EFFECT OF *SHISHA* (WATER-PIPE) SMOKING ON HUMAN SERUM LIVER ENZYMES, LIPID PROFILE AND ANTIOXIDANT VITAMINS AMONG SMOKERS IN KANO METROPOLIS

BY

IBRAHIM HARUNA MUHAMMED

SPS/17/MBC/00027

BEING A DISSERTATION SUBMITTED TO THE DEPARTMENT OF BIOCHEMISTRY, FACULTY OF BASIC MEDICAL SCIENCES, BAYERO UNIVERSITY, KANO IN PARTIAL FULFILMENT OF THE AWARD OF MASTER OF SCIENCE IN BIOCHEMISTRY (MEDICAL BIOCHEMISTRY).

AUG, 2021

DECLARATION

I hereby declare that this work is the product of my research efforts undertaken under the supervision of Prof. M. K. Atiku and has not been presented anywhere for the award of degree of MASTER OF SCIENCE in BIOCHEMISTRY (medical). All Sources have been duly acknowledged.

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Ibrahim Haruna Muhammed

SPS/17/MBC/00027

CERTIFICATION

This is to certify that the research work for this dissertation and the subsequent write up of this report by 'Ibrahim Haruna Muhammed SPS/17/MBC/00027' were carried out under my supervision.

_____

Prof. M. K. Atiku

Supervisor

APPROVAL

This dissertation has been examined and approved for the award of the degree of MASTER OF SCIENCE in BIOCHEMISTRY (Medical).

EXTERNAL EXAMINER	**DATE**
Malam Murtala Ya'u **INTERNAL EXAMINER**	**DATE**
Prof. M. K . Atiku **SUPERVISOR**	**DATE**
PROF A.J. ALHASSAN **HEAD OF DEPARTMENT**	**DATE**

ACKNOWLEDGEMENTS

To the Almighty Allah, The all knowing whose infinite unearned grace turned this project into reality.

My special appreciation of kindness goes to my supervisor Prof. M K Atiku for his tremendous and continuous guidance and suggestions from the beginning to the completion of this work, May almighty Allah rewards him abundantly.

Also my sincere appreciations goes to Dr. Bashir Isa Waziri of Physiology Department Bayero University Kano for his tireless effort toward the completion of this work may almighty Allah reward him abundantly.

My sincere appreciation goes to the Head of Department and the entire staff of Biochemistry Department for their support and encouragement throughout my programme.

My special appreciation of kindness goes to my supervisor Malam Murtala Ya'u for his tremendous and continuous guidance and suggestions from the beginning to the completion of this work, May almighty Allah rewards him abundantly.

I also acknowledge and appreciate the effort of my guardians Dr. Nafiu Yakubu Muhammed and Dr. Jinaidu Yakubu Muhammed for their supports morally and financially may almighty Allah reward them abundantly.

Finally, I want to express my sincere gratitude and appreciation to my friends: Sulaiman Kabir, Aisha Shehu and Abbas Sani.

DEDICATION

I dedicate my work to my Late father Malam Muhammad Ala Bello may his gentle soul rest in perfect peace and may almighty Allah grant him Jannatul Firdausi “amen”. I will never forget your love and support, you will forever remain in my memory.

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ABSTRACT

Shisha Smoking is a risk factor for liver and coronary heart diseases. It contains large amount of chemicals which are capable of generating reactive oxygen species which play an important role in oxidative stress which in turn leads to the development and progression of many disorders such as hypertension, cancer, diabetes mellitus and cardiovascular diseases. Monitoring the activities of serum liver enzymes (AST, ALT and ALP), lipid profile (HDL-cholesterol, LDL-cholesterol and triacylgycerides) and antioxidant vitamins (A, C and E) levels is very important to give an insight on the effect of shisha smoking on liver and lipid profile. This study aimed to determine the effect of shisha smoking on serum liver enzymes, lipid profile, and antioxidant vitamins in fifty (50) apparently healthy shisha smokers (exposed group) in Kano metropolis. A corresponding fifty (50) apparently healthy non-smokers were used as controls (non exposed group). The shisha smoking was significantly associated with increased levels of serum liver enzymes (ALP, ALT, and AST), total cholesterol (TC), triglyceride, LDL-cholesterol ($p>0.05$) in smokers compared to control group. However, there was a significant ($p>0.05$) decreased in HDL-cholesterol and serum antioxidant vitamins (A, C and E) in exposed group compared to non-exposed group. The results of this study also indicated that exposure of human being to shisha smoke over a period of time causes slight increased in some liver function indices, lipid profile and antioxidant vitamins but the relation was statistically not significant ($p>0.05$). The findings suggested that shisha smoking causes damage to the hepatocytes, dyslipidaemia and oxidative stress.

CHAPTER ONE

INTRODUCTION

1.1 Tobacco Smoking.

Tobacco smoking is a global epidemic phenomenon especially in developing countries, and considered as the earliest example of a noninfectious disease that causes preventable deaths in the world. It has been estimated that tobacco smoking is responsible for killing more than six million people in 2010 (WHO, 2014). Aden *et al.,.*(2013) believed that it is expected that by 2030 the death will exceed million per year in developing countries. There are different methods to smoke tobacco including cigarettes, cigars, chew, pipes or water pipe. Water-pipe smoking and cigarette smoking are currently considered a fashionable way of tobacco leaves consumption, especially among young and middle aged males and females (Aden *et al.,* 2013).

Primarily, there are two types of narghile tobacco mixtures: the flavored one could be either *moassel* (also known as *tobamel*) or *jurak*, and the unflavored type called *tumbak* (or *ajamy*). The tobacco used in water-pipe system (WPS) typically weighs 10 to 20 g. "*Muessel*" or "*maassel*" (literally, 'honeyed') contains 30% tobacco and 70% honey or molasses (treacle). *Tumbak* or *ajami* is a pure, dark paste of tobacco. *Jurak,* mainly of Indian origin, is an intermediate form that often contains fruits or oils but that may also be unflavored. *Muessel* is usually flavored with apple, mango, banana, strawberry, orange, grape, mint, cappuccino, or other additives. It is generally sold in cardboard boxes or plastic jars decorated with fruit or alcohol are often added to the tobacco (Melissa *et al.,* 2011). Furthermore, smoking is considered the second leading cause of death and the fourth major risk factor for disease worldwide (WHO, 2005). It has been estimated that tobacco contains more than 400 chemical compounds of which many compounds are toxic and tumorigenic in nature (Shevchenko, 2012). It is believed that nicotine, the chemical agent of

cigarette smoking, is beyond the behavior of addiction, and with other chemicals participate to smoking related diseases (Shihadeh and Saleh, 2005).

Even though hookah has been present for a millennium, far less studies have examined its chemical constituents/ air quality relatives to cigarette. With tobacco being the main source of smoke in both hookah and cigarettes, hookah users are exposed to many of the same toxic compound/ by products as cigarette users but at dramatically higher levels, which might in fact produce worsened health effects in users (Eissenberg and Shihadeh, 2009). According to the size of the heads of the water-pipe, there are two kinds: large or small. The large head holds nearly 20 g of tobacco, the small head contains about 10 g of tobacco. Smoking1 g of Ajami produces 35.65 mg (range of 30.0-413 mg) of nicotine,but 1 g of *Maassel* produces 3.35 mg (range of 1.8-6.3 mg) of nicotine (Hadidi and Mohamad, 2004). *Maassel* use contains small but not negligible amounts of the addictive substance nicotine (Shafagoj and Mohamad, 2002). Moreover, the World Health Organization study group on tobacco product regulator (Tob Reg)(2006) stated that heat sources are coomonly to burn the tobacco, such as wood cylinders or charcoal, are likely to increase health risks since when such fuels combusted produce toxicants, including high levels of carbon monoxide, metals and cancer-causing chemicals (Pryor and Stone, 1993) . It has been reported that one puff of a cigarette exposes the smoker to more than 10^{15} free radicals and other oxidants. Free radicals are highly unstable molecules that are naturally formed during energy metabolism or sometimes, the biological system can be exposed to these radicals from avariety of environmental sources, such as Cigarette smoke, air pollution, and sunlight among others (Bensasson *et al.,* 1993; Droge, 2002; Valko *et al.,* 2007). Free radicals can cause “oxidative stress,” a process that can trigger cell damage. Oxidative stress is thought to play a role in a variety of diseases including Cancer, cardiovascular diseases, diabetes,Alzheimer’s disease, Parkinson’s disease, and eye diseases such as cataracts and age related muscular degeneration (Klein *et al.,* 1998; Gandhi *et al.,* 2009; Vardavas *et al.,* 2012).The defence system

against radicals consists of enzymatic and non-enzymatic; essential and nonessential; endogenous and exogenous radical scavengers; Antioxidant vitamins with cigarette smoking (Eduardu*et al.,* 1999). Vitamin A, C and E are among the essential free radical scavengers, hence there are called antioxidants (Burton *et al.,* 1982; Halvevy and Sklan, 1987).Vitamin E is a major lipophilic antioxidant that inhibits free radical mediated peroxidation in cell membrane (Wallstrom *et al.,* 2001; Sahin *et al.,* 2001; Veysel *et al.,* 2013). Vitamin A is thought to function as an electron scavenging antioxidant and as a precursor of retinol (Wallstrom *et al.,* 2001; Gallicchio *et al.,* 2008). Vitamin C is a radical scavenger that has an important role in maintaining cellular functions and differentiation (Greg, 2003; Faure *etal.,* 2006). The concentrations of these essential antioxidants, in contrast to those of other antioxidant defence systems, are determined mainly by dietary intake (Eduardu *et al.,*1999; Greg, 2003; Walter, 2010). Smokers usually have poor eating habits, consuming less fruits and vegetables, and thus, may ingest lower levels of essential antioxidants (Wallstrom *et al.,* 2001; Young and Woodside, 2001).

1.2 Statement of the Problem

Water-pipe smoking has been practiced extensively for over 400 years and it has been referred by public health officials as a global tobacco epidemic (Chaouachi, 2009). In Nigeria*, shisha* smoking is common among youths as it has been the simplest way of tobacco smoking.

Evidences have shown that *shisha* smoking is associated with several health effects. The most commonest hazards associated with *shisha* smoking is exposure to nicotine, carbon monoxide and heavy metals which are lethal on different body organs and system such as liver, respiratory system, cardiovascular (WHO, 2014)**.**

Despite the health implication, its prevalence and popularity are increasing especially among youth in Nigeria (WHO, 2014). Researches on the use and health effects of *shisha* smoking on serum enzymes, lipid profile and antioxidants vitamin in this environment are scanty.

1.3 **Justification**

Understanding and having knowledge of the effect of *shisha* smoking among youths will help in generating data to be used in the prevention and timely intervention of *shisha* smoking. In the north western part of Nigeria, particularly kano state the rate of *shisha* smoking among youths is alarming and there is no data indicating the effect of *shisha* smoking on human serum liver enzymes, lipid profile and antioxidant vitamins, so there is need for the assessment of these biochemical parameters (serum liver enzyme, lipid profile and antioxidant vitamins) among young *shisha* smokers in our environments.

1.4 AIM AND OBJECTIVES

The aim of this research is to determine the effect of *shisha* smoking on serum liver enzymes, lipid profile and antioxidant vitamins in human subjects (males and females) in Nasarawa, kano Municipal, Tarauni, Ungogo Dala kumbotso , fagge and Gwale local governments of kano State.

OBJECTIVES:

The specific objectives include

I. To determine the serum activities levels of liver enzymes Alkaline Phosphatase (ALP), Alanine aminotransferase (ALT) and Aspartate aminotransferase (AST) of *Shisha* smokers and non-smokers.

II. To determine the serum level of Total cholesterol (TC), HDL-Cholesterol, LDL- Cholesterol, and Triglycerides in the serum of *shisha* smokers and non-smokers.

III. To determine the serum level of natural antioxidants vitamins (A, C and E) in *Shisha* smokers and non-smokers.

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 HISTORICAL BACKGROUND OF SHISHA SMOKING

The use of tobacco is steadily increasing and the most common form is cigarette smoking (Poyrazoglu, *et al.,* 2010). However, there are several other ways to use tobacco and one of them is hookah smoking (water-pipe smoking, *Shisha*, hubble-bubble, narghile, *goza,* Indian water-pipe). Attention has been focused on cigarette smoking, hookah smoking has lesser emphasis (Ahmed *et al.,* 2011) and also it has not been governed by any law. According Chaouachi (2009), Water-pipe smoking has been practiced extensively for over 400 years and It has been referred by public health officials as a global tobacco epidemic. There are 100 million daily shisha smokers worldwide (Poyrazoglu *et al.,* 2010). It is common practiced in the Arabian Peninsula, China, India, Pakistan, Bangladesh, Turkey, Europe and North America. Although, the users believed hookah smoking to be less addictive and less harmful to health than cigarette smoking, the researchers draw diametrically opposite conclusions (Martinasek, 2011). A sharp increase in the popularity of the water-pipe use has been noted in recent years (Radwan *et al.,* 2003). Water-pipe terminology can depend upon the region and includes names such as "*shisha"*, "*boory*" or *"goza"* in Egypt and Saudi Arabia (El-Hakim *et al.,*1999); "*narghil*e" or "*arghile*" in Jordan, Lebanon and Syria (Varsano *et al.,* 2003) and "hookah" in Africa and India. Besides terminology, there is also regional variation in shape, size and appearance of tobacco smoked (Radwa *et al.,* 2003). In this study the term "water-pipe" refers to tobacco use methods in which smoke passes through water. Generally, water-pipes consist of a head, body, water bowl and hose (Chaouachi, 2006.). Tobacco is placed in the head and often covered with perforated aluminium foil,burning charcoal is placed on top of the foil. Water half-fills the bowl,

submerging a tube through which smoke enters but not the hose-connected tube through which smoke leaves. Thus, an inhalation at one end of the hose produces a vacuum in the air-filled space of the water bowl, causing smoke to pass through the water (producing bubbles and the onomatopoetic moniker "hubble bubble") into the hose-connected tube and to the smoker. Disposable plastic mouthpieces can be added to limit the spread of disease (Chaouachi, 2006). The most common type of tobacco used in the water-pipe is called *Maassel* (Sajid *et al.,*2008), which is sweetened and flavoured (forexample, apple, mint and cappuccino). Other forms of tobacco may contain less sweeteners or flavours and are called Ajami, Tumbâk or Jurâk (El-Setouhy *et al.,*2008).

According to the size of the heads of the water-pipe, there are two type: large or small. The large head holds nearly 20 g of tobacco, the small head contains about 10 g of tobacco. Smoking 1 g of Ajami produces 35.65 mg (range of 30.0-413 mg) of nicotine, but 1 g of Ma'ssal produces 3.35 mg (range of 1.8-6.3 mg) of nicotine (Al-Fayez *et al.,* 1988). *Maassel* use contains small but not negligible amounts of the addictive substance nicotine (Daher *et al.,*2010). Moreover, the World Health Organization(2005) study group on tobacco product regulator (TobReg) 24 states that "commonly used heat sources to burn the tobacco, such as wood cylinders or charcoal, are likely to increase health problems when such fuels combusted produce toxicants, including high levels of carbon monoxide, metals and cancer-causing chemicals.

The misperceptions of safety, affordability, parental acceptance and practices of parents smoking along with them, reduces the impact of hookah smoking as a health risk behavior (Knishkowy and Amitai, 2005) among the youth. Cigarette smoking produces 500-600 ml of smoke whereas one hookah session produces 50,000 ml of smoke (Maziak, 2009). A study has demonstrated that

numerous toxic agents, including carcinogens, heavy metals, particulate matter and high levels of nicotine, are efficiently delivered through hookah smoking (Martinasek, 2011).

2.1.1 *SHISHA* SMOKING

Shisha smoke contains large quantities of flavoured nicotine, carbon monoxide, polycyclic aromatic hydrocarbons, volatile aldehydes, phenolic compounds, carcinogenic poly aromatic hydrocarbon (PAH), and heavy metals, including arsenic and lead (Aslam *et al.,* 2014). Waterpipe smoke and cigarette smoke contain the same toxic and carcinogens (Aslam *et al.,* 2014). And these chemicals undergo complex interactions with human biological systems.

2.1.2 HEALTH IMPLICATION OF *SHISHA* SMOKING

Smoking *shisha* is associated with three main detrimental health effects: cardiovascular damage, infection and cancer formation. Much of the morbidity and mortality associated with *shisha* smoking can be attributed to impairment of the cardiovascular system.

Shisha smoking has been reported to disrupt the autonomic regulation of the cardiac cycle, by causing an acute reduction in heart rate variability.(Cobb *et al.,* 2012). This may be associated with an increased susceptibility to arrhythmia, systemic inflammation and risk of coronary heart disease (Cobb *et al.,* 2012).

A positive association between coronary artery disease and *shisha* smoking was reported although the study did not demonstrate statistical significance (Eissenberg and Shihadeh, 2009).

Interestingly, serum concentrations of high-density lipoprotein (HDL)-cholesterol and apolipoprotein (apo) A-1 were found to be significantly lower in shisha smokers than in non-smokers (Al Numair, 2007). Moreover, low-density lipoprotein (LDL)-cholesterol, apo B, triglycerides and malondialdehyde were significantly higher in shisha smokers than in non-

smokers (. Al Numair, 2007). Total natural antioxidant vitamins were also found to be significantly lower in tobacco smokers than in non-smokers (Al Numair, 2007). These findings may implicate shisha smoking as a risk factor for coronary heart disease.

Blachman et al., (2014) reported that there is a substantial risk of infection with herpes, hepatitis and tuberculosis (TB) after smoking shisha. Often, *shisha* is smoked in large groups, rather than as individuals By sharing mouthpieces, various commensal and pathogenic organisms may be transmitted between the smokers through saliva (Blachm *et al.,* 2014). Recently, *shisha* cafes provide a plastic disposable mouthpiece to every customer, which aims to limit the spread of communicable diseases. The risk of infectious disease also increases due to the moist nature of *shisha* molasses, creating an environment that promotes growth of many different microorganisms (Blachman *et al.,* 2014). Although well-run *shisha* cafes regularly wash their shisha pipes, the relatively rigid and complicated structure of the shisha apparatus makes it virtually impossible to efficiently wash the internal parts (Blachman *et at* ., 2014). For example, tuberculosis may grow and survive on the internal surface of the shisha pipe and water, significantly increasing risk of transmission *(http://www.quitshisha.com/*). In the Middle East, outbreaks of infectious disease have been correlated with shisha smoking (Akl *et al*., 2010). Two outbreaks in 2010, revealed a possible association between TB and sharing a shisha pipe (Akl *et al.,* 2011).

Similarly, cigarette smoking, shisha smoking introduces many harmful chemicals and free radicals into the body, many of which have been contributed in cancer development. For example, one study revealed that smoking shisha quadruples the risk of lung cancer, when compared to non-smokers (Akl *et al.,* 2010). Other studies also reported that there was an association of shisha smoking with bladder cancer, prostate cancer, squamous cell carcinoma and

keratoacanthoma of the lip, nasopharyngeal cancer, oesophageal cancer and oral dysplasia (El-Hakim and Uthman, 1999). How ever these associations were not statistically significant.

Harmful effects of water pipe tobacco smoking on lung functions are similar to those of cigarette smoking (Daher *et al.* ,2010). It has already been shown that the risks to develop COPD, cardiovascular problems, lung cancer and many other types of cancers are not less than cigarette smoking.

There is a misperception that the water part of water pipe equipment filters the tobacco tar completely, thus presumably making this kind of smoking harmless. This is totally wrong and the scientific data show that it is at least as harmful as cigarette smoking (One session of water pipe smoking lasts around one hour, and the users take very deep breaths compared to cigarette smoking while inhaling it). Although there is a big variation between their results, the studies revealed that a single session of waterpipe tobacco smoking may be equal to smoking 20-200 puffs of cigarettes (Shihadeh *et al.,* 2004).

The use of *shisha* has been made fashionable by adding flavours such as apple, grapes and mint (Murtaza *et al.,* 2015). The use of flavours makes it have a pleasant smell unlike cigarettes.

The health effect of *shisha* has been documented in several studies. Tobacco which is the main ingridient of shisha contains over 4800 different chemicals, of which 69 are carcinogenic (Sajid *et al.,* 2008). Indeed, smoking *shisha* causes a wide range of cancers including oral cancer, lung cancer and stomach cancer among others (Alk *et al.,* 2010). The World Health Organization (WHO, 2005) notes that contrary to popular believe, the smoke of water- pipe contains toxicants known to cause cancer, heart disease and other diseases. WHO further reported that the intensity of smoking *shisha* is higher than that of smoking cigarette considering that cigarette smokers typically take 8-12, 40-75 ml puffs over about 5-7 minutes and inhales 0.5 to 0.6 litres

of smoke. In contrast *shisha* smoking sessions typically take 20 to 80 minutes during which a smoker inhale 50- 200 puffs which range from about 0.15- 1 litre each. WHO (2005) reported that a *shisha* smoker inhales an equivalent of 100 cigarettes. A similar view is held by the British Heart Foundation. Due to the mode of smoking shisha and the frequency of puffing, the lengh of the smoking sessions, *shisha* smokers absorb higher concentration of toxins found in tobacco thereby increasing the hazards to the body.

It is confirmed that like cigarette, *shisha* contains nicotine, tat, carbon monoxide and heavy metals such as arsenic and lead. As a result, shisha smokers are at risk of the same kind of diseases as cigarette smokers that include heart diseases, cancer, respiratory diseases and problems during pregnancies. According to The Center for Disease Control and Prevention, *shisha* tobacco and smoke contains many toxic agents that can cause clogged arteries and heart disease

According to the American Lung Association (2011) *shisha* is typically smoked in groups with the same mouth piece passed from person to person. This is very likely to pass infections from one smoker to another. Although disposable mouth pieces are available but they are scarcely used therefore infections spread unabated.

2.2 LIVER ENZYMES

There are many organs in Human body which are not in direct contact with tobacco smoking but they are affected greatly, One of these organs is the liver which is very important for the metabolism, storing the Glycogen and it is also important for the process of eliminating the harmful compounds, alcohol, toxic compounds, and drugs from the Human body (Pessione, 2001).

The liver has the capability to store glucose as glycogen. An association exists between patient that has been smoking and liver injury including fatty liver fibrosis and cirrhosis (Zein *et al.,* 2011).

Serum level of liver enzymes increased according to the damage of the liver cell, these enzymes include transferase enzymes, Aspartate transferase (AST), Alanine transferase (ALT) and Alkaline phosphatase (ALP).

Alkaline phosphatase is the most frequently measured indicator for liver bile ducts disease (American Gastroenterological Association, 2002). AST and ALT enzymes frequently appear in the serum following liver cell injury or sometimes in smaller amounts from degraded cells (Raja *et al.,* 2011). The liver has a central and critical biochemical role in metabolism, digestion, detoxification and blood from intestinal tract initially passes through the liver.

The enzyme that is important for producing energy is Alanine Aminotransferase (ALT), this enzyme is mainly found in the liver and other tissues beside the liver but in less concentrations than its concentration in the liver, it is found for example in the heart and the skeletal muscles. This enzyme beside other liver enzymes can be used for the detection of liver disorders, for example in the diagnosis of hepatitis and cirrhosis (Aubin, 2012).

Elevated liver enzymes may indicate inflammation or damage to cells in the liver (Farrell and Larter, 2006).

Most people are well aware about the effects of smoking on the heart and lungs. However what you may not know is that smoking *shisha* can also severely affect your liver, the numerous toxins found in tobacco smoke lead to chronic inflammation and scarring in the liver, which in turn, increases your risk for liver damage including diseases such as Hepatitis B, and C, liver cancer and liver fibrosis (Karl and Johnson, 2010). Additionally smoking affects the way your

liver processes alcohol and medications, which can increase your risk for alcoholism as well as your overall drug and alcohol tolerance level (Jang *et al.,*2012).

Morality risk and the death risk in the smoking individuals are depending on how long the persons are exposed to the tobacco smoke and how many times they smoke every day (Rahmioglu, 2009).

the elevation in the levels of ALT and AST because of the effectiveness of the tobacco smoke and its harmful chemical compounds on liver cells that lead to the liver cell secretion to the ALTand AST enzymes through inflammatory pathways (Farsalinos, 2013).

Several investigations concerning osteoporosis proved the increasing levels of the ALP in blood serum in tobacco smokers, and this can be used as a marker for the Bone Turnover disease (Bertholon, 2013).

2.2.1 Liver Enzyme Tests

Alanine Transaminase (ALT) is an enzyme mainly found in your liver. The ALT test measures the level of ALT in your blood. Consistently high levels of ALT in your blood can be a sign of liver damage. It catalyzes chemical reactions by transfer the amino group from alanine to 2-oxoglutrate forming pyruvate and glutamate.

Aspartate Transaminase (AST) is an enzyme found in large amounts in your liver and other parts of your body. The AST test measures the level of AST in your blood. High levels of AST can be a sign of liver damage. Alkaline Phosphatase (ALP) is an enzyme found in large amounts in your liver, bile ducts, and other parts of your body. The ALP test measures the level of ALP in your blood. High levels of ALP can be a sign of liver or bile duct damage.

2.3 LIPID PROFILE

This is a group of tests that are often ordered together to determine risk of coronary heart disease. They are tests that have been shown to be good indictors of whether someone is likely to have heart attacks or stroke caused by blockage of blood vessels or hardening of the arteries (atherosclerosis).

Lipoprotein is small spherules that transport fats in the body and consist of protein, cholesterol, triglycerides, and phospholipids. The term ‘’good’’ and ‘’bad’’ cholesterol refers to high density lipoprotein (HDL-cholesterol) and Low density lipoprotein (LDL-cholesterol), respectively. High level of LDL-cholesterol is associated with coronary atherosclerosis, whereas high levels of HDL-cholesterol appear to protect against cardiovascular diseases (Vance and Vance, 2002)

2.3.1 CHOLESTEROL

Despite its poor reputation, cholesterol is a naturally occurring, fatty substance in our bodies that is produced by the liver and helps with hormone production and food digestion. Cholesterol moves through the bloodstream inside two distinct proteins that work in tandem (Huff and Jialal, 2019)

Low-density lipoprotein (LDL-cholesterol), the so-called "bad cholesterol," delivers cholesterol throughout the body(Ference *et al.,* 2017), and high-density lipoprotein (HDL-cholesterol) known as "good cholesterol," collects fatty deposits and returns them to the liver. (Kosmas *et al.,* 2018). To maintain a healthy heart, the American Heart Association recommends keeping LDL-cholesterol levels below 100 mg/dL, HDL-cholesterol levels above 40 mg/dL, and below 200 mg/dl for total cholesterol *(National cholesterol education program,* 2005)

Eating too many high-fat foods can tip this balance, and recent research suggests smoking can as well. Acrolein interferes with the cleansing ability of HDL by attacking the protein (Chadwick, *et al.,* 2015)

Cardiovascular disease (CVD) is a class of diseases that involve the heart, and/or blood vessels (arteries, capillaries, and veins). It involves diseases that affect the cardiovascular system, primarily cardiac diseases, vascular diseases of the brain, kidney, and peripheral arterial disease (Amusa, 2002). Currently,coronary heart disease and stroke are the leading causes of morbidity and a leading contributor to mortality worldwide (WHO, 2013). CVD prevalence is stable in the developed countries and rapidly raising in the developing countries (Alberti, 2001). According to World Health Organization statistics, approximately 80% of deaths caused by CVD occurred in developing countries (Gaziano, 2007). Dyslipidemia, hypertension, obesity, insulin resistance, and hyperhomocysteinemia are the major CVD risk factors (Dzau and Braunwald , 1991).

Dyslipidemia, which is a very well established risk factor of CVD, is characterized by elevation of plasma total cholesterol (TC), low density lipoproteins (LDL-cholesterol), and triacylglycerides (TAGS); and decrease of plasma high density lipoprotein (HDL-cholesterol) concentrations. Several studies reported a relationship between tobacco use and dyslipidemia(Kimijima *et al.,* 2011).

Smoking is associated with a more atherogenic lipid profile(Gossett and Johnson, 2009). It increases the concentration of serum total Cholesterol, triglycerides, LDL-Cholesterol, VLDL-Cholesterol and decreases the level of good Cholesterol i.e., HDL-Cholesterol (Gepner *et al.,* 2011). Thus, smoking is a major risk factor for atherosclerosis and coronary artery disease (Fagerström, 2002). Various mechanisms leading to lipid alteration by smoking are: (a)nicotine results in increased secretion of hepatic free fatty acids and triglycerides along with VLDL-C in

the blood stream by increasing the secretion of catecholamines and thus stimulating sympathetic adrenal system resulting in increased lipolysis (Simons *et al.,* 1984); (b)consumption of a diet lacking in fibre and cereal content but enriched with fat and cholesterol by smokers as compared to non-smokers (Wynder *et al.,* 1989); (c) cigarette smoking is known to be associated with raised plasma Homocysteine level (. Pagán *et al.,* 2001) which causes oxidative modification of LDL-Colesterol and decreases HDL-Cholesterol (Austin *et al.,* 2004) several studies reported homocysteine inhibited Apo A-I protein expression and decreased HDL Cholesterol (Mikael *et al.,* 2006).

Nicotine and other toxic substances from Tobacco smoke are absorbed through the lungs into the blood stream and are circulated throughout the body. These substances damage the blood vessel walls, which allow plaques to form at a faster rate than they would in a non smoker (Mitchell, 1999). Nicotine increases the amount of bad fats (total cholesterol (TC), low-density lipoprotein cholesterol (LDL-Cholesterol), and triglycerides (TG)) circulating in the blood vessels and decreases the amount of good fat (high- density lipoprotein cholesterol (HDL-Cholesterol) availability (Mitchell B et al.1999). Nicotine induces oxidative stress, generates free radicals that attack on the membrane lipids resulting in the formation of malondialdehyde (MDA), which causes peroxidative, tissue damage(Suleyman *et al.,* 2002). Lipoprotein oxidation is presumed to occur in the artery that may generate superoxide radicals, hydrogen peroxide or lipid peroxides outside the cell may contribute to the oxidation of LDL-cholesterol (Khurana *et al.,* 2000). These silent effects begin immediately and greatly which increase the risk for heart disease and stroke(U.S. Department of Health & Human Services (HHS), 1994).An increased level of MDA has been documented in smokers by several authors (Binder *et al.,* 2005). An evidence of intensification of lipid peroxidation processes which may cause chronic stress for endothelial

cells. On the other hand, it can also re-orientate enzymatic systems of the arachidonic acid cascade towards intensified TXA2 synthesis (Venkatesan,*et al.,* 2006). In this way tobacco smoking substantially hastening the risk of coronary heart disease and ischemic stroke (Brischetto *et al.,* 1983). To date, no statistical data are available on tobacco smoking among Sudanese people. Moreover, there have been no studies showing the relationships of tobacco smoking according to the number of shisha smoked per day and the duration of smoking and their influence on serum lipid profile. Globally there is much controversy about which components in the lipid profile are mainly altered in response to cigarette smoking, and whether those lipid profile components influence other parts directly or indirectly and vice versa.

2.4 BIOLOGICAL IMPLICATIONS OF FREE RADICALS

Free radicals are highly reactive, and are capable of damaging almost all types of biomolecules (proteins, lipids, carbohydrates, nucleic acids). The fact that free radicals are generated from normal compounds which continues as a chain reaction. Below are some of their effects on biological macromolecules.

i. **PROTEINS:** Free radicals cause oxidation of sulfhydryl groups, and modification of certain amino acids (e.g. Methionine, Cysteine, Histidine, Tryptophan, and Tyrosine). Reactive oxygen species may damage proteins by fragmentation crosslinking and aggregation. The net result is that free radicals may often result in the loss of biological activity of proteins like enzymes (Tribble and Jones, 1990; Budeli *et al.,* 2006).

ii. **LIPIDS:** Polyunsaturated fatty acids (PUFA) are highly susceptible to damage by free radicals (Budeli *et al.,* 2006; Reejamol and Swaminathan, 2013).

iii. **CARBOHYDRATES:** At physiological pH, oxidation of monosaccharides (like glucose) can produce H_2O_2 and Oxoaldehydes. It appears that the linkage of carbohydrates to proteins

(glycation) increases the susceptibility of proteins to the attack by free radicals (Christine and David, 2007).

iv. **NUCLEIC ACIDS:** Free radicals may cause DNA strand breaks, fragmentation of bases and Deoxyribose. Such damages may be associated with cytotoxicity and mutations (Christine and David, 2007; Reejamol and Swaminathan, 2013).

Free radicals have been implicated in the causation and progress of several diseases including: Cardiovascular diseases, Cancer, Cataract, Male infertility and Aging process.

Others include Parkinson's disease, Alzheimer's disease, Multiple sclerosis, liver cirrhosis, Muscular Dystrophy and Toxemia of Pregnancy among others (Gades *et al.,* 2005; Tverdal and Bjartveit, 2010; Hackshaw, 2011; Thum *et al.,* 2014).

2.5 ANTIOXIDANT

Antioxidants are believed to play a very important role in the body defense system against reactive oxygen species (Boxin *et al*. 2002; Vivek and Surendra , 2006).

Halliwell (2007) reported that an antioxidant is "any substance that delays, prevents or removes oxidative damage to a target molecule. Antioxidants are an inhibitor of the process of oxidation, even at relatively small concentration and thus have diverse physiological role in the body. Antioxidant constituents of the plant material act as radical scavengers, and helps in converting the radicals to less reactive species. A variety of free radical scavenging antioxidants is found in dietary sources like fruits, vegetables and tea, etc.

Antioxidants are our first line of defense against free radical damage, and are critical for maintaining optimum health and well being. Regular consumption of anti-oxidative vegetables

and fruits has been recognized as reducing the risk of chronic diseases. (Dembinska *et al.,* 2008). In present time various antioxidant found in food viz.

1. **Natural antioxidants:** Natural antioxidants are constituents of many fruits and vegetables and they have attracted a great deal of public and scientific attention.(Diwani *et al.,* 2009). Natural antioxidants occur in all parts of plants tissues because they are (or were) living, under constant oxidative stress from free radicals, reactive oxygen species, and pro-oxidants generated both exogenously (heat and light) and endogenously (H2 O2 and transition metals). For this reason, many of these tissues have developed antioxidant systems to control free radicals, lipid oxidation catalysts, oxidation intermediates, and secondary breakdown products (Nakatani ,2003), Agati and others (2007), Brown and Kelly (2007), Chen (2008), Iacopini and others (2008). These antioxidant compounds include flavonoids, phenolic acids, carotenoids, and tocopherols that can inhibit Fe3 induced oxidation, scavenge free radicals, and act as reductants Khanduja (2003), Ozsoy and others (2009). Spices and herbs, used in foods for their flavor and in medicinal mixtures for their physiological effects, often contain high concentrations of phenolic compounds that have strong H-donating activity Lugasi and others (1995), Muchuweti and others (2007). Natural antioxidants are those oxidants that are found in natural sources, such as fruits, vegetables and meats. There are several common natural antioxidants which are found in everyday foods, the most common of which being Vitamin C (ascorbic acid), Vitamin E (tocopherols), Vitamin A (carotenoids), various polyphenols including flavonoids, and Anthocyanins (a type of flavonoid), Lycopene (a type of carotenoid), And Coenzyme Q 10, also known as Ubiquitin, which is a type of protein.

2. **Synthetic antioxidants:** Synthetic antioxidants are chemically synthesized since they do not occur in nature and are added to food as preservatives to help prevent lipid oxidation(Shahidi *et*

al. , 1992). These antioxidants fall into two major categories depending on their mode of action Primary antioxidants and Secondary antioxidants. The primary antioxidants, which prevent the formation of free radicals during oxidation, can further include three major categories

3. **Dietary antioxidant**: Dietary antioxidants: The dietary antioxidants such as ascorbates, tocopherols and carotenoids are well known and there is a surplus of publications related to their role in health (Boskou *et al.* 2005). Vitamin C, vitamin E, beta carotene, carotenoids and oxycarotenoids, (e.g., lycopene and luteinare) are among the most widely studied dietary antioxidants. In extracellular fluids vitamin C is considered the most important water-soluble antioxidant. It is capable of neutralizing ROS in the aqueous phase before lipid peroxidation is initiated. Vitamin E, a major lipid-soluble antioxidant, is the most effective chain-breaking antioxidant within the cell membrane where it protects membrane fatty acids from lipid peroxidation. Vitamin C has been cited as being capable of regenerating vitamin E (Sies, 1992). Beta carotene and other carotenoids are also believed to provide antioxidant protection to lipid-rich tissues. Research suggests beta carotene may work synergistically with vitamin E (Jocab, 1995). In plants, flavonoids serve as protectors against a wide variety of environmental stresses while, in humans, flavonoids appear to function as "biological response modifiers." Flavonoids have been demonstrated to have anti-inflammatory, antiallergenic, anti-viral, anti-aging, and anti-carcinogenic activity (Cody *et al.,* 1986; (Kuhnau *et al.* 1976; Havsteen , 1983 and Middleton , 1984).

4. **Endogenous antioxidant:** Exogenous antioxidants can derive from natural sources (vitamins, flavonoids, anthocyanins, some mineral compounds), but can also be synthetic compounds, like butylhydroxyanisole, butylhydroxytoluene, gallates, etc. (Litescu *et al.* 2011). There is an increasing interest in antioxidants, particularly in those intended to prevent the presumed

deleterious effects of free radicals in the human body, as well as the deterioration of fats and other constituents of foodstuffs (Molyneux , 2004)

2.6 **ANTIOXIDANT VITAMINS**

Vitamins are organic compounds which are needed in small quantities to sustain life, hence considered as micronutrients (Halliwell, 1994). They are essential nutrients that cannot be synthesized by human body therefore must be consumed as part of their diet. Vitamins can be classified broadly into fat and water soluble. Fat soluble vitamins include: Vitamins A, D, E and K while water soluble include Vitamin C and B complex (B1, B2, B3, B6, B9 andB12) (Iain *et al.,* 2007). Antioxidant vitamins are vitamins with a defence or protective nature against free radicals and oxidative stress. They are Vitamin A,Vitamin C and Vitamin E (Halliwell, 2007). They protect the biological system by scavenging free radicals either produced in the body or consumed from exogenous sources like tobacco smoking (Young and Woodside, 2001).

Epidemiologic studies showed that tobacco smokers consume fewer fruits, vegetables and vitamin supplementation than do nonsmokers and this leads to low serum P/S ratio (polyunsaturated fatty acids/saturated fatty acids (Dietrich *et al.,* 2003). which in turn results in a status of increased oxidative stress and decreased antioxidant capacity, (Miller *et al.,* 1997) .as observed in the present study.

Several nutrition surveys indicate that smokers in general consume less antioxidant than nonsmokers (Hu and Cassano, 2000). As a result attempts have been made to alleviate the adverse effects of smoking by increasing the intake of dietary antioxidants, either singly or in combinations that include vitamin C, vitamin E, β-carotene, glutathione, α-lipoic acid, and/or coenzyme Q10.

2.6.1 **VITAMIN A**

Vitamin A is a fat-soluble vitamin that belongs to a group of organic compounds called "Carotenoids". About 500 different carotenoids have been identified, including Retinal, Retinaldehyde, Retinol and Retinoic acid. It is ingested from diet as β-carotene (retinyl ester also called provitamin A) (Gallicchio *et al.,* 2008).

Dietary retinyl esters are hydrolyzed by pancreatic or intestinal brush border hydrolases in the intestine, releasing retinol and free fatty acids. Carotenes are hydrolyzed by β-carotene l5-15'-dioxygenase of intestinal cells to release 2 moles of retinal which is reduced to retinol. In the intestinal mucosal cells, retinol is re-esterified to long chain fatty acids, incorporated into chylomicrons and transferred to the lymph. The retinol esters of chylomicrons are taken up by the liver (Satyanarayana and Chakrapani, 2006).

As and when needed, vitamin A is released from the liver as free retinol. Retinol is transported in thecirculation by the plasma retinol binding protein (RBP) in association with pre-albumin. The retinol-RBPc complex binds to specific receptors on the cell membrane of peripheral tissue and enters the cells. Many cells of target tissues contain a cellular RBP that carries retinol to the nucleus and binds to the chromatin (DNA). It is here that retinol exerts its function in a manner analogous to that of a steroid hormone (Satyanarayana and Chakrapani, 2006).

Vitamin A is necessary for a variety of functions such as vision, proper growth and differentiation, in the maintenance of proper epithelial tissues and proper immune system, iron transport and synthesis of cholesterol. It is thought to function as both an electron-scavenging and a free radical- binding antioxidant (Stahl *et al.,* 1995;Luchoomun and Hussain, 1999).

Vitamin A activity in the diet derives from two sources: preformed vitamin A as retinyl esters in foods of animal origin and Provitamin A carotenoids, such as β-carotene, α-carotene, and β-

cryptoxanthin, found in plant derived foods including: liver, sweet potato,spinach, egg, tomato, carrots and a more recent studies have shown similar associations with fruit intake like mango and pea nut (Johnson *et al.,* 1997).

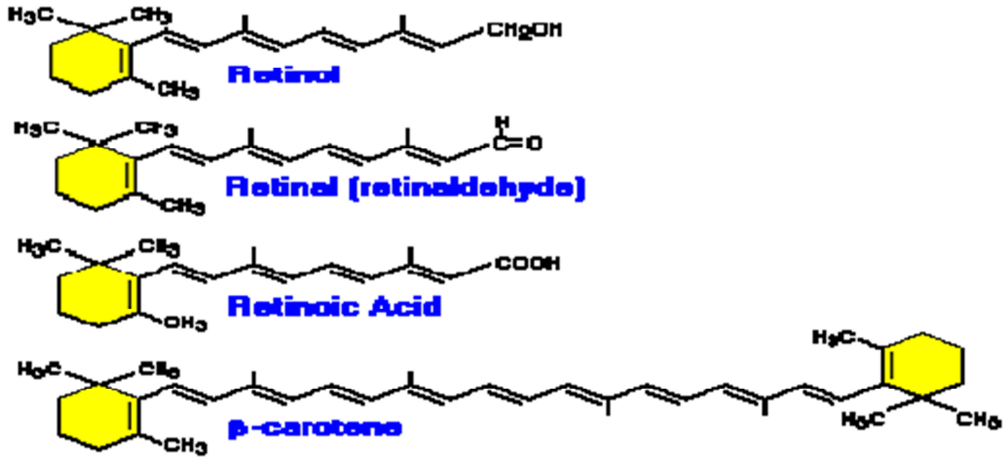


Fig 1 Carotenoids (Carcamo and Golde, 2005)

2.6.2 MECHANISM OF ACTION

The mechanism of the protective action against diseases of Vitamin A in vivo has not yet been elucidated (FNB, 2000). But evidences have shown that at low oxygen tension (2–20mm Hg), Vitamin A acts as a chain-breaking antioxidant scavenger whereas at oxygen tension >150 mm Hg, it is much less effective as an antioxidant and can show pro-oxidant activity (FAO and WHO , 2002).

2.6.3 VITAMIN A AND *SHISHA* SMOKING

Non-essential lipid-soluble antioxidants carotenoids such as β-carotene are suggested to have antioxidant properties capable of quenching free radicals such as singlet oxygen (1O2) (Stratton, *et al.,* 1993). It was also suggested that a cooperative interaction exists between fat-soluble antioxidants; the relation between β -carotene and vitamin E was reported to be synergistic. (

Niki *et al.,* 1995). In humans, the protective effects of β -carotene can be seen in those experiencing the stress of smoking, but not in non-smokers. For examples, in a randomized, double blind, controlled, interventional study, and 4 weeks of supplementation with 20-mg/day β-carotene had no effect on lipid peroxidation in non-smokers, but caused a 37 % reduction in smokers (Hennekens *et al.,* 1996).

2.6.4 VITAMIN C

Vitamin C also called Ascorbic acid is an important water soluble vitamin in biological fluids. It participates in many normal metabolic reactions of the body including vision, maintenance of proper growth, differentiation, in reproduction, maintenance of epithelial cells, in the synthesis of transferrin, maintenance of proper immune system and synthesis of Cholesterol (Satyanarayana and Chakrapani, 2006).

Vitamin C is a potent water soluble antioxidant because, by donating its electrons, it prevents other compounds from being oxidized. The species formed after loss of one electron is a free radical, semi-dehydroascorbic acid or ascorbonyl radical, a reactive and possibly harmful free radical. Many studies have demonstrated low plasma concentration of vitamin C in smokers and acute myocardial infarction (AMI) patients (Bloomer, 2007)

Vitamin C intake is inversely related to cancer, with protective effects shown for cancer of the lung, breast, pancreas, stomach, cervix, rectum and oral cavity (Simon *et al.* 2001). In stressful situations adrenal glands react by releasing hormones that trigger the “fight or flight” reaction. It has been reported that 200mg of vitamin C a day may reduce the levels of stress hormones. Stress suppresses the immune system. Mega doses of vitamin C increase the levels of antibody that fights against germs and viruses in both stressed and unstressed rats, with greater antibody increase in the unstressed rats (Block, 1999).

2.6.5 MECHANISM OF ACTION

Vitamin C oxidizes with loss of one electron to form a radical ion and then will loss a second electron to form dehydroascorbic acid. It typically reacts with oxidants of the reactive oxygen species such as the reactions by electron transfer. It can also transfer a single electron, owing to the resonance stabilized nature of its own radical ion called Semi-dehydroascorbate. The oxidized forms of ascorbate are relatively unreactive and do not cause lipid peroxidation (Droge, 200)

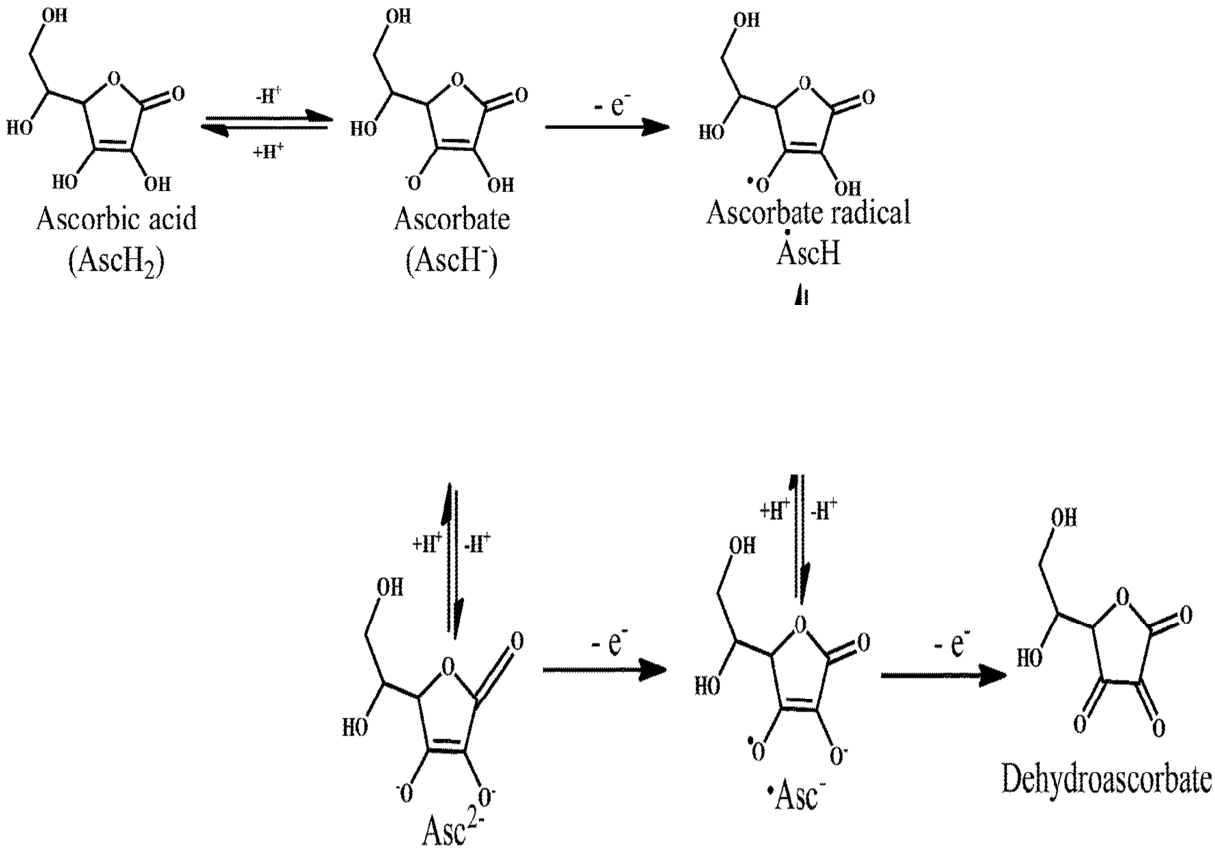


Fig 2 Mechanism of action of vitamin *C* (Carcoma and Golde, 2005)

2.6.6 VITAMIN C AND *SHISHA* SMOKING

Essential water-soluble antioxidants vitamin C: Vitamin C is the major essential water-soluble antioxidant in human serum, (Bendich, 1993). It is present in relatively high concentrations extracellularly in the blood plasma and in the RTLF overlaying the epithelial cell surface. Vitamin C can function as an antioxidant and scavenge the superoxide anion radical (O2-•), singlet oxygen (1O2), hydroxyl radicals (•OH), neutralize hypochlorous acid (HOCl), and prevent lipid peroxidation, (Anderson, 2001), but cannot scavenge or neutralize hydrogen peroxide (H_2O_2) (McCall *et al.,* 1974), rather it may potentiate its toxicity by inhibiting catalase activity(Anderson, 1981). Vitamin C can protect DNA from oxidant-mediated damage (Anderson, 1981). It has been reported to neutralize phagocyte-derived oxidants protecting the α1-protease inhibitor (API) from oxidant-mediated functional inactivation (Theron and Anderson, 1988). The vitamin C concentration is lower in blood plasma of tobacco smokers compared to non-smokers possibly due to increased turnover of the vitamin, increased oxidant stress in smokers, or other unknown mechanism (Schectman and Ann, 1993).. It could also be due to its increased consumption during recycling of vitamin E or b- carotene that is directly oxidized in the course of scavenging free radicals and reactive oxidant species

2.6.7 VITAMIN E

Vitamin E is a fat-soluble vitamin. It is a chiral compound with eight stereoisomers: α, β, γ, δ tocopherol and α, β, γ, δ tocotrienol. Only α-tocopherol is the most bioactive form in humans (Veysel *et al.,* 2013).Vitamin E is absorbed along with fat in the small intestine.

Bile salts are necessary for the absorption. In the liver, it is incorporated into lipoproteins (Very low density lipoprotein and Low density lipoprotein) and transported. Vitamin E is stored in adipose tissue, liver and muscle (Sahin *et al.,* 2001).

Vitamin E mainly resides in the cell membrane because it is highly fat-soluble; it safeguards the lipid molecules in the membrane from damage that can be caused by free radicals present in the tobacco smoke (Satyanarayana and Chakrapani, 2006). Dietary sources of vitamin E include: unprocessed vegetable oils, (Cotton seed oil, peanut oil, sunflower oil) whole grains, leafy vegetables, legumes, eggs and is especially abundant in wheat (Young and Woodside, 2001; Satyanarayana and Chakrapani, 2006).

2.6.8 MECHANISM OF ACTION

Since α-tocopherol is thought to be the major non-enzymatic antioxidant present in the lipid structures of cells, it appears to react with peroxyl radicals to inhibit the propagation cycle of lipid peroxidation (Halliwell and Gutteridge, 1985).

When α-tocopherol donates hydrogen, it becomes a free radical; converting fatty acid radicals(-OO·) to fatty acids (-OOH), but it is relatively unreactive and does not attack adjacent fatty acids, because the unpaired electron on the oxygen atom becomes delocalized into the aromatic ring structure. The concentration of α-tocopherol in cell membrane is low relative to the amount of lipid, about 1 mole for every 200 phospholipid molecules Therefore, to maintain α- tocopherol in its active form, it must be rapidly regenerated, possibly by Vitamin C which is present in the aqueous phases surrounding all membranes (Duthie, 2000).

Vitamin E is one of the most important lipid-soluble primary defense antioxidants Handan *et al.* (2007); Paul and Sumit (2002); Abdalla (2009). It is a generic term used for several naturally occurring tocopherols and tocotrienols. In its function as a chain-breaking antioxidant, vitamin E rapidly transfers its phenolic H-atom to a lipid peroxyl radical, converting it into a lipid hydroperoxide and a vitamin E radical (Bashir *et al.* 2004). Tocopherols (vitamin E) and Tocotrienols (provitamin E) are powerful antioxidants that confer oxidative stability to red palm

olein (RPO) as well as help to keep the carotenoids and other quality parameters of the oil stable (Veysel *et al.,* 2013). Vitamin E scavenges peroxyl radical intermediates in lipid peroxidation and responsible for protecting Poly Unsaturated Fatty Acid (PUFA) present in cell membrane and density lipoprotein (LDL), against lipid peroxidation (Vivek and Surendra, 2006). A fat-soluble vitamin that can be stored with fat in the liver and other tissues, vitamin E (tocopherols, tocotrienols) is promoted for a range of purposes from delaying aging to healing sun burn. The various function are maintains normal conditions of cells, and healthy skin and tissues, Protects red blood cells, antioxidation and enhance immunity. The important sources of vitamin E include wheat germ, nuts, seeds, whole grains, green leafy vegetables, vegetable oil and fish-liver oil.

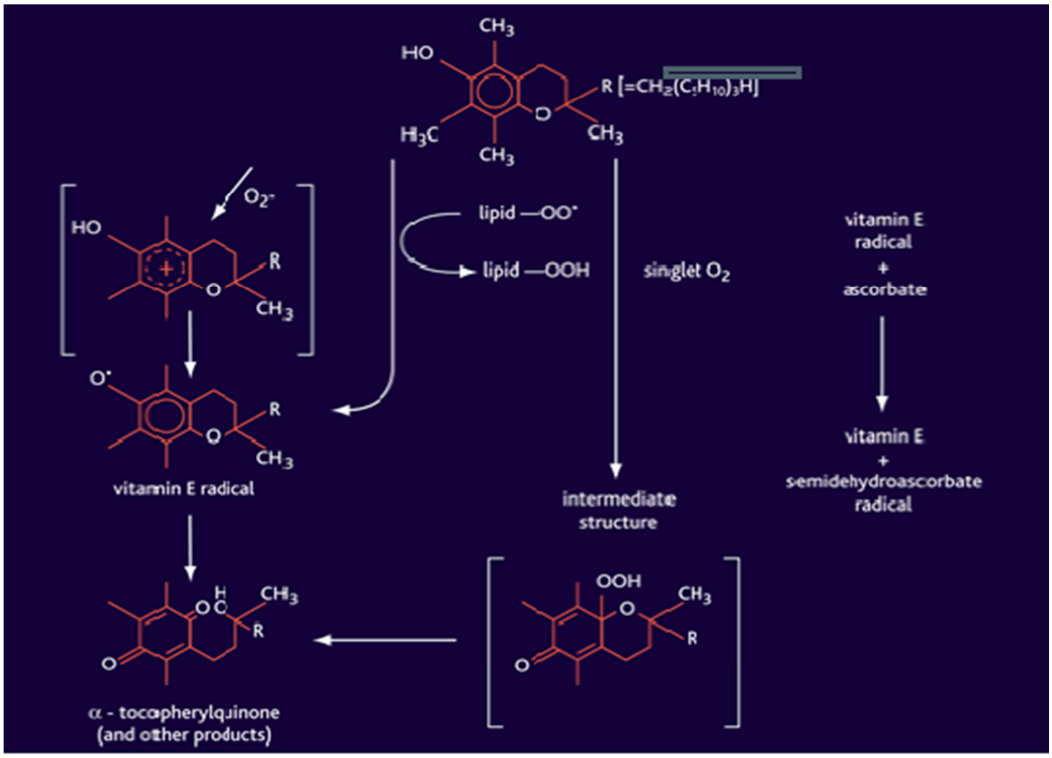


Figure 3 Mechanism of action of Vitamin E and its regeneration by Ascorbate (Halliwell, 1985)

2.6.8 VITAMIN E AND SHISHA SMOKING

Essential lipid-soluble antioxidants vitamin E: Vitamin E (α-tocopherol) is the major intracellular lipophilic, chain breaker, and efficient antioxidant capable of trapping peroxyl radicals and quenching free radicals and reactive oxygen species. It is also essential for structural membrane stability. (Sen and Packer, 2000).. Vitamin E concentration in the lung, however, does not decrease in response to tobacco smoke or inhaled environmental oxidants, rather it wasfound to increase following exposure to tobacco smoke in rats and guinea pigs (Chow *et al.,* 1989)., exposure to nitrogen dioxide (Elsayed and Mustafa, 1982), ozone (Elsayed and Mustafa, 1990)., and also increased in human blood after intensive exercise (Pincemail *et al.,*1988). This increased in vitamin E concentration was described due to mobilization by the lung in response to oxidative stress (Elsayed and Mustafa, 1990), reflecting a dynamic adaptive response of the lung to injury. It has been shown that upon lung injury by inhaled oxidants or chemicals, that ciliated cells in the airways and epithelial type I cells in the alveoli are damaged and sloughed off exposing the basement membrane (Paul and Sumit, 2002). This is then followed by a process of cell renewal characterized by proliferation of progenitor clara cells in the airways and epithelial type II cells in the alveoli to replace the damaged ciliated cells in the airways, and type I cells in the alveolar region. The proliferating cells particularly, epithelial type II cells are rich in lipids and possibly in vitamin E too, which may explain at least in part, the increase in lung vitamin E content (Kolleck *et al.,* 1999). However, it should be noted that such increases are nonetheless manifestations of the damage from smoking and reflect an adaptive response to an insult. Therefore, complete smoking cessation remains the best protective measure.

Studies conducted by different scientist including: (Abdulrahman *et al.,*1997; Young and Woodside, 2001 , Greg *et al al.,* 2003, Faur *al.,*2005, Gallicchio *et al.,* 2008).

CHAPTER THREE

MATERIALS AND METHODS

3.1 MATERIALS

Chemicals reagents and equipments

All the reagents used were of analytical grade (appendix II) and List of equipment used is shown in appendix I

3.2 Study population

A total of one hundred subjects took part in this study consisting of test group (*Shisha*-Smokers) and the control group (Non-Smokers). Fifty (50) apparently healthy Smokers in Kano metropolis constituted the smokers group. Fifty (50) apparently healthy subjects who do not smoke *Shisha* constituted the non smokers group and this group consisted largely of students from School of Health Technology, Kano and Shop attendants selected from Nasaraw, Kano municipal and Gwale local governments of Kano state metropolis.

3.3 **Data Collection**

A structured questionnaire eliciting information about the age, socio-economic status, health status, occupational history among others was issued to all the participants as shown in the appendix IV

3.4 Ethical Consideration

The ethical committee of Kano State Ministry of Health approved the study protocol. (MOH/Off/797/T.1/1639). Ethical consideration and confidentiality were respected. An informed consent (consent form) (appendix V) was obtained from all participants of this study. The nature and purpose of the study was explained to the participants, following which they willingly consented to participation in the study.

3.5 Determination of Sample Size

The sample size was calculated according to Cochran (1975), using the formular:

$$n = \frac{n_o}{1+(n_o-1)/N}$$

Where n= sample size

N= population size

$n_o = Z^2pq/e^2$

Z^2 is the abscissa of the normal curve that cuts off an area α at the tails

e is the desired level of precision

p is the estimated prevalence which is 7.1%

q is 1-p

We use $n_o = (1.96)^2 \times 0.071 \times 0.929 / (0.05)^2 = 67.65$

$$n = \frac{0.2533}{0.0025}$$

n=100

3.6 Sample Collection

Venous blood (5 ml) was taken from a peripheral vein of each participant by a qualified medical personnel and immediately transferred into a sterile and labeled sample container for biochemical analysis. The samples in the sample containers were allowed to stand and clot for 30 minutes and then centrifuged at 4000 rpm for 5 minutes to obtain serum which were used for biochemical analysis.

3.7 Experimental Design

Apparently healthy volunteers (n=100) from Kano metropolis between the age of 15-30 years were recruited after collection of their respective data using Questionnaires and their serum were used for biochemical analysis. The fifty (100) apparently healthy volunteers were divided into two groups; apparently (50) healthy smokers and apparently (50) healthy non-smokers. The smokers were also divided into three groups (>2year ,2-5 years and < 5years) depends on the period of *shisha* smoking. Blood samples were drawn in a fasting state (no food or drink, except water, for at least 12 hours) and allowed to coagulate. The coagulated blood was centrifuged to separate the serum from the whole blood at 4000 rpm for 5 minutes. The supernatant was pipetted into plain bottles and stored at 0^{o}C until required for analysis. The analyses include estimation of liver enzymes activities, lipid profile (TC, HDL-CH, and LDL;-CH) and antioxidant vitamins (Vitamins A, C and E) among shisha smokers and non-smokers

Exclusion Criteria

- Subjects outside of the age group (15 – 30) years
- Involvement in any form of smoking other than shisha
- Volunteers under lipid lowering drugs
- Volunteers outside Kano state.
- Subjects that decline consent

3.8 METHODS

3.8.1 Analysis of Biomakers for Liver Function

3.8.1.1 Determination of Activity of Serum Aspartate Aminotransferase (AST) by Reitman And Frankel, (1957**)**

Principle

Aspartate aminotransferase (AST), previously known as glutamate oxaloacetate transaminase (GOT) catalyses the transamination reaction between aspartate and α-ketoglutarate to produce oxaloacetate, the oxaloacetate formed reacts with 2,4-dinitrophenylhydrazine to produce hydrazone.

α-ketoglutarate + L-aspartate $\xrightarrow{\text{AST}}$ Glutamate + oxaloacetate

oxaloacetate + 2,4-dinitrophenylhydrazine → hydrazone

AST is measured by monitoring the concentration of oxaloacetate hydrazone formed with 2, 4-dinitrophenylhydrazine.

Procedure

Two test tubes were labelled as sample and sample blank. Buffered substrate (0.5ml) was added into both tubes. The sample (0.1ml) and distilled water (0.1 ml) were pipetted into the sample and sample blank tube respectively. They were then mixed and incubated for 30 minutes at 37℃ and also 2, 4-dinitrophenylhydrazine (0.5 ml) was pipetted into each tube. They were mixed and allowed to stand for 20mins at 25℃. Sodium hydroxide (0.5 ml) was pipetted into both tubes, they were mixed and the absorbance of sample against the sample blank after 5 minutes was read at 546 nm in a spectrophotometer. The activity of AST was obtained by comparing the absorbance with the activity value given in the Randox kit manual.

3.8.3 Determination Of Activity Of Serum Alanine Aminotransferase (ALT) Describe By Reitman And Frankel, (1957)

Principle

ALT catalyses the transfer of the amino group from alanine to α-ketoglutarate forming pyruvate and glutamate. The pyruvate reacts with 2, 4-dinitrophenyl hydrazine (DNPH) to form 2, 4-dinitrophenyl hydrazone which in an alkaline medium gives a red-brown colour.

α-ketoglutarate + L-alanine $\xrightarrow{\text{ALT}}$ Glutamate + pyruvate

pyrutvate + 2,4-dinitrophenylhydrazine → 2,4-dinitrophenylhydrazone

ALT is measured by monitoring the concentration of pyruvate hydrazone formed with 2,4-dinitrophenyl hydrazine.

Procedure

Two test tubes were lebelled as sample and sample blank, buffered substrate (0.5 ml) was added into both tubes. Serum (0.1ml) was pipetted into the sample tube. They were then mixed and incubated for 30 minutes at 37℃ and 2, 4-dinitrophenyl hydrazine (0.5 ml) was added into both tubes followed by 0.1 ml of distilled water into the sample blank. They were then mixed and allowed to stand for exactly 20min at 25℃. Sodium hydroxide (5 ml) was then pipetted into the tubes and mixed. The absorbance of the sample against the sample blank was read and recorded after 5 minutes at 546 nm in a spectrophotometer. The activity of ALT was obtained by comparing the absorbance with the activity value given in Randox kit manual.

3.8.4 Determination of activity of serum Alkaline phosphatase (ALP) by Rec, (1972)

Principle

ALP catalyzes the hydrolysis of P-nitrophenylphosphate to give p-nitrophenol and inorganic phosphate.

P-nitrophenyl phosphate + H_2O $\xrightarrow{ALP}$ Phosphate + P- nitrophenol

Procedure

Two test tubes were lebelled as sample and sample blank, Phenyl phosphate (1 ml) was added into both tubes and 0.1 ml each of serum and distilled water then added to the sample and sample blank respectively. The solutions were mixed and incubated for 15 minutes at 37℃. Absorbance ($A_{initial}$) was read and recorded immediately and timing was started simultaneously. The absorbance was read after first, second and third minutes against sample blank at 405nm in a spectrophotomer.

Activity

The activity of ALP was obtained by using the formula

U/L = 2760 × ΔA 405nm/minutes

Where ΔA: Difference in absorbance

3.8.5 Determination of Lipid Profile Using Cardiochek Strip (John,1991)

The lipid panel test system is intended to measure total cholesterol, HDL-cholesterol, LDL-cholesterol and triglycerides in whole blood on a CardioChek PA or a CardioCliek Plus professional analyzer. The test strips are intended to be used by Healthcare professionals and scientists to measure three blood analytes: total cholesterol, HDL-cholesterol, and triglyceride: Cholesterol measurements are used in the diagnosis and treatment of disorders involving excess cholesterol in the blood and lipid and lipoprotein metabolism disorders. Lipoprotein measurements are used in the diagnosis and treatment of lipid disorders (such as diabetes mellitus) atherosclerosis, and various liver and renal diseases. Triglycerides measurements are used in the diagnosis and treatment of patients with diabetes mellitus, nephritis, liver obstruction, other diseases involving lipids metabolism, or various endocrine disorders.

PTS Panels test strips are designed for use with fresh capillary and fresh venous whole blood collected in "EDTA or heparin tubes. A memo Chip" is provided with each package of test strips and must be properly inserted into the analyzer before any test can be run. The *MEMo* Chip contains the test name, calibration curve, lot number, and test strip expiration date. After the test strip is inserted into the analyzer and blood applied to the test strip, test results are displayed before or after 90 seconds.

Principle of the Test

When blood is applied to a test strip, the blood reacts to produce color that is read by the analyzer using reflectance photometry. The amount of colour produced is proportional to the concentration. The enzymatic reactions that occur are listed below.

Cholesterol

Cholesterol ester + H_2O $\xrightarrow{\text{Cholesterol esterase}}$ cholesterol + fatty acid

Cholesterol + H_2O + O_2 $\xrightarrow{\text{Cholesterol Oxidase}}$ cholesterol + 4-en-3-one + H_2O_2

$2H_2O_2$ + 4- APP + Disubstituted Aniline $\xrightarrow{\text{Cholesterol peroxide}}$ Quinoneimine dye + 4 H_2O

HDL Cholesterol

VLD, LDL, HDL Plasma $\longrightarrow$ VLDL, LDL, depleted Plasma

Cholesterol ester + H_2O $\xrightarrow{\text{Cholesterol esterase}}$ cholesterol + fatty acid

Cholesterol + H_2O + O_2 $\xrightarrow{\text{Cholesterol ooxidase}}$ cholesterol – 4 – en-3-one + H_2O_2

$2H_2O$ + 4-APP + Disubstituted Aniline $\xrightarrow{\text{peroxide}}$ Quinoneimine dye + 4 H_2O

Triglycerides

Triglyceride + $3H_2O$ $\xrightarrow{\text{lipoprotein lipase}}$ Glycerol + 3 Fatty acid

Glycerol + ATP $\xrightarrow{\text{glycerol kinase}}$ Glycerol -3-PO + ADP

Glycerol-3-PO_4 + O_2 $\xrightarrow{\text{glycerophosphate oxidase}}$ Dihydroxyacetone-PO_4 + H_2O_2

$2HO_2O_2$ + 4 – APP + N, N-Disubstituted aniline $\xrightarrow{\text{peroxide}}$ Quinoneimine dye + 4 H_2O

Procedure

All instructions were carefully read before testing for best results. Participants were tested in a fasting state (no food or drink, except water, for at least 12 hours).

The *MEMO* Chip inserted that matches the lot number on the test strip vial and one of the buttons pressed to turn the analyzer ON. The test strip was held by the end marked "PTS" and the opposite end of the test strip was inserted into analyzer. The test strip was pushed in as far as it will go. When applying the sample, a capillary blood collector or pipette was used to apply 35-40 |uL of blood serum to the test strip blood application window. After 90 seconds, the results appeared on the display automatically on the analyzer: the button marked NEXT was pressed to view additional results. The test strip was removed and discarded.

3.8.6 Determination of Vitamin C using the method by (Rutkowski and Grzegoezyk, 1998).

Analyzed liquid (1ml) was added into the centrifugal test-rube and 1 ml of the PR was added and the contents were mixed thoroughly and left in a room temperature for 30 minutes.

The tubes were centrifuged (7000 xg) for 10 minutes, and the supernatants were collected for spectrophotometric measurements. The standard sample was prepared as above (using 1 ml of the standard solution instead of the analyzed liquid), without centrifugation. The absorbance of the test sample and standard sample were measured at 700 urn against the mixture PR: 50 mM solution of oxalic acid = 1:1 (v/v) as a reference sample. Concentration of vitamin C (uM) in the analyzed liquid was calculated using the formula:

$$Cx = \frac{Ax}{As} \times Cs$$

Where:

C_s - Concentration of the standard solution.

Cx- concentration of the sample

Ax- Absorbance of the sample

As- Absorbance of the standard

3.8.7 Determination of Vitamins E Using the Method by (Rutkowski *et al.* 2005)

Analysed sample (0.5ml) was added into each test-tubes and 0.5ml of anhydrous ethanol was also added, the contents were shaken vigourously for 1minute. Xylene (3 ml) was added into the test tubes and shaken vigourouly for another 1 minute. The contents of the test tubes were centrifuged to separate the extract (150 xg, 10 minutes), and 0.25 ml solution of batophenantroline was measured simultaneously into usual test tube II.The extract (upper layer) (1.5 ml) was collected and transfered into the test tube II, the contents were mixed. $Fecl_3$ solution (0.5 ml) was added to the test tube II, the contents were then mixed and 0.25 ml of H_3PO_4 solution was added and the contents were mixed again and the test sample was obtained for spectrophotometric. The standard sample was prepared (0.5 ml of the standard solution instead of the analysed liquid). De-ionized water (0.5 ml) was added instead of anhydrous ethanol at the beginning of the analysis and sample was not centrifuged. The absorbance of the test sample and standard sample was measured at 539 nm against the blank test in a spectrophotomer (preparation –as the test sample but using blank instead of the analyzed liquid).

Calculation

Concentration C_x of vitamin E (um) in the analyzed liquid was calculated using the a/a presented formula.

$$Cx = \frac{Ax}{As} \times Cs$$

Where:

C_s - Concentration of the standard solution.

Cx- Concentration of the sample

Ax- Absorbance of the sample

As- Absorbance of the standard

3.8.8 **Determination of Vitamin A Using the Method by (Rutkowski Et Al. 2006)**

Analyzed sample (0.5ml) was measured into the test-tube I and 1 ml of the KOH solution was added and the tube shaken vigorously for 1 minute. The tube was heated in a water bath (60^{O}C for 20 minutes), then cooled. Xylene (1 ml) was added and the the tube shaken vigorously again for 1 minute. The tube (1500xg, 10 minutes) was centrifuged at 1500 xg for 10 minutes and the whole of the separated extract (upper layer) was collected and transferred to the test-tube II. The absorbance A_1 of the obtained extract was measured at 335 nm against xylene in a spectrophotomer. The extract in the test tube II was irradiated in the UV light for 30 minutes and the absorbance A_2 was measured.

The concentration Cx of vitamin A (uM) in the analysed liquid was calculated using the formula.

$Cx = (A_1 - A_2) = 22.23$

Cx- concentration of the sample

A1- Absorbance before UV light

A2- Absorbance after UV light

3.9 DATA ANALYSIS

Data were expressed as mean ± standard deviation (SD). The parameters were analyzed statistically using student's t-test(Table1, 2 and 3) and One-way Analysis of Variance (ANOVA) (Figures 1, 2 and 3) using SPSS software; version 20.0 and Microsoft Excel; 2010 version. Differences were considered statistically significant at $p<0.05$.

CHAPTER FOUR

RESULTS AND DISCUSSION

4.1 RESULT

4.1.1 Table 1. Show the effect of *Shisha* Smoking on Serum Liver Enzymes Activities in *Shisha* Smokers and Non Smokers. From the table, it can be seen that the mean ALP, ALT and AST activities were significantly higher at $p<0.05$ in smokers than non-smokers.

Parameter	Smokers	Non-smokers
ALP (U/L)	35.19 ± 8.56^{a}	28.59 ± 5.84^{b}
ALT(U/L)	29.28 ± 10.99^{a}	25.16 ± 12.08^{b}
AST(U/L)	50.18 ± 8.34^{a}	42.85 ± 12.61^{b}

Values are expressed as mean ± SD; values followed by different superscript letters in the same row are considered significantly different at $p < 0.05$.

4.1.2 Table 2

Table 2 indicates the effect of *Shisha* Smoking on Serum Lipid Profile in Smokers and Non Smokers. From the table it can be seen that the mean value of total cholesterol (TC), triacylgycerides (TAG), low density lipoprotein (LDL) were significantly higher ($p<0.05$) in smokers than in non-smokers and the mean of high density lipoprotein (HDL) was decreased ($p<0.05$) in smokers than in non smokers.

Parameter	**Smokers**	**Non-smokers**
TC(mmol/L)	185.26 ± 9.15^{a}	166.54 ± 7.25^{b}
HDL(mmol/L)	49.94 ± 8.56^{a}	55.56 ± 6.08^{b}
TAG(mmol/L)	71.84 ± 14.24^{a}	66.44 ± 6.67^{b}
LDL(mmol/L)	83.78 ± 9.17^{a}	64.34 ± 4.66^{b}

Values are expressed as mean ± SD; values followed by different superscript letters in the same raw are considered significantly different at $p < 0.05$.

4.1.3 Table 3

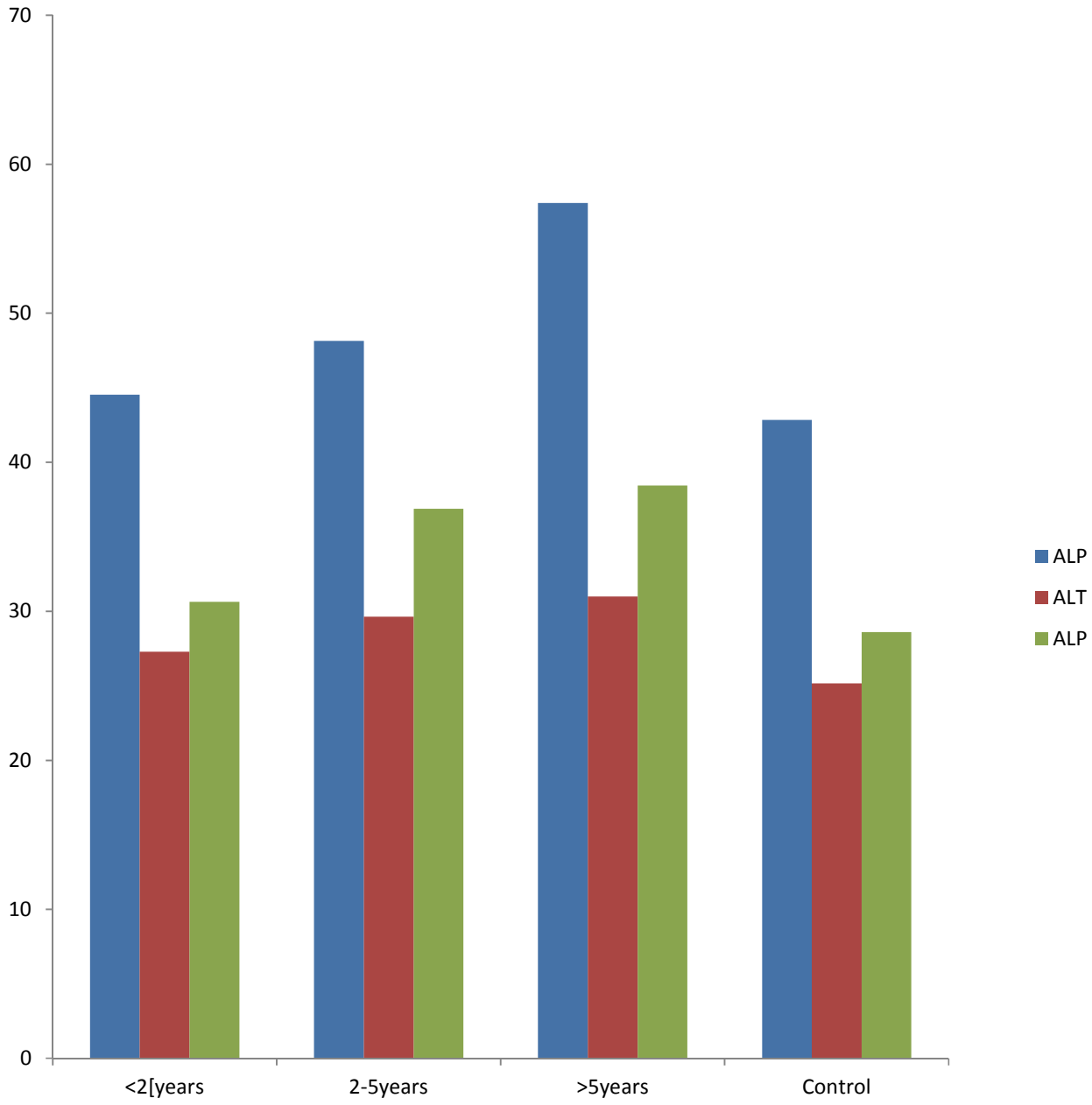
Table 3 shows the effect of Shisha Smoking on human Serum Antioxidant Vitamins among *Shisha* and Non-Smokers. From the table it can be seen that the mean of vitamin A, C and E were significantly higher ($p<0.05$) in smokers than non-smokers.

Parameter	Smokers	Non-smokers
Vitamin A(mg/dl)	11.98 ± 3.04^{a}	18.04 ± 1.88^{b}
Vitamin C(mg/dl)	51.81 ± 15.35^{a}	73.18 ± 18.01^{b}
Vitamin E(mg/dl)	50.61 ± 8.83^{a}	56.67 ± 7.44^{b}

Values are expressed as mean ± SD; values followed by different superscript letters in the same raw are considered significantly different at $p < 0.05$.

Co

Figure


70
60
50
40
30
20
10
0
<2[years
2-5years
>5years
Control
ALP
ALT
ALP

4. Effect of Shisha Smoking on Serum Liver Enzymes in Smokers of different period of smoking.

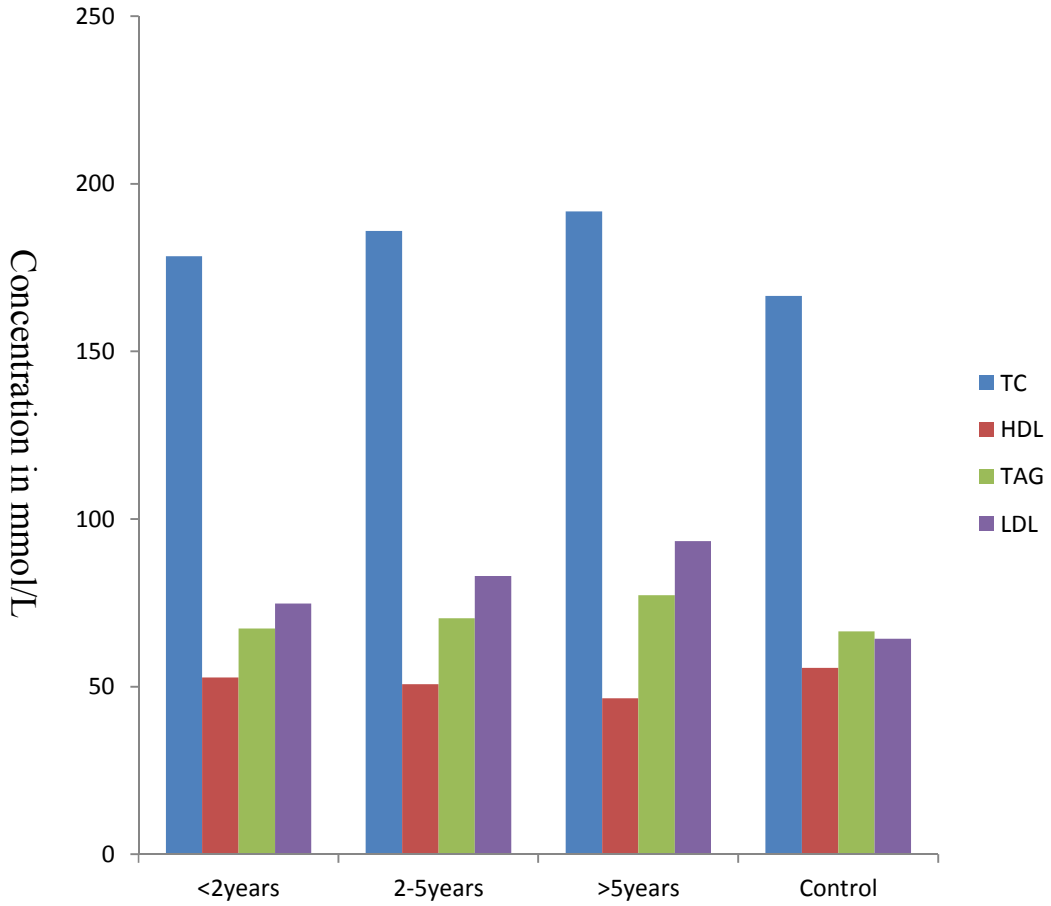


Figure 5. Effect of *Shisha* Smoking on Serum Lipid Profile in Smoker of different age groups

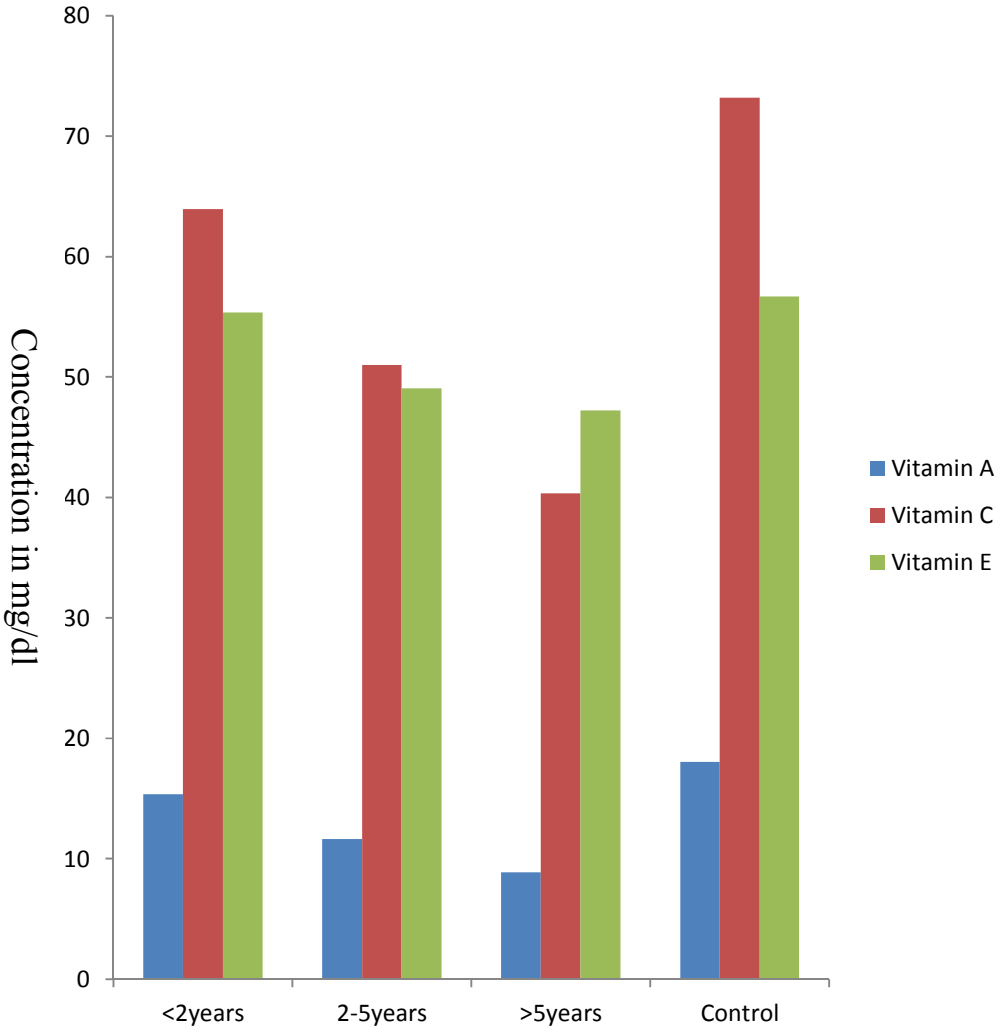


Figure 6. Effect of *Shisha* Smoking on Serum Antioxidant Vitamins in Smoker of different period of smoking.

4.2 DISCUSSION

Shisha smoking was significantly associated with increased levels of AST, ALT and ALP compared with non-smokers (Table1). Enzymes AST, ALT and ALP levels were significantly higher in smokers compared non-smokers ($P< 0.05$). This is in line with research reported by Burstyn (2014). According to the Bridges(1979) shisha smoke contains considerable amount of toxic substances such as free radicals which are highly reactive atoms that damage the biological membrane through lipid peroxidation. Base on the result found in (Table 1), the elevation may be due to free radicals present in the shisha smoke which damage the biological membrane of the hepatocytes causing the enzymes leaked out into the blood. (Rochling, 2001)

This finding suggested that water-pipe smoking induces oxidative damage in the liver (Charab *et al.,* 2016). Several studies have reported the pathologic effects of smoking in general on liver enzymes (Olivella *et al.,*1985). The findings of the present study may assist in understanding the effect of smoking shisha on liver.

On the other hand, analyzing the results of the current study with regard to the period of smoking, it was observed that there was an increased in the activities of serum AST, ALT and ALP with regard to the increased in period of *shisha* smoking (figure 1). But, the relation with regard to period of *shisha* smoking was not statistically significant ($p<0.05$) (figure 1).

Shisha smoking is associated with coronary heart diseases compared non-smokers. This may explained by by various ways such as impairedment in the integrity of arterial wall, derangement in the blood lipid and lipoproteins concentreation, alteration in blood coagulation.

This study revealed significantly high concentration of total cholesterol, Triglycerides, LDL-cholesterol in *shisha* smokers campared non-smokers (Table 2).The increased in lipid levels

may be explained by the following mechanism*: shisha* smoking causes absorption of nicotine into the body which leads to lypolysis and release of free fatty acids into the blood stream via action of adenyl cyclase in adipose tissue by nicotine stimulated secretion of catecholamines. These increased free fatty acids in liver give rise to increased hepatic triglycerides and VLDL-cholesterol synthesis, thus increasing the concentration of Triacyglycerides and VLDL-C in blood (Devaranavadgi *et al.,*2012).

The present results agreed with many earlier reports (Kong, *et al.,* 2001; Zhu, *et al.,* 2011 and Devaranavadgi, 2012). Similar findings were found in the study done by (Trupti, *et al.*, 2014). The present study also showed significant decreased in level of HDL-cholesterol ($P<0.05$) in smokers than in non-smoker (Table 2). Several studies reported high levels of plasma homocysteine in chronic smokers (O' Callaghan *et al*., 2002). Plasma homocysteine is negatively correlated with HDL-C and Apo A-1. Decreased in HDL-Cholesterol in smokers may be due to smoking induced increase catecholamine release, causing increased in VLDL-cholesterol and decreased in HDL-cholesterol concentration. The present study findings are in line with the finding of Trupti *et al*., (2014) who found that the mean of HDL-cholesterol was significantly lower and LDL-cholesterol was significantly higher among smokers compared control. Furthermore, the results of the current study with regard to the period of *Shisha* smoking, it was observed that there was an increased in the levels of serum total cholesterol (TC), triacylgycerides (TAGs) and LDL-cholesterol with a slight decrease in HDL-cholesterol with regard to the increase in the period of *shisha* smoking. But the relation was statistically not significant (figure2).

Vitamin A is a strong antioxidant and is the best quencher of singlet oxygen. Its lipophilic nature allows it to pass across the membrane and scavenge free radicals (Wallstrom *et al.,* 2000).

Several observations were reported on the effects of tobacco smoking and serum levels of vitamin A. Some reported a significant decrease (Chui *et al.,* 2009) while others observed a significant increased in the levels of Vitamin A (Biesalski *et al.,* 1986). This research however revealed that smokers had significantly lower levels of serum Vitamin A than non-smokers at $p<0.05$(Table 3) which might be due to destruction of this antioxidant vitamin during neutralization of free radicals present in *shisha* smoke which contains more free radicals than the biological system can handle (Abdulrahman *et al,.* 1997).

Vitamin C is a water-soluble vitamin that efficiently scavenges free radicals. It also promotes the regeneration of the active form of vitamin E (α-tocopherol) from α-tocopheroxyl radical produced during scavenging of ROS (Satyanarayana and Chakrapani, 2006).

This research showed that there was significantly ($p<0.05$) lower concentrations of vitamin C in the serum of *shisha* smokers compared to non-smokers (Table 3) which might be due to a combination of impaired vitamin C absorption and an increased turnover due to oxidative stresses affected by *shisha* smoking.

There are conflicting reports about the effects of tobacco smoking and serum Vitamin C levels. Some reports revealed that there was effect showing that smokers have statistically significant lower levels than non-smokers (Greg *et al.,* 2003) while others reported a reverse effect (Mezzetti *et al.,* 1995). A significant amount of research indicates that smokers may have higher requirements for vitamin C (Weber *et al.,* 1996; Burri and Jacob, 1997). These results confirmed that smoking is associated with decreased serum vitamin C concentrations.

Alberg (2002) reported that the average vitamin C concentrations were 27% lower in tobacco smokers compared with non-smokers.

The biological activity of vitamin E is almost entirely due to its antioxidant properties. In addition to its antioxidant role, vitamin E might also have a structural role in stabilizing membranes (Christine *et al.,* 2006)

In the same way, this study showed that the serum level of Vitamin E was found to be significantly lower in smokers than in non-smokers at $p<0.05$(Table 3). This was in line with the findings of another study by Palanisamy *et al.*,(2009).

On the other hand, analyzing the results of the current study with regard to the period of smoking it was observed that there is decreased in the levels of serum A, C and E with regard to the increased in period of *shisha* smoking (figure3) and relation was not statistically significant (figure3).

4.3 CONCLUSION

The present study showed that *shisha* smoking causes damage to the hepatocytes of smokers group which increases the secretion of the liver enzymes AST, ALT and ALP, compared to none-smokers. It also revealed increase in total cholesterol, Triacylglycerides and LDL-cholesterol with a decreased in serum HDL-cholesterol which reflect a great significance since the findings associated with coronary heart disease. Serum antioxidants vitamins (A, C, and E) decreased significantly compared to non-smokers.

This study also showed increase in the activities of serum liver enzymes, Total cholesterol, Triacylglycerides, LDL-cholesterol with a decreased in HDL- cholesterol and antioxidant vitamins as the period of *shisha* smoking increased.

RECOMMENDATION

This study finding recommends increase in dietary intake or supplements of the appropriate amount of essential antioxidant vitamins which may be used to prevent oxidative stress caused by free radicals present in the *shisha* smoke.

It is also strongly recommended to avoid smoking for the benefit of liver and cardiac health. It is important to establish a visible and audible communication aids and through schools and colleges explaining risks of smoking *shisha* on the liver, cardiovascular system and other systems.

It is recommended that studies on the effect of shisha cessation in human should be carried out.

However, studies pertain to this line can be undertaken which include: estimation of the levels of biological enzymatic oxidants like glutathione peroxidase, catalase and Superoxide dismutase in relation to shisha smoking as well as heavy metals that may be present in the smoke.

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APPENDIX 1

Equipments

S/NO	equipment	Model number	Manufacturer
1.	A centrifuge	800D	Shanghai medicalinstruments (group)Ltd.,China
2.	test-tubes shaker	5716	Stuart scientific corp. Ltd., Great Britain
3.	water bath	GD100	Grant instrument LtdCambridge,England
4.	Spectrophotometer	SP300 Optima,	Tokyo, Japan
6.	Lipids panel test strips.		
7.	Memo Chip		

8. CardioChek PA or CardioChek Plus professional analyzers

9. Lancets for finger stick

10. Alcohol wipes and gauze

11. Capillary blood collector

APPENDIX II

REAGENTS

Reagents used in the research

s/no	reagent	purity%	manufacturer
1.	phosphotungstate (PR)	60.0	Kermel chemical reagents Co., Ltd., China
2.	Thiobarbituric acid (TBA)	99.0	LabTech chemicals, Australia
3.	Deionized (DI) water,		
4.	Sulphuric acid (VI);	98.1	Fisons plc. scientific equipment, England
5.	Xylene	98.5	Kermel chemical reagents Co., Ltd., China
6.	Anhydrous ethanol;	99.7	JDH chemicals Ltd., Bangladesh
7.	Vitamin E standard	50.0	Titan Biotech, India
8.	L-ascorbic acid standard	99.7	LabTech chemicals, Australia
9.	Anhydrous iron chloride (III)	96.0	Avis laboratories and Access chemicals, Australia.
10.	Crystalline orthophosphoric acid		
11.	Potassium hydroxide		
12.	sodium hydroxide		

APPENDIX III

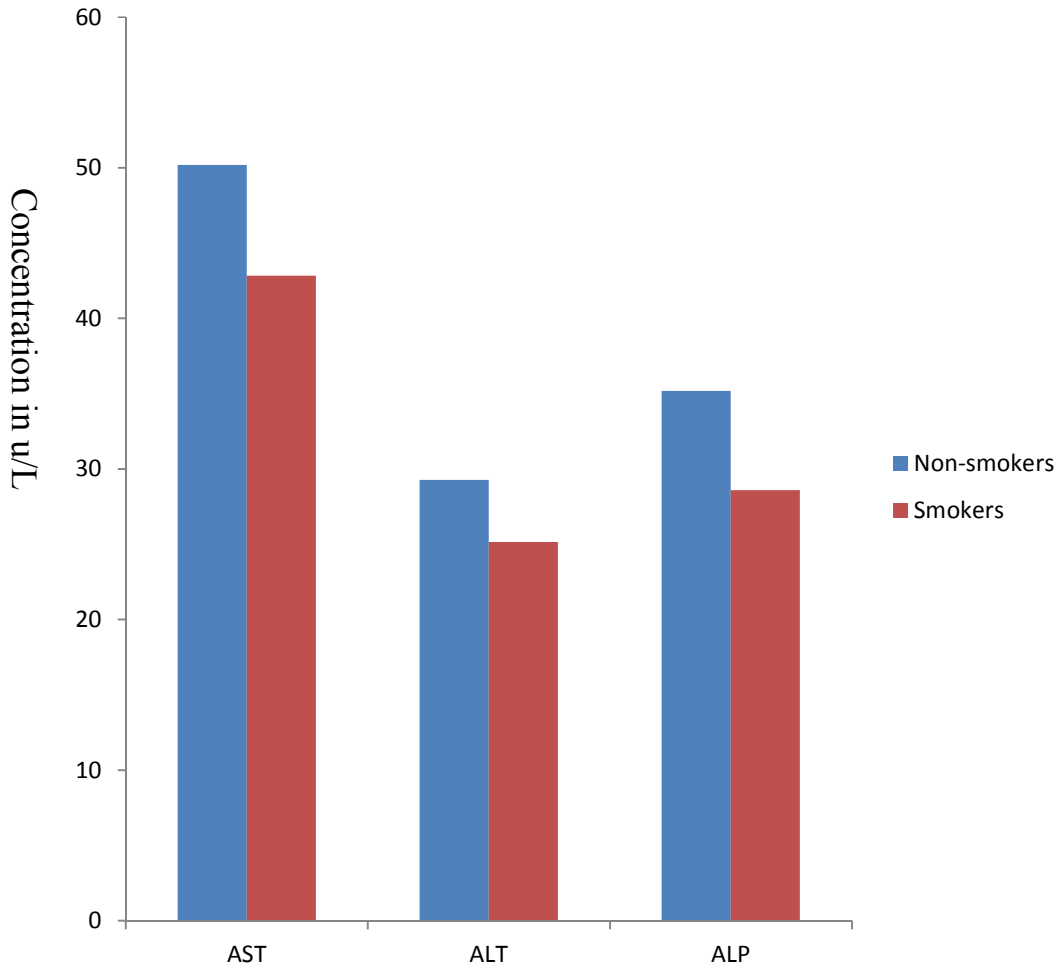

Figure 1. Effect of Shisha Smoking on Serum liver enzymes among *Shisha* and Non-Smokers

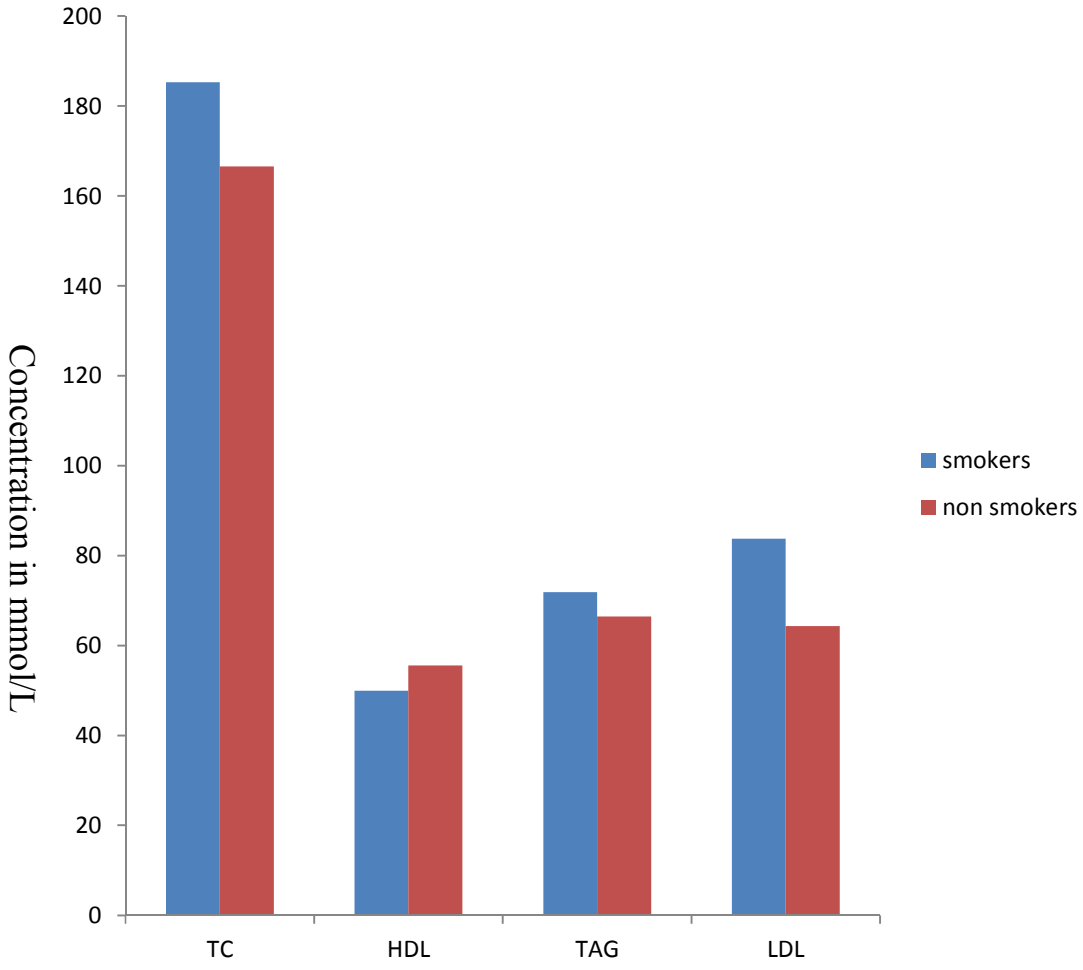

Figure 2. Effect of Shisha Smoking on lipid profile among *Shisha* and Non-Smokers

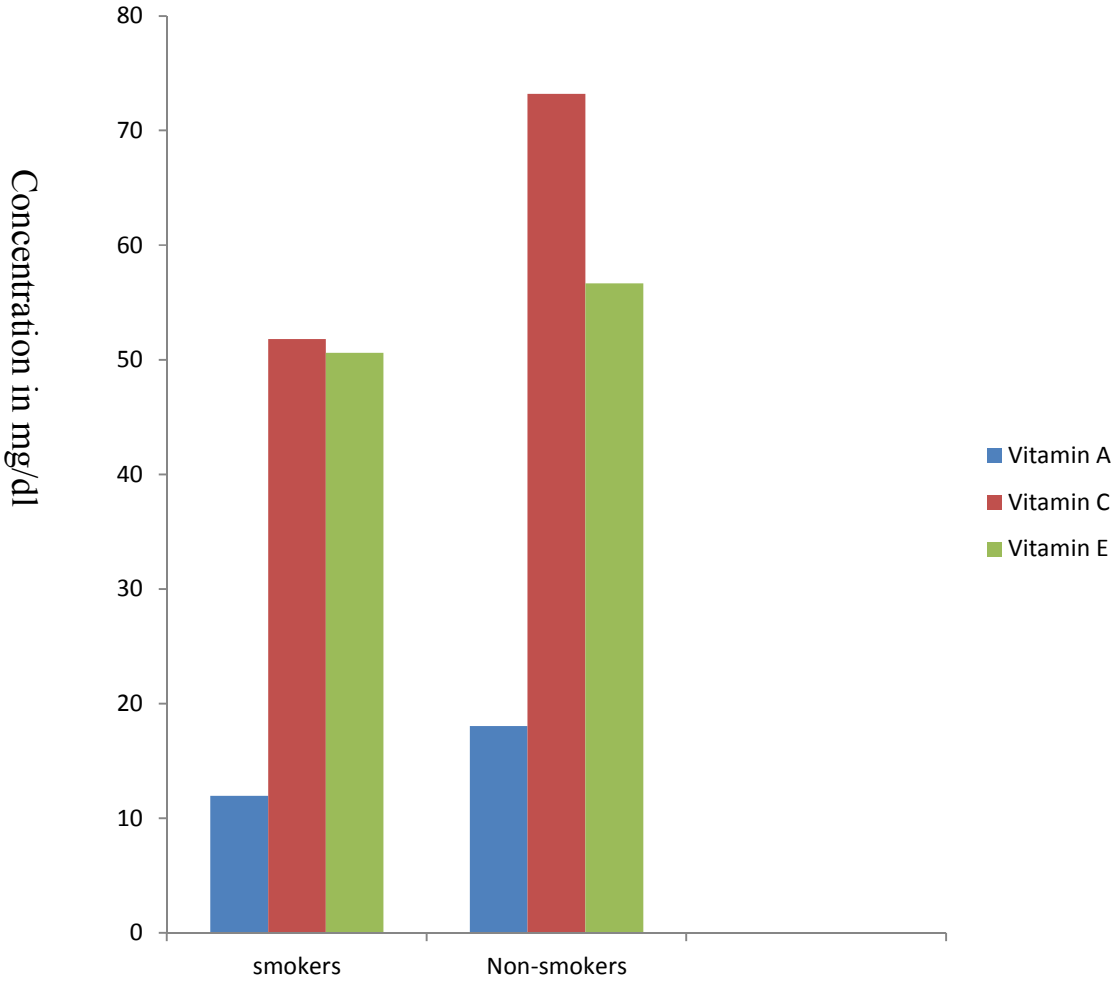

Figure 3. Effect of Shisha Smoking on Serum Antioxidant Vitamins among *Shisha* and Non-Smokers

Appendix IV

ETHICAL APPROVAL

APPENDIX V

QUESTIONNAIRE ON EFFECT OF SHISHA SMOKING ON SERUM LIVER ENZYMES, LIPID PROFILE AND ANTIOXIDANT VITAMINS IN KANO METROPOLIS.

Good day!

My name is Ibrahim Haruna Muhammed. Iam a final year MSc. Biochemistry (medical) student conducting a study on the above mentioned topic in partial fulfilment for the award of master's degree certificate. Your participation in this research is voluntary. The information you will provide will be treated as confidential. I want you to answer my questions to the bestof your knowledge and it will not last more than 5-10mins. The result will be utilized by the government and healthcare authorities to know where to tailor their intervention in promoting awareness on effect of Shisha smoking on human health.

QUESTIONNARE ID

1. Name…….....

2. Sex: Male ☐ Female ☐

3. Age……

4. Ethnic group: Hausa/Fulani ☐ Yoruba ☐ Igbo ☐ others ☐

5. Marital status: Single ☐ Married ☐

6. Employment status: Employed ☐ Unemployed ☐

7. Level of education: None ☐ Read and write ☐ Secondary ☐ High school ☐ University ☐

8. Do you smoke Shisha? Yes ☐ No ☐

9. For how long you have been smoking shisha? 2years ☐ 2-5years ☐ more than 5years ☐

10aDo you engage in cigarette smoking or other form of smoking: Yes ☐ No ☐

When did you start shisha smoking? Less than 20 years 20-25years 26-30years

10b.How many times do you smoke Shisha per day? One two three more than three

11a Do you have any disease? Yes No . If yes,Please specify……

11b.Are you under lipid lowering drugs? Yes No