrapment during storage. All these ingredients contain water and increase consequently the moisture level of the lozenges. Moreover, instead of using the water percentages mentioned above, since not all the percentages are mentioned by the manufacturers, the water content will be assumed equal to the volume of the ingredients used and that is to assume the worst-case scenario of the water content in the ingredients. The total liquid volume for 5.5 lozenges include; 7.15 ml distilled water, 0.5 ml strawberry essence (10 drops), 2.61 ml pomegranate juice, 2.86 ml Black Maca liquid extract, and 0.894 ml honey. The total liquid volume is equal to 14.014 ml. This volume is much higher than the distilled water volume which is equal to 7.15 ml. As such taking into account the total liquid volume, the actual percentages of glycerin, gelatin, and liquid content are different from the initially targeted percentages; liquid content (39.52%), glycerin (48.34%), and gelatin (12.13%). The high liquid content can explain the stickiness observed in batches “2” and “3”. And the low gelatin percentage can explain the softness of the lozenges. As such, this can be fixed by subtracting the volumes of the liquid ingredients from the volume of water; 7.15 ml distilled water – (2.61+2.86+0.5+0.894) = 0.286 ml distilled water for 5.5 lozenges. This volume of water is used instead of the previous water volume to add up to 7.15 ml of total liquid content, which simulates the 25% liquid content.

However, using this method alone leads to a significant problem: The amount of water used for the preparation of the lozenges might be too low, and will cause early solidification of the mixture while compounding using the equipment available in the laboratory. This could be fixed by increasing the mixing speed and heating

temperatures, however, this increases the risk of damaging the active ingredients content and the glycerin base formation.

To fix the problem discussed, the amounts of distilled water, glycerin, and gelatin will have to be recalculated to maintain an adequate glycerin base volume and adequate percentages. Instead of subtracting all active ingredients' liquid volumes from the distilled water volume, only the volumes of Black Maca liquid extract and Pomegranate juice will be subtracted since they have densities and textures closer to water compared to honey and strawberry essence (Ethyl methylphenylglycidate has a density of 1.1 g/cm3). (National Center for Biotechnology Information. 2022)

More specifically Black Maca liquid extract and pomegranate juice volumes were subtracted from the distilled water volume to obtain a distilled water volume of 1.68 ml (7.15 – (2.61+2.86)) for 5.5 lozenges or 0.305 ml per one lozenge. The total liquid volume becomes equal to 8.544 ml. This volume allows the determination of the glycerin and gelatin quantities to use by assuming that 8.544 ml is equal to 25% of the total glycerin base for 5.5 lozenges. More specifically it simulates the 25% distilled water content previously assumed. Assuming that the percentages of glycerin and gelatin are respectively 60% and 15%, the quantities obtained for glycerin and gelatin are respectively 20.7 ml and 5.3 g for 5.5 lozenges, or 3.763 ml and 0.964 g per lozenge. More specifically the percentages were respectively 24.73%, 59.93%, and 15.34%. The total liquid content percentage remained slightly lower than 25% on purpose to reduce the stickiness of the lozenges as much as possible without affecting the other physical properties of the lozenges. As for the firmness of the lozenges, the ones obtained in batch “3” were considerably softer in comparison to the ones in batch “1”. This proves that the initially targeted percentages of the glycerin base should ideally offer similar physical characteristics when adding the active ingredients. However, as mentioned previously when the total liquid content was assumed instead of the distilled water content alone, the percentages shifted in favor of the total liquid content, reducing the gelatin percentage to 12.13% which is a significantly lower percentage compared to the initially targeted 15%. This low gelatin percentage was observed to reduce the firmness of the lozenges from batches “2” and “3”. By adjusting the total liquid content and reducing the volume of distilled water, the new gelatin percentage was recalculated to obtain a 15.34%, this percentage is slightly bigger than the targeted 15%, and that is to increase as much as possible the firmness of the lozenges without affecting the other

physical properties of the lozenges. It was also observed that the chewiness of the lozenges was proportionate to the softness of the lozenges. It was theorized that the higher the moisture content is, the softer the lozenges are, and the more chewable they are. Although the chewiness of the lozenges was higher in both batches "2" and "3" compared to batch "1", this difference was only slightly noticeable. This proves that higher moisture content can increase the chewiness of the lozenges,

In addition, the lozenges from batch "3" still had Vitamin C coating residue that was still significantly visible. As such, a mesh number 60 was used for the development of batch "4".

Moreover, it was observed that using multiple drops of a liquid red food coloring agent gave a red-pink coloration instead of the bright red color targeted even when using the recommended amount of drops according to the manufacturer of the liquid red food coloring. It was suspected that adjusting the compounding procedure would also increase the incorporation of the red coloration into the mixture by reducing foam formation and air entrapment. However, it was shown in batch "3" (figure 8) that the coloration obtained did not differ from the coloration obtained in batch "2". In addition, due to the concern over using multiple liquid ingredients that could increase the moisture content of the lozenges, the red liquid food coloring was switched with a red powder food coloring to increase the intensity of the red coloration while reducing the number of liquid ingredients used to prepare the chewable lozenges.

Moving on to the taste of the lozenges in batch "3", it was observed that although the taste of the lozenges was refreshing with a strong mint flavor, the lozenges suffered from a bitter after taste. It was theorized that the use of peppermint oil is a major causative agent that can accentuate the bitter flavor of the lozenges. Consequently, for the subsequent batches, the peppermint oil was switched with strawberry essence as a flavoring agent.

All these adjustments led to the compounding of batch "4", as seen in figure 9. The lozenges obtained were much less sticky and much more firm compared to the lozenges from batches "2" and "3". The coating residues from vitamin C tablets were non-visible, proving that using a mesh number 60 is suitable for subsequent batches preparation. The shiny and smooth appearance of the lozenges proved that the moisture content of the lozenges is an essential factor and directly linked to the physical

appearance and stickiness of the lozenges. In addition, the use of strawberry essence flavor proved to be more effective than peppermint oil in offering better-tasting lozenges, however, it was unable to completely cover up the bitter after taste. Another ingredient was suspected to cause the bitter after taste of the lozenges, namely the ethanol content of the Black Maca liquid extract. In the latter, ethanol is used as a solvent with a range of 52-62% according to its manufacturer. By removing the ethanol content, it is assumed that the bitter taste of the lozenges would be eliminated.

When the rotary evaporation technique was used, the ethanol volume extracted was calculated to be 6.7 ml or 55.83% of the total volume of the Black Maca liquid extract. The efficiency of this technique was calculated to be 90%, proving that the use of rotary evaporation to remove ethanol is an efficient technique. Consequently, this technique can be used for the extraction of ethanol for the compounding of future batches.

Moving on to batch "5", the lozenges obtained showed overall the best properties targeted for the preparation of the chewable lozenges, as observed in figure 10. Particularly in terms of stickiness, chewiness, firmness, taste, color, and appearance. The lozenges had a strong strawberry flavor with a slightly noticeable bitter after taste proving that the removal of ethanol from the Black Maca liquid extract is directly proportional to the reduction of the lozenges' bitter taste. However, the remaining bitter taste can be due to the residual ethanol that was not extracted from the Black Maca liquid extract.

In addition, the removal of ethanol from the Black Maca liquid extract using the rotary evaporation technique significantly lowered the stickiness levels when compared to batch "4". Batch "5" lozenges had a slightly noticeable stickiness when held in hand. This proves that eliminating solvents other than water might also reduce the stickiness of the lozenges. Moreover, during the rotary evaporation procedure, the heating bath temperature was changed from 40˚C to 50˚C since no ethanol was being extracted, and after raising the heating bath temperature, ethanol was evaporated and extracted. As such, the switch in temperature was used instead of 40˚C for subsequent batches preparations.

5.1 Limitations

It is important to keep in mind that the development of the lozenges, the equipment used, and the compounding procedure developed was only used for a "laboratory scale" demo product development, and do not represent the actual conditions, equipment, and procedure that will be used when producing the lozenges in an up-scale or industrial level. In addition, it is important to note that the compounding procedure was optimized for the development of 5 lozenges for each batch, taking into account that the doses selected were for 5.5 lozenges to account for losses during preparation. Nonetheless, the findings and the product developed are in line with the physical properties targeted and are a good representation of a product that could be developed at an up-scale level.

The equipment used during the compounding of the lozenges is, as mentioned earlier, laboratory-scale equipment that is used to compound a small-scaled quantity of chewable lozenges. Due to the limitations of the equipment used, specifically the magnetic stirrer, the compounding steps and conditions used for the development of the lozenges such as the heating temperatures were adjusted. For instance, a recurrent problem encountered during the compounding of the batches was the rapid solidification of the mixture due to the weak force of the magnetic stirrer even at higher mixing speed. This problem was resolved on-site by constantly adjusting the heating temperatures and mixing speed to eliminate the risk of early solidification. However, this problem could happen at random moments during the compounding procedure and consequently lead to non-adequate batches if not resolved instantly. The compounding procedure, steps, and conditions could still be improved, optimized, and refined to obtain more adequate chewable lozenges if the equipment used has a stronger mechanical mixing force or has another more precise and robust stirring mechanism such as Overhead stirrers.

Another limitation includes the remaining stickiness of the lozenges. During the development of the chewable lozenges, the stickiness of the lozenges was resolved by adjusting the moisture level by taking into account the liquid content, which includes distilled water used in addition to the water content of the liquid active ingredients, instead of only taking into account the distilled water content. Although the chewable lozenges of batch "5" reach a nearly non-sticky state, it is possible to assume that the stickiness of the lozenges is not attributable to the water and moisture content only. Interestingly, the difference between batches "4" and "5" demonstrated that eliminating

the ethanol content from the Black Maca liquid extract noticeably reduced the stickiness of the lozenges. As such, the assessment of the presence of other solvents, such as ethanol, need to be further studied.

In addition, for the development of the demo product, some of the active ingredients were used in their liquid form instead of using active ingredients in their powder form. This resulted in calculating the moisture content of all liquid ingredients. For the development of future batches or even at an up-scale or industrial level, it would be more advantageous to use active ingredients in their powder form instead of liquid form and limit the use of liquid ingredients as much as possible to reduce the stickiness of the chewable lozenges, and to be able to optimize the compounding procedure more efficiently.

5.2 Implications

This work has implications, and it serves as a guide for potential future research and product development. The selection and incorporation of multiple active ingredients that cover different mechanisms of action and target multiple uses for different population groups in a single dosage form proved to be feasible. It is important to note that this work is innovative in this aspect. This demo product paves the way for the development of an industrial-grade product that covers multiple indications and treats multiple diseases more specifically erectile dysfunction and post-menopausal symptoms.

In addition, throughout the development of the chewable lozenges, some properties from the active constituents were shown to affect the physical properties of the compounded lozenges. For instance, the Black Maca liquid extract was shown to be one of the causes leading to two important problems: stickiness and bitter taste. One was due to the water content leading to increased moisture content, and the second was due to the presence of ethanol used as a solvent in the liquid extract. These findings although simple, might be of importance when the formulation and compounding procedures will be optimized during up-scale production of the chewable lozenges.

This work also reveals the results of the initial steps in the development of chewable lozenges. As stated previously this work is made for the development of laboratory-scaled chewable lozenges, however, the results obtained confirm that the compounding procedures and steps used and the parameters taken into account are valid

and can be used for the development of industrial-scaled chewable lozenges. For instance, the steps followed and the compounding procedure optimizations were done to reduce the foam formation during preparation were valid and reduced air entrapment leading to shinier chewable lozenges. With further research and development, it is possible to refine the compounding procedure and use its main elements and steps for the development of industrial-grade chewable lozenges with batches of higher quantity and more importantly quality.

CHAPTER SIX

CONCLUSION

6.1 Conclusion

This work illustrates the development and production of chewable lozenges used for the treatment of erectile dysfunction. The selection of the natural active ingredients was based on their specific mechanisms of action and benefits towards males and females. The formulation was based on two major parts; the combination of the active ingredients and the glycerin base, which itself follows proportions of 60%, 15%, and 25% for glycerin, gelatin, and water respectively. The compounding procedure was based on preparing the glycerin base separately and then adding to it the aqueous and non-aqueous phases. Subsequent adjustments were made to adjust the physical parameters of the lozenges; namely the stickiness, firmness, taste, color, and appearance of the lozenges. The compounding of the different batches led to the formation of successful lozenges with adequate physical properties. The selection of the active ingredients form, and the compounding procedure steps and parameters all affect and impact significantly the characteristics of the lozenges causing them to have inadequate properties such as being too sticky, too firm, or too soft. It is important to adjust the proportions of the ingredients and excipients to obtain adequate lozenges. Additionally, these characteristics can be further improved and adjusted by optimizing the glycerin base percentages, specifically the moisture and gelatin content of the lozenges.

6.2 Perspectives

As a follow-up to this work, observational trials could be used to assess the efficacy and safety of chewable lozenges. For the efficacy outcomes, the International Index of Erectile Function (IIEF) questionnaire can be used for males as described in clinical trials that used arginine as a natural treatment for the treatment of erectile dysfunction (Rhim HC, et. al. 2019). The Greene Climacteric Scale can be used for females; according to some studies, this test was used to measure the efficacy of Maca in treating menopausal symptoms in females. (Shin B, et. al. 2010) It is expected that

males taking the chewable lozenges would have an increase in the erectile domain scores of the IIEF questionnaire, whereas females would have a reduction in the Greene Climacteric Scale scores, specifically in the area of psychological symptoms. The safety outcomes could be assessed by simple patient self-observation and reporting. It is expected that the chewable lozenges would have a low incidence of side effects. These results are important since they would be able to provide data that confirms that the doses used per lozenge and the frequency used are adequate to obtain statistically significant results. They would also confirm that the use of multiple active ingredients that cover multiple mechanisms of action work well in combination with each other. This observational trial will form a basis from which further clinical trials could be initiated to assess more accurately the extent of the lozenges' efficacy compared to currently used treatments similar to their safety.

Other studies should also be done to ensure and confirm the active ingredients' doses and frequency selection. For instance, further studies about the synergistic effects of the combination used in this work should be studied on different cells in an in-vitro setting to evaluate and better reflect how the different mechanisms of action would work in-vivo. (Yang Y, et. al. 2014) In addition, toxicological studies, where the combination of the active ingredients' effect is studied in an in-vitro setting on either human or animal cells. (Ibrahim R, et. al. 2021) Another study that should be covered is stability; where the lozenges are inserted into different conditions for a specific duration of time. For instance, the most commonly used stability chambers include the long-term chamber which includes normal temperature and humidity conditions (30˚C/65% humidity) for 3, 6, 9, 12, 18, 24, and 36 months. And the accelerated chamber includes harsh temperature and humidity conditions (45˚C/75% humidity) for 3, and 6 months. (Arunachalam A, Shankar M.2017) Another stability study that could prove important for the evaluation of the lozenges is photostability. Other tests that could be done in vitro also include; dissolution, disintegration, pH, assay, and moisture content. And physical tests that could be done are thickness, diameter, and weight uniformity. (Rao M, et. al. 2018) It is imperative to note that the selection of the administration frequency of the lozenges was used to build a concept or model product with the rational backbone of the dose selection. To be able to confirm the doses that will be used with the administration frequency, all these studies should be covered.

The use of active ingredients in the form of nanoparticles would be an interesting additional innovation in the development of the chewable lozenges to improve the delivery of some of the active ingredients and to increase the drug loading and entrapment levels, consequently increasing the bioavailability of the active constituents. The use of nanoparticles has been discussed as a tool to aid in controlling the sustained release of active constituents. (Singh M. 2014) One of the main concerns during the formulation of the chewable lozenges and the selection of the active ingredients was the bioavailability of these ingredients. Although some combinations were selected based on the advantage of increasing bioavailability among other benefits, the use of nanoparticles can help in the development of chewable lozenges whose active ingredients have a longer duration of action while reducing the daily intake frequency. More specifically, nanoparticles of herbal ingredients have been used in formulations as anti-cancer; for instance, Berberine-loaded nanoparticles for the treatment of nasopharyngeal carcinoma by inducing apoptosis to the epithelioid cell line CNE-1. (Javed Iqbal M, et. al. 2017) Other herbal ingredients have been used in oral formulations to offer hepatoprotective effects, cream formulations such as sunscreens and UV-filters, and hair products to increase the strength of hair follicles. The combination of the chewable lozenge formulation with nanoparticles can theoretically increase the bioavailability of the active ingredients by offering a double protective layer; the first being the glycerin base, and the second being the nanoparticle layer which could be a liposome layer. (Vani M, et. al. 2020)

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