INVESTIGATION OF THE EFFECTS OF COMMONLY USED TOBACCO AND ITS VARIETIES ON HUMAN PHYSIOLOGY SYSTEM

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Thesis advisor
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"I declare that all the information within this thesis has been gathered and presented in accordance with academic regulations and ethical principles and I have according to the requirements of these regulations and principles cited all those which do not originate in this work as well."

Abdarazag ALMGADMI

ÖZET

Yüksek Lisans Tezi

YAYGIN OLARAK KULLANILAN TÜTÜN VE YAPISAL ÇEŞİTLERİNİN İNSAN FİZYOLOJİSİ SİSTEMİNE ETKİLERİNİN İNCELENMESİ

Abdarazag ALMGADMI

Karabük Üniversitesi
Lisansüstü Eğitim Enstitüsü
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Günümüzde, bir infertilite kliniğini ziyaret eden insanların toplam sayısı artış göstermekte ve sigara içmek erkek kısırlığına yol açan en önemli yan etkilerden biri olarak kabul edilir. Bu alanda pek çok çalışma, sigara dumanı içeriğinin sperm niteliğini, sperm plazmasını ve diğer çeşitli üreme faktörlerini olumsuz etkilediğini bildirmiştir. Bununla birlikte, sigara içmenin erkek üreme üzerindeki gerçek etkisi belirsizdir. Sigaranın semen parametreleri üzerindeki etkisi, sigaranın oksidatif strese (OS) yol açan reaktif oksijen türlerinin varlığını arttırdığı kesin biyolojik sonucuna ile ilişkilidir.

Çalışma sistemi, fizibilite ve morfoloji gibi sperm parametreleri üzerinde tahrip edici etkilere sahiptir ve erkek doğurganlığını azaltan sperm fonksiyonunu zayıflatıcı bir rol oynamaktadır. Ancak, tüm bu çalışmalar aynı sonuçlara ulaşmamıştır. Bu çalışmada, sigara ve erkek fertilitesi arasındaki korelasyon vurgulanmakta ve sigara içmeyen

bireylerin tütün kullanımı üzerine erkek infertilitesi üzerine etkisini incelenecektir. SEM, XRD ve FTIR gibi uzman analizleri de dahil olmak üzere, sigarayı erkek kısırlığına bağlayan kanıtlar vurgulanacak ve farklı tütün türlerinin klinik deneysel etkileri tartışılacaktır. Çalışmanın sonuçlarının gösterdiği gibi, tütünlerin tüm yapıları oda sıcaklıklarında benzer mikroyapısal dağılımlar göstermektedir. T15 tütün malzemelerinin mikroyapısı diğer tütünlerden farklı olarak kristal bir yapıya sahiptir.

Anahtar Sözcükler : Kısırlık, Sigara, Spermatogenez, SEM, XRD, FTIR, Spermatozoa.

Bilim Kodu : 92504

ABSTRACT

M. Sc. Thesis

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The number of couples attending an infertility clinic is rising, and smoking is one of the most common causes of male infertility, according to researchers. Cigarette smoke has been shown to adversely impact sperm standards, sperm plasma, and other reproductive parameters in many investigations. The precise impact of smoking on male fertility is unknown, though. There is a strong scientific conclusion that smoking increases the presence of reactive oxygen species, resulting in oxidative stress, which has an impact on semen parameters (OS).

Sperm characteristics including feasibility and morphology are harmed by the operating system, which also decreases sperm function. However, the results of the many research were not all in agreement. This study examines the link between tobacco use and male fertility, as well as the impact of non-smoking alternatives on human physiology. Evidence connecting smoking to male infertility, including

expertmental analysis such as SEM, XRD and FTIR, will be highlighted, and clinical experimental implications of different tobacco kinds will be discussed. As the results of the study shows all the structures of the tobaccos show the similar microstrucal distributions at room temperatures. The miscrostructure of the T15 tobacco materials has a crystalline structures which is different from other tobaccos.

Key Word **:** Infertility, Smoking, Spermatogenesis, SEM, XRD, FTIR, Spermatozoa.

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CONTENTS

Page

Page

Page

LIST OF FIGURES

Page

LIST OF TABLES

Page

PART 1

INTRODUCTION

The continuous increase in the rate of infertility has raised serious concerns about human reproduction in recent years. Depending on the incidence of infertility, the budget spent on assisted reproductive techniques is increasing day by day. It is known that there are many factors that cause a decrease in sperm quality. Depending on the developing industrialization, the needs of the increasing population, the needs of changing lifestyles, the number of toxic substances exposed is increasing day by day and each toxic substance (alcohol, cigarette, pesticides, insecticides, heavy metals, radiation, cosmetics, cleaning materials, pharmaceutical products, agents encountered in occupational exposures and all xenobiotics) affect all steps of the ecological system. The presence of dangerous agents is one of the important factors that cause malfunctions in the male reproductive system.

Lifestyle factors such as increasing levels of environmental pollutants such as smoking, alcohol consumption, and drug use are also suggested as the most important factors responsible for the decrease in semen quality. Sperms are highly sensitive indicators of environmental, occupational and vital toxic exposure and, accordingly, toxic effects on the individual manifest themselves as hormonal disorders. Couples going to infertility clinics are more numerous than ever before, and smoking is one of the most common causes of male infertility, according to the study. Smoking has been linked to lower sperm counts, sperm plasma, and other reproductive issues, according to research. However, it's unclear if smoking has an impact on male fertility. As a result of the well-established scientific conclusion that smoking increases reactive oxygen species and oxidative stress, the impact of smoking on semen parameters must be determined (OS).

In terms of sperm characteristics, such as feasibility and morphology, the operating system has a catastrophic impact. It also decreases sperm function, which lowers male fertility. However, the findings varied from study to study. This study examines the link between smoking and male fertility and the impact of non-smoking techniques on human physiology when it comes to tobacco usage. Additionally, new genetic and genetic data connecting smoking to male infertility will be discussed, as well as the clinical experimental implications of various tobacco kinds.

First chapter of the thesis focuses on the problem that infertility has raised serious concerns and the effect of smoking on human health. Second chapter of the thesis reviews literature to explain the general structure and content of tobacco, nicotine addiction, infertility, diagnostic tests in male infertility and tobacco addiction. Then, material and method of the experiment is explained in the third chapter of the thesis. Fourth chapter of the thesis informs about the data and analysis these data. The fifth chapter provides results of analysing the data with discussion.

PART 2

THEORETICAL CONCEPTS AND BACKGROUND

2.1. TOBACCO

The World Health Organization (WHO) reports tobacco use as one of the main risk factors for global mortality. More than 7 million people die prematurely each year as a result of smoking, according to WHO statistics from 2017.

More than 7.1 million people died as a result of tobacco smoking in the year 2016 alone (5.1 million in men, 2.0 million in women). Smoking accounted for 6.3 million of these fatalities, with passive smoking accounting for the remaining 884 thousand. With the decrease in cigarette consumption since the 1960s in developed countries, the rate of deaths related to smoking has decreased, but it is expected that smoking-related deaths will increase with the increase of smoking in developing countries since the 1980s. More than 1 billion adults over the age of 15 smoke tobacco worldwide. 82% of these people (768 million) are male. There are far fewer women who smoke than men. 35% of the male population and 6% of the female population smoke [1].

The Global Youth Tobacco Survey (GYTS) is a study conducted with the participation of 16,000 students aged 13-15 across the country. In the GYTS held in 2003, information was collected on the frequency of tobacco use among young people, their knowledge levels and attitudes towards tobacco use. Three out of every 10 students (26.3%) stated that they had smoked in any period of their life. Tobacco smoking is higher in males (31.7%) than females (19.7%), and 30.7% of smokers started smoking before the age of 10 (34.9% for males, 23.7% for female students). 6.9% of the students are still smoking. Current smoking is higher in males (9.4%) than females (3.5%). (4) According to the Global Youth Tobacco Survey (GYTS) 2017 data; 17.9% of the students (23.2% of the boys, 12.1% of the girls) still use a tobacco or tobacco product.

7.7% of the students (9.9% of the boys and 5.3% of the girls) are still smoking. Those who smoked at least once were 28.0%, while those who used hookah at least once were 24.6%. While the use of tobacco and tobacco products was 8.4% in 2009 GYTS, it increased to 10.4% in 2012 GYTS and decreased to 7.7% in 2017 GYTS [2]. Unfortunately, the period when the smoking rate is highest in young adult males is observed in the reproductive period. When it comes to males, smoking has been linked to infertility because of reduced sperm concentrations, poorer sperm motility, as well as a smaller proportion of morphologically normal sperm. Cigarette smoking has been linked to reduced sperm quality and mechanisms that affect testosterone metabolism and spermatogenesis have been proposed.

2.1.1. General Structure and Content of Tobacco

There are four types of tobacco that can be consumed in four ways: sun-dried, fire-dried, ventilated and dried indoors from time to time, and dried with heat. Two percent of foreign origin tobacco, namely tobacco and Burley, are produced in our country. Tumbak tobacco; although nicotine content is almost the highest, it is the most commonly used type of tobacco in hookah making. Tobacco is a plant belonging to the Nicotiana genus from the Solanaceae family. It is usually grown annually. The Nicotiana genus includes more than seventy species, but only Nicotiana tobacco and Brazilan tobacco are varieties grown as delightful plants. The properties of chemical components in tobacco varieties differ according to production conditions and tobacco genetics. Coarse fibers such as tobacco leaf ash, cellulose and lignin, pentosanes, ether soluble compounds, polyphenol, phenolic acids and derivatives, organic acids soluble in ether such as oxalic acid, citric acid and malic acid, proteins, amino acids, amides, ammonia and nitrate compounds contains water-soluble nitrogen compounds such as alkaloids and dynamic carbohydrates such as sugar, starch and dextrin. When the tobacco obtained by various methods is burned; It turns into smoke form, containing more than four thousand harmful substances with gas phase and particle part. These harmful substances; It is found in the main stream containing harmful substances produced by tobacco, which is the main product of the person, and in the side stream containing the combustion products of the surrounding substances. For example, tar,

which has serious biological effects; there is a large amount in the side stream. Some harmful substances in cigarette smoke are shown in Table 2.1 [3].

Table 2.1. Some harmful substances in cigarette smoke [3].

Gas Phase	**Main Effect**	**Particle Section**	**Main Effect**
Carbon monoxide	It prevents oxygen from binding to hemoglobin	Nicotine	Dose-dependent stimulant or parasympathetic N depressor on cholinergic receptors
Nitrogen oxides	Irritant, proinflammatory, ciliotoxic	Tar	Mutagenic / carcinogenic
Aldehydes	Irritant, proinflammatory, ciliotoxic	Phenol	Irritant, Mutagenic / carcinogenic
Ammonia	Irritant, proinflammatory, ciliotoxic	Cresol	Irritant, Mutagenic / carcinogenic
Acrolein		Aromatic hydrocarbons	Mutagenic / carcinogenic
Hydrocyanic Acid	Irritant, proinflammatory, ciliotoxic	b-Naphthylamine	Mutagenic / carcinogenic
Nitrosamines	Mutagenic / carcinogenic	Catechol	Mutagenic / carcinogenic
Hydrazine	Mutagenic / carcinogenic	Benzopyrene	Mutagenic / carcinogenic
Vinyl Chloride	Mutagenic / carcinogenic	Indole	Tumor acceleration
		Carbazole	Tumor acceleration

2.1.2. History of Tobacco Use

Spanish seafarer Cristopher Columbus, who set foot on the American island of San Salvador on October 12, 1492, came across many plant species that were not found in Europe with his friends on a cruise. The most common was tobacco, which the Indians consume by chewing or burning in their mouths, called "tobacco," a medicinal bitter herb used in the treatment of diseases. Tobacco, which emerged about 8 thousand years ago, was first used in South America by Maya and Aztec natives. Maya and Aztec Priests using tobacco as incense in religious ceremonies and rituals; Because of the pleasurable effect of tobacco, they started to use it outside of rituals. Tobacco gradually spread among the population and later in Central and North America [4].

Tobacco reaching Europe is difficult to obtain, so it was valuable and could only be used by wealthy people. Tobacco, which was presented to the queen with headache by Jean Nicot de Villemain, the ambassador of Portugal to Portugal, eliminated the headache of the queen and tobacco was named "queen herb" in those years. The trade of tobacco, which has spread so much and has increased consumption demand, was first made in the State of Virginia in 1612. And in the following years, tobacco trade became the most important source of income. Tobacco, which was first used in religious ceremonies and rituals of primitive tribes, in the treatment of illnesses and later in society as a pleasurable substance; The cigarette obtained by wrapping the shredded tobacco in paper was first used in Brazil in the 18th century. However, the cigarette in the form used today was invented in France in 1843. In 1881, when John Bonsack took the patent of the first cigarette rolling machine in the USA, about 120,000 cigarettes a day began to be produced. And this was revolutionary in cigarette production. As such, cigarette consumption increased significantly as a result of reduced costs as a result of mass production, easier distribution and transportation. In 1895, 66 million cigarettes were sold in Canada [5]. Geographical discoveries, the most important milestone of tobacco trade; caused the presence of the east to pass to the west. With the opening of the Suez Canal in 1869, trade turned from west to east this time, and the Mediterranean, North Africa and the Middle East regained their economic and political power. Jews in the 15th century. With the positioning of the

Ottoman Empire in important trade settlements such as Thessaloniki, Istanbul, Izmir and Samsun, tobacco trade gained momentum by blending.

2.1.3. Types of Tobacco Usage

The term that appears in minds when talking about "tobacco products"; These are products that are used by burning and smoking such as cigarettes (wrapped and manufactured cigarettes), cigars, pipes and hookahs. But the most widely used tobacco products for the finished cigarette; The findings obtained as a result of the studies carried out are mainly related to smoking. In addition, they are packaged and made ready for different usage purposes by going through various technological processes [6].

- Direct smokeless use of tobacco: Chewing, snuff, use in nicotine preparations (nicotine water, gum, lollipop, band, tablet, granules, spray, electronic cigarette).
- Usage types of smoke caused by burning tobacco: Usage in the form of incense, cigarette, cigar, pipe, hookah.
- Industrial use of tobacco for other purposes: It is used as oil obtained from its seed, as a fertilizer, in the cellulose industry to obtain paper, as an insecticide. Also; Nicotine is obtained from its leaves, essence and cologne from its flowers, and potassium carbonate from its ash.

Although tobacco has such a wide area of use, it is used as the most enjoyable in the world. And the fixture of the tobacco economy is cigarettes.

2.1.3.1. Cigarette

Cigarettes ready for consumption in the form of special cylinders obtained by placing shredded tobacco on special papers; Papyrus is collected in three groups as unfiltered and filtered. Papyrus types; A cardboard mouthpiece was placed on one end and chopped tobacco was placed on the remaining part. And its size reaches approximately

75 mm. In filtered cigarettes; It consists of a short filter rod with cellulose acetate. Unfiltered cigarettes also contain shredded tobacco completely inside the special roll.

2.1.3.2. Electronic Cigarette

Electronic cigarette; It consists of mouthpiece, cartridge and battery part. Working principle; It is based on the heating and gasification of the liquid nicotine in the cartridge upon inhalation. The cartridge contains flavoring propylene glycol along with nicotine. There are varieties such as disposable e cigarettes, refillable e cigarettes and tank-type e cigarettes [7].

2.1.3.3. JUUL

JUUL, which was first launched in the USA in 2015 and has a very low nicotine ratio; It was produced to prevent the rapid spread of electronic cigarettes among adolescents. However, since it is an expensive product, it could only be used by the wealthy and the expected benefit could not be achieved.

2.1.3.4. Heat-Not-Burn Cigarette (IQOS)

IQOS (I Quit Ordinary Smoking), which was first introduced to the market in Japan and Italy in 2014; As of July 2017, it is on the market in 30 countries. IQOS; It consists of a thin and small cigarette containing tobacco and a device that the cigarette is placed in and used by heating. The manufacturer claimed that tobacco is much safer than other tobacco products in the market, since it is not burned but heated to approximately 300-350 degrees and does not smoke. However, many studies have been published showing that there is pyrolysis and 'thermogenic degradation' in tobacco as a result of incomplete combustion reaction, and additional carcinogenic and irritant substances such as acetaldehyde and benzopyrene, which is a polycyclic aromatic hydrocarbon, have emerged.

2.1.3.5. Hookah Tobacco Product

Hookah; It is based on inhaling burning tobacco smoke through cold water. There are two types of natural hookah tobacco and flavored hookah tobacco. While traditional tobacco for hookah is used in natural hookah tobacco; flavored hookah tobacco contains 20% -30% tobacco and 70% -80% aromatic substances and other chemicals. Since hookah can be used repeatedly, there is a risk of tuberculosis, herpes simplex, viral infections, and eczema [8].

2.1.3.6. Cigar and Sigarillo

Cigars classified as sigarillo, panatella, cigar and corona according to their size; It is mostly produced in Marmara, Istanbul and Ankara in our country. After blending, machine production, wrapping of the outer wrapper, shaping, cutting, baking and quality control, the packaged cigars are made ready for consumption.

2.1.3.7. Pipe

Pipe; It differs in that it has a wider chopping width depending on the type of tobacco it contains, as well as its variety and quality. Tobaccos obtained by saucing and flavoring Virginia, Kentucky, Burley and Maryland tobaccos are used. Snuff used by nasal inhalation; It is formed by blending powdered high nicotine tobacco with aromatic substances such as bergamot, clove and cinnamon.

2.1.3.8. Chewing Tobacco

It is made by saucing and flavoring Virginia, Kentucky and Burley tobaccos and then thickly minced and pressed [9].

2.1.4. Nicotine

Nicotine is dopamine from the nucleus accumbens in the central nervous system, which makes the individual feel good; Locus coeruleus, on the other hand, acts by

causing the release of norepinephrine, which is responsible for withdrawal symptoms such as restlessness and seeking behavior [4].

Natural alkaloid nicotine is found in tobacco and Duboisia hopwoodii, among other nightshade plants, and is commonly used as a stimulant and anxiolytic. As a pharmaceutical medicine, it is used to help people quit smoking by reducing the effects of nicotine withdrawal. When it comes to most of the receptors (nAChRs), nicotine operates as an agonist. When it comes to two nAChR subunits, however, it acts as an antagonist. About 0.6–3.0 percent of tobacco's dry weight is nicotinic. The culinary family Solanaceae, which includes potatoes, tomatoes, and eggplants, contains nicotine at ppb-concentrations, however sources dispute on whether this has any biological importance for human consumers. Neonicotinoids, such as imidacloprid, are some of the most effective and frequently used insecticides, and nicotine was employed as an insecticide in the past [6].

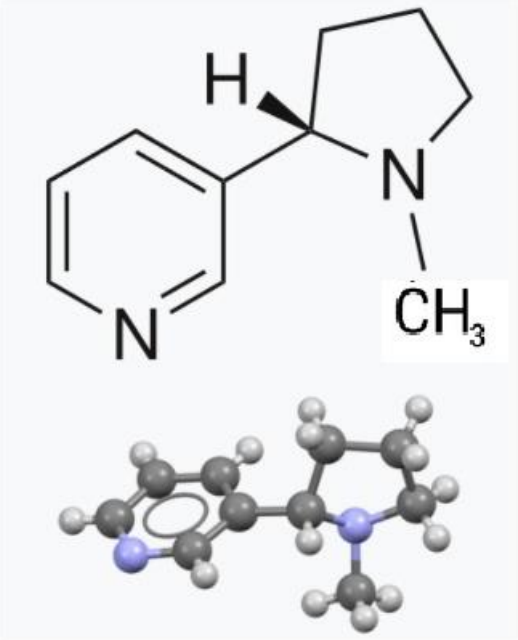

Figure 2.1. Chemical structure of nicotine.

2.1.5. Nicotine Addiction

The constant repetition of a behavior is called a habit. If a behavior called habit causes an individual to experience negativity in his social relationships, business life or

prevents him from spending time for other activities in his daily life, if abandonment of the behavior causes mental or physical problems, the habit turns into addiction. Most of the substance addicts start using the substance with the assumption that they can control the substance use they want at any time and that they will not be addicted, but become addicted without realizing it. Depending on the type and purity of the substance used, and the physical and mental state of the person using it, the course of addiction may change. Due to the intense effect of some addictive substances, even one use can pose a risk. In order to be diagnosed with a drug addict, at least three of the following criteria must be found in a 12-month period in a way that causes significant discomfort. These criteria can be listed as follows [10]:

- Taking the substance for longer and at higher doses than intended.
- To have developed a tolerance to the addicted substance within the last year (the effect of the substance decreases when the substance is taken in the same amount continuously).
- When the substance is not taken, showing withdrawal symptoms and continuing to take the addicted substance or similar in order to get rid of these symptoms,
- To be in constant effort to get rid of or control substance use, to prevent substance use or to fail in attempts to reduce the substance taken.
- To acquire the drug, use it, and recover from its effects, it will take an increasing amount of time.
- Reducing or quitting social, professional, or recreational activities as a result of drug or alcohol addiction.
- Using the drug despite the fact that it is causing bodily or psychological harm.

Tobacco Use Disorder is classified as follows under the heading of "Tobacco-Related Disorders" in the diagnostic criteria of the American Psychiatric Association Diagnostic and Statistical Manual of Mental Disorders (DSM–5): A problematic pattern of tobacco uses over a period of twelve months, manifested by at least two of the following, leading to clinically significant distress or reduced functionality [11]:

- Often tobacco is taken to a greater extent or longer than desired.

- Tobacco usage is something that many people want to quit, yet attempts to do so have been fruitless.
- Much time is spent on actions that are required to acquire tobacco, use tobacco, or relieve oneself of the effects of tobacco.
- Compulsiveness to smoke or use tobacco is a strong urge.
- Inability to meet the primary responsibilities of one's position at work, school, or home due to the use of tobacco on a regular basis.
- Using tobacco despite the fact that it is causing or exacerbating social or interpersonal difficulties.
- Because of smoking, people stop or cut down on many of their favorite social, professional, and leisure activities.
- Use of tobacco on a regular basis in circumstances where it may be hazardous (e.g. smoking in bed).
- To keep using tobacco even if you are aware that you have a chronic or recurrent medical or mental condition that may be worsened or caused by tobacco use.
- Tolerance developed as defined by one of the following [12]:

  a) Lack or need to use tobacco to a significantly increasing extent to achieve the desired effect.
  b) Achieving significantly less impact despite continuing tobacco use at the same level.

- Deprivation developed as defined by one of the following [12]:

  a) Developed symptoms of tobacco withdrawal syndrome
  b) Taking tobacco to get rid of deprivation.

Tobacco abstinence is described as follows under the heading of "Tobacco-Related Disorders" in the diagnostic criteria of the American Psychiatric Association Diagnostic and Statistical Manual of Mental Disorders (DSM-5).

- Smoking tobacco every day for at least a few weeks

- Within 24 hours of cessation or reduction in tobacco use, the development of at least four signs or symptoms from the following findings [12]:

    1. Easily irritated, frustrated, or anger
    2. Anxiety
    3. Difficulty focusing
    4. Increased desire to eat
    5. Unrest
    6. Depressed mood
    7. Insomnia

- Clinically severe distress or decreased functioning in social, work-related, or other key functional domains are caused by symptoms on the B diagnostic scale.
- This set of signs and symptoms can't be explained by any other medical ailment or mental illness.

Taking tobacco or tobacco products themselves or their smoke into the lungs creates psychic and physical dependence in the person over time. The substance that causes addiction in the structure of tobacco is nicotine. Cigarette; It is described as more "addictive" rather than "pleasurable". The American Psychiatric Association has classified addictive or abused substances into 9 groups in DSM-5-TR as follows [13]:

- Alcohol
- Caffeine
- Hemp (cannabis)
- Hallucinogens (LSD, mescaline, phencyclidine etc.)
- Volatiles (thinner, gasoline, gasoline, etc.)
- Opiates (morphine, heroin, codeine, methadone, etc.)
- Soothing, sedative and anxiety relievers (Diazepam, clorazepate, etc.)
- Stimulants (amphetamine, ecstasy, cocaine, etc.)
- Tobacco
- Other unknown substances.

2.1.6. Health Effects of Tobacco Use

To smoke; It is the habit that threatens humanity the most and is the most frequently observed, as it causes premature deaths and preventable diseases. Nicotine contained in cigarettes; By acting on the central nervous system, it is first enjoyable and then addictive. Smoking; It has a wide range of effects from aesthetic impairment in the mouth and teeth to death, such as cancer, stroke [14].

Cigarette smoke that occurs with the burning of cigarettes and contains many carcinogenic substances such as nitrosamine, aromatic amine and benzopyrene; It is a serious threat to not only active but also passive smokers. Cancers caused by smoking directly, without smoke; esophagus, oral cavity, larynx, pharynx and pancreas cancers. Passive smoking causes larynx, pharynx and lung cancer; People who actively smoke and are also exposed to smoke; In addition to myeloid leukemia, malignancies of the cervix and colorectum may be found as well as kidney, larynx and paranasal sinuses as well as of the esophagus and oral cavity, as well as of the liver, lung, pharynx, stomach and ureter.

A significant portion of indoor cigarette smoke can be held by hair, skin, clothing, furniture, flooring, walls, beds, carpets, dust and other surfaces and can remain on these surfaces for a long time, and these residues are considered third-hand smoke. Third-hand cigarette smoke components can pass back into the gas phase and diffuse into the air or react with oxidants in the environment to form secondary pollutants. Practices such as general cleaning methods such as wiping, airing the room, opening the windows, using a fan or air conditioner, or only smoking in certain areas do not prevent or eliminate third-hand smoke. When active smokers enter the home immediately after putting out their cigarettes, their breath and clothing continue to emit harmful chemicals from smoking [15].

Several mechanisms are dominant in the effect of smoking on the cardiovascular system. The most effective of these is endothelial damage. In almost all vascular structures that make up the cardiovascular system, endothelium damages the coronary and peripheral vascular structures and prevents circulation and nutrition. Endothelial

damage is caused by oxidizing substances and nicotine. Another mechanism is increased thrombopoietic activity. As if there is a chronic inflammation, inflammation markers increase and atherosclerosis occurs. At the same time, it has an atherogenic effect by causing an increase in triglyceride and a decrease in HDL. As an acute effect, it creates myocardial ischemia as a result of decreased oxygenation and blood supply in the heart [16].

COPD is the most common disease caused by smoking in the respiratory system. COPD (Chronic Obstructive Pulmonary Disease); It gradually increases and causes deaths. Nowadays, women get more COPD and die. In addition, it causes exacerbation of asthma attacks, severe course of existing tuberculosis and relapses. It causes many diseases in the gastrointestinal system from the beginning to the end, including cancer in the oral cavity, larynx, pharynx, esophagus, pancreas, stomach, colorectal system. It accelerates the formation of reflux disease, ulcerative colitis, dyspepsia, stomach and duodenal ulcers, Crohn's disease, gastrointestinal cancers (esophagus cancer, stomach cancer, colorectal cancers, pancreatic cancer, hepatocellular carcinoma) by relaxing the sphincter tone in the stomach, increasing acid secretion and epithelium damage [17].

Smoking causes fertility problems in both men and women in the urogenital system. In women; While causing problems such as menstrual irregularity, infertility, early menopause; It causes impairment in sperm count and motility in men.

2.2. INFERTILITY

Infertility has been documented after 12 months or more of frequent unprotected sexual intercourse, according to the World Health Organization. Approximately 15 percent of couples who have unprotected intercourse for a year cannot conceive. The incidence of infertility, which was around 7-8% in 1960 in industrialized countries, has increased to around 20-35% today. In total, Carlsen and colleagues, who compiled several studies of about 15,000 men, showed that in 50 years the human sperm concentration decreased by almost 50% and the sperm volume decreased. It is among other studies that have been done. The steady increase in infertility has raised serious

concerns about human reproduction in recent years. These significant shifts in fertility are critical, and they must be thoroughly investigated in order to keep assisted reproductive technology affordable [18].

2.2.1. Male Infertility

Infertility is mentioned in men who cannot have children within a year as a result of sexual intercourse with normal frequency and without protection, although their spouse is normal. The male factor; plays a role in 50% of all infertile couples. As male infertility can be idiopathic; It is known to be under the influence of many factors such as systemic and genital infections, chromosomal disorders, undescended testicles, urogenital infections, systemic diseases, sexual or ejaculatory dysfunction, immunological factors, varicocele, hypogonadism, and obstructions. The causes of male infertility are shown in Table 2.2 [19].

Table 2.2. Causes of male infertility [19].

Sperm production problems	• Chromosomal or genetic causes • Undescended testicle • Infections • Testicular torsion • Varicocele • Pharmaceuticals and chemicals • Radiation • Unknown reasons
Obstruction of the sperm ducts	• Infections • Prostate related problems • Absence of vas deferens • Vasectomy
Sexual problems (erection and ejaculation problems)	• Retrograde and premature ejaculation • Ejaculation failure • Erectile dysfunction • Sparse relationship • Spinal cord injury • Prostate surgery • Nerve damage • Some medications
Hormonal problems	• Pituitary tumors • Congenital LH / FSH deficiency (pituitary problem from birth) • Anabolic (androgenic) steroid abuse
Sperm antibodies	• Vasectomy • Injury or infection in the epididymis • Unknown reasons

Basically, in male infertility research, taking the patient's history, applying semen tests, traditional tests and histological examinations for the evaluation of testicular tissue

samples, detection and classification of spermatogenesis disorders are followed. In the diagnosis of male infertility, sperm analysis (spermogram) is an essential diagnostic investigation, and it is frequently abnormal in male infertility sufferers. The most common sperm abnormalities that can occur with the effect of one or more of these factors; It counts as oligozoospermia, asthenozoospermia and oligoastenozoospermia. Oligozoospermia according to World Health Organization parameters; sperm count is below 15 million / ml. Asthenozoospermia; This means that the progressively motile spermatozoa are <50% or those with forward motility are <25%. Oligoastenozoospermia; It is expressed as sperm being below normal values in terms of both number and movement [20].

2.2.2. Incidence of Male Infertility

Semen quality is decreasing due to a variety of reasons, including changes in lifestyle and increasing exposure to contaminants like alcohol and drugs [21].

In the West, drinking alcohol is extremely prevalent. 76 percent of people have used alcoholic drinks in the past 12 months, according to the latest available statistics; rates are increasing from the southern areas (lowest in Portugal at 58 percent) to the northern regions, according to this data (highest 93 percent in Denmark). According to recent statistics, 70.7% of Americans drank alcohol in the past year, and 56% did so in the month before. [22].

Some research has indicated a link between alcohol use and poor semen quality, although this has not been verified by others. It's tough to draw similarities across research since people and alcohol use vary so widely. The average amount of alcohol consumed in most trials has only been a few questions, and intake within response categories varies widely and seems to be insufficient.[23].

It's been proposed that alcohol intake and poor sperm quality share pathways that affect testosterone metabolism and spermatogenesis negatively. In addition to altering the free estradiol/testosterone ratio, alcohol use has been linked to an increased risk of spermatogenetic arrest and Sertoli cell-like syndrome.

WHO estimates that approximately a third of people over the age of 15 use cigarettes worldwide. Smoking has been shown to have a greater impact on reproductive health in men and women than coffee or alcohol use. There is still a lot of mystery around how tobacco affects spermatogenesis. The connection between smoking and semen analysis, factors including concentration, morphology, and motility have been studied in several research.[24].

Male infertility may be diagnosed using sperm analysis, which is a critical step in the process. Sperm analysis is often abnormal in infertile males. Sadly, infertility in most men is idiopathic, with the underlying cause believed to be a distinct aspect of testicular function. Heat, smoking, radiation, heavy metals, and other environmental variables may all have an impact on spermatogenesis. A substantial but reversible drop in sperm count may be caused by febrile illnesses. According to some ideas, occupations that require a lot of sitting (like driving) may increase the risk of infertility, although this has not been proved in clinical trials.[25].

Smoking has been shown to have negative impacts on sperm function, although the exact mechanism has yet to be discovered. When it comes to research on smoking and sperm analysis criteria (such as molecular morphology, motility, or concentration), results vary widely, but it is difficult to know for sure if smoking is a cause of infertility or whether it has a connection to it. Smoking reduces inflammatory agents and their impact on the sperm genome and gonads, resulting in sperm-ovum infertility and inability to reproduce.[26].

For infertile couples, the initial diagnostic test is usually a sperm analysis, which is a simple, low-cost laboratory test. Infertility in men is often accompanied with abnormal sperm tests. Diagnostic methods that are more in-depth are required for abnormal tests.[27].

2.2.3. Diagnostic Tests in Male Infertility

Male infertility may be assessed using a variety of diagnostic techniques. While semen analysis comes first among these methods, evaluation of non-sperm cells and function tests are very important for diagnostic tests.

2.2.3.1. Morphological Evaluation of Spermatozoa

The morphological characteristics of healthy sperm with normal functions were determined by Kruger criteria and WHO criteria (WHO, 2010).

Kruger Criteria

Head: Acrosome should be found in 40-70% of the sperm head. The head should be seen in a smooth oval structure. The width of the head should be around 2-3μ and its length should be between 3-5μ. Such head shapes are considered abnormal even if there is no apparent deformity in the dimensions within the boundaries.

Neck: It should be along the long axis of the head and intact.

Middle Part: Its width is about 1μ and its length is around 1.5 times its width. It should be in the form of a cylinder and connected to the head in the long axis direction. Cytoplasmic residues greater than half the size of the head are considered abnormal.
Tail: Length should be between 35-45μ. It should generally be of smooth texture and should not have any fine bends or bends in the middle. Other than that, those with other morphological features are considered abnormal.

WHO Criteria

Head; The acrosomal part should not be more than one third of the head surface. should be smooth-edged and oval in shape. Head measurements should be between expected normal length 3-5μ and width 23μ. The width of the head should be between half and one third of the height.

Neck: An evaluation on the neck is not included in the WHO criteria.

Middle part: It should not be less than one third of its width. Its shape should be cylindrical. It should be tidy around. Cytoplasmic residues exceeding half the size of the head are considered abnormal.

Tail: There should be no bends or twists. Its structure is cylindrical. length should be 35- 45µ. Its external structure must have a smooth form. Apart from that, other values are considered abnormal.

At least 100, if possible 200, sperm should be examined in order to make a definite and healthy morphological evaluation. The morphological structure of the entire sperm should be evaluated. Evaluation is carried out at 100X magnification area. Sperms in areas of cell agglomeration should not be taken into account during the evaluation. At least 100 cells should be evaluated in different regions of the preparation.

2.2.3.2. Semen Analysis

Semen analysis has an important place in the evaluation of infertility. For semen analysis, fresh semen obtained after at least 48 hours of sexual abstinence is evaluated. At this stage, sperm morphology, number and motility are evaluated. Semen analysis has a very important place in evaluating the infertile male individual in determining the fertility potential.

2.2.4. Tobacco Addiction

Tobacco obtained from tobacco plants called Nicotiana rustica and Nicotiana ketum is known worldwide as the only source of nicotine. The active ingredient of the tobacco plant was first discovered by Nicolas Vauquelin in 1809. After the leaves of the tobacco plant are shredded and then wrapped in the form of a cigarette, it is the most common use of burning the smoke. Today, smoking emerges as an important public health problem.

2.2.4.1. Neurophysiological Effects of Nicotine

There are two types of cholinergic receptors: one for nicotine and one for muscarinic stimuli. Antimuscarinic drugs like atropine inhibit muscarinic receptor stimulation caused by muscarine. Nicotine stimulates Nicotinic receptors and Nicotinic antagonists inhibit them. Nicotinic receptors are located in the bodies of dopaminergic and neuradrenergic neurons in the brain and cause the release of dopamine and neuradenine upon their stimulation (Figure 2.2). In addition, nicotinic receptors are found in the cerebral cortex, ventral tegmental area, and nucleus acumben [28].

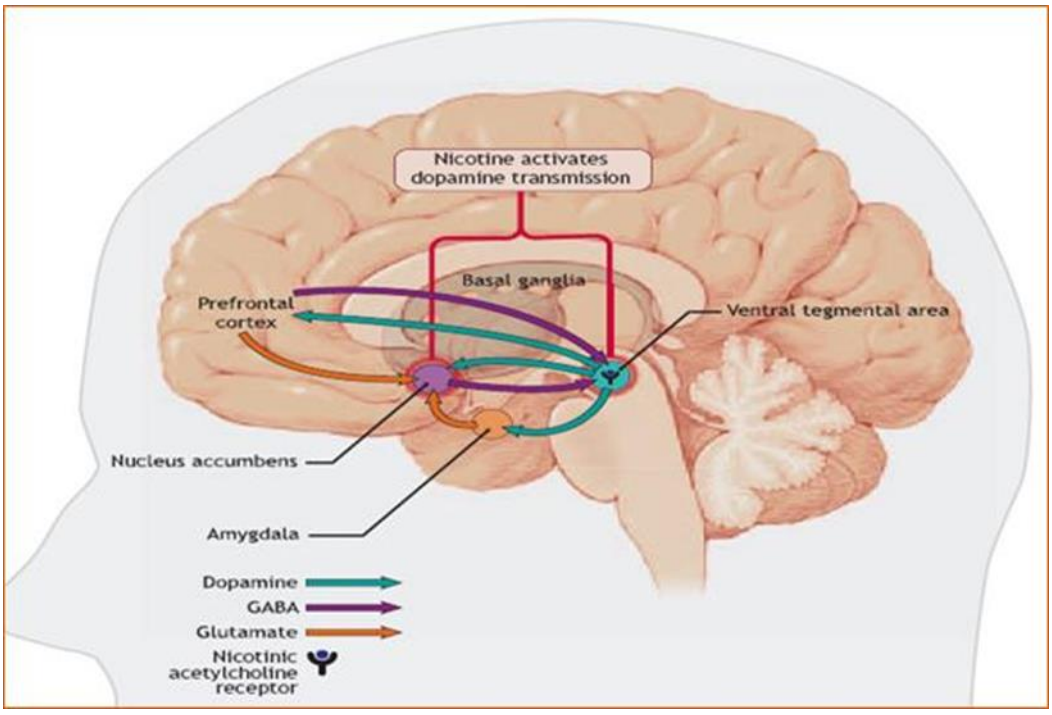

Figure 2.2. Effect of nicotine on the brain reward pathway.

2.2.4.2. Harmful Effects of Tobacco Use

Tobacco usage, such as smoking cigarettes, is well-known to raise one's chance of contracting many illnesses and to hasten mortality. Cigarettes contain about 4000 different chemical constituents, including carbon monoxide, nitrous oxide, hydrocarbons, tar, hydrogen cyanide, and nicotine. Some 40 different kinds of these additives have been shown to be carcinogenic. About 25 illnesses have been linked to smoking, including cardiovascular disease, lung cancer, bronchitis, and cancers of the

urinary system. Cigarette addiction is a risk factor for over 60% of noncommunicable illnesses, and tobacco use and passive smoking are responsible for over 6 million fatalities per year. The World Health Organization's latest statistics show that despite mounting proof of its negative consequences, smoking remains a widespread practice. More than a third of men over the age of 18 use cigarettes worldwide. [25]; In the same way, around 30% of reproductive smoking women are tobacco users. In terms of tobacco usage, Europe continues to dominate the world, although the United States has seen a decline in smoking rates in recent years. Smoking may have a negative impact on men's and women's fertility, and the consequences of smoking can even be felt by the smoker's spouse. [29].

2.2.4.3. The Effect of Smoking Addiction on Fertility

Worldwide, the infertility rate is between 10 and 15 percent, and recent research show that smoking is one of the risk factors for male infertility that affects 50 to 80 million couples. As a result of inhaling all the poisons such as nicotine, carbon monoxide, cadmium, and other mutagenic chemicals that are produced when tobacco is burned, smoking may have detrimental consequences on male germ cells. carcinogens from cigarette smoke may decrease the mitochondrial function of human sperm, which in turn damages the chromatin structure [30].

Several criteria frequently employed in the clinical environment to evaluate fertility such as motility, concentration, and morphology have been linked to smoking's negative impact on sperm quality. Some studies have shown no impact on the quality of semen, while others have found inconclusive data. There is evidence to indicate that smoking has a greater impact on women's reproductive health than caffeine or alcohol use. There is still a lot of mystery around how tobacco affects spermatogenesis. In a few studies, researchers looked at the link between smoking and a variety of semen analytic characteristics, including morphology, concentration, and mobility [31].

PART 3

MARTIALS AND METHOD

3.1. MARTIALS

3.1.1. The X-Ray Diffraction Technique (XRD)

As each crystallographic phase has its own unique atomic sequence, the order in which the diffraction of X-rays occurs determines the X-Ray Diffraction technique (XRD). Diffraction profiles for each crystal phase are like fingerprints since they identify the crystal. Since the sample isn't destroyed during analysis using X-Ray Diffraction, it's possible to analyze even a small number of samples. The X-Ray Diffraction device may be used to conduct qualitative and quantitative studies on rocks, crystalline minerals, thin films, and polymers. Rigaku Ultima IV X-Ray Diffractometer serves with its multi-purpose units. The X-ray tube is made of copper and includes a water chiller to keep it from overheating. With this technology, monochromatized X-rays may be obtained using a high-resolution graphite monochromator (HGM). The cross-beam optical mechanism (CBO) in the Ultima IV XRD device provides the opportunity to work in focus or parallel beam geometry without a new setting and adjustment. Although quite strong diffraction bands were obtained from well-crystallized and smooth-surfaced samples with the routinely used "Bragg-Brentano focal beam geometry" method; "Parallel focus beam geometry" is used in the phase definition of samples with rough surfaces, weakly crystallized and especially thin films. In addition, although a weak signal is usually received from thin films of different thicknesses with the standard Θ / 2Θ (2Θ = 2-90 ° range) scanning method, a stronger signal is obtained with the 2Θ scanning method and a fixed grazing angle (GIXD-minimum 0.1 °). signal can be obtained.

With this technique, very precise measurements can be made on thin film and polycrystalline samples [32].

Figure 3.1. Rigaku Ultima IV X-Ray Diffractometer (Karabuk University).

3.1.2. The X Fourier-Transform Infrared Spectroscopy (FTIR)

Vibration spectroscopy, such as FTIR, uses a Fourier Transform Infrared Spectrometer (FTIR). The Fourier transform technique, used in FTIR, quantifies the number of waves compared to the infrared light's intensity. Between 14000 cm-1 and 10 cm-1, the electromagnetic light array's infrared area includes near infrared (NIR; 4000 14000 cm-1), medium infrared (MIR; 400 4000 cm-1) and far infrared (FIR; 400 10 cm-1) wavelengths. Wavelength infrared (FIR; 4 400 cm-1) is one of three major zones. The vibrational motions of the molecule absorb IR photons. The luminous intensity is taken into account as a time-dependent variable in mathematical Fourier transform

spectroscopy. It is possible to acquire fast and high-resolution spectra without scanning each size individually. With this technique, it is possible to characterize molecular bonds in the structure of organic compounds in solids, liquids, gases, or solutions and identify whether functional groups are aromatic or aliphatic and whether or not two compounds are the same [33].

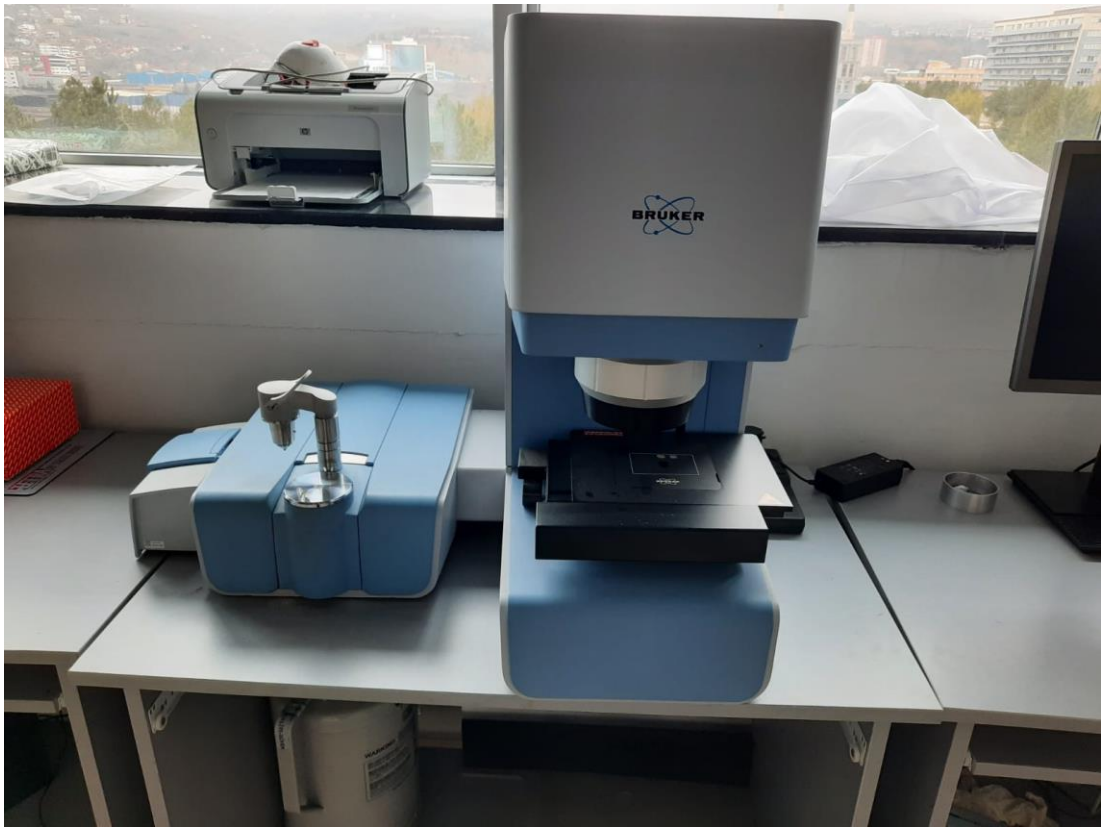

Figure 3.2. FT-IR device (Karabuk University).

3.1.3. The Scanning Electron Microscopy (SEM)

With a scanning electron microscope, a focussed electron beam is used to take pictures of a sample's surface, which is referred to as a SEM (scanning electron microscopy). Different signals containing information about the sample surface topography and composition are generated when electrons contact with atoms in the sample. Using raster scanning, an electron beam scans the surface, and the beam's location is matched with the received signal to produce a picture. SEM allows for a resolution of more than 1 nanometer. For analyzing high vacuum, dry, and conductive surfaces, standard SEM

equipment are well suited. Even in humid circumstances (such as an environmental scanning electron microscope), there are specialized devices that can work in low vacuum (such as an extreme-temperature scanning electron microscope). Most of the pictures in SEM are created by secondary electrons (SE) produced by electron-excited sample atoms. When a beam meets a surface, the angle at which it meets the surface determines how many secondary electrons are detachable from various areas of it. Additionally, secondary electrons, distinctive X-rays, light (electron beam), sample current, and transferred electrons are all collected from the sample, and suitable topographical and composition analyses are conducted [34].

Figure 3.3. SEM device (Karabuk University).

3.2. TOBACCO TYPES USED IN THE STUDY

In the Figure 3.4, the Karelia Slims brand of tobacco. This brand contains; Tar 6 mg, Nicotine 0.6 mg.

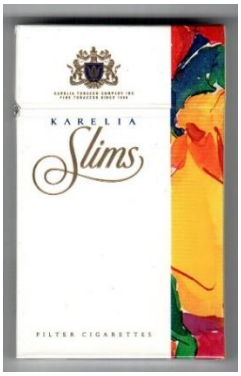

Figure 3.4. Karelia Slims tobacco.

In the Figure 3.5, the Bon international Ultra Light brand of tobacco. This brand contain; Tar 8 mg, Nicotine 0.6 mg.

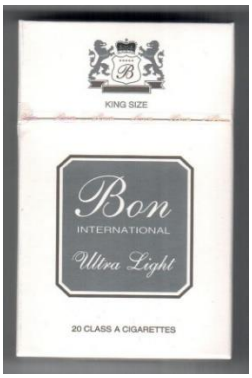

Figure 3.5. Bon international Ultra Light tobacco.

In the Figure 3.6, the Milano Skyline brand of tobacco. This brand contains; Tar 1 mg, Nicotine 0.1 mg.

Figure 3.6. Milano Skyline tobacco.

In the Figure 3.7, the Oris Slims brand of tobacco. This brand contains; Tar 10 mg and Nicotine 0.9 mg.

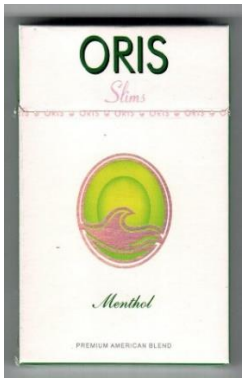

Figure 3.7. Oris Slims tobacco.

In the Figure 3.8, the Kent Blue HD brand of tobacco. This brand contains; Tar 8 mg and Nicotine 0.7 mg.

Figure 3.8. Kent Blue HD tobacco.

In the Figure 3.9, the Manchester Ultra Lights brand of tobacco. This brand contain; Tar 3 mg and Nicotine 0.4 mg.

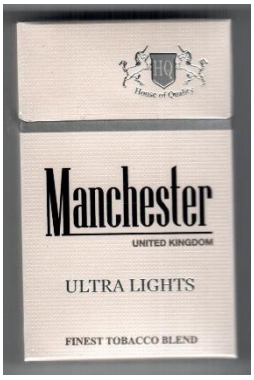

Figure 3.9. Manchester Ultra Lights tobacco.

In the Figure 3.10, the Winston Blue brand of tobacco. This brand contains; Tar 8 mg and Nicotine 0.6 mg.

Figure 3.10. Winston Blue tobacco.

PART 4

STATISTICAL ANALYSIS

4.1. RESULTS

RQ1: Is there an association between smoking and liquefaction?

Table 04.1. Liquefaction and Smoking Crosstabulation

			Smoking		Total
			No	Yes	
Liquefaction	30 min	Count	46_a	64_b	110
		% Within smoking	100.0%	81.0%	88.0%
	Above 30 min	Count	0_a	15_b	15
		% Within smoking	0.0%	19.0%	12.0%
Total		Count	46	79	125
		% Within smoking	100.0%	100.0%	100.0%
Each subscript letter denotes a subset of smoking categories whose column proportions do not differ significantly from each other at the .05 level.					

A chi-square test of independence, with Yates Continuity Correction, was performed to examine the relation between smoking and Liquefaction. The relation between these two variables was significant, χ^2 (1, n = 125) =8.209, p = .004, Cramer's V = .282. According to Cohen, 2013 a Cramer's V of (.282) for two categories indicates a small effect size. Results indicate that 19% of smokers fall in the Liquification period of "Above 30 minutes" while 0% of non-smokers fall into the same category Table 4.1.

Based on this result I conclude that there is an association between smoking and liquefaction.

RQ2: Is there an association between smoking and round cell count?

Table 4.2. Round Cell Count and Smoking Crosstabulation.

			smoking		Total
			No	Yes	
Round Cell Count	10 and below	Count	40_a	59_a	99
		% Within smoking	87.0%	88.1%	87.6%
	11 and above	Count	6_a	8_a	14
		% Within smoking	13.0%	11.9%	12.4%
Total		Count	46	67	113
		% Within smoking	100.0%	100.0%	100.0%
Each subscript letter denotes a subset of smoking categories whose column proportions do not differ significantly from each other at the .05 level.					

A chi-square test of independence, with Yates Continuity Correction, was performed to examine the relation between smoking and Round Cell Count. The relation between these two variables was not significant, χ^2 (1, n = 113) = 0.0, p = 1.00, Cramer's V =.016. According to Cohen, 2013 a Cramer's V of (.016) for a two categories table indicates a small effect size. Results indicate that 11.9% of smokers have 11 and above round cell count while 13% of non-smokers fall into the same category Table 4.2. Based on this result I conclude that there is no association between smoking and Round Cell Count.

4.2. TESTING NORMALITY OF DEPENDENT CONTINUOUS VARIABLES

Table 4.3. Tests of Normality.

	smoking	Kolmogorov-Smirnov[a]			Shapiro-Wilk		
		Statistic	df	Sig.	Statistic	df	Sig.
volume/ml	No	.174	46	.001	.929	46	.008
	Yes	.157	78	.000	.911	78	.000
COUNT *million /ml	No	.165	46	.003	.878	46	.000
	Yes	.191	77	.000	.815	77	.000
MOTILITY total %	No	.212	46	.000	.867	46	.000
	Yes	.154	69	.000	.928	69	.001
G1 %	No	.153	46	.009	.909	46	.002
	Yes	.107	74	.035	.950	74	.005
G2 %	No	.222	46	.000	.833	46	.000
	Yes	.138	74	.001	.937	74	.001
G3 %	No	.518	46	.000	.221	46	.000
	Yes	.463	69	.000	.403	69	.000
normal form %	No	.297	46	.000	.622	46	.000
	Yes	.282	74	.000	.725	74	.000
a. Lilliefors Significance Correction							

Tests of normality of dependent continuous variables were performed using Kolmogorov-Smirnov; and Shapiro-Wilk tests, table 4.3. All variables are not normality distributed according to Kolmogorov-Smirnov; and Shapiro-Wilk tests, $p < .05$, therefore, nonparametric tests should be used when testing the impact of categorical variables on these dependent continuous variables.

RQ3: What is the impact of age on volume/ml, COUNT *million /ml, MOTILITY total**%** , G1**%** , G2**%** , G3 % and normal form %?

Table 4.4. Descriptive Statistics of dependent continuous variables.

	N	Mean	Std. Deviation	Minimum	Maximum
Volume/ml	124	2.7460	1.40692	.50	8.00
COUNT *million /ml	123	47.2829	51.78297	.00	300.00
MOTILITY total %	115	49.7000	26.97333	.00	100.00
G1 %	120	33.9917	21.44506	.00	100.00
G2 %	120	56.0583	24.82182	.00	100.00
G3 %	115	2.6000	8.93721	.00	50.00
Normal form %	120	9.4500	14.77669	.00	70.00

Table 4.4 shows descriptive Statistics (Mean, Standard Deviation, Minimum and Maximum scores) of volume/ml, COUNT *million /ml, MOTILITY total %, G1 %, G2 %, G3 % and normal form.

Table 4.5. Mean Ranks of volume/ml, COUNT *million /ml, MOTILITY total %, G1 %, G2 %, G3 % and normal form according to age groups.

	Age group	N	Mean Rank
volume/ml	30 to 39 years	61	59.39
	40 to 49 years	53	68.80
	50 years and above	10	48.05
	Total	124	
COUNT *million /ml	30 to 39 years	60	58.00
	40 to 49 years	53	64.29
	50 years and above	10	73.85
	Total	123	
MOTILITY total %	30 to 39 years	57	58.50
	40 to 49 years	49	60.30
	50 years and above	9	42.33
	Total	115	
G1 %	30 to 39 years	58	62.69
	40 to 49 years	52	56.53
	50 years and above	10	68.45
	Total	120	
G2 %	30 to 39 years	59	59.36
	40 to 49 years	51	64.98
	50 years and above	10	44.40
	Total	120	
G3 %	30 to 39 years	57	57.57
	40 to 49 years	49	58.56
	50 years and above	9	57.67
	Total	115	
normal form %	30 to 39 years	59	61.33
	40 to 49 years	51	60.94
	50 years and above	10	53.35
	Total	120	

Table 4.5 shows mean ranks of volume/ml, COUNT *million /ml, MOTILITY total %, G1 %, G2 %, G3 % and normal form according to age groups.

Table 4.6. Kruskal-Wallis Test statistics of age groups for volume/ml, COUNT *million /ml, MOTILITY total %, G1 %, G2 %, G3 % and normal form.

Test Statistics [a, b]							
	volume/ml	COUNT *million /ml	MOTILITY total %	G1 %	G2 %	G3 %	normal form %
Chi-Square	3.772	2.081	2.248	1.450	3.088	.071	.470
df	2	2	2	2	2	2	2
Asymp. Sig.	.152	.353	.325	.484	.214	.965	.791
a. Kruskal Wallis Test							
b. Grouping Variable: Age group							

A Kruskal-Wallis Test revealed no statistically significant difference in volume/ml, COUNT *million /ml, MOTILITY total %, G1 %, G2 %, G3 % and normal form levels across three different age groups (Gp1: 30–39yrs, Gp2: 40–49yrs, Gp3: 50+yrs), χ^2 (2, n = 115-124) = .152-.965, $p < .05$. Based on these results I conclude that age has no impact on any of the above-mentioned variables, therefore, age could be excluded as a confounding variable when testing independent variables impact on volume/ml, COUNT *million /ml, MOTILITY total %, G1 %, G2 %, G3 % and normal form %.

RQ4: What is the impact of smoking on volume/ml, COUNT *million /ml, MOTILITY total**%** , G1**%** , G2**%** , G3 % and normal form %?

Table 0.2. Mann-Whitney U Test statistics for volume/ml, COUNT *million /ml, MOTILITY total %, G1 %, G2 %, G3 % and normal form.

	Volume/ ml	**COUNT *million /ml**	**MOTILITY total %**	**G1 %**	**G2 %**	**G3 %**	**Normal form %**
Mann-Whitney U	1711.00	1707.500	1338.50	1518.00	1426.50	1406.00	843.00
Z	-.433	-.332	-1.424	-1.000	-1.496	-1.766	-4.665
Asymp. Sig. (2-tailed)	.665	.740	.155	.317	.135	.077	.000
N	124	123	115	120	120	115	120
Non-smokers number and Median	46	46	46	46	46	46	46
	2.50	30.50	45.00	25.00	67.50	.00	2.00
Smokers number and Median	78	77	69	74	74	69	74
	2.50	33.00	60.00	40.00	60.00	.00	6.50

A Mann-Whitney U Test revealed no significant difference in the volume/ml levels of non-smokers (Md = 2.5, n =46) and smokers (Md = 2.5, n = 78), U = 1711, z = -.433, p = .665, r = .04, the COUNT *million /ml levels of non-smokers (Md = 30.5, n =46) and smokers (Md =33, n =

77), U = 1707.50, z = -.332, p = .740, r = .03, the MOTILITY total % levels of non-smokers (Md = 45, n =46) and smokers (Md =60, n = 69), U = 1338.50, z = -1.424, p = .155, r = .13, the G1 % levels of non-smokers (Md = 25, n =46) and smokers (Md =40, n = 74), U = 1518, z = -1.00, p = .317, r = .09, the G2 % levels of non-smokers (Md = 67.50, n =46) and smokers (Md =60, n = 74), U = 1426.50, z = -1.496, p = .317, r = .14 and the G3 % levels of non-smokers (Md = 00, n =46) and smokers (Md =00, n = 69), U = 1406, z = -1.766, p = .077, r = .16. According to Cohen, 1988 an r value of less than .3 is considered a small effect size. A Mann-Whitney U Test revealed a significant difference in the Normal form % levels of non-smokers (Md = 2.00, n =46) and smokers (Md = 6.50, n = 74), U = 843, z = -4.665, p < .001, r = .43 which is considered a medium effect size according to Cohen,1988. Based on these results I conclude that smoking has an impact on normal form % only in the above variables.

RQ5: What is the impact of Duration in years and Amount per day on volume/ml?

Table 4.8. Impact of Duration in years and Amount per day on volume/ml.

Independent Factors	**R^2**	**F**	**β**	**t**	**p**
Duration/years	.116	4.609*	-.303	-2.786-	.007
Amount per day			.162	1.492	.140

[a] Dependent variable: volume/ml * p <.05

Multiple regression was used to assess the ability of two factors (Duration in years and Amount per day) to predict levels of volume/ml. The total variance explained by the model was 11.6%, F (2, 75) = 4.609 ,p < .05. The Duration/years was a significant predictor of volume/ml, with a beta value = -.303, p = .007. Results can be interpreted as One Standard Deviation in Duration/years brings about -.303 standardized change in volume/ml. Amount per day does not have a significant impact on volume/ml, p= .140.

RQ6: What is the impact of Duration in years and Amount per day on COUNT *million /ml?

Table 4.9. Negative binomial regression model.

Parameter Estimates							
Parameter	B	Std. Error	95% Wald Confidence Interval		Hypothesis Test		
			Lower	Upper	Wald Chi-Square	df	Sig.
(Intercept)	3.346	.4010	2.560	4.132	69.633	1	.000
Duration/years	.026	.0151	-.003	.056	3.019	1	.082
Amount\day	.008	.0093	-.010	.026	.755	1	.385
(Scale)	1[a]						
(Negative binomial)	1[a]						
Dependent Variable: COUNT *million /ml Model: (Intercept), Duration/years, Amount\day							
a. Fixed at the displayed value. Likelihood Ratio χ^2 (2) =3.620, p=.160							

A negative binomial regression model (Table 4.9) was used to examine the ability of two factors (Duration in years and Amount per day) to predict COUNT *million /ml. Together the predictors did not account for any significant amount of variance in the outcome, likelihood ratio χ^2 (2) =3.620, p=.16. Duration in years and Amount per day were not significant predictors of COUNT *million /ml, B = .026, SE_B = .015, p = .082, 95% CI [-.003, -.056] and B =.008, SE_B = .009, p = .385, 95% CI [-.010,.026], respectively. Based on these results I conclude that duration in years and amount per day do not have an impact on COUNT *million /ml

RQ7: What is the impact of Duration in years and Amount per day on MOTILITY total %?

Table 4.10. The impact of Duration in years and amount per day on MOTILITY total %.

Independent Factors	**R^2**	**F**	**β**	**t**	**p**
Duration/years	.008	.275[!]	-.090	-.736	.465
Amount per day			.013	.110	.913

[a] Dependent variable: MOTILITY total %, p =.760

Multiple regression was used to assess the ability of two factors (Duration in years and Amount per day) to predict levels of MOTILITY total %. The total variance explained by the model was very low, .8%, F (2, 66) = .275 ,p = .760. Based on these results I conclude that duration in years and amount per day have no significant impact on MOTILITY total %.

RQ8: What is the impact of Duration in years and Amount per day on G1**%** ?

Table 4.11. The impact of Duration in years and Amount per day on G1%.

Independent Factors	**R^2**	**F**	**β**	**t**	**p**
Duration/years	.009	.338[!]	-.070	-.589	.558
Amount per day			-.066	-.561	.577

[a] Dependent variable: G1 %,[!] p =.715

Multiple regression was used to assess the ability of two factors (Duration in years and Amount per day) to predict levels of G1%. The total variance explained by the model was very low, .9%, F (2, 71) = .338 ,p = .715. Based on these results I conclude that duration in years and amount per day have no significant impact on G1%.

RQ9: What is the impact of Duration in years and Amount per day on G2**%** ?

Table 4.12. The impact of Duration in years and Amount per day on G2%.

Independent Factors	**R^2**	**F**	**β**	**t**	**p**
Duration/years	.002	.087[!]	.031	.263	.793
Amount per day			-.039	-.330	.742

[a] Dependent variable: G2 %,[!] p =.917

Multiple regression was used to assess the ability of two factors (Duration in years and Amount per day) to predict levels of G2%. The total variance explained by the model was very low, .2%, F (2, 71) = .087 ,p = .917. Based on these results I conclude that duration in years and amount per day have no significant impact on G2%.

RQ10: What is the impact of Duration in years and Amount per day on G3**%** ?

Table 4.13. The impact of Duration in years and Amount per day on G3%.

Independent Factors	**R^2**	**F**	**β**	**t**	**p**
Duration/years	.002	.068[!]	-.002	-.019	.985
Amount per day			-.045	-.369	.713

[a] Dependent variable: G3 %,[!] p =.934

Multiple regression was used to assess the ability of two factors (Duration in years and Amount per day) to predict levels of G3%. The total variance explained by the model was very low, .2%, F (2, 66) = .068 ,p = .934. Based on these results I conclude that duration in years and amount per day have no significant impact on G3%.

RQ11: What is the impact of Duration in years and Amount per day on normal form **%**?

Table 4.14. The impact of Duration in years and Amount per day on normal form %.

Independent Factors	**R^2**	**F**	**β**	**t**	**p**
Duration/years	.020	.724[!]	-.141	-1.200	.234
Amount per day			.013	.111	.912

[a] Dependent variable: normal form %, p =.488

Multiple regression was used to assess the ability of two factors (Duration in years and Amount per day) to predict levels of normal form %. The total variance explained by the model was very low, 2%, F (2, 71) = .724 ,p = .488. Based on these results I conclude that duration in years and amount per day have no significant impact on normal form%.

RQ12: What is the impact of tar levels on volume/ml, COUNT *million /ml, MOTILITY total**%** , G1**%** , G2**%** , G3 % and normal form %?

Table 4.15. Descriptive statistics of volume/ml, COUNT *million /ml, MOTILITY total %, G1 %, G2 %, G3 % and normal form according to tar levels.

Tar Level		volume/ml	COUNT *million /ml	MOTILITY total %	G1 %	G2 %	G3 %	Normal form %
Low Tar	N	23	22	23	22	23	21	23
	Mean	3.0435	85.4364	69.7826	30.2273	63.5217	3.5238	19.9565
	Median	2.5000	75.0000	75.0000	22.5000	70.0000	.0000	10.0000
	Std. Deviation	1.79591	78.17135	23.60462	19.67578	24.40914	9.13575	21.35517
Moderate Tar	N	24	24	18	24	24	23	22
	Mean	2.9083	40.4917	56.6667	36.5000	51.9167	7.9565	20.0455
	Median	2.5000	34.0000	59.5000	40.0000	54.5000	.0000	15.0000
	Std. Deviation	1.14623	37.05297	7.45181	18.17787	17.96353	16.47720	18.23518
High Tar	N	31	31	28	28	27	25	29
	Mean	2.6677	32.6226	36.4464	37.4286	49.6296	.4800	3.4138
	Median	2.0000	18.0000	25.7500	40.0000	55.0000	.0000	2.0000
	Std. Deviation	1.65960	41.14063	29.07695	24.45620	26.34556	1.68622	3.47971
Total	N	78	77	69	74	74	69	74
	Mean	2.8526	50.1649	52.8333	34.9865	54.6892	3.8986	13.5000
	Median	2.5000	33.0000	60.0000	40.0000	60.0000	.0000	6.5000
	Std. Deviation	1.55327	57.33498	27.23005	21.13200	23.76997	11.10398	17.44129

Table 4.15 shows descriptive statistics (mean, median and standard deviation) of volume/ml, COUNT *million /ml, MOTILITY total %, G1 %, G2 %, G3 % and normal form according to three different tar levels.

Table 4.16. Kruskal-Wallis Test statistics for impact of tar levels on volume/ml, COUNT *million /ml, MOTILITY total %, G1 %, G2 %, G3 % and normal form.

Test Statistics [a,b]							
	volume/ml	COUNT *million /ml	MOTILITY total %	G1 %	G2 %	G3 %	normal form %
Chi-Square	1.467	8.711	20.419	3.696	8.046	2.834	26.682
df	2	2	2	2	2	2	2
Asymp. Sig.	.480	.013	.000	.158	.018	.242	.000
a. Kruskal Wallis Test							
b. Grouping Variable: Tar Level							

A Kruskal-Wallis Test revealed no statistically significant difference in volume/ml, G1%, G3% levels across three different tar levels (Low Tar, Moderate Tar and High Tar), χ^2 (2, n = 77) = 1.467, p =.480, χ^2 (2, n = 74) = 3.696, p =.158 and χ^2 (2, n = 69) = 2.834, p =.242 respectively. Kruskal-Wallis Test, also, revealed a statistically significant difference in COUNT *million /ml, MOTILITY total % G2 %, and normal form levels across three different tar levels (Low Tar, Moderate Tar and High Tar), χ^2 (2, n = 77) = 8.711, p =.013, χ^2 (2, n = 69) = 20.419, p <.001, χ^2 (2, n = 74) = 8.046, p =.018 and χ^2 (2, n = 74) = 26.682, p <.001 respectively.

Table 4.17. Mann-Whitney U Test statistics of tar levels for COUNT *million /ml, MOTILITY total %, G2 % and normal form.

Test Statistics of Tar Levels (Low V. Moderate)				
	COUNT *million /ml	MOTILITY total %	G2 %	Normal form %
Mann-Whitney U	167.000	61.000	149.000	248.500
Asymp. Sig. (2-tailed)	.033	.000	.007	.918
Z	-2.134-	-3.857-	-2.720-	-.102-
Test Statistics of Tar Levels (Low V. High)				
Mann-Whitney U	189.000	131.500	198.000	78.000
Asymp. Sig. (2-tailed)	.006	.000	.027	.000
Z	-2.745-	-3.614-	-2.207-	-4.738-
Test Statistics of Tar Levels (Moderate V. High)				
Mann-Whitney U	309.000	144.500	310.500	110.500
Asymp. Sig. (2-tailed)	.285	.015	.798	.000
Z	-1.070-	-2.431-	-.256-	-3.997-

A Mann-Whitney U Test revealed a significant difference in the COUNT *million /ml levels between low tar and moderate tar, U = 167, z = -2.134, p = .033, r = .31 and between low tar and high tar, U = 189, z = -2.745, p = .006, r = .38. The Mann-Whitney U Test, also, revealed a significant difference in the MOTILITY total % levels between low tar and moderate tar, U = 61, z = -3.857, p < .001, r = .60, between low tar and high tar, U = 131.5, z = -3.614, p < .001, r = .51, and between moderate tar and high tar, U = 144.5, z = -2.341, p = .015, r = .35. In the G2% results there was significant difference between low tar and moderate tar, U = 149, z = -2.720, p = .007, r = .40, and between low tar and high tar, U = 198, z = -2.207, p =.027, r = .31. Finally, there

was a significant difference in the normal form% between low tar and high tar, U = 78, z = -4.738, p <.001, r = .66 and between moderate tar and high tar, U = 110.5, z = -3.997, p < .001, r = .56. According to Cohen, 1988 r values of .3 represent a medium effect size and .5 represent a large effect size. Based on these results I conclude that tar has an impact on COUNT *million /ml, MOTILITY total % G2 %, and normal form levels.

RQ13: What is the impact of nicotine levels on volume/ml, COUNT *million /ml, MOTILITY total**%** , G1**%** , G2**%** , G3 % and normal form %?

Table 4.18. Descriptive statistics of volume/ml, COUNT *million /ml, MOTILITY total %, G1 %, G2 %, G3 % and normal form according to nicotine levels.

Nicotine Level		volume/ml	COUNT *million /ml	MOTILITY total %	G1 %	G2 %	G3 %	normal form %
Low Nicotine	N	20	19	20	19	20	19	20
	Mean	3.1250	90.8737	71.7500	33.1053	66.8500	1.7895	16.2000
	Median	2.7500	80.0000	72.5000	30.0000	70.0000	.0000	10.0000
	Std. Deviation	1.78351	80.51938	18.91498	19.36748	20.77264	3.86694	16.88849
Moderate	N	27	27	21	27	27	25	25
	Mean	2.7556	38.8444	53.8571	33.5926	48.7407	8.9200	22.9200
	Median	2.5000	30.0000	60.0000	40.0000	50.0000	.0000	20.0000
	Std. Deviation	1.27470	39.08569	21.03636	19.35152	22.69123	17.12678	21.60810
High Nicotine	N	31	31	28	28	27	25	29
	Mean	2.7613	35.0742	38.5536	37.6071	51.6296	.4800	3.5172
	Median	2.3000	18.0000	32.5000	40.0000	55.0000	.0000	2.0000
	Std. Deviation	1.64412	41.39548	28.46939	24.18959	24.41177	1.68622	3.41865
Total	N	78	77	69	74	74	69	74
	Mean	2.8526	50.1649	52.8333	34.9865	54.6892	3.8986	13.5000
	Median	2.5000	33.0000	60.0000	40.0000	60.0000	.0000	6.5000
	Std. Deviation	1.55327	57.33498	27.23005	21.13200	23.76997	11.10398	17.44129

Table 4.18 shows descriptive statistics (mean, median and standard deviation) of volume/ml, COUNT *million /ml, MOTILITY total %, G1 %, G2 %, G3 % and normal form according to three different nicotine levels.

Table 4.19. Kruskal-Wallis Test statistics for impact of nicotine levels on volume/ml, COUNT *million /ml, MOTILITY total %, G1 %, G2 %, G3 % and normal form.

Test Statistics [a, b]							
	volume/ml	COUNT *million /ml	MOTILITY total %	G1 %	G2 %	G3 %	normal form %
Chi-Square	.527	8.330	18.871	1.441	10.972	3.144	24.175
df	2	2	2	2	2	2	2
Asymp. Sig.	.768	.016	.000	.486	.004	.208	.000
a. Kruskal Wallis Test							
b. Grouping Variable: Nicotine Level							

A Kruskal-Wallis Test revealed no statistically significant difference in volume/ml, G1%, G3% levels across three different nicotine levels (Low nicotine, Moderate nicotine, and High nicotine), χ^2 (2, n = 78) = 527, p =.768, χ^2 (2, n = 74) = 1.441, p =.486 and χ^2 (2, n = 69) = 3.144, p =.208 respectively. Kruskal-Wallis Test, also, revealed a statistically significant difference in COUNT *million /ml, MOTILITY total % G2 %, and normal form levels across three different nicotine levels (Low nicotine, Moderate nicotine, and High nicotine), χ^2 (2, n = 77) = 8.330, p =.016, χ^2 (2, n = 69) = 18.871, p <.001, χ^2 (2, n = 74) = 10.972, p =.004 and χ^2 (2, n = 74) = 24.175, p <.001 respectively.

Table 4.20. Mann-Whitney U Test statistics of nicotine levels for COUNT *million /ml, MOTILITY total %, G2 % and normal form.

	COUNT *million /ml	MOTILITY total %	G2 %	normal form %
Test Statistics of Nicotine Levels (Low V. Moderate)				
Mann-Whitney U	147.500	73.000	122.500	227.000
Asymp. Sig. (2-tailed)	.015	.000	.001	.598
Z	-2.432	-3.593	-3.195	-.527
Test Statistics of Nicotine Levels (Low V. High)				
Mann-Whitney U	161.000	104.500	154.000	72.000
Asymp. Sig. (2-tailed)	.008	.000	.012	.000
Z	-2.669	-3.677	-2.515	-4.469-
Test Statistics of Nicotine Levels (Moderate V. High)				
Mann-Whitney U	393.500	200.500	321.000	138.000
Asymp. Sig. (2-tailed)	.697	.058	.450	.000
Z	-.390	-1.897	-.756	-3.921

A Mann-Whitney U Test revealed a significant difference in the COUNT *million /ml levels between low nicotine and moderate nicotine, U = 147.5, z = -2.432, p = .015, r = .36 and between low nicotine and high nicotine, U = 161, z = -2.669, p = .008, r = .38. The Mann-Whitney U Test, also, revealed a significant difference in the MOTILITY total % levels between low nicotine and moderate nicotine, U = 73, z = -3.593, p < .001, r = .56, between low nicotine and high nicotine, U = 104.5, z = -3.677, p < .001, r = .53, and between moderate nicotine and high nicotine, U = 200.5, z = -1.897, p = .058, r = .27. In the G2% results there was significant difference between low nicotine and moderate nicotine, U = 122.5, z = -3.195, p = .001, r = .47, and between low nicotine and high nicotine, U = 154, z = -2.515, p =.012, r = .37. Finally, there was a significant difference in the normal form% between low nicotine and high nicotine, U = 72, z = -4.469, p <.001, r = .64 and between moderate nicotine and high

nicotine, U = 138, z = -3.921, p < .001, r = .53. According to Cohen, 1988 **r** values of .3 represent a medium effect size and .5 represent a large effect size. Based on these results I conclude that nicotine has an impact on COUNT *million /ml, MOTILITY total % G2 %, and normal form levels.

4.3. DISCUSSION

4.3.1. XRD

Seven samples were taken from different types of cigarettes and were manually crushed and then sent to the laboratory for examination, and the results were as follows:

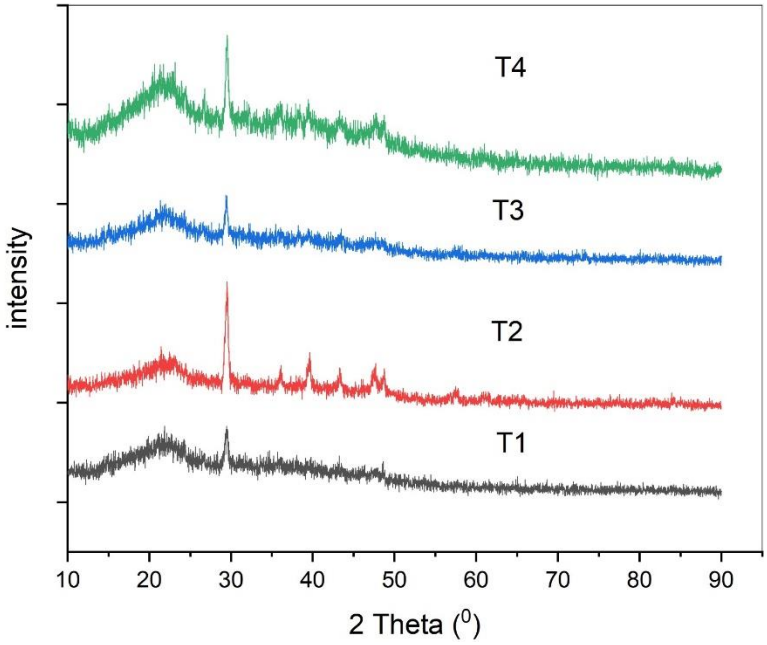

Figure 4.1. XRD Spectrum for the tobacco materials.

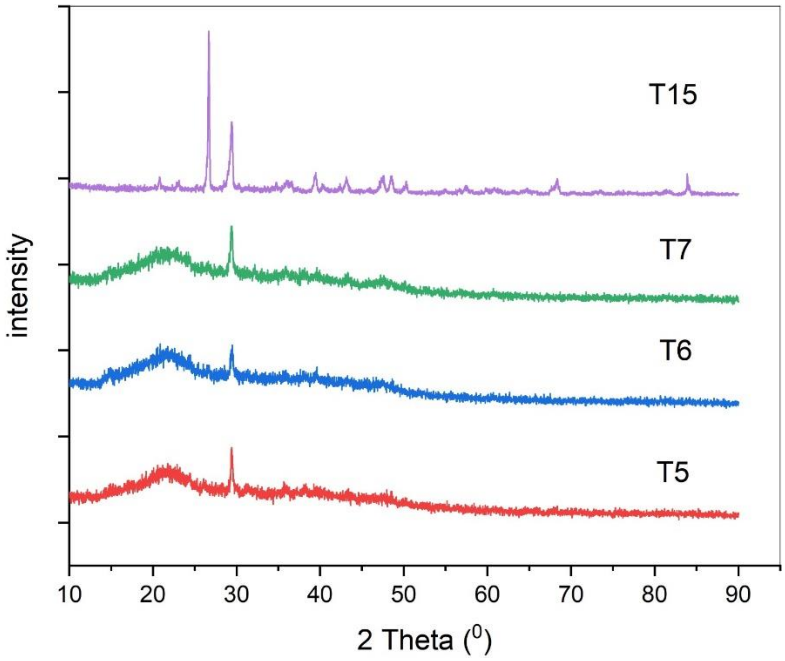

Figure 4.2. XRD Spectrum for the tobacco materials.

All of the tobacco's materials have amorphous structures due to the broad band as seen from the Figure 4.1 and Figure 4.2. All the structures of the tobaccos show the similar microstrucal distributions at room temperatures.

4.3.2. FTIR

Seven samples were taken from different types of cigarettes and were manually crushed and then sent to the laboratory for examination, and the results were as follows:

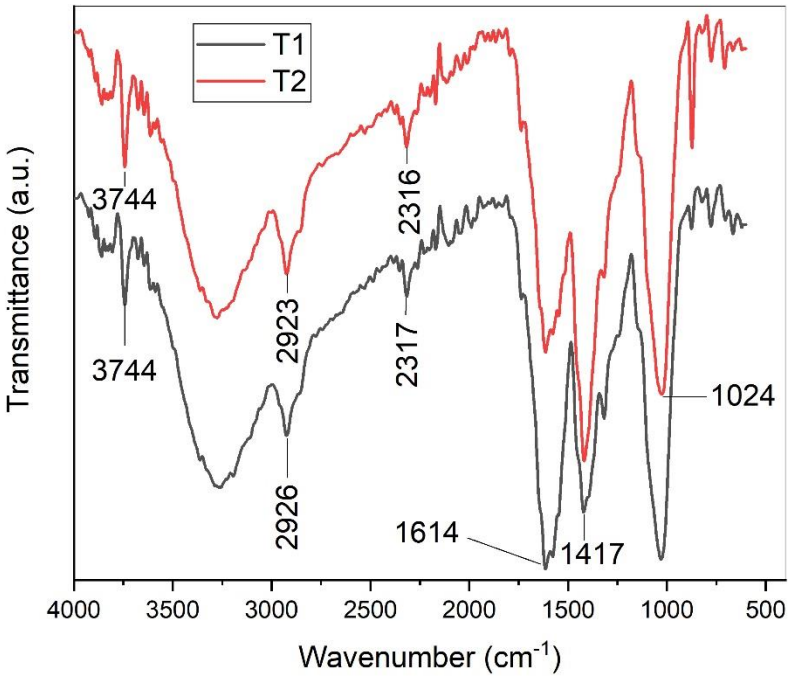

Figure 4.3. FT-IR Spectrum for the tobacco materials.

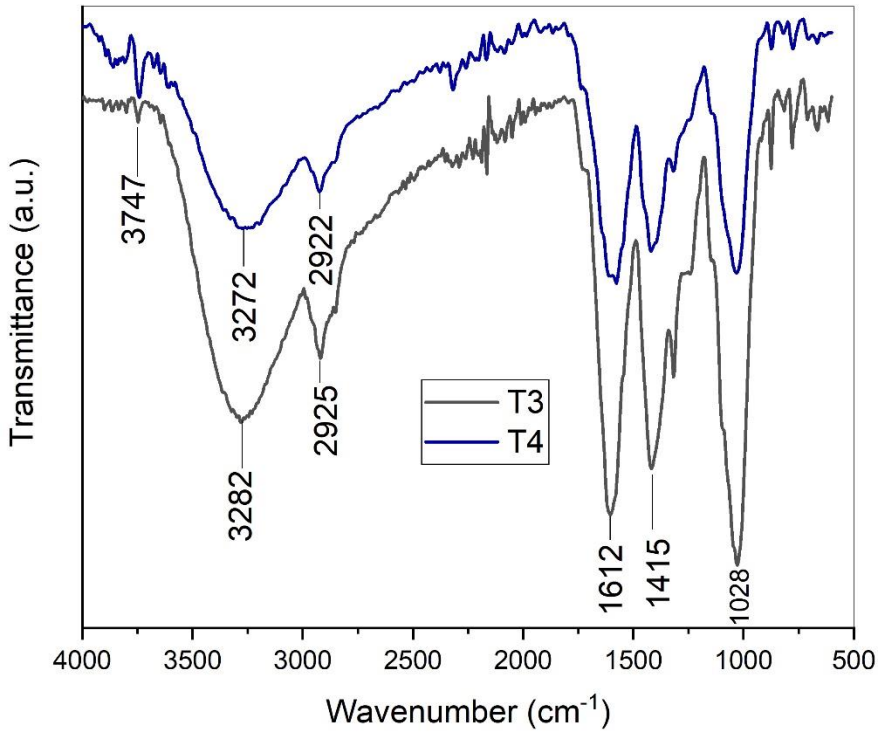

Figure 4.4. FT-IR Spectrum for the tobacco materials.

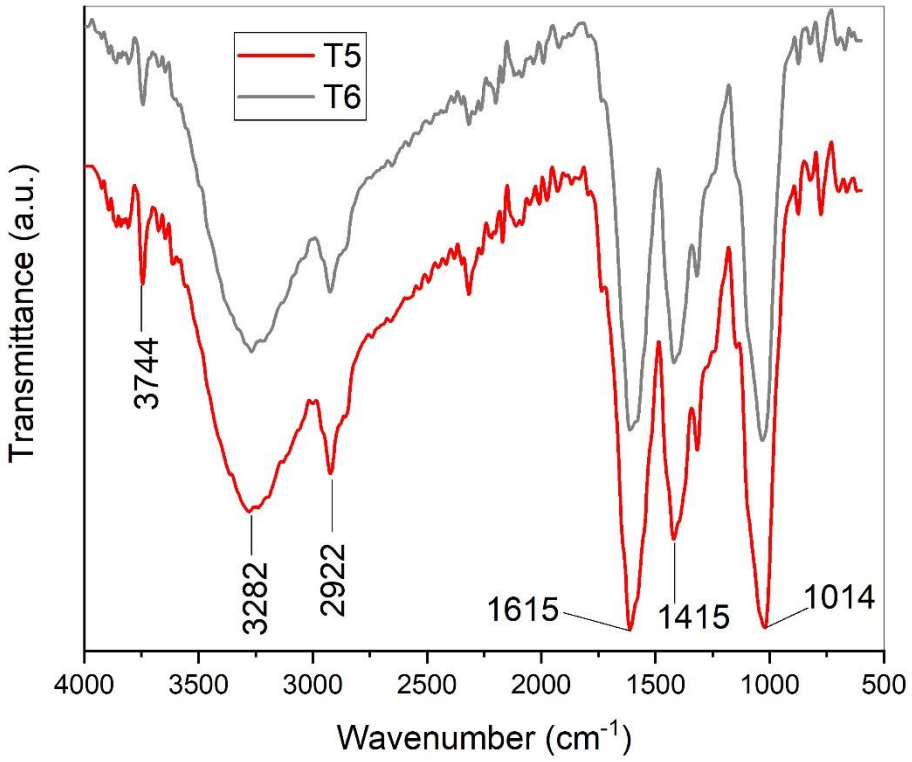

Figure 4.5. FT-IR Spectrum for the tobacco materials.

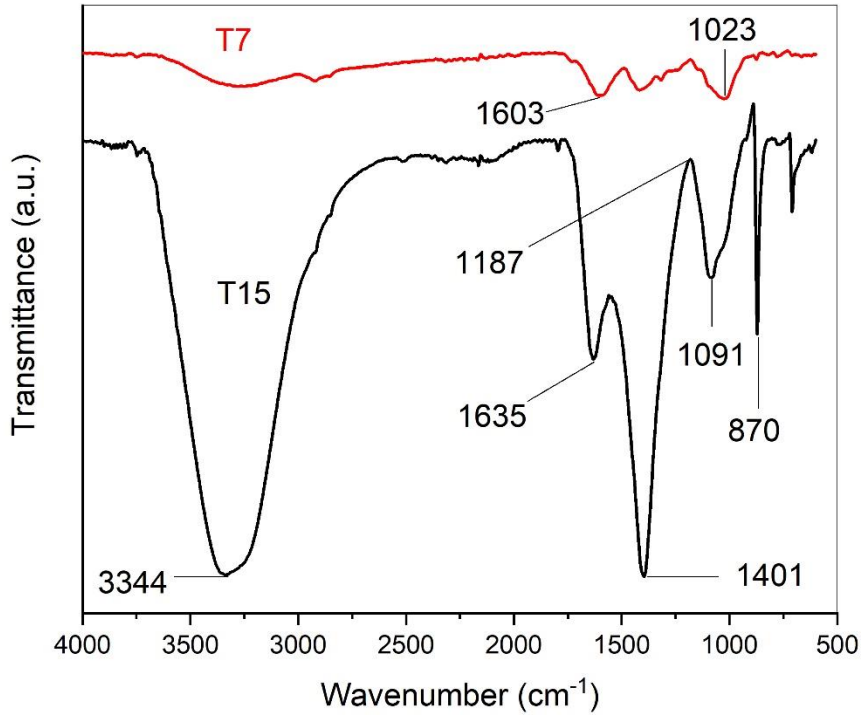

Figure 4.6. FT-IR Spectrum for the tobacco materials.

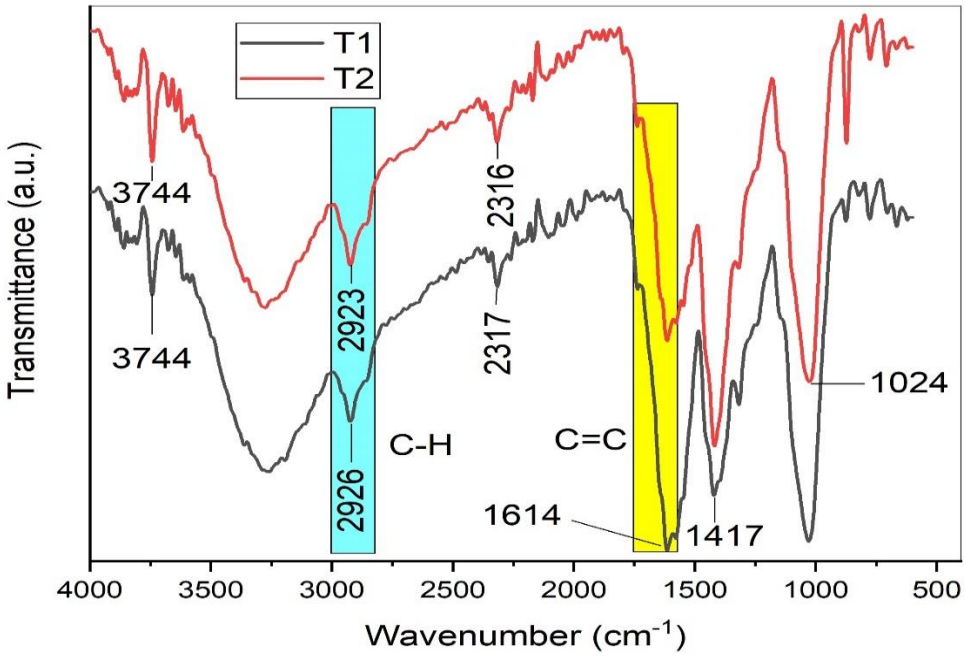

Figure 4.7. FT-IR Spectrum for the tobacco materials.

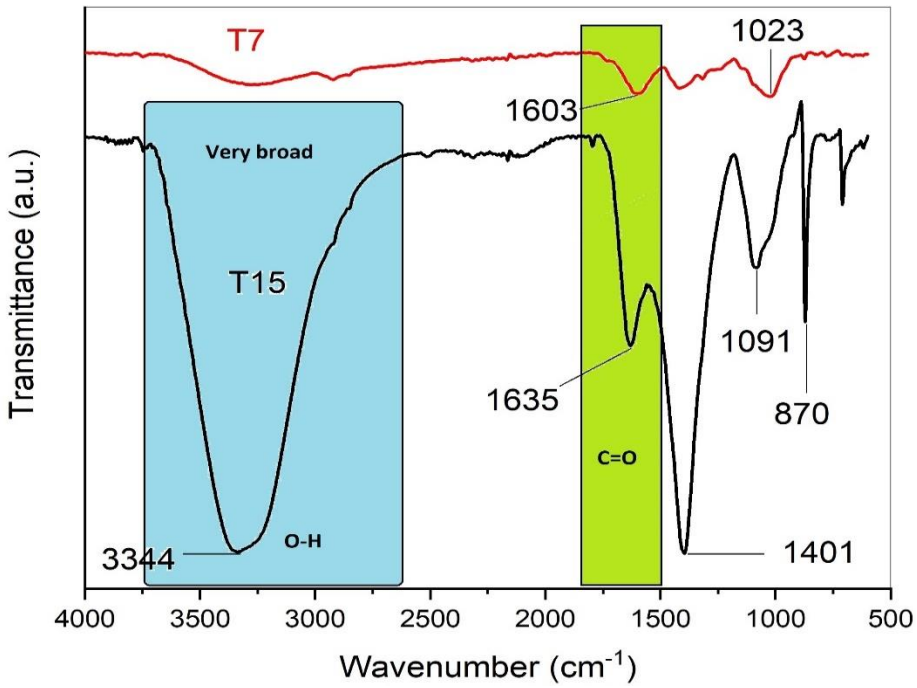

Figure 4.8. FT-IR Spectrum for the tobacco materials.

The FT-IR spectra shows the tobacco structures has a various absorption band at around different frequency. As seen from the Fig. 4.3 to Fig. 4.8, ft-ir has a broad bands frequency bbbbcm-1, ddcm-1, ppp cm-1, respectively. The ft-ir spectral Bands were not shifted for various tobacco materials might be they all have similar

microstrural phases. All samples have absorbtion between 2990 to 2850 which is C-H group, but in T15 the O-H group is not clear which appears at 3550 to 3200.

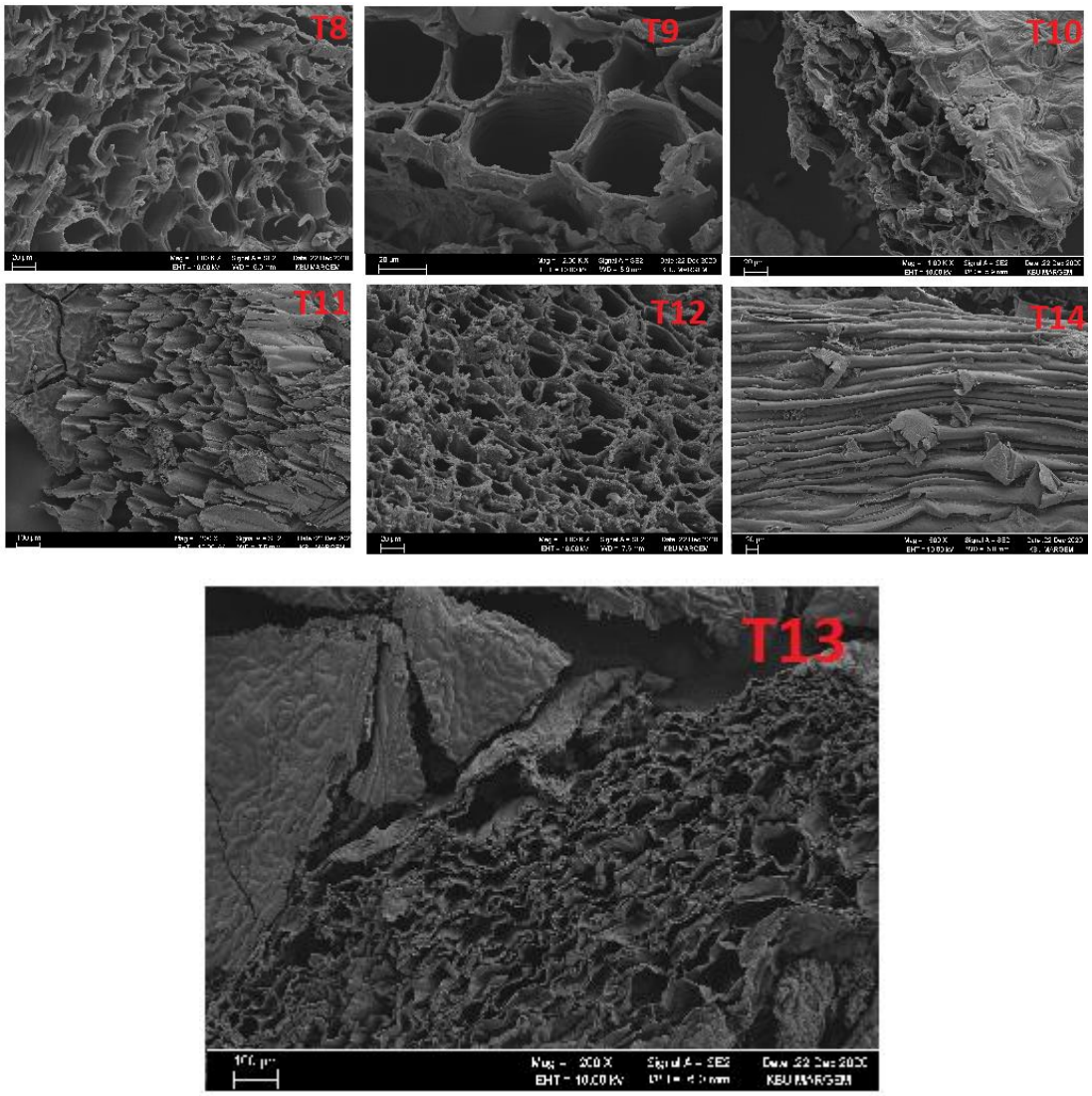

Figure 4.9. SEM morphologies for the tobacco materials.

The microstructure of the tobaccos as seen from the Fig. 4.9, shows structures for the different brands which were analyzed after the well powdered and all of them has a holly cylindrical honey styles as microtubes protected by the wall. The miscrostructure of the T15 tobacco materials has a crystalline structures which is different from other tobaccos. Due to the holly honey comb cylindrical tobaccos strictures probably increase the efficiency of the smokes during the firing and smokes clouds followed the way to go outside.

PART 5

CONCLUSION AND DISCUSSION

Throughout human history, tobacco has been used in different ways and for different purposes; consumption is widespread. Produced using all or part of the tobacco leaf as raw material; The substances consumed by smoking (drinking), chewing, sucking or sniffing are called "tobacco product". The main tobacco products used in different parts of the world are cigarettes, hookahs, pipes, cigars, bidi, snuf, snus, kreteks, guthka, rolled tobacco, chewing tobacco. The most widely used of these products is cigarettes, and therefore, while the data obtained in many studies on tobacco products are related to cigarette use; The words tobacco and cigarette are also often used interchangeably.

Using tobacco means being exposed to a lethal chemical mixture that will harm all of organs. There are more than 7000 toxic chemicals in this chemical mix, of which at least 70 are known carcinogens. Children of smokers have a higher chance of congenital abnormalities, cancer, lung illnesses and sudden death than nonsmokers' children. Smoking has been linked to new dangers such as renal failure, intestinal ischemia, and hypertension in the heart, according to recent research. Despite the fact that tobacco usage raises the danger of mortality and illness the more cigarettes you smoke, the more harm you do the less you smoke. A regular smoker in his life loses 10-11 years prematurely.

A smoker's risk of coronary heart disease is 2-4 times greater than someone who doesn't smoke. It is known that tobacco use can impair atherosclerosis, clot formation and blood flow in the veins, and this effect starts from the twenties. Damage or clot formation in cerebral vessels may cause stroke, and extremity veins may cause limb loss.

Lung cancer is the leading cause of cancer-related mortality for both men and women worldwide. Smoking is the leading cause of lung cancer-related death. Lung cancer is 20 times more likely in those who smoke. In addition, it raises the chance of developing oral, pharynx, larynx, and esophageal cancers as well as cancers of the stomach, colon, pancreas, kidneys, bladder, cervix, and breasts, as well as AML. About one-third of all human malignancies are caused by smoking.

In our study, semen analysis results were evaluated in infertile men due to cigarette use. Smoking addiction have many known negative effects on human health. It is known that increasing types of addiction have negative effects on fertility. It is thought that cigarettes, one of the most widely used and easiest to access tobacco products in the world, cause changes in the parameters used in semen analysis as a result of use. Throughout research; Among the couples who applied for infertility treatment, information about the age, body mass index, duration and type of infertility, smoking use of the male spouses were scanned. A urology consultation was made and semen analysis parameters such as hormonal evaluation, sperm count, volume and motility were evaluated.

The idea of the thesis came as a result of the significant increase in the number of infertility cases in Libya immediately after the events of the war. What is the reason for this problem, or what has changed in the Libyan state, causing these cases to rise? The reason is the smoke that enters Libyan territory illegally and without state control, and also its very cheap price, as it is sold in Libya at a price not exceeding 2 dinars, or the equivalent of $0.4 per pack. In this thesis, the smoke in Libya will be examined by taking 7 random samples, in addition to examining a type of weed called (Al-Nafeh) in Libya and its scientific name: Haloxylon.

At the same time, we made an official correspondence with the Misurata Infertility Center to obtain a set of samples from smokers and non-smokers who had problems in semen analysis.

The importance of the research lies in knowing the extent of the effect of smoking on semen, or whether the smoke in Libya is unfit for use and has an effect in increasing the number of infertility cases in Libya.

One of the difficulties that we encountered during the research is the presence of a group of smokers who use different types of smoke, as well as some of them suffer from varicoceles, which have a major role in the occurrence of infertility or delayed childbearing.

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RESUME

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