POSSIBLE EFFECT OF MARIJUANA CONSUMPTION ON LIVER FUNCTION AMONG YOUNG ADULTS IN BENIN CITY

BY

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SCHOOL OF BASIC MEDICAL SCIENCES,

COLLEGE OF MEDICAL SCIENCES,

UNIVERSITY OF BENIN,

BENIN CITY.

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THE DEPARTMENT OF MEDICAL LABORATORY SCIENCE,

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UNIVERSITY OF BENIN,

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DEPARTMENT OF MEDICAL LABORATORY SCIENCES,

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SUPERVISED BY PROF M. A. EMOKPAE

SEPTEMBER, 2023.

CERTIFICATION

This is to certify that this project work was satisfactorily carried out by **ORODE ESEOGHENE DISTINCTION** with matriculation number **BMS1702121** in the Department Of Medical Laboratory Science, School of Basic Medical Sciences, and University of Benin City, Benin City, under my supervision in partial fulfillment of the requirement for the award of Bachelor of Medical Laboratory Science (BMLS) Degree.

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EXTERNAL EXAMINER **DATE**

DEDICATION

This project work is dedicated to God almighty, who is the author of knowledge and wisdom.

ACKNOWLEDGMENTS

I give thanks to God almighty for His grace upon my life and for seeing me through the process of my academic pursuit.

My sincere gratitude goes to my supervisor Prof M. A. Emokpae for his immerse contribution and for his constructive and supportive ideas which aided this research effectively. Special thanks to the Head of Department, Medical Laboratory Science, Dr. B. I. G. Adejumo. My heartfelt gratitude also goes to the entire staff of the department for investing so much in my academic development.

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ABSTRACT

Recently, social attitude toward the use of marijuana has changed and some have been advocating for legalization of its use. In the same vein, there has been an increasing interest relating to the health risk associated with it and how it affects several organs in the human body including the liver which is a key metabolic organ of the body. The aim of this study was to determine the possible effects of marijuana consumption on liver function parameters. Sixty adult marijuana smokers and 60 age-matched non-marijuana/cigarette smokers were recruited in the study. Socio-demographic data were collected using structured questionnaire. Serum aspartate amino transferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP), gamma glutamyl transferase (GGT), total protein (TP) and albumin were determined by spectrophotometric method. Data were compared using appropriate statistical tool. The results indicate that AST (42.4±8.67), ALT (41.67±14.15) and GGT (29.56±8.48) activities were significantly higher ($p<0.05$) in marijuana smokers than in non-smokers (26.34±4.95 24.00±5.97, 14.91±3.36) respectively. Conversely, total protein and albumin concentrations were significantly lower ($p<0.05$) in marijuana smokers than non- smokers. Serum ALP activity was however not significantly different when compared with controls. Some 48/60 (80%) of marijuana smokers had AST values (44.8±8.44) above the upper limit of the reference range while 34/60 (56.7%) had GGT values (61.3±7.60) above upper limit of the reference range. Marijuana consumption may predispose individuals to liver injury independent of quantity consumed and duration of use therefore the public should aware of the liver health risk associated with marijuana consumption.

CHAPTER ONE

INTRODUCTION

1.1 Background of Study

In Nigeria little information is known about the effect of Cannabis on the liver. What is known is that Cannabis is an illicit drug that is largely considered as a social evil (Dumbili, 2020). The government also uses repressive (war on drugs) measures to regulate it (Nelson, 2018). But despite the negative perception of cannabis and the government paternalistic approach to drug regulation, available data show that cannabis remains the most-sort after illegal drug in Nigeria (United Nations Office on Drugs and Crime, 2018)

Cannabis, which is colloquially referred to as marijuana, is an component of the Cannabis sativa plant. Marijuana originates from Central Asia, and is widely distributed in temperate and tropical areas (Bonini *et al*., 2018). Cannabis Sativa contains compounds known as cannabinoids. The most well-known cannabinoid is tetrahydrocannabinol (THC), which is responsible for the plant's psychoactive effects. Cannabidiol (CBD) is another important cannabinoid, which does not have psychoactive effects but has been shown to have therapeutic potential for a variety of conditions (Fusar-Poli *et al*., 2009). Other compounds found in marijuana include terpenes, which are responsible for the plant's distinct aroma, and flavonoids, which are responsible for the plant's color and flavor.

In recent years, there has been a dramatic increase in the number of marijuana users. The use of marijuana is particularly on the rise among adolescents and young adults, making marijuana one of the most commonly abused substances in the world after alcohol and tobacco (Suerken *et al.,* 2014).

Marijuana works by interacting with the body's endocannabinoid system (ECS). The ECS is a complex system of receptors and neurotransmitters that plays a role in regulating a wide range of physiological processes, including pain, mood, appetite, and sleep. Young adults are the most vulnerable group of people than any age group to cannabis dependence and related problems which produce more years lived with disabilities (Degenhardt *et al*., 2013). Results of recent studies regarding how the drug affects human health have resulted in a number of conflicting conclusions. Some studies found a significant therapeutic effect of the drug on human health, including a positive effect on the liver, whereas other research warned of marijuana's adverse health effects (Volkow *et al*., 2014; Adejumo *et al*., 2017). In the medical field, many questions related to the actual impact of the drug on different organs of the human body, the impact of the drug on less frequent users, and the effect of the age of initiation and method of consumption of the drug remain unanswered (Sznitman and Room, 2018).

In recent years, more studies related to marijuana use have emerged (Maier *et al*., 2017). Researchers have begun to investigate the risk factors associated with marijuana and how it affects different human organs including the liver (Gudsoorkar and Perez Jr., 2015). Although many findings are still in their early stages, they appear to point in many different directions, including reports of positive and negative health effects, as well as no significant health effect (Adejumo *et al*., 2017).

1.2 Statement of Problem

Cannabis is the most abused substance which is still illicit in most countries globally (United Nations, 2012). In Nigeria, the burden of drug abuse is on the rise and becoming a public health concern. The recent legalization of the recreational use of

marijuana in some parts of the world and ease of availability has resulted in a gradual rise in marijuana use among various age groups (Cerda *et al.*, 2012). In recent years, there has been a change in perceived risk associated with the use of marijuana, and people who believe that marijuana use is associated with health risk is decreasing (Okaneku *et al*., 2015). With the recent change in social attitudes toward the use of marijuana and its continued decriminalization (Maier *et al*., 2017), there has been an increase in interest related to risk factors associated with use of the drug and how it affects several organs in the human body (Gudsoorkar and Perez Jr., 2015). It is therefore necessary to do a comprehensive research on how Marijuana consumption affects the liver.

1.3 Justification of Study

The consumption of marijuana may have effects on different organs in the body. Volkow *et al.* (2014) published a report that addressed the adverse effects of marijuana use, including its effects on the brain development, the risk of cognitive impairment, altered mental health, diminished life satisfaction, poor educational outcomes, and the development of symptoms of chronic bronchitis. The report also acknowledges the health benefit of marijuana use, including its ability to relieve the symptoms of glaucoma, nausea, chronic pain, inflammation, multiple sclerosis, and epilepsy. The necessity involved in carrying out this study on population consuming marijuana with emphasis on its health risk especially on the liver cannot be overlooked. There is little to no awareness on the effect of Marijuana consumption on liver function. Many young adults are at risk of developing liver disease as a result of marijuana consumption. Understanding the effects on marijuana consumption on liver

function may generate an intriguing data that can alert government and certain bodies into taking actions to control marijuana use.

1.4 Aim of Study

The aim of this study is to determine the possible effects of Marijuana consumption on Liver function parameters among young adults in Benin City.

1.4.1 Specific Objectives

The specific objectives of this study are to:

1. Determine the serum levels of liver function parameters (Aspartate amino transferase, Alanine aminotransferase, gamma glutamate transferase, alkaline phosphatase, serum albumin and total protein) in people who consume marijuana and those who do not.
2. Calculate De Ritis ratio (AST:ALT ratio) and compare between marijuana consumers and non-consumers.
3. Determine the effect of duration and age of initiation of marijuana and effect on the liver function parameters

1.5 Research Questions

1. Are the liver function parameters raised among marijuana consumers than in non-Marijuana consumers?
2. Is De Ritis ratio raised among marijuana consumers than non-consumers?

3. Is there any significant association between duration/the amount of marijuana used and serum levels of liver function parameters?

1.6 Hypotheses

1.6.1 Null Hypothesis

1. The liver function parameters are low among marijuana consumers than in non-Marijuana consumers?
2. De Ritis ratio is normal among marijuana consumers than non-consumers?
3. There is no significant association between duration/the amount of marijuana used and serum levels of liver function parameters?

1.6.2 Alternate Hypothesis

1. The liver function parameters are raised among marijuana consumers than in non-Marijuana consumers?
2. De Ritis ratio raised among marijuana consumers than non-consumers?
3. There is a significant association between duration/the amount of marijuana used and serum levels of liver function parameters?

CHAPTER TWO

LITERATURE REVIEW

2.1 Marijuana

Marijuana refers to the dried leaves, flowers, stems, and seeds from the Cannabis sativa or Cannabis indica plant. The plant contains the mind-altering chemical THC and other similar compounds. Marijuana is highly lipophilic which leads to its storage in adipose tissue, liver, muscle and spleen and redistributed into the users blood stream long after ingestion. Because of the persistence in the body, marijuana can cause highly potent mental, physical and toxic effects in the users that are hard to control or predict (Sharma *et al*., 2012). In addition, Marijuana causes dependence, tolerance and addiction. Anxiety, sadness, decreased appetite, headaches, insomnia, irritability, muscle tension, nausea, nightmares, and unpleasant vivid dreams are among the withdrawal symptoms that result from trying to stop using it (Grotenhermen, 2007; Lamarine, 2012).

Marijuana, as used in the general population for smoked consumption, is an extract of the plant Cannabis sativa. It consists of more than 421 components and more than 60 pharmacologically active cannabinoids. The two most well described cannabinoids in marijuana are delta9-tetrahydrocannabinol (THC) and cannabidiol (CBD) (Grotenhermen and Muller-Vahl, 2012). Marijuana is a plant that grows best in temperatures of about 20°C or 68°F but the plant has a characteristic of being "flexible" meaning it can potentially grow in extreme conditions. Typical growing regions include Mexico, Nepal, Northern India, many parts of Africa, Afghanistan, the United States and Australia. Marijuana can also be planted in homes and it is therefore possible to plant marijuana almost anywhere in the world. The duration of

the growth of the plant depends of the conditions and can vary from 8 weeks to more than 7 month, the average growth being 3-4 month for indoor growers. After the harvest process the flower buds need to be dried for 3-10 days (Hillig and Mahlberg, 2004)

Cannabis is also the most widely used illicit drug in the world and its use has been associated with various mental health problems, particularly in the young (Hall and Degenhardt, 2007, Degenhardt et al. 2010). The dried leaves and flowers (buds) of the cannabis plant are known as marijuana. Marijuana use is on the rise compared to the other illicit drug use. This significant increase has been observed across all population subgroups (sex, age, race/ethnicity, education, marital status, income, urban/rural, and region) (SAMHSA, 2014). There is also a significant increase in marijuana use disorders (Hasin *et al.,* 2015). According to the National Survey on Drug Use and Health, cannabis (marijuana) is one of the most used drugs in the United States, and its use is widespread among young people. In 2021, 35.4% of young adults aged 18 to 25 (11.8 million people) reported using marijuana in the past year. Despite the fact that numerous studies have discovered negative impacts of marijuana use, many more studies have revealed benefits for one's health. Volkow *et al*., (2014) published a report that addressed the adverse effects of marijuana use, including its effects on the brain development, the risk of cognitive impairment, altered mental health, diminished life satisfaction, poor educational outcomes, and the development of symptoms of chronic bronchitis. The study also notes that marijuana use has health benefits, including its capacity to ease the signs and symptoms of multiple sclerosis, glaucoma, nausea, chronic pain, inflammatory process, and epilepsy. Volkow and his colleagues, however, recommended maximizing marijuana's medical advantages in order to protect those who are ill from the drug's inherently harmful consequences.

The issues related to the health impacts of marijuana on the human body continue to be the subject of contentious discussions among scientists and decision-makers, despite the fact that some studies have revealed marijuana's positive health effects and other studies have reported its negative health consequences.

2.1.1 Tetrahydrocannabinol

Delta-9-tetrahydrocannabinol (THC) is the most abundant and the major psychoactive phytocannabinoid. THC has a tri-cyclic 21-carbon structure without nitrogen and with 2 chiral centers in transfiguration. THC is volatile viscous oil with high lipid solubility and low water solubility. The primary active metabolite of THC is 11-hydroxy-delta-tetrahydrocannabinol (11-OH-THC) and the primary inactive metabolite is 11-nor-9- carboxy-delta-9-tetrahydrocannabinol (THC-COOH) (Sharma et al., 2012). THC is a high-affinity partial agonist of both CB1R and CB2R (Ligresti et al., 2016). The majority of THC's well-known effects include its psychotropic effects and impact on appetite stimulation since CB1R is extensively expressed in the CNS (Lannotti *et a*l., 2016). THC also has anti-inflammatory properties by inhibiting the proinflammatory response and shifting the immune response toward an anti-inflammatory phenotype, inducing apoptosis, and suppressing cell proliferation, mainly through the activation of CB2R (Kopustinskiene *et al*., 2022). The delta-9-tetrahydrocannabinol doses needed in order to produce effects in humans vary from 2 to 22 mg per inhalation. Marijuana does not build tolerance, although its psychoactive effects are dose-dependent. Of the 60 natural cannabinoids, delta-9-tetrahydrocannabinol is the only one that binds to a membrane receptor which is receptor is present in every cell. In binding to it, delta-9-tetrahydrocannabinol displaces its natural ligand, anandamide, and persistently disrupts the physiological

signaling of the receptor. The anomalies observed clinically when delta-9-tetrahydrocannabinol is present in the cell membrane are associated with the molecular disruption of membrane signaling. Because of the disruption of this molecular mechanism by delta-9-tetrahydrocannabinol, it has not been possible to separate the adverse effects of delta-9-tetrahydrocannabinol and marijuana from their therapeutic properties (Nahas *et a*l., 2020).

2.1.2 Cannabidiol

Cannabidiol (CBD) is the second most abundant and is the main non-psychoactive compound of the cannabis plant (Ligresti *et al*., 2016). Cannabidiol is a non-psychoactive phytocannabinoid, and it possesses analgesic, neuroprotective, anticonvulsant, antiemetic, spasmolytic, and anti-inflammatory properties (Kopustinskiene et al., 2022). CBD has been proposed by some to act as a negative allosteric modulator of CB1R, and it has been shown to exert its anti-inflammatory properties through non-CBRs by inhibiting the activity of the NF-kappa B pathway, the activator protein-1 and nuclear factor of activated T-cell transcriptional activity, and leading to the suppression of proinflammatory cytokines (IL-2, TNF-α, INF-γ) and immune-cell activation and proliferation (Devi *et al*., 2022). Both THC and CBD have also been found to exert antioxidant activity by scavenging free ROS and blocking ROS generation (Kopustinskiene *et al*., 2022).

2.1.3 Mechanism of Action

Marijuana's main sites of action are in the brain and the spinal cord. It binds to two types of G-protein-coupled receptors, CB1 and CB2. CB1 receptors are predominantly expressed in the brain and located in the basal ganglia, cerebellum,

hippocampus, association cortices, spinal cord and peripheral nerves (Hosking and Zajicek, 2008; Pertwee, 2008). Marijuana produces its psychological and behavioral effects through its antagonistic effect on the CB1 receptor. Marijuana affects the user's perceptions and mood, interferes with memory and learning, and impairs judgment via its action on the CB1 receptor. Additional central effects of marijuana are a disruption of psychomotor behavior, psychosis and loss of time perception as well as impairment in movement coordination (Grotenhermen, 2007). Originally, marijuana was categorized as a hallucinogen due to these perceptual anomalies. Marijuana over-activates the endocannabinoid system, also known as "the body's own cannabinoid system" causing addiction and a major part of the behavioral abnormalities (Iversen, 2003).

The CB2 receptors are mainly found in the peripheral tissues on cells in the immune system, the hematopoietic systems and in the spleen. These receptors may play a role in the immune-suppressive activity of cannabis (Pertwee, 2008). The G-protein-coupled CB1 and CB2 cannabinoid receptors are both activated by the inhibition of adenylate-cyclase. When these receptors are activated, the release of the neurotransmitters acetylcholine and glutamate is inhibited, and serotonin, y-aminobutyric acid, N-methyl-D-aspartate, and opioid receptors are also indirectly affected. The cannabinoid receptors are predominantly located presynaptic rather than postsynaptic which means that cannabinoids modulate the neurotransmitter releases (Iversen, 2003).

2.1.4 Routes of Administration

1. **Inhalation/Smoking**: Smoking marijuana has the highest addictive potential due to rapid and efficient drug delivery from the lungs to the brain (Strougo et

al., 2008). Pyrolysis destroys a significant portion of THC, which accounts for the difference in systemic bioavailability between heavy and infrequent users (Sharma *et al*., 2012).

2. **Oral Route**: When compared to inhalation, oral usage has a delayed onset of effects, lower peak concentrations, but longer-lasting pharmacokinetic effects and a delayed return to baseline (Huestis, 2007). First pass metabolism means that only a fraction of the marijuana ingested actually becomes available in the blood stream (Borgelt *et al*., 2013)
3. **Transcutaneous**: The hydrophobic properties of cannabis hinder the transport across the skin layers during transcutaneous absorption, making this process the rate-limiting step. A transcutaneous carrier selected from the group consisting of water, short carbon chain alcohols, dimethysulfoxide, polyethylene glycol, polypropylene glycol, glycerin, mineral oil and mixtures thereof (Lamarine, 2012). The delivery to the brain when administered transcutaneously is much slower compared to smoking. Steady state plasma concentrations were found to be maintained for at least 48 hours (Huestis, 2007).

Table 2.1: Tabular representation of the various routes of administrations

Route	Absorption	Peak concentration	Factors impacting absorption	Bioavaila y
Inhalation/ Smoking	Quick Fast, rapid drug delivery to the brain	22 min	Depth of inhalation, frequency of puffs, breath hold	Varies 2- percent Heavy us 23-27 per Occasion users 10- percent
Oral	Slow	1-2 h, can be delayed to up to 8 h	Degradatio n of the drug in the stomach and First-pass	Ranges fr 10-20 per
Oralmucosal/sub lingual	Fast	30mins	High first past metabolism	Ranges fr 10-20 per
Rectal	Fast	15mins	Low first past metabolism	Twice of route

2.1.5 Metabolism

THC is metabolized in the liver by microsomal hydroxylation and oxidation via CP450 enzymes. The hydroxylation of THC through CP450 enzymes leads to the production of the equipotent active metabolite 11-OH-THC. Cytochromes involved in the oxidation of THC are CYP 450 2C9, 2C19 and 3A4. More than 100 THC metabolites including di- and trihydroxy compounds, ketones, aldehydes, and carboxylic acids, have been identified. The inactive metabolite THC-COOH is created when the psychoactive 11-OH-THC is oxidized. Together with its conjugates, THC-COOH is the main byproduct of biotransformation. Extra-hepatic sites in the brain, intestine and lung might contribute to the metabolism of cannabis (Sharma et al., 2012). Marijuana has been shown to affect the pharmacokinetics of other drugs in several ways through inhibition of the CP450 enzyme. It mediates the absorption of other drugs and may also slow down the absorption of others and to enhance or delay the penetration of drugs into the brain. Marijuana interacts with drugs like warfarin and theophylline through the CP450 enzyme and is associated with toxic levels or INR values that are above the therapeutic range. Also antidepressants for example SSRIs inhibit the hepatic CP450 enzymes. Prozac and Paxil extend the availability of marijuana in the serum due to competition with the enzyme (Thompson, 2015)

2.1.6 Pharmacokinetics Of Marijuana

It is essential to know the pharmacokinetic effects of marijuana to understand the onset and magnitude of its effects as well as the toxicity and extended potency. In addition, marijuana has many documented acute and chronic toxic effects on brain and behavior (Thompson, 2015). The primary factor contributing to marijuana's toxicity is that it is a highly lipophilic substance that is absorbed by tissues with high

blood flow, including the heart, lungs, brain, muscle, and liver, as well as adipose tissues. With prolonged marijuana exposure, fatty acid conjugates of THC and 11-OH-THC are formed, increasing the stability of these compounds and allowing even its extended storage in the tissues. Studies with radioactive tracers for THC have shown a large volume distribution of the active form of THC and its slow elimination from body stores long after use for continued pharmacological effects. The half-life of infrequent users is 1.3 days and for frequent user it is 5 to 13 days (Sharma et al., 2012). As a result, marijuana's pharmacological effects and toxic effects might linger for a long time after the last intake or exposure, making them very unpredictable. Additionally, marijuana's effects become more potent in regular and long-term users because cannabis releases from storage in the tissues and is combined with newly ingested marijuana. (Alexander, 2016).

2.2 Structure and Functions of the Liver

A person's liver is in the upper right section of the abdomen and sits below the diaphragm. It typically weighs around 3 pounds, but this can vary between people. The skin is the only organ heavier and larger than the liver. The liver is roughly triangular and consists of two lobes: a larger right lobe and a smaller left lobe. The falciform ligament separates the lobes. This ligament is a band of tissue that keeps the liver anchored to the diaphragm. A layer of fibrous tissue called Glisson's capsule covers the outside of the liver. The peritoneum, a membrane that forms the lining of the abdominal cavity, then covers this. This helps hold the liver in place and protects it from physical damage.

2.2.1 Blood Supply

Unlike most organs, the liver has two major sources of blood. The portal vein brings in nutrient-rich blood from the digestive system, and the hepatic artery carries oxygenated blood from the heart. The blood vessels divide into small capillaries, with each ending in a lobule. Lobules are the functional units of the liver and consist of millions of cells called hepatocytes. Three hepatic veins remove blood from the liver

2.2.2 Functions of the Liver

The liver is a gland that has many functions in the body. It is difficult to give a precise number, but it may have more than 500 distinct roles.

The major functions of the liver include:

1. Bile production: Bile helps the small intestine break down and absorb fats, cholesterol, and some vitamins. Bile consists of bile salts, cholesterol, bilirubin, electrolytes, and water.
2. Absorbing and metabolizing bilirubin: The breakdown of hemoglobin forms bilirubin. The liver or bone marrow stores iron released from hemoglobin, which makes the next generation of blood cells.
3. Supporting blood clots: Vitamin K is necessary to create coagulants that help clot the blood. Bile is essential for vitamin K absorption and forms in the liver. The liver must produce enough bile to make clotting factors.
4. Fat metabolization: Bile breaks down fats and makes them easier to digest.

5. Metabolizing carbohydrates: The liver stores carbohydrates. The body can break down stored carbohydrates in the liver, known as glycogen, into glucose. Glucose, or sugar, is released into the bloodstream to regulate blood sugar levels and for a quick burst of energy.
6. Vitamin and mineral storage: The liver stores fat-soluble vitamins, known as vitamins A, D, E, K, and B12. It keeps significant amounts of these vitamins stored. The liver stores iron from hemoglobin in the form of ferritin, ready to make new red blood cells. The liver also stores and releases copper.
7. Helps metabolize proteins: Bile helps break down proteins for digestion.
8. Filters the blood: The liver filters and removes compounds from the body, including hormones, such as estrogen and aldosterone, and compounds from outside the body, including alcohol and other drugs.
9. Immunological function: The liver is part of the mononuclear phagocyte system. Cells involved in immune activity, Kupffer cells, are found in high numbers in the liver. These cells destroy disease-causing viruses, bacteria, or other microorganisms that might enter the liver through the gut.
10. Production of albumin: Albumin is the most common protein in blood serum. It transports fatty acids and steroid hormones to help support the correct pressure and prevent the leaking of blood vessels.

11. Synthesis of angiotensinogen: This hormone raises blood pressure by narrowing the blood vessels when alerted by production of an enzyme called renin in the kidneys.

2.3 Liver Diseases

The liver is the largest organ in the body. The liver performs a variety of functions that range from cleaning toxins from the blood and providing the body with nutrients to storing energy by participating in the metabolism of carbohydrate, lipid, and protein (Snyder, 2016), a deviation from the normal functioning of the liver can lead to diseased states. The major causes of liver disease are related to a variety of factors, include an increase and continued consumption of alcohol, autoimmune disorders, viral infections, drug-related causes and non-alcoholic accumulation of fat in the liver cells (Gao and Wang, 2014; Fumeaux *et al*., 2018). It is important to note that liver disease can be acute or chronic. Acute liver disease can develop into chronic. For example, viral hepatitis such as hepatitis A through E are considered acute viral diseases. However, Hepatitis B and C can progress into chronic hepatitis due to the continued and longtime inflammation of the liver (Snyder, 2016). In addition, heavy alcohol consumption can also progress from acute liver disease to a chronic liver disease which is manifested by cirrhosis of the liver (Woldin, 2014; Snyder, 2016). Common liver diseases are hepatitis B virus (HBV), HCV, alcoholic and nonalcoholic liver disease, cirrhosis and hepatocellular carcinoma (Wang *et al*., 2014).

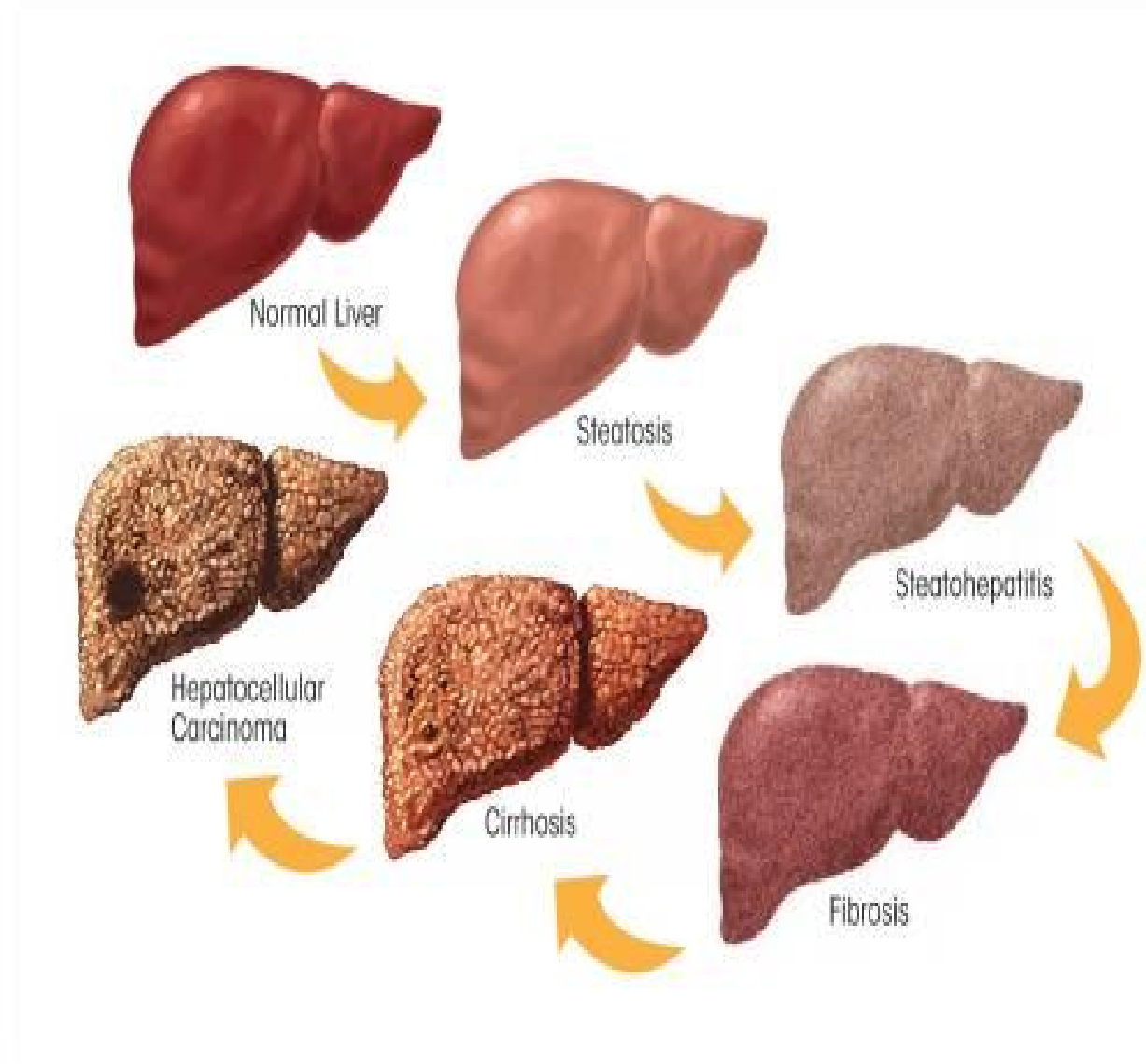

FIG 2.1: Spectrum of Alcoholic Liver Disease (Osna et al., 2017)

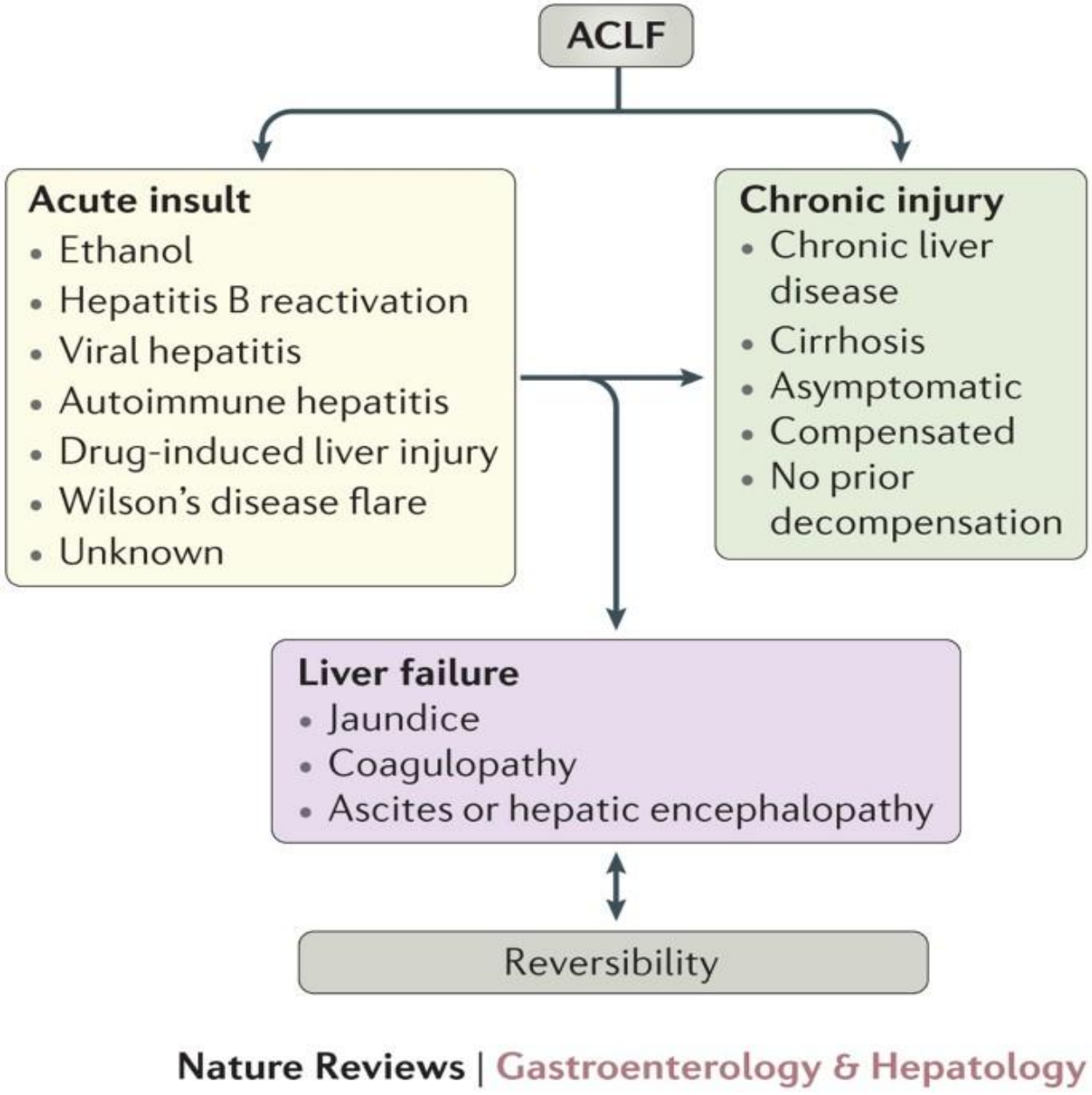

FIG 2.2: Acute and Chronic Liver diseases (Sarin and Choudhury, 2016)

2.4 Marijuana and Liver Function

In determining the effect of marijuana on liver function some factors need to be considered such as the quantity and duration of Marijuana use, age of Marijuana users and pre-existing liver diseases. Discussions about the impact of marijuana on human health center on the age of marijuana users and the age at which marijuana usage began (Johnson *et al*., 2015). Age has been found to be a risk factor for many chronic diseases including liver diseases. For example, Cheng *et al*., (2013) found increased prevalence of metabolic syndrome and fatty liver disease among the elderly population. When assessing how marijuana affects the prevalence of NAFLD in two different subgroups (less than 40 years versus greater than 40 years), the study found that the younger population (less than 40 years old) who heavily used marijuana displayed a 35% reduction in risk of prevalence of NAFLD compared to a 26% reduction in the older population (greater than 40 years old) (Kim *et al.,* 2017). Kotan *et al*. in 2017 presented the results of the effect of cannabis use on 34 cannabis users who used cannabis for the first time at age 21.8 years after an average of more than 30.5 months of use, the patients still displaying close to normal liver function parameters. In contrast, Quraishi *et al*. (2013) discovered that 51% of cannabis-dependent patients displayed abnormal liver-related parameters at the age of commencement, which was 15.31 years old and after consuming cannabis for more than 9.53 years. These findings suggested that the age of marijuana users, the age of initiation and the duration of the substance use introduced some degree of variation in the effects of marijuana use on the health condition of the liver.

2.4.1 Duration of Marijuana Use and Liver Function

The knowledge of the duration of use is important in determining the effect of marijuana consumption on the normal liver functioning. Kotan *et al.* (2017) have found no negative health outcome at a mean duration of cannabis use of 30.5 months while Quraishi *et al.* (2013) discovered negative health impacts from cannabis use after 9.53 years. In addition, a Sudanese case-control study showed a strong correlation between the duration of marijuana use and liver enzyme activity (Mohamed *et al*., 2015).

2.4.2 Quantity of Marijuana Use and Liver Function

Another element at the core of marijuana users' health impacts is the amount of marijuana they use (Terry-McElrath *et al*., 2017). Out of 14,080 NHANES participants, Kim *et al*., (2017) found that 56.1% never used marijuana, 36.9% used in the past, and 7% current users of marijuana. Light users made up 4.9% of the 7% who said they were presently smoking marijuana, while strong users made up 2.1%. The prevalence rates of suspected NAFLD were 30.5%, 38.0%, and 40.7%, respectively, in present light users, previous users, and those who had never used marijuana when the study evaluated the relationship between dose-dependent marijuana use and suspected NAFLD. It has become apparent that current or past marijuana use has been significantly associated with a lower risk of the suspected NAFLD. Additionally, when the study evaluated only current users, light users were apparently shown to be negatively associated with NAFLD, but due to the small number of heavy users among the participants, there was no significant association with heavy users. By contrast, Liu *et al*., (2014) found no significant difference in biopsy fibrosis, liver inflammation, and steatosis in 21 HCV positive patients

classified as “high daily marijuana users” (greater than 1 g /day for marijuana use) compared to non-current marijuana users.

2.5 Liver Function Markers

The liver, located in the right upper quadrant of the body and below the diaphragm, is responsible for several functions, including primary detoxification of various metabolites, synthesizing proteins, and producing digestive enzymes (Iluz-Freundlich, 2020). Typically when reviewing liver function tests, the discussion includes alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP), gamma-glutamyl transferase (GGT), 5'nucleotidase, total bilirubin, conjugated (direct) bilirubin, unconjugated (indirect)bilirubin, prothrombin time (PT), the international normalized ratio (INR), lactate dehydrogenase, total protein, globulins, and albumin. These tests can help in locating the site of the liver damage, and the elevation pattern can assist in organizing a differential diagnosis (Ribeiro et al., 2019). Many of these tests do not comment on the function of the liver but rather pinpoint the source of the damage. Elevations in ALT and AST in out of proportion to ALP, and bilirubin denotes a hepatocellular disease. An elevation in ALP and bilirubin in disproportion to ALT and AST would characterize a cholestatic pattern. A mixed injury pattern is defined as an elevation of alkaline phosphatase and AST/ALT levels. Isolated hyperbilirubinemia is defined as an elevation of bilirubin with normal alkaline phosphatase and AST/ALT levels (Vagvala and O'Connor, 2018).

2.5.1 Aminotransferases

Aminotransferase includes AST and ALT. They are markers of hepatocellular injury. They participate in gluconeogenesis by catalyzing the transfer of amino groups from

aspartic acid or alanine to ketoglutaric acid to produce oxaloacetic acid and pyruvic acid, respectively. AST is present as cytosolic and mitochondrial isoenzymes and is found in the liver, cardiac muscle, skeletal muscle, kidneys, brain, pancreas, lungs, leucocytes, and red cells. It is not as sensitive or specific for the liver as ALT, and elevation in AST may be seen as secondary to non-hepatic causes as well. AST activity in neonates and infants is approximately twice that in adults, but these decline to adult levels by approximately six months (Oh *et al.*, 2017). ALT is a cytosolic enzyme that is found in high concentrations in the liver. The half-life of ALT is approximately 47 ± 10 hours. ALT is usually higher than AST in most types of liver disease in which the activity of both enzymes is predominantly from the hepatocyte cytosol. Hepatocellular injury and not necessarily cell death triggers the release of these enzymes into circulation. Both AST and ALT values are higher in normal males than females (Prati *et al*., 2002).

2.5.1.1 De Ritis Ratio

This is described as the ratio between aspartate Transaminase and Alanine Transaminase. De Ritis described the AST/ALT ratio as being a useful indicator of the aetiology of hepatitis anh his work was confirmed and extended by Wroblewski. In acute viral hepatitis, ALT is usually higher than AST with the AST/ALT ratio usually well below 1.0 and typically in the range of 0.5 to 0.7. in Chronic viral hepatitis, AST/ALT ratios are also below 1.0. however ratios above 1 may be found when progression to fibrosis and cirrhosis is present. AST/ALT ratios greater than 1.5 is highly suggestive of alcoholic hepatitis (Cohen and Kaplan, 1979)

2.5.1.2 Marijuana and Alanine aminotransferase

ALT is a circulating transaminase in the human body and a specific marker for liver dysfunction (Huang *et al.*, 2017). Clinical factors such as hepatitis-related diseases, alcohol intake, disease states like NAFLD, specific drugs, and physiological factors like extreme physical exercise affect the enzyme activity (Liu et al., 2014). Socio-demographic factors such as age, gender, and ethnicity may also interfere with the enzyme activity (Kim et al., 2017; Ruhl and Everhart, 2012). Although the enzyme is measured to assess overall health (Liu *et al*., 2014), the elevated level is often observed in liver dysfunction meaning that the enzyme is more specific to liver disease (Marshall et al., 2014). Mohamed *et al.* in 2015 proposed that chronic marijuana use is associated with the hepatic enzymatic alteration. A case-control study of 60 people with a history of cannabis use and 60 samples in a control group was carried out by Mohamed et al. (2015). Age and sex were matched, with the range of ages being 18 to 60. The study excluded people with liver cirrhosis, hepatitis, jaundice, hepatomegaly, and liver carcinoma and discovered a substantial statistical distinction between the two groups' ALT values. The study concluded that cannabinoids are possible hepatotoxic substance. By contrast, Kotan *et al*. (2017) found a normal ALT level in 118 Indians male cannabis users who used cannabis for more than 30 months. Cannabis may have a therapeutic impact by bringing the level of ALT in NAFLD back to normal, in contrast to its cytotoxic effect as seen in persons without liver disease. (Kim *et al.*, 2017).

2.5.1.3 Aspartate aminotransferase and Marijuana

Like ALT, the serum level of AST also increases during liver dysfunction. However, AST is less specific to the liver compared to ALT. The activity of the enzyme is

affected by the specific condition, such as myocardial infarction, muscular dystrophy, pulmonary emboli, and acute pancreatitis, in addition to being raised in liver diseases (Marshall et al., 2014). Demographic factors such as age, gender and ethnicity are also important when evaluating the activity of AST (Ruhl and Everhart, 2012; Kim *et* al., 2017). It is also known that marijuana alters the effect of AST (Mohamed et al., 2015). Mohamed *et al*. (2015) elucidated in a case-control study involving 60 subjects that the AST level in marijuana users is significantly different compared to the control group in a Sudanese population. The study then concluded that a possible alteration effect of marijuana on AST exists. Contrary to the above finding, Kotan et al. (2017) discovered a normal level of AST in 118 Indians male cannabis users who used cannabis for more than 30 months. Other studies like the one conducted by Muniyappa *et al.* (2013) found no effect of cannabis on AST.

2.5.2 Alkaline Phosphatase and Marijuana

Alkaline phosphatase is part of a family of zinc metalloenzymes that are highly concentrated in the microvilli of the bile canaliculus as well as several other tissues (e.g., bone, intestines, and placenta) (Iluz-Freundlich *et al.,* 2020). There are four isozymes: placental ALP or hPLALP (human placental ALP), germ cell ALP (GCALP or PLALP-like), intestinal ALP (IALP), and tissue-nonspecific ALP (TNALP). Of these four, PLALP and GCALP are the most heat stable at 65 C, and the bone ALP component of TnALP is the least. In healthy, non-smoking individuals, the PLALP and GCALP represent less than 1% of total ALP activity in the serum (Sharma et al., 2014). ALP is another enzyme used as part of liver function tests to evaluate possible dysfunction of the liver (Bishop et al., 2018; Lowe and John, 2018). In most adult patients, an elevated ALP is an indicator of liver disease (Lowe and

John, 2018). The enzyme serves as a marker of extrahepatic cholestasis such as stone in the bile duct or intrahepatic cholestasis such as drug-induced cholestasis or biliary cirrhosis (Bishop et al., 2018; Lowe and John, 2018; Sharma et al., 2014). Congestive heart failure, related bone disorders, primary and metastatic cancer, liver cirrhosis, chronic hepatitis, viral hepatitis, and other conditions that do not cause liver damage can all cause variations in the enzyme activity, making it challenging to interpret ALP. (Bishop et al., 2018; Lowe and John, 2018). Age, gender, and ethnicity are demographic factors associated with variations in ALP activity. The ALP level is slightly higher in men compared to women, and it decreases in the 15 to 50 age group, then and increases again in the old age (Lowe and John, 2018). Marijuana has been shown to be strongly correlated with an increase in ALP level in a case-control study in the Sudanese population using 60 patients who used cannabis for more than ten years matched with 60 controls non-smoker subjects. Chronic smokers showed a significant increase in ALP compared to non-smokers (Mohamed et al., 2015). A study conducted in India in 34 cannabis users (mean duration of cannabis use was 30.5 months) showed normal ALP serum level (Kotan et al., 2017). Quraishi et al. (2013) discovered, in contrast to the findings above, a significant rise in ALP in cannabis-dependent patients, suggesting an anomaly in liver function brought on by cannabis use. For the study, a total of 51 substance abusers with a mean cannabis use history of 9.53 years and 30 control subjects were selected. Findings revealed an elevation of 37.25% in substance-using subjects compared to the control group.

2.5.3 Gamma-Glutamyltransferase and Marijuana

Glycoprotein gamma-glutamyltransferase (GGT) is located on membranes of cells with high secretory or absorptive activities. Its primary function is to catalyze the

transfer of a gamma-glutamyl group from peptides to other amino acids. It is also abundant in many other sources of the body (kidney, pancreas, intestine, prostate, testicles, spleen, heart, and brain) but is more specific for biliary disease when compared to alkaline phosphatase because it is not present in bone. GGT levels are reported to be increased by an average of 12-fold in obstructive liver disease compared to ALP, which increased only 3-fold, so GGT is slightly more sensitive than ALP in this regard. GGT activity level in children may be a reliable index of bile duct damage. It is a useful indicator in separating the two forms of idiopathic cholestasis, with or without bile duct involvement. When biliary atresia in a child is identified and surgically treated, the GGT levels in the blood remain elevated as long as the infant is nursed. This is due to the high level of GGT in human breast milk for at least four weeks postpartum (Koenig and Seneff, 2015). There is a relationship between plasma GGT activity and weight, with values being 50% higher in individuals with a BMI greater than 30. This is believed to be due to fat deposition in the liver (steatosis) in obese subjects. Steatosis with a raised plasma GGT also occurs in diabetes mellitus, non-alcoholic steatohepatitis, and non-alcoholic fatty liver disease. Any liver disease that results in fibrosis and/or cirrhosis, such as alcoholic cirrhosis, hemochromatosis, α1-antitrypsin deficiency, and Wilson disease, will cause a raised plasma GGT. Space-occupying lesions, including malignancy (HCC or metastases secondary to malignancy elsewhere in the body), and granulomatous disease, for example, sarcoidosis and TB, are also associated with a raised plasma GGT. Drugs such as warfarin, phenobarbital, and phenytoin are noted to increase the enzyme level. Marijuana consumption is also noted to affect the level of GGT. Wani *et al.* (2017) noted in a case- control study of 250 male participants (125 cannabis abusers and 125 control group, mean age 25.32 years) that cannabis abusers exhibited

a higher level of GGT compared to non-users. However, 34 participants, who had used cannabis for an average of 30.5 months and had a mean age of 21.8, showed a normal level of GGT, with an average GGT of 24.6. (Kotan *et al*., 2017).

2.5.4 Albumin and Marijuana

Albumin is synthesized by the hepatic parenchymal cells at a rate dependent on colloidal osmotic pressure and dietary protein intake. Albumin is the main form of protein in human serum and is synthesized by the liver. It's involved in maintaining proper osmotic pressure and in the transport of various substances through the body (Bishop et al., 2018; Morman and Varacallo, 2018). The rate of albumin synthesis is also subject to feedback regulation determined by the plasma albumin concentration. The half-life of albumin is 21 days. Traces of albumin can be found in almost all extracellular body fluids. Little is lost from the body by excretion (Rozga *et al.,* 2013). It is catabolized in various tissues, which are taken up by cells by pinocytosis. With any liver disease, there is a fall in serum albumin, reflecting decreased synthesis. If liver function is normal and serum albumin is low, this may reflect poor protein intake (malnutrition) or protein loss (nephrotic syndrome, malabsorption, or protein-losing enteropathy) (Chen *et al.*, 2021).

A low concentration of albumin is most commonly associated with potential liver disease (Morman and Varacallo, 2018). Illegal drug use such as cannabis has been shown to decrease the level of albumin in human serum. In a recent case-control study, Quraishi *et al.* (2013) demonstrated the presence of a low albumin level in cannabis dependent patients compared to non-users with a mean duration of cannabis use being 9.53 years and the mean age of initiation being 15.31 years. Like the previous study, Wani et al. (2017) also found a lower score of albumin level in cannabis users

compared to non-users. In a different cohort, Kotan *et al*. (2017) discovered that 34 patients had normal levels of albumin, with an average ALB level of 4.20g/dl and initiation age of 21.8 years and 30.5 months of cannabis usage.

2.5.5 Total Protein and Marijuana

The serum TP, which is mainly synthesized by the liver, is of great importance because it serves in the regulation of several physiological functions, maintaining the osmotic pressure, transport of various metabolites, and participation in the activity of the immune system (Bishop, 2018). The level of serum TP gradually decreases with age and varies across gender (Tian *et al*., 2014). The evaluation of serum TP is useful to assess the synthetic ability of the liver. Although the protein level is not a sensitive marker for liver damage, it's useful in quantifying the severity of liver dysfunction (Bishop *et al*., 2018). Findings of the health effect of marijuana on the Total serum protein in adults varies across studies. A case-control study conducted in India which assessed the level of serum TP in 125 cannabis abusers, compared 125 non- cannabis smokers and found a decreased level of serum TP in cannabis abuser compared to non-smokers (Wani *et al*., 2017). Unlike the previous study, Quraishi *et al*. (2013) found in another case-control study involving 51 cannabis-dependent participants and 30 control subjects that smoking cannabis increased the level of serum TP by 15.68%.

In conclusion epidemiological study findings of the health effects of marijuana are conflicting. Several studies reported the therapeutic effect of the drug with respect to certain diseases, while others are still warning about its adverse health effects and have recommended more epidemiological investigations. The available literature on the effect of marijuana on the liver of healthy participants is rare hence this study aims at determining the possible effect of marijuana consumption on normal liver

function. Several studies have shown that the quantity of marijuana smoked, the age of initiation and the duration of marijuana use are critical factors in assessing the health effects of marijuana on the liver. These studies revealed that marijuana consumption causes some variations in the enzymatic activity of the liver.

CHAPTER THREE

MATERIALS AND METHODS

3.1 Study Design and Population

This is a case-control study aimed at determining the level of serum liver function test parameters among young adults who consume marijuana. The study was carried out in the Department of Medical Laboratory Science, University of Benin, Benin City. The subjects include smokers of marijuana in Benin City, while control subjects were non-smokers of marijuana. Evidence of marijuana smoking was confirmed at smoking joints where volunteers, certified by co-smokers as consumers for at least two years were enrolled. Only subjects who gives consent and meets with inclusion criteria will be enrolled in the study. A total of 120 participants were recruited for this study. The participants included 60 marijuana smokers and 60 non marijuana smokers.

3.2 Study site

The study was conducted in the department of Chemical Pathology in the University of Benin Teaching Hospital, Benin City. Benin City is the capital of Edo state, Nigeria and has a population of about 1,147,188 (National population Census, 2000). The people living in this area are mainly involved in trading and majority of them work in the public sector.

3.3 Sociodemographic Data

Participants were notified several days before the commencement of the study and were given appropriate instructions. The age and sex of the participants was recorded. All subjects were interviewed to establish their level of literacy and were assisted in filling out the questionnaire to minimize errors. Sociodemographic data were

collected by an interviewer who administered structured questionnaire to determine the age, educational levels, socioeconomic status, duration of marijuana use and quantity of marijuana consumed. Information on general health and history of past disease(s) and habits like consumption of alcoholic beverages and addictions were collected according to the British Medical Research Council questionnaire (BMRC, 1960).

3.4 Inclusion and Exclusion Criteria

Study group includes all adult male and female smokers of marijuana who gave consent to participate in this study. Only individuals who tested positive for the presence of cannabinoids in their urine using cannabis test strips (ACRO BIOTECH diagnostics) were recruited. Control group was non-smokers of marijuana who give consent to participate in this study. Individuals who already have pre-existing metabolic or system diseases like liver disease, viral hepatitis, fatty liver disease or alcoholic liver disease were excluded. Also, those with HIV and chronic infectious diseases, those who smoked both marijuana and cigarette, individuals with history of bleeding or clotting disorders and those less than 18 years or above 65 years of age were excluded.

3.5 Ethical Approval

Approval was sought and obtained from the Ethics committee of the College of Medical Sciences, University of Benin, Edo State. Informed consent was obtained from each of the participant.

3.6 Sample Size Determination

The sample size for this study was determined using the sample size determination formula for health studies (Lwanga and Lemeshow, 1991) and 2.5% world-wide annual prevalence of marijuana use (WHO, 2023).

$$N = \frac{z^2 p (1-p)}{d^2}$$

Where

N = required sample size

Z = confidence level interval 95% (standard value of 1.96)

P = estimated prevalence of 2.5% of the world population who use marijuana

D = margin of error at 5% (standard value of 0.05)

$$N = \frac{1.96^2 * 0.025 (1 - 0.025)}{0.05^2} = 37.5$$

n = 38

However, 60 adults were recruited for the study while 60 healthy subjects who do not smoke marijuana or cigarette were enrolled as controls.

3.7 Sample Collection

Prior to the collection of blood specimen, random urine specimen was collected and tested for the presence of cannabinoids using cannabis test strips (ACRO BIOTECH diagnostics). Only those with positive strip test were further evaluated. Under aseptic

conditions, 5mL of venous blood was collected via venipuncture and dispensed in lithium heparin sample container. The sample was centrifuged at 4000 rpm for 10 minutes to separate plasma from the red cells. After centrifugation, the plasma was decanted into another clean and dry plain tube. The plasma sample was stored at -20^{o}C until analyzed.

3.8 Laboratory Analysis

The liver function test parameters estimated in this study include alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP), Gamma Glutamyl-transferase (GGT), total protein and albumin.

3.9 Estimation of plasma Alanine Amino Transferase (Reitman and Frankel, 1957)

3.9.1 Principle of test:

Alanine aminotransferase catalyzes the transfer of Amino group from L-alanine to alpha ketoglutarate forming pyruvate and L-glutarate. Pyruvate reacts with 2,4-dinitrophenylhydrazine to form 2,4-dinitrophenylhydrazone whose concentration is proportional to ALT activity.

Oxoglutarate + L-alanine $\xrightarrow{\text{AST}}$ glutarate + pyruvate

3.9.2 Reagent composition

The R1 reagent contains 100mmol/L Phosphate buffer at pH of 7.4, 100mmol/L L-alanine and 2mmol/L oxoglutarate

The R2 reagent contains 2mmol/L of 2,2,4-dinitrophenylhydrazine

3.9.3 Procedures

Exactly 0.5mL of Reagent 1 was added to clean dry test tubes prepared for sample, standard and blank. Then, 0.1mL of samples were added to the respective tubes and 0.1mL of standard was added to the tube labeled standard using an automatic pipette, while 0.1mL of distilled water was then added to the tube labelled blank. The tubes were mixed and incubated at 37^{o}C for 30minutes. Thereafter, 0.5mL of Reagent 2 was then added to tubes containing samples, standard and blank respectively. The tubes were mixed and allowed to stand for 20 minutes at 25^{o}C. The reaction was stopped by the addition of 5mL of NaOH to the tubes containing samples, standard and blank respectively. The tubes were mixed and absorbance read against reagent blank after 5 minutes.

3.9.4 Calculation

The activity of ALT was obtained from the table below. It is such that the absorbance obtained is matched with that on the table to get the activity. The absorbance obtained is matched with that on the table to get the activity. For absorbance value between values on the table, the activities of both are added and the average taken.

Absorbance	U/I	Absorbance	U/I
0.025	**4**	**0.275**	**48**
0.050	**8**	**0.300**	**52**
0.075	**12**	**0.325**	**57**
0.100	**17**	**0.350**	**62**
0.125	**21**	**0.375**	**67**
0.150	**25**	**0.400**	**72**
0.175	**29**	**0.425**	**77**
0.200	**34**	**0.450**	**83**
0.225	**39**	**0.475**	**88**
0.250	**43**	**0.500**	**94**

3.9.5 Reference Range: 0 – 55 U/L

3.9.6 Quality Control

The reagent's expiry date was verified. Clinical Chemistry Quality Controls sera (Acusera, Randox Laboratories) were included in the assay and the values obtained were compared with the values provided by the manufacturer.

3.10 Estimation of Aspartate aminotransferase (Reitman and Frankel, 1957)

3.10.1 Principle

Aspartate aminotransferase catalyzes the transfer of amino acid groups from aspartate to ketoglutarate, forming oxaloacetate and glutamate. The oxaloacetate reacts with

2,4-dinitrophenylhydrazine to form 2,4-dinitrophenylhydrazone which in alkaline pH is reddish brown and whose concentration is proportional to the AST activity.

Consider,

Oxoglutarate + L-aspartate $\xrightarrow{\text{AST}}$ glutamate + oxaloacetate

3.10.2 Reagent Composition

The R1 reagent contains 100mmol/L Phosphate buffer at pH of 7.4, 100mmol/L L-aspartate and 2mmol/L oxoglutarate

The R2 reagent contains 2mmol/L of 2,2,4-dinitrophenylhydrazine

3.10.3 Procedure

Exactly 0.5mL of Reagent 1 was added to the tubes prepared for samples, standard and blank. Then, 0.1mL of samples were added to the respective tubes and 0.1mL of standard was added to the tube labeled standard using an automatic pipette. Thereafter, 0.1mL of distilled water was then added to the tube labelled blank. The tubes were mixed and incubated at 37^{o}C for 30 minutes. Then, 0.5mL of Reagent 2 was added to tubes containing samples, standard and blank respectively. The tubes were mixed and allowed to stand for 20 minutes at 25^{o}C. Then, the reaction was stopped by the addition of 5mL NaOH to the tubes containing samples, standard and blank respectively. The tubes were mixed and absorbance read against reagent blank after 5 minutes.

3.10.4 Calculations

The activity of AST was obtained from the table below. It is such that the absorbance obtained is matched with that on the table to get the activity. The absorbance obtained is matched with that on the table to get the activity. For absorbance value between values on the table, the activities of both are added and the average taken.

Absorbance	U/L	Absorbance	U/L
0.020	**7**	**0.100**	**36**
0.030	**10**	**0.110**	**41**
0.040	**13**	**0.120**	**47**
0.050	**16**	**0.130**	**52**
0.060	**19**	**0.140**	**59**
0.070	**23**	**0.150**	**67**
0.080	**27**	**0.160**	**76**
0.090	**31**	**0.170**	**89**

3.10.5 Normal Range: 5-34 U/L

3.10.6 Quality Control

The reagent's expiry date was verified. Clinical Chemistry Quality Controls sera (Acusera, Randox Laboratories) were included in the assay and the values obtained were compared with the values provided by the manufacturer.

3.11 Estimation of Alkaline Phosphatase

3.11.1 Principle:

The alkaline phosphatase acts upon the AMP-buffered sodium thymolpthalein monophosphate. The addition of an alkaline reagent stops enzyme activity and simultaneously develops a blue chromogen which is measured spectrophotometrically at 590nm.

3.11.2 Reagent Composition

Alkaline phosphatase substrate: 3.6Mm sodium thymolpthalein monophosphate

Buffer: 0.2M 2-amino-2methyl-1-propanol

Magnesium chloride--------- 1.0mM

Colour developer: 0.1M sodium hydroxide

0.1Msodium carbonate

Alkaline phosphatase standard: Thymopthalein in n-propanol (0.5Mm/L).EQUIVALENT TO 50U/L enzyme activity when used according to the alkaline phosphatase procedure.

3.11.3 Procedure

Precisely, 0.5mL of the ALP substrate was added to tubes prepared for reagent blank, standard and test. The tubes were incubated at 37^{o}C for 3 minutes for equilibration. Then, 0.05mL Samples were added to the respective tubes and 0.05mL standard was added to the tube containing standard. Also, 0.05 mL of distilled water was added to the tube containing blank. The tubes were mixed and incubated at 37^{o}C for 10

minutes. Thereafter, 2.5 mL of ALP colour developer was added and the tubes were mixed and absorbance read at 590nm.

3.11.4 Calculations

Activity of test (IU/L) = Absorbance of test x activity of standard

Absorbance of standard

Where standard activity = 50U/L

3.11.5 Normal Range

: Adult: 40 – 150 U/L

3.11.6 Quality Control

The reagent's expiry date was verified. Clinical Chemistry Quality Controls sera (Acusera, Randox Laboratories) were included in the assay and the values obtained were compared with the values provided by the manufacturer.

3.12 Estimation of Serum Gamma Glutamyl transferase (GGT)

3.12.1 PRINCIPLE:

The substrate L-y-glutamyl-3-carboxy-4-nitroanilide, in the presence of glycylglycine is converted by y-glutamyltransferase in the sample to 5-amino-2-nitrobenzoate which is measured at 405nm.

L-y-glutamyl-3-carboxy-4-nitroanilide + glycylglycine L-y- ⟶ glutamylglycylglycine + 5-amino-2nitrobenzoate

3.12.2 Reagent Composition

R1 contains 100mmol/L of Tris, pH of 8.25, 100mmol/L of glycylglycine 100mmol/L of preservative and additive.

R2 reagent contains 22.5mmol/L of L-y-glutamyl-3-carboxy-4-nitroanilide, 10mmol/L acetate, pH of 4.5 , a stabilizer and preservative.

3.12.3 Procedure

The working reagent was made from a mixture of R1 and R2. Exactly 1ml each of the working reagents was placed in the tubes labelled for sample, blank and standard using a pipette. Precisely, 0.10mL of samples were placed in the respective tubes and 0.10mL of standard was placed in the tube for standard and 0.10mL of distilled water was added to the tube containing blank. The tubes were mixed, initial absorbance was read and timer was started simultaneously. The absorbance was read again after 1, 2 and 3 minutes.

3.12.4 Calculations

Activity (U/L) = 1158 x A 405 Δm/min

3.12.5 Normal Values: 0 – 49 U/L

3.12.6 Quality Control

The reagent's expiry date was verified. Clinical Chemistry Quality Controls sera (Acusera, Randox Laboratories) were included in the assay and the values obtained were compared with the values provided by the manufacturer.

3.13 ESTIMATION OF TOTAL PROTEIN

3.13.1 Method: Biuret Method

3.13.2 Principle

Proteins give an intensive violet-blue violet complex with copper salts in an alkaline medium. Iodide is included as an antioxidant. The intensity of the colour formed is proportional to the total protein concentration in sample.

3.13.3 Reagent Composition

Reagent 1 which is the Biuret reagent stock contains 15mmol/L sodium potassium tartate, 100mmol/L sodium iodide, 5mmol/L potassium iodide, 5 mmlol/L copper(III)sulphate, 100mmol/L sodium hydroxide

3.13.4 Procedure

Exactly I.0 mL of the biuret working reagent was pipetted in a series of glass tubes and 0.025mL aliquots of the standard and samples were added to their respective tubes. One tube was reserved for biuret reagent blank. The contents were mixed and incubated at 37^{o}C for 5 minutes. It was read spectrophotometrically at 540nm against reagent blank.

3.13.5 Calculation

$$\text{Protein concentration (mg/dl)} = \frac{\text{Absorbance of test x concentration of standard}}{\text{Absorbance of standard}}$$

3.13.6 Normal range: 60-83g/L

3.13.7 Quality Control

The reagent's expiry date was verified. Clinical Chemistry Quality Controls sera (Acusera, Randox Laboratories) were included in the assay and the values obtained were compared with the values provided by the manufacturer.

3.14. Estimation of Serum Albumin

3.14.1 Principle: This method is based on the specific binding of bromocresol green (BCG), an anionic dye, and the protein at acid pH to produce a colour change of the indicator from yellow-green to green-blue with the resulting shift in the absorption wavelength of the complex. The intensity of the colour formed is proportional to the concentration of albumin in the sample

BCG + albumin $\xrightarrow{\text{pH 4.2}}$ **BCG albumin complex**

3.14 .2 Reagent Composition

The Bromocresol green reagent was contained 83mmol/L Succinic acid, 167mmol/L Bromocresol green, 50mmol/L sodium hydroxide and 1g of polyoxyethylene monolauryl ether at a pH of 4.2

3.14.3 Procedure

Precisely 1.0mL of Bromocresol green was added to tubes prepared for test, standard and blank. Then, 0.005mL of the sample was added to the tubes containing the different samples and 0.005mL of standard was added to the tube labelled Standard. The tubes were mixed and absorbance was read at 630nm against reagent blank after 3 minutes.

3.14.4 Calculations

Serum albumin concentration (mg/dl) = Absorbance of test x concentration of standard

Absorbance of standard

3.14.5 Normal Range: 32-52g/L

3.14.6 Quality Control

The reagent's expiry date was verified. Clinical Chemistry Quality Controls sera (Acusera, Randox Laboratories) were included in the assay and the values obtained were compared with the values provided by the manufacturer.

3.15 Statistical Analysis

The values of the measured parameters are expressed as mean and standard error of mean (SEM) for both test and control. The data generated were compared using Students' paired t-test and Analysis of Variance (ANOVA) at 95% confidence intervals and $p < 0.05$ was considered significant. Correlation between parameters was done using correlation coefficient test.

CHAPTER FOUR

RESULTS

Table 4.1 shows the socio-demographic characteristics of study participants (marijuana smokers). Male participants were 54 (90%) while the female was 06 (10%). The mean ± SD age of study participants was 34.33±11.57 with a minimum age of 19 years and a maximum of 58 years. Majority of the participants (46.7%) were between 15-30 years followed closely by age group 31-45 (40%). Age group 46-60 had the lowest participation (13.3%). Some 56.7% of participants were single while the other 43.3% were married. 10% of participants had primary school education, 56.7% had secondary school education while 33.3% had tertiary school education.

Table 4.2 shows the duration and quantity of marijuana consumed by study participants. Participants who had been smoking marijuana for 1-10 years were the most frequent (66.7%), followed by 11-20 years (20%) and lastly 21-30 years (13.3%). It was also observed that 90% of participants consumed between 1-5 wraps of marijuana daily while the other 10% consumed between 6-10 wraps daily.

Table 4.3 shows the height, weight and body mass index between control and marijuana smokers in male and female participants. There was no statistically significant difference in the height, weight and BMI between control and marijuana smokers in male and female participants ($p<0.05$).

Table 4.1: Socio demographic Characteristics of Marijuana Smokers.

Variable	Category	Frequency	Percentage (%)
Gender	Male	54	90
	Female	06	10
Age(Years)	15-30	28	46.7
	31-45	24	40.0
	46-60	8	13.3
Marital Status	Single	34	56.7
	Married	26	43.3
Educational Status	Primary	6	10.0
	Secondary	34	56.7
	Tertiary	20	33.3

Table 4.2: Duration and Quantity of Marijuana Consumed by Participants

Variable	Category	Frequency	Percentage (%)
Smoking Duration (Years)	1-10	40	66.7
	11-20	12	20.0
	21-30	08	13.3
Quantity per day (Wraps)	1-5	54	90
	6-10	06	10

Tale 4.3. Height, Weight and Body Mass Index of Study Participants

Parameter		Control Subjects	Marijuana Smokers	p value
Height (cm)	Male	170.43±10.39	169.37±8.80	0.696
	Female	165±8.24	168±5.00	0.588
Weight (kg)	Male	66.65±8.69	67.26±7.43	0.786
	Female	61.83±7.83	64.33±7.57	0.662
BMI (Kg/m^2)	Male	22.87±0.84	23.45±1.86	0.188
	Female	22.63±1.14	22.74±1.46	0.913

Values are shown in Mean±SD, $p<0.05$ was considered significant.

Table 4.4 shows the comparison of Liver Function biomarkers activity between control subjects and Marijuana smokers. Aspartate Aminotransferase (AST) was significantly higher in male and female marijuana smokers (42.4±8.67, 41.67±14.15) when compared to controls (26.34±4.95, 24.00±5.97) ($p<0.05$). Alanine Aminotransferase (ALT) was significantly higher in male and female marijuana smokers (29.56±8.48, 20.33±1.15) when compared to control (14.91±3.36, 14.29±3.55) ($p<0.05$). De Ritis ratio (AST/ALT ratio) showed no statistically significant difference in males and females between smokers and non-smokers ($p<0.05$). Gamma Glutamyl- transferase (GGT) was significantly higher in male and female marijuana smokers (50.04±13.55, 57.33±17.68) when compared to controls (20.09±6.17, 20.14±2.91) ($p<0.05$). Male marijuana smokers (6.67±0.50) had a significantly lower total protein when compared to controls (7.05±0.28) ($p<0.05$). Albumin (ALB) was significantly lower in male and female marijuana smokers (3.19±0.38, 3.10±0.26) when compared to control (3.92±0.22, 3.82±0.16) ($p<0.05$). Alkaline phosphatase (ALP) showed no significant difference between male and female marijuana smokers and controls ($p>0.05$). Some 48/60 (80%) of marijuana smokers had AST values (44.8±8.44) above normal range while 34/60 (56.7%) had GGT values (61.3±7.60) above normal range.

Table 4.4. Comparison of Liver Function biomarkers between control subjects and Marijuana smokers

Parameters		Control Subjects	Marijuana Smokers	95% Confidence Interval	p value
AST(U/L)	Male	26.34±4.95	42.4±8.67	-20.1, -11.9	<0.001
	Female	24.00±5.97	41.67±14.15	-31.6, -3.7	0.019
ALT(U/L)	Male	14.91±3.36	29.56±8.48	-18.4, -10.9	<0.001
	Female	14.29±3.55	20.33±1.15	-11.0, -1.1	0.023
AST/ALT Ratio	Male	1.80±0.36	1.55±0.53	-0.1, 0.5	0.060
	Female	1.71±0.25	2.07±0.64	-1.0, -0.3	0.226
GGT(U/L)	Male	20.09±6.17	50.04±13.55	-36.1, -23.8	<0.001
	Female	20.14±2.91	57.33±17.68	-51.8, -22.6	<0.001
TP(g/dL)	Male	7.05±0.28	6.67±0.50	0.1, 0.6	0.002
	Female	6.91±0.46	6.43±0.45	-0.3, 1.2	0.166
ALB(g/dL)	Male	3.92±0.22	3.19±0.38	0.6, 0.9	<0.001
	Female	3.82±0.16	3.10±0.26	0.4, 1.0	0.001
ALP(U/L)	Male	86.70±9.69	84.85±6.15	-2.7, 6.4	0.419
	Female	79.00±7.14	75.33±5.03	-7.0, 14.2	0.449

Mean±SD of Liver Function Biomarkers of Marijuana Smokers above Normal Range

Parameter	Mean±SD	Normal Range
AST (U/L)	44.8±8.44	5-34
GGT (U/L)	61.3±7.60	0-49

AST=Aspartate aminotransferase; ALT=alanine aminotransferase, ALP=alkaline phosphatase, GGT=gamma glutamyltransferase, TP=total protein, ALB=albumin

Table 4.5 shows the correlation of gender, age, duration of smoking and quantity of smoking with liver function parameters. Quantity of marijuana consumed in wraps had a significant negative correlation with total protein (r=-0.392, p=0.032), while gender correlated negatively with alkaline phosphatase (r=-0.403, p=0.027).

Table 4.5. Correlation Between Age, Gender, Duration and Quantity of Smoking with Liver Function Parameters

Parameter		Gender	Age (Years)	Duration (Years)	Quantity (Wraps)
AST	r value	0.013	0.214	0.333	0.126
	p value	0.948	0.256	0.072	0.506
ALT	r value	-0.251	0.157	0.193	0.187
	p value	0.181	0.407	0.307	0.323
AST/ALT Ratio	r value	0.219	-0.047	0.022	-0.042
	p value	0.244	0.807	0.907	0.826
GGT	r value	0.137	0.200	0.286	0.316
	p value	0.472	0.289	0.125	0.089
TP	r value	-0.054	0.064	0.022	-0.392
	p value	0.777	0.738	0.910	0.032*
ALB	r value	-0.012	0.073	0.100	-0.098
	p value	0.948	0.701	0.597	0.607
ALP	r value	-0.403	0.020	0.180	-0.010
	p value	0.027*	0.918	0.340	0.960

AST=Aspartate aminotransferase; ALT=alanine aminotransferase, ALP=alkaline phosphatase, GGT=gamma glutamyltransferase, TP=total protein, ALB=albumin

CHAPTER FIVE

DISCUSSION, CONCLUSION AND RECOMMENDATION

5.1 Discussion

Marijuana is an illicit drug and its consumption has been found to have varying effects in the body especially in young adults (Fischer *et* al., 2013). Various studies have been carried out to access the effect of marijuana but the results appears to be inconsistent. Understanding the effects on marijuana consumption on liver function may generate an intriguing data that can alert government and certain bodies into taking actions to control marijuana use. This is particularly important in recent times where the consumption of marijuana is increasingly popular with legalization of consumption in certain countries (Cerda *et al.*, 2012). The change in perceived risk associated with the use of marijuana, with some people believing that marijuana use may not be associated with health risk (Okaneku *et al*., 2015).

From this study the possible effect of marijuana consumption on the liver was assayed for by measuring the levels of the various liver function parameters including the levels of AST, ALT, ALP, GGT, Total Protein and Albumin and comparing between Marijuana smokers and non smokers. This study also describes the Association between the duration of use and quantity of marijuana used with the various Liver Function Parameters. Abnormal level is indicative of various liver disease.

In this study, AST and ALT was significantly higher in Marijuana smokers when compared to controls. This is consistent with a previous study carried out by Bornini *et al*.,(2004). The authors observed that hepatic and enzymatic alterations were associated with chronic marijuana usage. They observed slightly elevated AST (42.3%) and ALT (34.6%). Elevation in the level of ALT and AST is often observed

in hepatocellular dysfunction meaning that these enzymes are specific to liver disease (ALT is more specific). Also, GGT activity was observed to be significantly higher in Marijuana smokers than control group. In 2017, Wani *et* al noted in a case control study of 250 male participants (125 cannabis users and 125 control group) that cannabis users had higher activities of GGT, ALT and AST compared to non smokers. According to the study, abnormal range in these enzymes could lead to destruction of red blood cells, hepatitis or cirrhosis, blockage of bile ducts, osteomylacia and cardiovascular diseases. Elevation in GGT levels could be due to any liver disease that results in fibrosis and/or cirrhosis, such as alcoholic cirrhosis, hemochromatosis or some certain drugs like Wafarin and Phenobarbital.

In this study, majority of marijuana smokers had elevated AST and GGT above the reference ranges. This is particularly important since GGT is an indicator of liver toxicity and damage to bile ducts. Elevated GGT can lead to red blood cell membrane damage thus causing the release of potentially toxic transition metals and increased oxidative stress (Koenig and Seneff, 2015). Elevated GGT is also associated with risk of coronary heart disease, type 2 diabetic mellitus and stroke. It has been established that GGT enables metabolism of glutathione and glutathionylated xenobiotics (Koenig and Seneff, 2015). GGT is very sensitive for the diagnosis and prognosis of liver disease beyong its association with biliary disease and the ALP. This may be attributed to its regulation of redox status. GGT can be used in differential diagnosis and prognostic algorithms or scores for liver disease. It is an important predictor of development and the presence of liver disease in subjects discovered to have first-time abnormal liver function tests (Dillon et al., 2016). The mean values of AST and GGT were far above the reference range. A raised level in AST values is associated with acute hepatocellular damage, myocardial infarction, circulatory collapse (shock) and

infectious mononucleosis (Bishop, 2018). Raised levels of GGT is a sensitive indicator of liver disease. Increased levels are seen in all types of liver diseases and therefore it does not help in the differential diagnosis of hepatic diseases (Bishop, 2018).

Albumin was significantly lower among smokers than than controls. This is consistent with a previous study(Morman and Varacallo, 2018). According to the authors, illegal drug use such as cannabis has been shown to decrease the level of albumin in human serum, therefore albumin is most commonly associated with potential liver disease. Illegal drug use such as cannabis has been shown to decrease the level of albumin in human serum and this can be due to liver diseases. If liver function is normal and serum albumin is low, this may reflect poor protein intake (malnutrition) or protein loss (nephrotic syndrome, malabsorption, or protein-losing enteropathy) (Chen *et al*., 2021).

Total protein was significantly lower in male marijuana smokers when compared to controls. This supports the case-control study conducted in India which assessed the level of serum TP in 125 cannabis abusers, compared 125 non- cannabis smokers and found a decreased level of serum TP in cannabis abuser compared to non-smokers (Wani *et al.,* 2017). The reduction in total protein levels may be due to deficiency in diet, stress exercise, liver disease or dehydration. The evaluation of serum TP is only useful to assess the synthetic ability of the liver because it is not specific for liver damage.

Alkaline phosphatase (ALP) showed no significant difference between male and female marijuana smokers and control. This result corresponded with a study conducted in India in 34 cannabis users (mean duration of cannabis use was 30.5

months) showed normal ALP serum level (Kotan *et al*., 2017). The results from this study contradicts that of Quraishi *et al*. (2013). They discovered that in contrast to the findings in this study, there was a significant rise in ALP in cannabis-dependent patients, suggesting an anomaly in liver function brought on by cannabis use.

De Ritis ratio (AST/ALT ratio) showed no statistically significant difference in males and females between smokers and non-smokers. This result shows that there is no significant relationship between marijuana consumption and chronic or toxic hepatitis.

Findings from this study shows that Quantity of marijuana consumed in wraps had a significant negative correlation with total protein (TP) but there was no significant correlation with the other liver function parameters. Bornini *et al*., in 2004 observed that there was no correlation between the amount of Marijuana used and the serum levels of ALT, AST, ALP and GGT which is consistent with this study. Bornini *et al* did not show any relationship between the quantity of Marijuana use and Total protein. Serum Total protein is mainly synthesized in the liver, it can be used to access the synthetic ability of the liver. Although the protein level is not a sensitive marker for liver damage, it's useful in quantifying the severity of liver dysfunction (Bishop et al., 2018). It is apparent that an increased consumption of Marijuana can lead to the impairment in the regulation of several physiological functions.

Results from this study shows no significant correlation between the duration of marijuana use and the liver function parameters. This is consistent with a study by Kotan *et al.* (2017). These authors found no negative health outcome at a mean duration of cannabis use of 30.5 months. The oucome of the effect of duration on liver enzymes can be affected by epidermiological factors. Factors like gender and age could also affect this result.

5.2 CONCLUSION

The study explored the possible hepatotoxic effects of marijuana consumption among young adults. The mean levels of AST, ALT and GGT were elevated in marijuana smokers. Total protein and Albumin levels were low in Marijuana smokers compared with controls. The findings suggest that there may be a potential link between marijuana use and compromised liver function. Awareness should be made on the effect of marijuana to promote better liver health in young adults.

5.3 RECOMMENDATION

Based on the results obtained from the research conducted on the effect of marijuana consumption on liver function parameters more research needs to be done to understand the direct biological mechanisms through which marijuana consumption can affect liver function. This could be done by exploring interractions between cannabinoids and liver enzymes. Government needs to explore potential interventions for improving liver health in young adults who used marijuana with abnormal liver markers. Government and NGOs should develop educational programs to inform young adults on the potential risk associated with marijuana use on liver function.

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APPENDIX I

Laboratory Apparatus

1. Cuvette
2. Test tube rack
3. Automatic micropipette

Laboratory Equipment

1. Spectrophotometer
2. Water bath

APPENDIX II

DEPARTMENT OF MEDICAL LABORATORY SCIENCE

SCHOOL OF BASIC MEDICAL SCIENCES

UNIVERSITY OF BENIN.

BENIN CITY.

QUESTIONNAIRE ON ESTIMATION OF DIGESTIVE ENZYMES, C-REACTIVE PROTEIN, CREATINE KINASE, FULL BLOOD COUNT, LIVER FUNCTION PARAMETERS AND LIPID PROFILE AMONG YOUNG ADULTS IN BENIN CITY

Dear Respondent,

This questionnaire is strictly to provide information regarding a research work on estimation of digestive enzymes, c-reactive protein, full blood count, liver function parameters, lipid profile and creatine kinase among marijuana consumers in Benin City. You are assured of full confidentiality of all information provided please. Please tick (√) or write your responses where appropriate.

Thanks for your co -operation

SECTION A:

Sociodemographic Data

1. Age: _____
2. Sex: Male: [ ] Female[ ]
3. State of origin: _____
4. Ethnicity: Hausa [ ] Igbo [ ] Yoruba [ ] Others _____
5. Marital status: Single [ ] Married[ ]
6. Occupational status: Employed [ ] Unemployed[ ] Retired[ ] Student [ ]
7. Level of formal Education: None [ ] Primary [ ] Secondary [ ] Tertiary[ ]

SECTION B

- Do you smoke? YES[ ] NO[ ]
- Do you drink alcohol? YES [ ] NO[ ]
- Extent of alcohol intake: Heavy [ ] Medium[ ] Mild[ ]
- b. If heavy, how many bottles per day? _____
- Are you conscious of your dietary intake? YES[ ] NO[ ]
- Do you use Marijuana? YES[ ] NO[ ]
- If yes, how often? _____
- When did you start using Marijuana? _____
- what route of administration do you use? SMOKING [ ] ORAL [ ]
- When last did you go for checkup? _____
- Do you have any pre-existing disease?_____
- Do you have any of your family members with any disease Yes [ ] NO[ ]
- Are you on medication(s)? YES[ ] NO[ ]
- What kind of medication(s)? _____
- Weight (kg):_____
- Height (m):_____

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