

**EFFECTS OF ALCOHOL ON SOME BIOCHEMICAL
PARAMETERS OF ALCOHOLICS IN NSUKKA, ENUGU
STATE, NIGERIA**

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**DEPARTMENT OF BIOCHEMISTRY
UNIVERSITY OF NIGERIA
NSUKKA**

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TITLE

**EFFECTS OF ALCOHOL ON SOME BIOCHEMICAL
PARAMETERS OF ALCOHOLICS IN NSUKKA, ENUGU
STATE, NIGERIA**

**A THESIS SUBMITTED IN PARTIAL FULFILMENT OF THE
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NSUKKA**

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NOVEMBER, 2014

CERTIFICATION

EZE, Chukwuka Wenceslaus, a postgraduate student with Registration Number PG/M.Sc/Ph.D/08/48299 in the Department of Biochemistry has satisfactorily completed the requirements for the research work for the degree of Doctor of Philosophy (Ph.D) in Medical Biochemistry. The work embodied in this report is original and has not been submitted in part or full for any other diploma or degree of this or any other university.

.....
PROF L.U.S. EZEANYIKA
(Supervisor)

.....
PROF O.F.C. NWODO
(Head of Department)

.....
EXTERNAL EXAMINER

DEDICATION

This work is dedicated to the Almighty God and to brighter future as personified by my wife Ebere and my little children Adaeze, Chisom and Chilomma and Chimdindu Weseze.

ACKNOWLEDGEMENT

Since I couldn't have accomplished this work all alone, I owe thanks to Almighty God and to some special people whose help have been instrumental to the success. In this respect, I wish to acknowledge in a special way the contributions of my supervisor Prof. L.U.S. Ezeanyika whose wisdom is inspiring and invigoratingly satisfying. His systematic channeling of my mind and efforts to the critical aspects of the topic was a rare ingenuity. I am grateful for his critical appraisal of this work which indeed was indelible.

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I can only plead that these few lines may not be adequate to fully mention all the names that helped in this work and at the same time give them their deserved measure of thanks.

ABSTRACT

This work was aimed at finding the effects of alcohol on some biochemical parameters. A total of one hundred and eighty (180) apparently healthy, non-hypertensive male alcoholics were used for the study. Forty (40) non-consumers of alcohol were used as control. The activity of alanine aminotransferase (ALT) in the control was 10.50 ± 2.00 IU/L while it was 16.50 ± 1.50 IU/L; 17.50 ± 2.00 IU/L and 18.31 ± 2.00 IU/L in alcoholics who showed preference for palmwine, beer and distilled spirit respectively. Also, the activity of aspartate aminotransferase (AST) in the control was 9.51 ± 0.35 IU/L while it was 18.44 ± 0.40 IU/L, 19.21 ± 0.19 IU/L, 20.32 ± 0.64 IU/L in alcoholics who showed preference for palmwine, beer and distilled spirit respectively. The ALT and AST activities of alcoholic subjects who showed preference for distilled spirit was significantly higher ($p < 0.05$) than those who showed preference for palmwine and beer. The activities of alcoholics who showed preference for palmwine was the lowest. Furthermore, the serum total bilirubin concentration of the alcoholics was significantly higher ($p < 0.05$) compared with the control. The serum total bilirubin concentrations were 18.65 ± 2.10 $\mu\text{mol/l}$, 19.40 ± 1.50 $\mu\text{mol/l}$ and 22.75 ± 1.60 $\mu\text{mol/l}$ for alcoholics who showed preference for palmwine, beer and distilled spirit respectively. The serum total bilirubin of the control was 8.30 ± 2.00 $\mu\text{mol/l}$. The alkaline phosphatase (ALP) activity of the alcoholic subjects was significantly higher ($p < 0.05$) compared with the control. The ALP activity of the control was 61.50 ± 30.00 IU/L while the ALP activity was 174.20 ± 2.50 IU/L, 175.10 ± 1.50 IU/L and 177.40 ± 1.00 IU/L in the three categories of alcoholics who showed preference for palmwine, beer and distilled spirit respectively. Moreover, the urine total protein concentration of the alcoholics was significantly higher ($p < 0.05$) compared with the control. Alcoholics who showed preference for distilled spirit had urine total protein of 153.96 ± 0.43 mg/dl followed by alcoholics who showed preference for beer and palmwine who had urine total protein of 152.74 ± 0.42 mg/dl and 151.34 ± 0.60 mg/dl respectively. The urine total protein of the control was 56.40 ± 0.40 mg/dl. Furthermore, the urine specific gravity, serum urea and creatinine of the alcoholics were significantly higher ($p < 0.05$) compared with the control. However, the plasma sodium, potassium and creatinine clearance of the alcoholics were significantly lower ($p < 0.05$) compared with the control. The body mass index (BMI) of the three groups of alcoholics fell within the range of 18.50 to 24.90. The blood pressure of both the alcoholic and control subjects were normal (below 140/90 mmHg). This work therefore shows that chronic alcohol use could induce both hepatic and renal dysfunctions in the alcoholics which manifested in form of adverse variations in some biochemical parameters of prognostic and diagnostic utility.

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LIST OF ABBREVIATIONS

ATP	Adenosine triphosphate
ALT	Alanine aminotransferase
AST	Aspartate aminotransferase
ALP	Alkaline phosphatase
HDL	High density lipoprotein
Cyt	Cytochrome
GBM	Glomerular basement membrane
GFR	Glomerular filtration rate
ACE	Angiotensin converting enzyme
ECF	Extracellular fluid
ADH	Alcohol dehydrogenase
NAD	Nicotinamide adenine dinucleotide
UDPG	Uridyldiphosphate glucuronyl
MAO	Monoamine oxidase
LDL	Low density lipoprotein
SOD	Superoxide dismutase
VLDL	Very low density lipoprotein

CHAPTER ONE

INTRODUCTION

Generally, alcohol designates a class of compounds that are hydroxyl derivatives of aliphatic hydrocarbons. However, in this study, the term alcohol used without additional qualifications refers specifically to ethanol. A variety of alcoholic beverages have been consumed by man in the continuing search for euphoria producing stimuli. Among some people, alcohol enjoys a high status as a social lubricant that relieves tension, gives self confidence to the inadequate, blurs the appreciation of uncomfortable realities and serves as an escape from environmental and emotional stress.

Alcohol has been loved and hated at different times by different people. Alcohol has been celebrated as healthful especially to the heart (red wine) and most pleasant to the taste buds; and then dismissed as "demon's rum" and "devil in solution" depending on the prevalent view.

In spite of the apparent divergent and sometimes conflicting opinions about alcohol, the consensus shared by drinkers and non drinkers alike is that excessive and chronic consumption of alcohol is a disorder. Like any other chronic disorder, it develops insidiously but follows a predictable course. The first or pre-alcoholic symptomatic phase begins with the use of alcohol to relieve tensions. The second (or prodromal) phase is marked by a range of behaviours including preoccupation with alcohol, surreptitious drinking and loss of memory (Hock *et al.*, 1992). In the third (or crucial) phase, the individual loses control over his drinking. This loss of control is the beginning of the disease process of addiction. The individual starts drinking early in the morning and stays up drinking till late in the night. Impairment in biochemical activities becomes manifest as the organs of the alcoholic begin to deteriorate. Other medical problems develop by the time the alcoholic gets into the final (chronic phase). Prolonged intoxications become the rule. Alcoholic psychosis develops, thinking is impaired, and fear and tremors become persistent (Klemin and Sherry, 1981). A previously responsible individual may be transformed into an inebriate – stereotype alcoholic.

Fear-instilling but thought-provoking terms such as the "coming epidemic", a "miserable trap", have been used to show concern for the potential hazard of widespread alcoholism.

In its 1978 revision of the international classification of diseases, the World Health Organization defined alcoholism as "a state, psychic and usually also physical, resulting from taking alcohol, characterised by behavioural and other responses that always include a

compulsion to take alcohol on a continuous or periodic basis in order to experience its psychic effects and sometimes to avoid the discomfort of its absence; tolerance may or may not be present. This definition emphasized the compulsive nature of drinking, the psychological and physical effects, and dependence (‘discomfort of its absence’) (WHO, 1978).

The kidney and liver could be particularly vulnerable to the chemical assault resulting from alcohol abuse because they receive high percentage of the total cardiac output. Also, the liver is pivotal in intermediary metabolism; so ingested alcohol must come in contact with the liver and kidney. Alcohol could produce many of its damaging effects by the formation of dangerous, highly reactive intermediates such as acetaldehyde which may lead to glutathione depletion, free radical generation, oxidative stress and cell dysfunction.

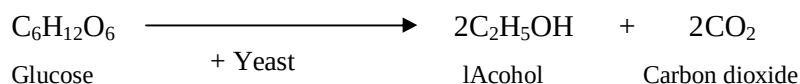
Alcohol dehydrogenase in the presence of a hydrogen acceptor nicotinamide adenine dinucleotide (NAD) oxidizes ethanol to acetaldehyde. This is the initial obligatory biochemical event in alcohol induced hepatotoxic and nephrotoxic effects. Thus, it is important to find out in quantitative terms the effects of different types of alcohol drinks on some principal biochemical parameters of diagnostic utility.

1.1 Alcohol

1.1.1 Chemistry of Alcohol

The term ‘alcohol’ refers to a class of compounds that are hydroxy (-OH) derivatives of aliphatic hydrocarbons. There are many common alcohols i.e. methanol or wood alcohol, isopropyl alcohol, the antifreeze diethylene glycol, and glycerine. In this study however, when the term alcohol is used without additional qualification, ethyl alcohol, a liquid also known as ethanol, is referred to. Alcohol can be considered as being derived from the corresponding alkanes by replacing the hydrogen atoms with hydroxyl groups. The hydroxyl group is the functional group of alcohols as it is responsible for their characteristic chemical properties. Monohydric alcohols contain only one hydroxyl group in each molecule. Monohydric alcohols form a homologous series with the general molecular formula $C_nH_{2n+1}OH$.

All alcoholic beverages arise from the process of fermentation. Indeed, ethanol, the alcohol in beverages, is the quantitative end product of yeast glycolysis. In the presence of water, yeasts are able to convert the sugar (glucose) of plants into alcohol, as depicted by the following chemical reaction:



A wide variety of plants have proved to be useful substrates for the action of yeast, and this is reflected by the different types of beverages used throughout the world.

1.1.2 Alcohol Production

1.1.2.1 Beer Production

Beer is generally considered to be of two types, the ale types, brewed with *Saccharomyces cerevisiae* and the lager type, brewed with *Saccharomyces carlsbergensis*. The main ingredients of beer are malted barley, the source of fermentable carbohydrates, proteins, polypeptides, minerals, and hops the primary purpose of which is to impart bitterness and the hop characteristic, but which also have anti-microbial properties, yeast and water. The basic processes for the brewing of beer include:

(a) Malting

Malting involves the mobilization and development of the enzymes formed during germination of the barley grain. The grain is permitted to germinate under controlled conditions of moisture and temperature, the starch/enzyme balance then being fixed by kilning at drying temperatures as high as 104°C

(b) Mashing

During mashing, ground malt is mixed (mashed) with hot water. This serves both to extract existing soluble compounds from the malt and to reactivate malt enzymes which complete the breakdown of starch and proteins.

(c) Wort boiling

Wort is drained from the mash tun into a copper and boiled to inactivate malt enzymes. In traditional brewing, hops are added at this stage, the humulones (α-acids) being extracted and chemically isomerized. The resulting iso-humulones have a greater solubility and contribute the characteristic bitter flavour to beer, while the hop character is derived from essential oils. In recent years, there has been a tendency to replace hop cones with various types of hop pellets, powders or extracts including pre-isomerized hop products which may be added after fermentation. Boiling serves two other functions: reducing the

potential for microbiological problems by effectively sterilizing the wort and coagulation of proteins followed by their removal as trub. Inadequate coagulation may adversely affect the subsequent fermentation due to interference with yeast:substrate exchange processes (membrane blocking) and lead to poor quality beer.

(d) Fermentation

Fermentations are considered to be of two distinct types: the top fermentation used in production of ales, in which CO₂ carries flocculated *Sacch. cerevisiae* to the surface of the fermenting vessel, and the bottom fermentation used in production of lagers, in which *Sacch. carlsbergensis* sediments to the bottom of the vessel. Differentiation on the basis of the behaviour of the yeast is, however, becoming less distinct with the increasing use of cylindro-conical fermenters and centrifuges.

(e) Maturation (Conditioning; Secondary fermentation)

Maturation may be considered to include all transformations between the end of primary fermentation and the final filtration of the beer. These include carbonation by fermentation of residual sugars, removal of excess yeast, adsorption of various non-volatiles onto the surface of the yeast and progressive change in aroma and flavour. During maturation, priming sugar may be added or amyloglucosidase used to hydrolyse dextrins.

1.1.2.2 The Production of Palm Wine

There are two main sources of palmwine namely: raphia palm particularly *Raphia vinifera* and *Raphia hookeri*; and the oil palm: *Elaeis guineensis*. Palmwine is an alcoholic beverage produced from the fermenting palm sap. The part tapped is the male inflorescence of a standing oil palm tree. The fermentable sugars present in palm wine are glucose, sucrose, fructose, maltose, and raffinose. The yeast species *Saccharomyces* spp are responsible mainly for the conversion of the sugars in palm sap into alcohol as well as oxidative fermentation of alcohol to acetic acid.

In the fermentation of natural palm wine, lactic acid bacteria, *Lactobacillus plantarium*, *Leuconostoc mesenteroides* and *Pediococcus cerevisiae* are also involved. All of them utilize meyerhof parnas pathway which results in the formation of alcohol as well as organic acids. The leuconostoc mesenteriode is a hetero-fermenter and ferments sugar to produce acetic acid., lactic acid, ethanol and carbondioxide. *Lactobacillus plantarium* is a homofermenter and ferments sugars to produce mainly lactic acid and small amount of

alcohol and carbondioxide. *Pediococcus cerevisiae* is also a homo fermenter and produces the same metabolites as *Lactobacillus plantarium*. Thus, the bacterial flora of palm wine contribute significantly to the fermentation of sugars to alcohol and the alcoholic constituent of palm wine varies with the species of palm tree from which the wine was tapped.

1.1.2.3 Production of Distilled Spirit

Nature alone cannot produce spirits or hard liquor by the simple process of fermentation. Yeast will continue to carry out fermentation until the alcoholic content becomes high. The process of distillation then helps to produce beverages with higher concentration of alcohol in form of distilled spirit.

1.1.3 Absorption, Distribution and Metabolism of Alcohol

1.1.3.1 Absorption

After its ingestion, alcohol is rapidly absorbed into the blood stream from the stomach and small intestines. The rate of alcohol absorption can be delayed by the presence of food or milk in the stomach. It is a common observation that when several drinks are taken on an empty stomach, a far more rapid and profound effect is observed than when an equivalent amount of alcohol is taken when there is food in the stomach.

1.1.3.2 Distribution

Alcohol gains access to all the tissues and fluids of the body. The concentrations of alcohol in the brain rapidly approach those levels in the blood because of the very rich blood supply to the brain and other organs such as the liver and the kidney. This is of obvious significance, because alcohol-induced dysfunctions in several organs depend on the concentration and duration of exposure of the organs to alcohol.

1.1.3.3 Alcohol Metabolism

Two major pathways of alcohol metabolism have been identified namely alcohol dehydrogenase pathway and microsomal ethanol oxidizing system (MEOS).

1.1.3.3.1 Alcohol Dehydrogenase Pathway

The primary pathway for alcohol metabolism involves alcohol dehydrogenase (ADH), a cytosolic enzyme that catalyzes the conversion of alcohol to acetaldehyde. This enzyme is

located mainly in the liver but small amounts are found in other organs such as the brain and stomach.

During conversion of ethanol by ADH to acetaldehyde, hydrogen ion is transferred from alcohol to the cofactor nicotinamide adenine dinucleotide (NAD^+) to form NADH. As a net result, alcohol oxidation generates an excess of reducing equivalents in the liver, chiefly as NADH. The excess NADH production appears to contribute to the metabolic disorders that accompany chronic alcoholism and to both the lactic acidosis and hypoglycaemia that frequently accompany alcohol poisoning.

1.1.3.3.2 Microsomal Ethanol Oxidizing System (MEOS)

This enzyme system, also known as the mixed function oxidase system, uses NADPH as a cofactor in the metabolism of ethanol and consists primarily of cytochrome P450 2E1, 4A2, and 3A4. At blood concentrations below 100mg/dl (22 mmol/l), the MEOS system, which has a relatively high K_m for alcohol, contributes little to the metabolism of ethanol. However when large amounts of ethanol are consumed, the alcohol dehydrogenase system becomes saturated owing to depletion of the required cofactor, NAD^+ . As the concentration of ethanol increases above 100mg/dl, there is increased contribution from the MEOS system, which does not rely on NAD^+ as a cofactor.

During chronic alcohol consumption MEOS activity is induced. As a result, chronic alcohol consumption results in significant increases not only in ethanol metabolism but also in the clearance of other drugs eliminated by the cytochrome P450s that constitute the MEOS system, and in the generation of the toxic by-products of cytochrome P450 reactions (toxins, free radicals H_2O_2). Metabolism occurs mainly via the zinc containing enzyme alcohol dehydrogenase (ADH). Other enzyme systems, such as the microsomal ethanol oxidizing system (MEOS) or catalase system are capable of metabolising alcohol.

Oxidation of alcohol by ADH involves the transfer of hydrogen via nicotinamide adenine dinucleotide (NAD), which is converted to nicotinamide adenine dinucleotide reduced (NADH). The result of this oxidation is the metabolite acetaldehyde. The subsequent oxidation of acetaldehyde by aldehyde dehydrogenase also involves the reduction of NAD. Acetaldehyde is metabolized to acetate and this is transformed into acetyl coenzyme A, which is then oxidized by the citric acid cycle to carbon dioxide and water. The rate limiting step in this metabolic process is the oxidation of alcohol to acetaldehyde since acetaldehyde is metabolized faster than it is formed.

1.1.4 Patterns of Alcohol Use and Abuse

Patterns of alcohol consumption may range from its occasional use to relieve emotional stress, to periodic 'spree' drinking, to extreme cases where the alcoholic has little or no control over the amount of alcohol consumed. Chronic and excessive consumption of alcohol is a health and psycho-social disorder characterized by obsessive pre-occupation with alcohol and loss of control over alcohol consumption such as to lead continuously to intoxication (Johansson *et al.*, 2003). Chronic abuse of alcohol is typically associated with physical disability, social maladjustments, emotional and occupational impairments.

The hallmarks of excessive and chronic alcohol abuse are:

1. Psychological dependence
2. Physical dependence
3. Tolerance (Martin *et al.*, 2008).

1.1.4.1 Psychological Dependence

Psychological dependence is typically characterized by intense and uncontrollable craving for alcohol. The alcoholics' desire for alcohol is intense, obsessive and overwhelming. The alcoholics are deeply concerned about how daily activities interfere with drinking than how drinking negatively militate against the performance of daily activities. Family, relationships, friends, profession and business are relegated to subordinate roles with full joy. Alcohol consumption becomes the driving and motivating force (Johansson *et al.*, 2003).

1.1.4.2 Physical Dependence

Excessive and chronic consumption of alcohol produces unequivocal physical dependence, with the intensity of the syndrome associated with withdrawal directly proportional to the level of intoxication and its duration. Excessive consumption of alcohol on chronic basis directly or indirectly adversely modifies the physical and mental health of the abuser. Intermediate levels of alcohol consumption produce withdrawal symptoms typified by tremors or 'shakes', anxiety, sleeplessness and gastrointestinal upset.

Delirium tremens is one of the potentially risky withdrawal symptoms experienced by chronic abusers, when physical dependence has set in. Alcoholics that have delirium tremens suffer from restlessness, tremors, weakness, nausea and anxiety few hours after the last drink. Generally, these effects experienced by alcoholics on momentary withdrawal from alcohol serve as an impetus driving them to initiate another drinking bout in order to feel 'normal'.

again; thus, potentiating the physical dependence. The tremors could be so severe that the alcoholic on resuming drinking finds it difficult to successfully navigate beer bottle or cup to his mouth yet he craves for more alcohol.

In the early stages of this withdrawing syndrome after the onset of physical dependence, the alcoholic is hyperactive and is a victim of auditory and visual hallucinations. The alcoholics could be heard shouting that cockroaches are crawling upon them; they see red lions and they may seriously believe that they are being attacked by dangerous animals or people. They are completely disoriented.

Progressively, the alcoholic becomes weaker, agitated and confused. These syndromes coupled with exhaustion and fever are called *áremulus delirium*. In physical dependence the intensity of the syndromes associated with withdrawal as typified by tremulus delirium is related to the duration and level of alcohol abuse. Physical dependence could develop from ethanol induced alterations in membrane components and functions (Cargiulo, 2007).

1.1.4.3 Tolerance

Alcoholics usually exhibit increased resistance to the intoxicating effects of alcohol and are often sober at blood alcohol concentrations that could be deadly in naïve occasional drinkers. Indeed, chronic alcohol abusers can readily ingest quantities of alcohol that would severely intoxicate the occasional drinker (Chiaochicy and Shijium, 2008).

Ethanol can cross the blood- brain barrier and enter the brain quickly. Blood alcohol level is almost always directly proportional to the concentration of alcohol in brain tissue (Oscarberman and Marinkovit, 2003). However, despite increasing levels of alcohol in the blood, alcoholics usually exhibit decreasing response to the intoxicating effect of alcohol.

This phenomenon known as tolerance could be explained in part by these mechanisms: first, tolerance could develop consequent upon alterations in the absorption rate, distribution, metabolism and elimination of alcohol from the body (Rottenburg, 1986). The resultant effect of these alterations is a reduction in the duration and intensity of alcohol's effects on the body tissues most remarkably the brain.

The second mechanism involves alterations in the properties or function of tissues rendering them less vulnerable to effects of alcohol (Wilson *et al.*, 1984). Tolerance to alcohol could develop as a result of adaptive alterations in the central nervous system. Alcohol changes many specific membrane dependent processes such as Na^+ , K^+ ATPase and adenylyl cyclase process in the cell precipitating ethanol-induced alterations in neural

functions. It has been observed that after chronic exposure to alcohol, cellular membranes often develop resistance to the fluidizing effect of alcohol (Goldstein, 1986). Ethanol-induced alterations also occur in membrane components and functions such as alterations in membrane lipids, receptors, phosphatidylinositol, GTP binding proteins, second messengers and neuro-modulator (Reynolds *et al.*, 1990). Alterations in ion channels and transporters are also some of the ethanol induced changes in human cell membranes related to tolerance (Chastain, 2006). Putting these observations in a functional perspective, it is salient to point out the fact that these adaptive changes in membrane components are exquisite phenotypic markers for genetic predisposition to alcoholism and its attendant problems (Das *et al.*, 2008).

1.1.5 Aetiology/Causes of Alcohol Abuse

1.1.5.1 Biochemical basis

(a) Monoaminergic System

The enzyme monoamine oxidase (MAO) is the major degradative enzyme for both catecholamines and indoleamines. It has been shown that reduced platelet monoamine oxidase concentrations are closely associated with a remarkable predisposition to alcoholism (Patsenka, 2004) and psychiatric vulnerability. It has also been proposed that a weak monoaminergic system causes predisposition to alcohol abuse (Raddatz and Parini, 1995).

Available evidence is becoming overwhelming in support of the view that sub class of alcoholics exists where genetic considerations are of etiological significance. These imposing factors appear to be reflected in low platelet monoamine oxidase (MAO). Low concentrations of platelet monoamine oxidase reflect a disturbance in the serotonergic system (Chastain, 2006). Thus, the biochemical basis of alcoholism seems to involve combined aberrations in some transmitter system. In essence, these aberrations have far reaching effects which are reflected in neuro-physiological, psychosocial and personality abnormalities.

(b) Tetrahydroisoquinolines

Biologically active chemicals called tetrahydroisoquinolines are formed during alcohol metabolism (Antkiewicz *et al.*, 2000). Catecholamines could also condense with aldehydes via a Pictet-Spengler reaction to form 1,4-Disubstituted tetrahydroisoquinoline (Raddatz and Parini, 1995). Tetrahydroisoquinoline such as tetrahydropapaveroline changes drinking behaviour from alcohol rejection to alcohol acceptance (Nappi and Vass, 1999).

The Picket-Spengler reaction provides a useful route for the synthesis of tefrahydrolsoquinoline (TIQ). Many tetrahydroisoquinoline are formed from dopamine and

carbonyl compounds (phenylpyruvic acids, aldehydes and ketones) two catecholamine norepinephrine or epinephrines could also react resulting in the formation of diastereomeric TIQ as shown below

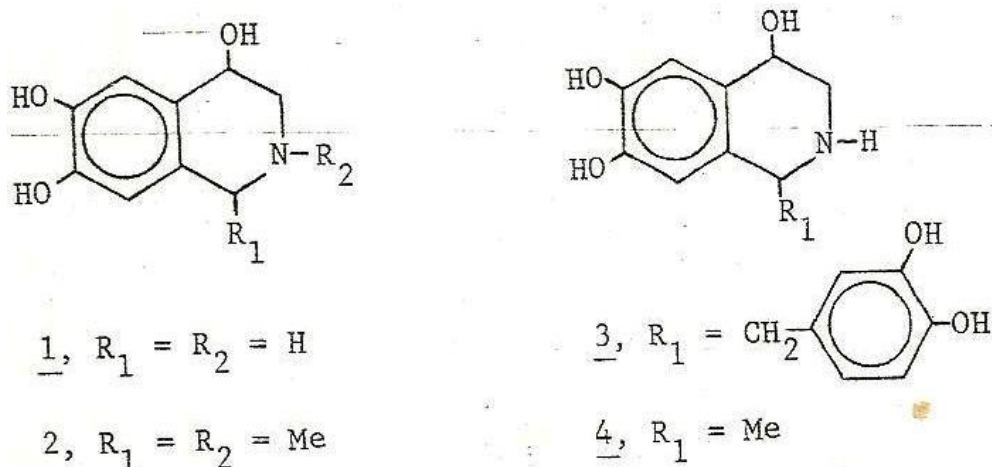


Fig. 1: 1,4-disubstituted TIQ occur in form of two diastereomers and their optical isomers

Furthermore, ethanol or its first oxidation product acetaldehyde can induce catecholamines to undergo an unusual form of metabolism that results in the formation of 1,2,3,4- tetrahydroisoquinoline. Tetrahydroisoquinoline compounds have biochemical and neuropharmacological properties. They possess abilities to interact with catecholaminergic and dopaminergic systems. They also exert a potent influence on Ca^{2+} binding in synaptosomes in a similar manner to morphine and ethanol. TIQs have biochemical associations with ethanol and is deeply involved in the aetiology of alcoholism (Raddtz and Parini, 1995).

Patsenka (2004) showed that excessive and chronic consumption of alcohol by human alcoholics could be caused by metabolic abnormalities that result in the formation of tetrahydroisoquinolines. He reasoned that because drinking generates tetrahydroisoquinolines and tetrahydroisoquinolines stimulate drinking, the positive feedback loop could be responsible for the obsessive, uncontrolled and habitual drinking by alcoholics. Thus, tetrahydroisoquinolines could be involved in the mediation of voluntary ethanol consumption habit that often degenerates to heavy intoxication, physical dependence and tolerance (Chastain, 2006).

(c) Alcohol: An Etiological Factor in Alcoholism and Alcohol Abuse

The positive reinforcing properties of alcohol are largely responsible for the cravings and tendency to ingest more and more alcohol. Alcohols depress the inhibition centres of the brain and produce a feeling of euphoria. By shattering the shackles of inhibition and self restraint, ingestion of alcohol leads to further ingestion; and feelings of excitement and self confidence continue to reign supreme. Laughter, lively gesticulation and loquacity then flow. Indeed, the intoxicating effects of moderate imbibing of alcohol is rewarding to the consumers (Fowkes and Steven, 2012).

Essentially, most studies of alcohol as a drug of abuse are based on the premise that humans ingest alcohol due to its reinforcing or rewarding properties. There are considerable interest in the similarities between alcohols reinforcing properties and those of other drugs of abuse. There is a marked resemblance between the reinforcing effects of alcohol and those of opiate drugs (Giflow, 2006).

It has been noted that the positive reinforcing potential of alcohol is partly the cause of the strong desire and inclination individuals have for alcohol consumption. However, it is actually a metabolite of ethanol called acetaldehyde rather than ethanol itself which mediates this reinforcing effect (Goedde and Agarwal, 1983).

Increased interest has been ignited in the biochemical pharmacology of acetaldehyde, the oxidation product of ethanol and in the part it may play in alcohol intoxicating effects, physical dependence and tolerance. Acetaldehyde is a toxic substance which when it occurs in a relatively high levels in the circulation induces a characteristic set of aversive physiological reactions (Harada *et al.*, 1983). So ordinarily, it is expected that the toxic effects of acetaldehyde should lead to attenuation of voluntary alcohol ingestion. However, contrary to expectations, there are some unfolding evidence that acetaldehyde may be directly involved in voluntary ethanol consumption (Thacker *et al.*, 1984).

While the accumulation of acetaldehyde, the oxidation product of ethanol, in circulation discourages ethanol ingestion, the presence of acetaldehyde in the brain strongly supports voluntary alcohol intake, physical dependence and tolerance (Harade *et al.*, 1983).

1.1.5.2 Psychosocial Basis of Alcohol Abuse

(a) Temperament

Myriads of definitions and characterizations of temperament have been attempted ever since ancient philosophers first considered the origins of psychological individuality. One

definition that embraces an individual's style of psychological functioning was furnished by Kandel *et al.* (2001) in which they showed that temperament is the characteristic phenomena of an individual's nature, including his susceptibility to emotional stimulation, his customary strength and speed of response, the quality of his prevailing mood, and all the peculiarities of fluctuations and intensity of mood, these being phenomena regarded as dependent on constitutional make up and therefore largely hereditary in origin (Kandel *et al.*, 2001).

This definition of temperament captures the central features of temperament, including behaviour and mood. In other words, one could proffer that individual differences in temperament reflect variability in neuro-biological processes that possess strong genetic link (Tarter *et al.*, 1994).

Aberration in temperament trait expression paves the way for a developmental trajectory that may culminate in non normative behaviour such as anxiety and alcohol abuse. A defect in temperament is closely associated with negative mood, slow adaptability, high intensity of emotional reactions and alcohol abuse (Cosci *et al.*, 2007). This constellation of temperament features is related to a heightened risk for conduct problems (Maziade *et al.*, 1990) and alcohol abuse (Lerner and Vicary, 1984; Windle, 1992; Tarter *et al.*, 1994).

Deviations in temperament are typically connected with high susceptibility to maladjustment and psychopathology. In fact, aberrant behaviour and alcoholism are common among individuals with defective temperament disposition (Maziade *et al.*, 1990). Significantly, a difficult temperament is intricately associated with a special predisposition to develop oppositional behavioural abnormalities (Maziade *et al.*, 1984). These aberrant behaviour manifestations usually occur in conjunction with chronic abuse of alcohol, physical dependence and tolerance to alcohol (Das *et al.*, 2008).

(b) Disruptive Home Environment

Defective marital adjustment influences the subsequent development of difficult behaviours (Esterbrooks and Ende, 1988). Disruptive home environment has deep impacts on the development of the family members. Marriages are almost always dysfunctional and conflict-laden where both or one of the partners is alcoholic. No doubt, good child rearing in such home is often adversely affected. It has been shown that children are perceived negatively after a heated quarrel between parents that are alcoholic (Markman and Jonesleonard, 1985). Under these unfortunate conditions, coercive and aversive child-rearing strategies are usually adopted by the disillusioned and near paranoid parents leading to maladjustment in the children. As a vicious-circle, such children develop to become well

entrenched edifice of violence and viable precursors of alcoholism. This in part accounts for the reasons why alcoholism seems to be perpetuated in some families (Blackson *et al.*, 1994).

(c) Adverse Environment

Adverse relationships with the environment and poor adjustments typically herald alcohol abuse. Indeed, adverse person-environment cohabitations could make an individual susceptible to aberrant psychological development. Those who experience aggression are often more likely than others to perpetrate aggression (Stewart and Dublois, 1981). Such individuals usually resist authority figures and are prone to alcohol abuse (Billman and McDevilt, 1980).

It is expedient to point out that environmental factors interact chronologically with behavioural traits to place an individual onto a trajectory toward alcohol abuse (Blackson *et al.*, 1994).

1.1.5.3 Genetic Factors

Alcoholism is rather a complex, heterogeneous disease. Familial aggregation of alcohol abuse has been noted for a long time and has led to the belief that the tendency to abuse alcohol is heritable (Nurnberger *et al.*, 2007). Some families do have a disproportionate amount of alcohol abuse.

Nurnberger *et al.* (2007) showed that twenty four percent of sons of alcoholics adopted into non-alcoholic homes themselves became alcoholic. It is indeed difficult to conceptualize anything but genes connecting the adopted-away son with his birth father. Furthermore, there are manifestations of genetic influences on specific aspects of alcohol-related behaviour. For instance, there are genetic influence on the quantity and frequency of alcohol consumption (Dick and Bierut, 2006).

1.1.6 Effects of Alcohol

1.1.6.1 The Effects of Ethanol on the Central Nervous System

The quantity and frequency of alcohol consumption are affected by the interaction of the rewarding and aversive experiences derived from drinking (Enoch, 2006). The central nervous system (CNS) is markedly affected by alcohol consumption. Alcohol causes sedation and relief of anxiety. At higher concentrations, alcohol can lead to slurred speech, impaired judgement and disinhibited behaviour. Like other sedative-hypnotic drugs, alcohol is a CNS depressant. Ethanol affects a large number of membrane proteins that participate in signalling pathways including neurotransmitter receptors for amines, amino acids, opioids and

neuropeptides enzymes such as phosphoinositide-specific phospholipase C, adenyl cyclase, Na^+ , K^+ ATPase, nucleoside transporter and ion channels (Sakmam, 1992).

Ethanol also has effects on neurotransmission by glutamate and GABA, the main excitatory and inhibitory neurotransmitters in the CNS. Ethanol exposure enhances the action of GABA_A at GABA receptors which are consistent with the ability of GABA_A antagonists to attenuate some of the actions of ethanol. Ethanol inhibits the cation channel associated with the N-Methyl-D- aspartate (NMDA) subtype of glutamate receptors. The NMDA receptor is involved in many aspects of cognitive function including learning and memory. "Blackouts" - periods of memory loss that occur with high levels of alcohol - results from inhibition of NMDA receptor activation (Michael *et al.*, 1998).

Although, alcohol is transported to all parts of the body after its consumption, the effects of alcohol on the brain are most evident. The human brain is extremely sensitive to the intoxicating effects of alcohol. Contrary to popular belief, alcohol is always a central nervous system depressant (Glavas and Weinberg, 2006). Some extremely sophisticated areas of the brain concerned with the restraint of natural impulses seem to have low threshold to alcohol-induced depression (Fergusson *et al.*, 2009). This accounts for the apparent stimulation observed with moderate alcohol consumption and for the depression evident after imbibing high concentration of alcohol. The increased activity, loud laughter and long unending speech observed after moderate drinking of alcoholic beverages should not be perceived as a consequence of central nervous stimulation. Alcohol rather depresses or inhibits the inhibitory centres of the brain. This shatters the shackles of self restraints and produces apparent stimulation (Glavas and Wemberg, 2006).

In small quantities, alcohol produces a feeling of euphoria, good fellowship, and increased, albeit unjustified self-confidence. For some individuals, modest alcohol ingestion serves as an effective and efficient catalyst or defroster for promoting physical activities and overcoming inhibitions that act as barriers to successful performance of certain tasks (Glavas and Weinberg, 2006). However, ingestion of large quantities of alcohol interferes with the normal process of information integration by the cerebral cortex (Crews *et al.*, 2007). Thinking ceases to be systematic and becomes disorganised and confused. With progressively greater quantities of alcohol, concentration, memory, judgement and perspective are grossly blunted. Excessive consumption of alcohol leads to impairment of motor coordination, movement become uncertain leading to staggering and total inability to move. The alcoholics' personality is thus compromised (Kushner *et al.*, 2000). Indeed, alcohol reduces both mental and physical efficiency.

Alcohol acts on virtually every single cell of the body, but the central nervous system is the target most affected. In fact, alcohol affects almost every level of the nervous system. It affects the neuro-chemicals within single cells as well as the macro-functions controlling thought processes and behaviour (Guerri and Pascual, 2010). Alcohol distorts judgement and impairs information processing and reaction time. Actually, alcohol can lead to alteration in brain response to visual and auditory stimuli (Verbaten, 2009).

Chronic and excessive consumption of alcohol can lead to two biochemically and neuro-pathologically distinguishable chronic organic brain syndromes namely: alcohol amnestic syndrome (also called Korsakoff's psychosis) and alcoholic dementia (Panza *et al.*, 2009).

1.2 Microanatomy of the Kidney

The kidneys are paired organs situated on either side of the vertebral column extending from the twelfth thoracic to the third lumbar vertebrae (Tisher and Madsen, 1996). Principally, the kidneys excrete the waste products of metabolism, precisely regulates the body's concentration of water and salt, maintain the appropriate acid-base balance of plasma and serve as endocrine organs secreting such hormones as prostaglandins erythropoietin and renin (Glodny *et al.*, 2009).

The physiologic mechanisms that the kidneys have evolved to carry out these functions require a high degree of structural complexity. Basically, the ureter enters the kidney at the hilum and dilates into a funnel-shaped cavity, the pelvis, from which is derived two or three main branches, the major calyces; each of these subdivides again into three or four minor calyces (Knight *et al.*, 2003).

Essentially, the kidney is made up of a cortex and a medulla. Within the cortex, two main zones can be distinguished namely:

- (1) The cortical Labyrinth, composed of the glomeruli, convoluted tubules and associated vessels; and
- (2) The medullary rays composed of parallel groups of the straight segments of proximal tubule (pars recta) and thick ascending (distal) tubules as well as collecting ducts.

The medulla consists of renal pyramids, the apices of which are called papillae. Each papilla projects into a minor calyx. Cortical tissue extends into spaces between adjacent pyramids as the renal columns of Bertin.

The outer medulla can be divided into an outer (Juxta ï Cortical) stripe and an inner stripe. The outer stripe contains the proximal straight tubule and the medullary thick

descending limb. The inner stripe contains the thin segments of the descending limb tubule and the thick ascending limb (loop Henle) (Greger, 1985). The vascular bundle consists of descending and ascending vasa recta surrounded by pars recta, the descending limb and the thick ascending limb. Further away are the collecting tubules. The inner medulla contains the thin limbs of long loops (of Henle) both ascending and descending.

1.2.1 The Nephron

The nephron is the basic structural and functional unit of the kidney. Each nephron consists of a renal corpuscle, comprising Bowman's capsule, the glomerular capillary tuft and a renal tubule. Each capsule communicates with the tubule and then the renal calyx via the collecting tubule and duct (Howie and Rollason, 1993).

The tubular segments are structurally, cytologically and functionally distinct. The renal tubule extends into or towards the medulla and loops (the loop of Henle) back to its own renal corpuscle before draining into a collecting tubule. The loops may be long or short. The short loop of Henle turns at the junction of outer and inner medulla. The long loops extend into the inner medulla (Jamieson and Kriz, 1982).

The renal corpuscle is the term properly used to describe Bowman's capsule and the capillary tuft (the glomerulus). Bowman's capsule consists of parietal epithelial cells and a basement membrane which is reflected onto the glomerulus becoming continuous with the glomerular basement membrane in the point of entry of the afferent and efferent arterioles (the vascular pole). Bowman's capsule invests the glomerulus and is continuous with the proximal tubule at the tubular orifice (pole) which is found opposite the vascular pole. The Bowman's capsule is lined with a layer of flattened epithelium the parietal podocytes (Gibson *et al.*, 1992).

The glomerular capillary tuft arises from the afferent arteriole. The capillary loops are lined with an endothelium that has a highly specialized basement membrane and visceral epithelial podocytes. The capillaries then recombine to form the efferent arteriole. The capillary loops are supported by the mesangium which is composed of basement membrane such as matrix in which are embedded mesangial cells. At the vascular pole, mesangial cells form part of the juxtaglomerular apparatus (Jamieson and Kriz, 1982).

The juxtaglomerular apparatus is largely concerned with blood pressure and circulating fluid volume control (Schnermann and Briggs, 1985). The components of the juxtaglomerular apparatus are

- (1) The terminal portion of the afferent arteriole and efferent arteriole.

- (2) The macula densa, a specialized segment of the distal tubule.
- (3) The extraglomerular mesangial cells at the vascular pole and
- (4) The sympathetic nerve supply.

The tubule of the nephron comprises the following segments from the glomerulus:

- (1) The proximal tubule (convoluted section), the proximal tubule (straight part), also known as pars recta,
- (2) The descending thin limb of Henle's loop (in long loop nephrons only).
- (3) The medullary thick ascending limb of Henle's loop.
- (4) The cortical thick ascending limb of Henle's loop.
- (5) The distal convoluted tubule.

The collecting duct system begins with the connecting tubule. This segment, which is poorly defined in the human kidney, resembles the distal convoluted tubule (Howie and Rollason, 1993). The connecting tubule drains into the cortical collecting duct, which passes from the cortex into the outer medulla. On reaching the inner medulla, paired fusions of collecting ducts occur forming the inner medullary collecting ducts which drain into the renal papillae which form the apices of the renal pyramids (Gosling *et al.*, 1982).

The basement membrane of Bowman's capsule is a multilayered structure which is continuous with the glomerular basement membrane at the vascular pole and with the basement membrane of the proximal tubule. The visceral podocytes, the glomerular, the glomerular basement membrane and the endothelial cell lining, together function as a complex filter in the Bowman's capsule (Batsford *et al.*, 1987). The podocytes are responsible for formation of the glomerular basement membrane.

Cell body of the podocyte has a number of wide processes which embrace the capillary. The pedicels arise from the subdivisions of these processes. The pedicels from adjacent podocytes are arranged alternately. The filtration slit diaphragm separates adjacent pedicels. This is considered to be the site of ultra filtration (Shikata *et al.*, 1990).

The glomerular basement membrane (GBM) is composed of three distinct layers: the central lamina densa and on the side; less dense zones, the lamina interna rara and lamina externa rara. The main structural component of the glomerular basement membrane is collagen type 4 which is tightly packed in the lamina densa, and has a looser arrangement in the laminae interna and externa. Importantly, collagen type 4 contains a non collagenous domain in which is found the good-pasture antigen (Pusey *et al.*, 1987). Other adhesion molecules such as laminin, fibronectin, indictin, amyloid P substance (Kaufman *et al.*, 1995)

and the negatively charged heparin sulphate proteoglycan are present. The lamina extena has a net negative charge from numerous polyanionic sites (Gibson *et al.*, 1992).

The glomerular capillaries are lined by endothelial cells. The cytoplasm of the endothelial cell is perforated by numerous fenestrations. The endothelin -1 produced by the glomerular endothelial cells influences adjacent mesangial cells (Ballerman and Marsden, 1991). The glomerular mesangium is analogous to the intestinal mesentery; it provides support for the glomerular capillary loops. The presence of the actin and myosin in the mesangial cells indicates a contractile function. And the contraction of the mesangium influences glomerular capillary flow and filtration (Kreisberg *et al.*, 1985). The cells of the proximal tubule vary in structure corresponding to the convoluted segment, a transition zone and the straight part. A typical proximal tubular cell is columnar with a basal nucleus.

Finally, the collecting ducts are composed of a mixture of intercalated cells and principal (collecting duct) cells. The main feature of the polygonal principal cell is the numerous regular infoldings (basal labyrinth) of the basal cell membrane (Kloth *et al.*, 1993).

1.2.2 Vulnerability of the Kidney to Alcohol Toxicity

There exist a good number of reasons why the kidney is uniquely vulnerable to the hazards of alcohol toxicity. First, it receives a high percentage of the total cardiac output. Indeed, the kidney has a rich blood supply. This means that the kidney could readily be exposed to large quantities of alcohol even if peak circulating levels are only maintained momentarily (Chung, 2005).

Secondly, the hypertonicity of the medullary interstitium which is generated by operation of the counter-current mechanism operates to concentrate alcohol and its toxic metabolite called acetaldehyde in the relatively hypovascular area of the kidney. Thus renal tubular cells could become exposed to alcohol concentrations which are more than those found in any other tissue or cells (Busse *et al.*, 2002).

Also, the pivotal role of the kidney as an obligatory route for the passage of some drugs including alcohol implies that in renal dysfunctions with less perfusion, excretion of alcohol is slowed and if other extra renal mechanisms of elimination are not mobilized, alcohol accumulation will occur. The high concentrations of alcohol in circulation therefore render the kidney more vulnerable to direct and unmitigated damage (Cushman, 2001).

1.2.3 Renal Function Tests as Indicator of Alcohol Induced Kidney Injuries

Laboratory tests play crucial roles in the diagnosis and assessment of renal dysfunction under excessive and consistent alcohol consumption because clinical signs and symptoms may be vague or absent. Some of the renal function tests reveal primarily disturbances in glomerular filtration (Kluth, 1999) while others reflect dysfunction of the tubules. However, it is important to highlight the fact that renal damage is seldom confined solely to a particular portion of the nephron because the anatomic portions of the nephron are closely related and have common blood supply so damage to one portion gradually involves the nephron as a whole (Remuzzi *et al.*, 1997). Eventually both glomerular and tubular portions of the nephron become involved irrespective of the site of the initial damage. The kidney has a high reserve capacity; hence early toxicities may not be immediately notice by clinical examination. Since the kidney is a common target of toxic chemicals such as alcohol (Fergusson *et al.*, 2009), a need exist for the inclusion of sensitive and reliable tests to ascertain the level of alcohol induced renal dysfunctions (Levey, 1990).

1.2.4 How Alcohol Induced Nephropathies Affect Glomerular Filtration

The glomerular filters usually retain within the circulation all cells and all proteins of the plasma, but allow free passage of water and solutes. The glomerular capillary wall is highly specialized in that the endothelial cells are reduced to a thin fenestrated sheet of cytoplasm, while on the external surface of the capillary, unique cell, the podocyte epithelial cell, whose surfaces are directed towards the capillary basement membrane form an intricate series of interlocking foot processes; thereby constituting a filter (Tryggyason and Wartiovaara, 2001).

The glomerular filter also functions as a charge-selective and size-selective barrier. Smaller molecules and those with a small negative or even a net positive charge at physiological pH will pass more easily through the glomerulus. However, since at the pH of plasma most proteins carry a net negative charge, charge selectivity is most important in determining the retention of plasma protein molecules. Thus the penetration of negatively charged macromolecules is hindered and that of positively charged molecules facilitated. So the glomerular filtrate is almost devoid of protein. The charge and size selective barrier of the glomerular filter is mostly disturbed in alcohol induced nephropathies (Tryggyason and Wartiovaara, 2001).

1.2.5 Determinants of Glomerular Filtration

As in all capillary beds, the determinants of glomerular ultrafiltration are first the net ultrafiltration pressure, second the hydraulic permeability of the capillary wall and the third is the area of available filtering surface (Brenner *et al.*, 1986). The hydraulic permeability of the glomerular capillaries is much higher than that found in other capillary beds, emphasizing the specialized function of the glomerulus as a filter. The ultrafiltration pressure depends in turn upon the hydrostatic pressure operating across the glomerular capillary wall and the osmotic pressure of the plasma proteins (Deen and Satrat, 1981).

One of the other crucial determinants of the glomerular filtration rate is of course the renal blood flow and hence plasma flow (Schnermann and Briggs, 1985). Obviously dilation of the efferent arteriole will increase glomerular blood flow, decrease the net ultrafiltration pressure and hence reduce glomerular filtration rate (GFR). Similarly efferent arteriolar constriction will raise the net ultrafiltration pressure and increase GFR. Exactly opposite effects will be seen from afferent arteriolar dilation or constriction, the former leading to an increase in GFR and the latter to a reduction. The interplay between these pre and post capillary sphincters can permit exquisite regulation of glomerular blood flow and glomerular filtration rate; this can be affected by alcohol ingestion (Steinhausen *et al.*, 1988).

1.2.6 Creatinine Clearance as a Measure of Glomerular Filtration Rate (GFR)

Ever since the useful suggestion of Popper and Mandel (1937) that the clearance of endogenous creatinine approximates to the glomerular filtration rate, creatinine clearance has been very popular in the assessment of renal dysfunctions. Creatinine is a waste product of muscle metabolism formed by the non-enzymatic dehydration of muscle creatine. Creatine itself is synthesized in the liver and transported to the muscle (Levey *et al.*, 1988); the main determinant of the creatine pool, therefore, is muscle mass. The only other source of creatine is, of course, meat from dietary sources.

Creatinine is freely filtered and is not reabsorbed within the renal tubule. However, in some animal species including humans there is limited tubular secretion of creatinine. Essentially, renal clearance is the volume of plasma that is completely cleared of a particular substance in a given time period (usually one minute), by the kidney (Chung *et al.*, 2005).

The clearance (C) of a substance (X) can be calculated as shown below:

$$C_X = \frac{U_X \bullet V}{P_X}$$

Where U_X and P_X are the urine and the plasma Concentrations of X and V is urine flow rate.

The clearance of a substance that is freely filtered at the glomerulus but once within the tubule is neither reabsorbed nor secreted can be used as a marker for measurement of glomerular filtration rate (GFR) (Chung *et al.*, 2005). The 24-hour creatinine clearance (obtained by collection of complete 24- hour urine) is widely used as a measure of GFR. In normal non pregnant adult, the rate of creatinine excreted by the kidneys is equal to the rate of creatinine produced by muscle metabolism. Thus plasma creatinine concentration remains constant when renal function begins to deteriorate due to alcohol mediated nephropathies, the excretion of creatinine falls, leading to an increase in plasma creatinine. Mild to moderate levels of renal impairment usually lead to small increases in plasma creatinine, but with severe renal disease, plasma creatinine changes markedly and thus the plasma creatinine value can be used as a clinical index glomerular filtration rate (GFR) (Nielson *et al.*, 1999).

The amount of a substance excreted through the kidneys is the product of the urine flow rate (V), and the concentration of that substance in the urine (U). The quantity (UV) is the excretion per unit time. The concept of renal clearance (C) expresses the relationship between the excretion per unit time and the concentration in the plasma, which is obviously an index of the kidney's ability to 'clear' the blood of any substance (Chung *et al.*, 2005).

The power of this concept of renal clearance is that it can be used to express the relative ability of the kidney to excrete any substance and this could be used to ascertain the structural and functional integrity/status of the kidney under excessive and consistent alcohol consumption (Hallan *et al.*, 2004).

1.3 Serum Electrolyte and Alcohol-Induced Renal Dysfunctions

Actually, filtration occurs as the blood flows through the glomerulus. During the passage of glomerular filtrate through the convoluted tubule, the medullary loop and the collecting tubule, selective reabsorption occurs leading to alteration in the volume and composition of the glomerular filtrate. The general purpose of this process is to reabsorb into the blood those filtrate constituents required by the body to maintain fluid, nutrient, electrolyte balance and the pH of the blood (Verbalis, 1990). Active transport is carried out at carrier sites in the epithelial membrane using chemical energy to transport substances against their concentration gradients (Sun *et al.*, 2004).

Some constituents of glomerular filtrate do not normally appear in the urine because they are completely reabsorbed. The kidney's maximum capacity for reabsorption of substances is called the transport maximum or renal threshold. If, the concentration of

substances rises above the transport maximum, the substance will start to appear in the urine because all the carrier sites are occupied and the mechanism for active transfer out of the tubule is overloaded (Wingo and Smolka, 1995).

However, the reabsorption of water is entirely passive; following osmotically the entirely active absorption of Na^+ . Sodium is the most common cation in extracellular fluid and potassium is the most common intracellular cation. Some complex mechanisms in the kidney assist to maintain the concentrations of sodium and potassium within physiological limit. The mechanisms of Na^+ reabsorption is heterogenous, differing in the various nephron segments. In the proximal tubular luminal absorptive process, Na^+ is taken up mainly in exchange for protons. In the early distal tubule, luminal NaCl reabsorption proceeds via a NaCl co-transport system. In the collecting duct, only a small percentage of the filtered NaCl is reabsorbed (Tannem, 1991).

The distal convoluted tubule is lined by a tall cuboidal epithelium and possesses the greatest $\text{Na}^+ \text{K}^+$ -ATPase activity (Greger and Valazquenz, 1987). Cells in the afferent arteriole of the nephron are stimulated to produce the enzyme renin by sympathetic stimulation, low blood volume or by low arterial blood pressure. Renin converts the plasma protein angiotensinogen, produced by the liver to angiotensin I. Angiotensin Converting Enzyme (ACE), formed in small quantities in the lungs and proximal convoluted tubules converts angiotensin I into angiotensin II. Angiotensin II stimulates aldosterone and aldosterone stimulates sodium reabsorption from the lumen of the distal renal tubule in exchange for either potassium or hydrogen ion. Alcohol-mediated distal tubule dysfunction leads to impaired response to aldosterone affecting the reabsorption of the sodium and the urine then contains an inappropriately high concentration of sodium (Tannem, 1991).

Potassium is the most prevalent ion in animal cells and the most crucial in generating the resting potential difference across the plasma membrane of cardiac, neuromuscular and polarized epithelial cells. Changes of a mere 1 or 2 mEq/L from the range of serum potassium concentration could progress unnoticed until suddenly and without warning the situation turns perilous. In view of the small quantity of K^+ in the extracellular fluid (ECF), ingestion of K^+ could lead to a dangerous elevation in serum K^+ , were it not for the mechanism that influence K^+ distribution between ECF and intracellular fluid (ICF); some of the ingested K^+ temporarily enter the cells. Eventually, the excess K^+ is excreted primarily by the kidney. Potassium undergoes simultaneous reabsorption and secretion by the renal tubule in the bidirectional transport (Giebisch and Wang, 1996). Different cells in the collecting duct are either potassium secretory or reabsorptive (Giebisch *et al.*, 1991).

There is a basic pump-leak system common to the epithelial cells lining the renal tubule (Bertorella and Katz, 1993). Na^+ , K^+ -ATPase is located on the lateral and blood-facing (basal) cell membrane only, i.e., not on the tubule lumen-facing (apical) membrane. The Basolateral Na^+ , K^+ - ATPase drives Na^+ out and 2K^+ in, creating a cell interior low in Na^+ , high in K^+ and electrically negative with respect to the tubule fluid (Blanco and Mercer, 1998).

The renin-angiotensin-aldosterone axis also plays a major role in regulating K^+ excretion. Precisely, aldosterone stimulates K^+ secretion (excretion/removal or loss). It is important to point out that alcohol-induced proximal tubular dysfunction among other features is significantly marked with impaired reabsorption of potassium leading to hypokalemia (Stoke, 1982). Hypokalemia in turn impairs nerve and neuromuscular transmission, cardiac conduction and muscular contraction. Muscular weakness and ileus of the intestine are typical clinical manifestations of hypokalemia resulting from proximal tubular dysfunction caused by chronic alcohol abuse (Wingo and Smolka, 1995).

1.4 Alcohol-Induced Pathological Proteinuria

Proteinuria represents the single most useful indicator of the presence of an injury to the kidney. Such injury impairs the barrier to protein passage imposed by the walls of glomerular capillaries (Shankar *et al.*, 2006). The glomerular capillary wall is highly specialized in that the endothelial cells are reduced to a thin fenestrated sheet of cytoplasm. The glomerular filter functions both as a charge- selective and size-selective barrier. Consequent upon this, the loss of most plasma proteins through the glomeruli is restricted by the size of the pores and by the charge on the basement membrane (Gausch *et al.*, 1993). Alteration of any one of these factors by alcohol-mediated glomerular dysfunction could allow albumin and larger proteins to enter the filtrate.

Actually, proteinuria could be precipitated as a result of glomerular dysfunction or impaired protein reabsorption of protein by the proximal renal tubule. The hallmark of tubular proteinuria is the presence of a number of proteins of a lower molecular weight than albumin in urine (Kaysen *et al.*, 1991). This contrasts with glomerular proteinuria in which albumin is the predominant species.

The proximal renal tubule is a major catabolic site for several plasma proteins which, by virtue of their small size and favourable isoelectric point, are filtered freely by the normal glomerulus (Binghan and Cummings, 1985). When there is proximal tubular dysfunction due to alcoholic toxicity, the reabsorption of these proteins could be impaired; and they appear in

increased quantities in urine. Put in a functional perspective for differential diagnostic purposes, it is important to point out the fact that the plasma levels of small molecular weight proteins rise if glomerular filtration rate is impaired as a result of chronic and excessive alcohol ingestion. However, if there is a selective proximal renal tubular impairment due to alcohol abuse, it is the urinary, not plasma levels, which rise (Knight *et al.*, 2003).

Essentially, glomerular damage is the most common cause of pathological proteinuria. Glomerular filter operating as both size-selective and charge-selective barrier normally prevents the passage of large protein molecules. So the large proteins commonly found in proteinuria could be as a result of glomerular dysfunction (Gausch *et al.*, 1993). Physical impedance to the passage of larger molecules through the filter arises through the ordered arrangement of type IV collagen and its glycol-protein matrix in the outer and inner *laminae rarae* and by the more tightly structured, principally type IV collagen of the central lamina densa of the glomerular basement membrane. The net effect is a rate dependent exclusion of some proteins. Globular proteins appear to be excluded more readily than those of elongated or tubular shape (Deen *et al.*, 1985).

Coupled with this structural barrier is the barrier due to the net negative charge on the sialoglycoproteins, particularly heparan sulphate and carboxyl residues, which constitute the matrix of the glomerular basement membrane (Bertolatus and Hunsicker, 1985). Those plasma proteins with isoelectric point equal to or higher than that of the glomerular basement membrane transverse through the barrier more easily than those of lower isoelectric point since at the pH of the plasma they possess a net positive charge (Daniels *et al.*, 1993).

The physical and electrical constituents of the glomerular barrier precipitate different, but related influences. If the structure of the glomerular basement membrane is altered, as in alcohol induced nephropathies, ordered arrangement of the negatively charged sialoglycoproteins will be disrupted. Since the physical structure adopted by collagen in the membrane is itself dependent on the ordered, negatively charged sialoglycoproteins of its matrix, it follows that, if these are adversely affected, the physical barrier itself is disrupted (Cecchin and DeMarchi, 1996).

Although, proteinuria is usually a manifestation of glomerular injury, it can also result from pathological process not involving the glomerulus. Defects in proximal tubule cells can prevent interiorization of the normal load of filtered protein, thereby permitting its escape into final urine. This is called tubular proteinuria. Essentially, tubular proteinuria could develop as a result of tubular or interstitial damage. Failure of the tubules to reabsorb some of the plasma proteins that have been filtered by the normal glomerulus due to alcohol-mediated

damage could readily lead to proteinuria. Characteristically, the proteins that are mostly excreted in tubular proteinuria have low molecular weight (Remuzzi and Bertani, 1990).

1.5 Urine Specific Gravity as an Index of Alcohol-Induced Renal Dysfunction

One of the earliest examinations of renal function used in clinical chemistry was the estimation of the concentrating ability of the kidney on the basis of the specific gravity of the urine. This test measures the density of urine relative to the density of water. Glucose and protein can contribute substantial increments to the specific gravity of urine. Excessive and chronic abuse of alcohol can lead to glomerular dysfunction. The resultant proteinuria could lead to a marked change in urine specific gravity (Michielson and VanRenterghen, 1983).

The specific gravity of urine varies directly with the quantity of solutes excreted per litre. It provides information on the ability of the kidney to concentrate the glomerular filtrate. In alcohol-induced renal tubular dysfunction, the concentration ability of the kidney is compromised (Verbalis, 1993).

The distal convoluted tubule and the collecting ducts perform a very crucial function in determining the concentration of urine that is ultimately excreted. This function can be monitored by renal concentration tests which examine the capacity of the kidney to perform osmotic work. This ability is reflected in urine specific gravity. It is salient to point out the fact that urine specific gravity is linearly related to osmolality which gives a measure of the number of particles in solution in the urine (Michielson and VanRenterghen, 1983).

1.6 Alteration in Plasma Urea Concentration as a Pointing Indicator of Renal Dysfunction Under the Nephrotoxic Assault of Chronic and Excessive Alcohol Abuse

Plasma urea concentration is a useful index of glomerular function. The ammonia generated from the deamination of amino acids is converted to urea in the liver by a cyclic mechanism known as the urea cycle (Halperin *et al.*, 1992). The first amino group to enter the urea cycle arises in form of free ammonia by the oxidative deamination of glutamate inside the mitochondria of the liver cell, catalyzed by glutamate dehydrogenase which requires NAD^+ . The free ammonia generated is utilized in conjunction with carbon (IV) oxide produced in the mitochondria by respiration to synthesise carbamoyl phosphate in the matrix in an ATP-dependent reaction catalyzed by carbamoyl phosphate synthetase 1. This enzyme is a regulatory enzyme and requires N-acetylglutamate as a positive modulator (Good and Knepper, 1990).

Next, carbamoyl phosphate donates its carbamoyl group to ornithine to form citrulline and release phosphate. This reaction is catalyzed by ornithine transcarbamoylase, a Mg^{2+} -requiring mitochondrial enzyme. The citrulline so formed then leaves the mitochondria and passes into the cytosol of the liver cell (Canaan *et al.*, 1993).

The second amino group now comes in, in the form of L α -aspartate which in turn acquired it from L-glutamate by the action of aspartate transaminase. The transfer of the second amino group to citrulline occurs by a condensation reaction between the amino group of aspartate and the carbamoyl carbon of citrulline in the presence of ATP to form argininosuccinate. The reaction is catalyzed by argininosuccinate synthetase of the liver cytosol (Good and Knepper, 1990).

Argininosuccinate is then reversibly cleaved by argininosuccinate lyase to form free arginine and fumarate. The fumarate so formed returns to the pool of citric acid intermediates. Lastly, the liver enzyme arginase cleaves arginine to yield urea and ornithine. Ornithine is thus regenerated and can enter the mitochondria again to initiate another round of the urea cycle (Halperin *et al.*, 1992).

Urea is freely filtered from the glomeruli and is not reabsorb nor secreted by the tubules. So the kidneys excrete most of the urea produced. Renal dysfunctions could cause a change in plasma urea concentration so urea can serve as a useful indicator of renal function under chronic alcohol abuse. However, results of plasma urea concentration should be interpreted with utmost caution because non renal factors such as high protein diet, dehydration, elevation in the catabolism of protein, starvation, decreased renal perfusion and reabsorption of blood after hemorrhage can elevate plasma urea concentration. This condition is known as pre-renal azotemia and should be taken into consideration while interpreting plasma urea concentration results (Obarzanek *et al.*, 1996).

On the other hand, obstruction of the outflow of urine as a result of tumors of the genitourinary tract or stones causes post-renal azotemia which must also be considered. These conditions notwithstanding, plasma urea concentration remains a very powerful indicator to monitor the structural and functional integrity of the kidney under chronic and excessive alcohol abuse (Cecchin and DeMarchi, 1996).

1.7 Microanatomy of the Human Liver

In order to put the numerous alcohol induced liver dysfunction in a good functional perspective, the anatomy of the liver is relevant (Duun *et al.*, 2006).

Microanatomy of the human liver is a prelude to capturing alcohol-mediated hepatotoxicity. The anatomic position and arrangement of the liver situate it well for maximum efficiency for receiving materials to be processed, as well as excreting undesirable products. The liver and biliary tree occupy the right upper quadrant of the abdomen (Saxena *et al.*, 1999) and is composed of complex parenchymal cells whose multifarious functions are vital for life.

Actually, the liver consists of lobes which are subdivided into lobules. The lobule comprises ramifying columns of hepatocytes (You and Crabb, 2004). The portal vein, hepatic artery, and bile-ducts, surrounded by a connective tissue capsule, enter the liver and branch repeatedly in the organ. The portal vein divides into branches, the interlobular veins, which surround the lobules; from these vessels, blood passes between the hepatocytes in vascular capillaries to reach the centre of the lobule where it drains into intralobular branches of the hepatic vein. The hepatic artery likewise divides into branches which accompany those of the portal vein between the lobules; ultimately the hepatic artery blood enters the vascular capillaries, where it mixes with the blood from the portal vein. At intervals along the vascular capillaries are the stellate cells of the kupffer which are part of the macrophage or reticulo-endothelial system (Menon *et al.*, 2001).

Indeed, the liver is unique in its fine anatomic architecture. It is prominently characterized by a series of plates of hepatic cells, one-layer thick, in contact with portal vein capillaries (Sinusoids) on two sides as well as with bile canaliculi that convey hepatic cell secretions to the bile ducts. Thus, each hepatocyte has a large surface area in contact with both nutrient intake system from the sinusoids and an outlet system, the bile canaliculi which carry away the secretions and excretions from the hepatocytes. The bile canaliculi combine to form bile ducts that carry the bile secretions into the small intestine (Arias *et al.*, 1993).

1.8 The Liver and Alcohol Metabolising System

The metabolism of ethanol is directly responsible for most of its toxic effects. Actually, ethanol is metabolized to acetaldehyde by alcohol dehydrogenase in the gastric mucosa and liver, and by Cytochrome P-450 (CYP 2E1) and catalase also in the liver (Eng *et al.*, 2007).

Metabolism of ethanol can be illustrated thus:

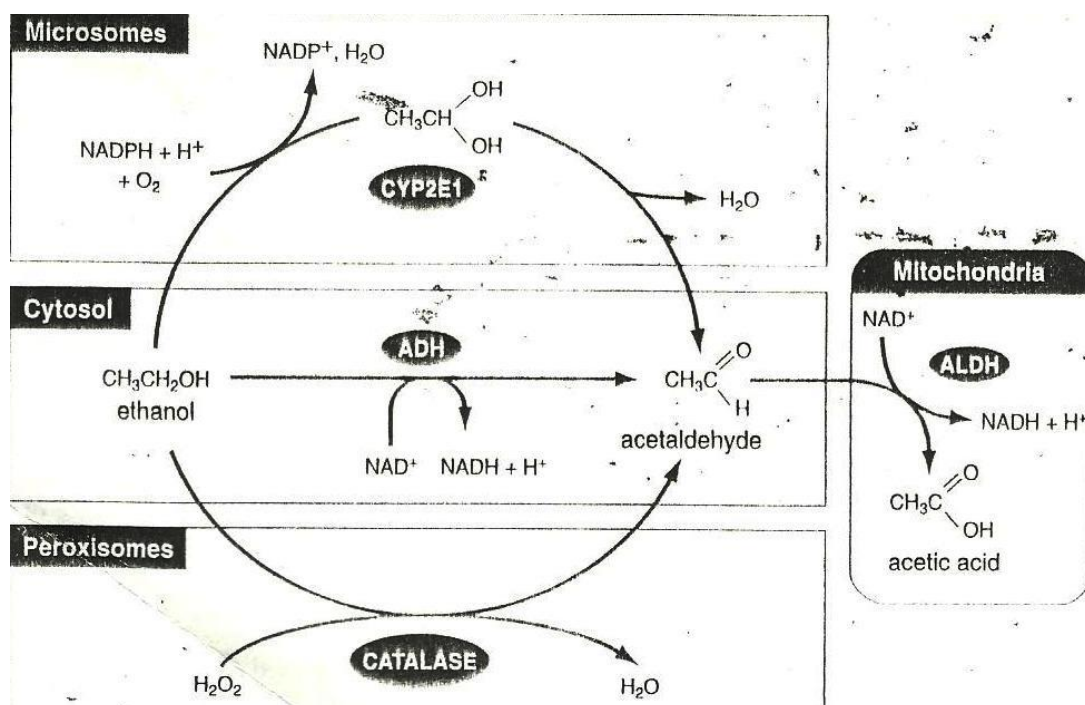
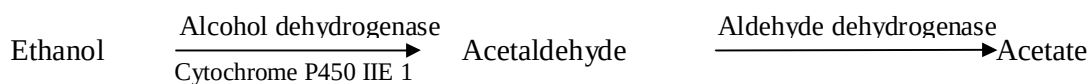


Fig. 2: Metabolism of ethanol ADH: alcohol dehydrogenase; ALDH: aldehyde dehydrogenase.

Essentially, ingested ethanol is first oxidized to acetaldehyde, which is then oxidized to acetate.

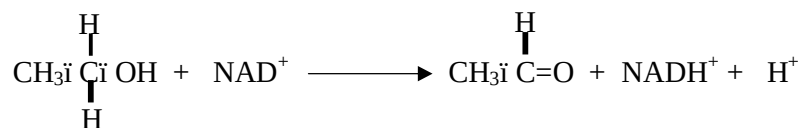


Quantitatively, the greatest proportion of alcohol is metabolized by the alcohol dehydrogenase pathway. Minor pathways of ethanol metabolism include the reaction catalyzed by catalase (Lieber, 1987). The minor pathways contribute little to the elimination of alcohol from the body. It cannot be over emphasized that the liver is the chief organ responsible for alcohol metabolism.

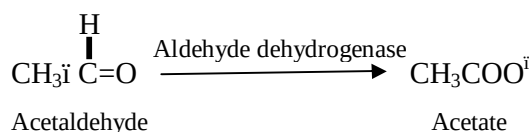
Actually, the breakdown of ethanol in the liver consists essentially of three separate steps:

1. The enzyme alcohol dehydrogenase, in the presence of the hydrogen acceptor nicotinamide adenine dinucleotide (NAD) oxidizes alcohol to acetaldehyde. This reaction is relatively slow and proceeds at constant rate, irrespective of the quantity of alcohol available.

The rate at which this reaction takes place determines the speed at which ethanol is cleared from the body. Thus this reaction is known as the rate-limiting step (Lieber, 2004).



2. In the second step, acetaldehyde is quickly converted to acetate by the enzyme aldehyde dehydrogenase (Rex *et al.*, 1985).



Significantly, the accumulation of acetaldehyde in the body is capable of generating extremely distressing and devastating toxic effects.

3. In the third step in the reaction sequence, the acetate generated from acetaldehyde combines with the acetate produced from the various other biochemical reactions and undergoes the same ultimate metabolic fate. It enters the Krebs cycle and in several steps later is converted to carbon dioxide and water (Crabb *et al.*, 1987). Carbon (iv) oxide is eliminated from the body in the exhaled air.

1.9 The Nature and Metabolic Roles of Some Alcohol Metabolizing Liver Enzymes

1.9.1 Alcohol Dehydrogenase

Alcohol dehydrogenase (ADH, E.C. 1.1.1.1), exists as dimers. There are up to five different classes of ADH. Class I isoenzymes are produced from three related subunits α , β , γ , which have the potential to hybridize giving rise to homo $\alpha\alpha$ and heterodimers with a low K_m for ethanol (Ehlers, 2007). Alcohol dehydrogenase could be found in the cytosol of cells of the liver, kidney, stomach, and lungs. However, the highest activity is in the liver. Class II ADH also exists in human liver and has a higher K_m (Michaelis Menten Constant) for ethanol.

Class III ADH exist in all tissues and appears to be inactive with ethanol, it acts on long-chain alcohols (Wagner *et al.*, 1984). Class IV enzymes have been isolated from the

stomach (Moreno and Pares, 1991). Class V is the last entrant to this group of enzymes (Yin *et al.*, 1990). Essentially Class I, II, and IV enzymes are the principal classes that take active part in the metabolic oxidation of ethanol (Moore *et al.*, 2007).

1.9.2 Cytochrome P450

Stripped to its barest essentials, cytochrome P450 IIEI (encoded by the Cyp 2E1 gene) is situated in the endoplasmic reticulum, particularly in hepatocytes of the centrilobular zone of the liver (Lieber, 1987; Tsutsumi *et al.*, 1989). Cytochrome P450 has relatively high K_m for ethanol. This enzyme is crucial because in addition to alcohol metabolism, it metabolizes many other xenobiotics.

1.9.3 Aldehyde Dehydrogenase

The enzymes, aldehyde dehydrogenases (ALDH, E.C. 1.2.1.3) are largely responsible for the oxidation of acetaldehyde. Many different classes of aldehyde dehydrogenase are available (Eng *et al.*, 2007). The class I and Class II enzymes occur as tetramers. These two classes (I and II) preferentially use NAD^+ as coenzyme even in the presence of abundant $NADP^+$. ALDH 1 is the main form of aldehyde dehydrogenase in the cytosol and has a low k_m for aldehydes. ALDH 2 is the major mitochondrial form of the enzyme. It has a very low k_m for acetaldehyde and exists principally in the liver (Enlers, 2007). It is important to note that ALDH 3 and ALDH 4 have substantially lower affinity for aliphatic aldehydes than ALDH 1 and 2 (Eng *et al.*, 2007).

The biochemical and medical role ALDH 2 plays in acetaldehyde elimination from the body is pivotal. This essential and indispensable role of ALDH 2 in the disposal of acetaldehyde is brought to the lime light by mutations in the ALDH 2 gene (Niemela *et al.*, 2000). A variant of the ALDH 2 gene leads to remarkable reduction in the activity of the enzyme (Scott and Taylor, 2007). When individuals with the abnormal form of the ALDH 2 ingest alcohol, acetaldehyde builds in the body. As a result of this observation, alcoholics who lack the functional ALDH 2 often have high incidence of alcoholic hepatitis and other complications of alcohol abuse (Day *et al.*, 1995). It is then necessary to point out here that ALDH 2 deficiency is one of the factors that usually render alcoholics more susceptible to liver complications. This derives from the fact that acetaldehyde accumulation is cardinal in the hepatotoxicity of alcohol (Menon *et al.*, 2001).

1.10 Some Alcohol-Induced Liver Problems

1.10.1 Ethanol-Induced Liver Dysfunctions

The most damaged organ in the body as a result of chronic and excessive ingestion of alcohol is the liver. Once absorbed, ethanol is rapidly distributed between the intracellular and extracellular compartments. This is because ethanol is completely miscible in water and thus has the ability to move to any part of the body where water moves. Ethanol can cross plasma membranes freely, but in so doing, alters them adversely. When ethanol is in contact with a protein, it denatures it. Thus, large and frequent ethanol exposures result in damage to proteins both within and around the liver cells (Sorensen *et al.*, 2003).

While gut cells are also adversely affected by excessive and consistent consumption of alcohol; these cells have such an accelerated turnover time that the damage resulting from chronic alcohol abuse is not as long-lasting as in the liver. In contrast, the liver is most affected partly because ethanol is carried directly to the tissues of the liver through the portal blood (Devor *et al.*, 1988). Liver cells also have a longer half-life and once the hepatic cells are damaged, they do not repair as readily. Indeed, while ethanol is distributed virtually throughout the body, the liver is the chief site for its oxidation. The first rate limiting reaction in ethanol oxidation is catalyzed by alcohol dehydrogenase which converts ethanol to acetaldehyde. Essentially, acetaldehyde is quite damaging to liver cells and mainly the hepatic injuries found in alcoholics is due to acetaldehyde (Day *et al.*, 1993).

There is a profound involvement of the metabolite of ethanol called acetaldehyde in the metabolic and toxic effect of ethanol (Moore *et al.*, 2007). The oxidation of acetaldehyde in the liver mitochondria is linked to the mitochondrial respiratory chain at the site of NAD-linked dehydrogenases. Chronic and excessive consumption of ethanol results in a significant reduction of the capacity of the liver mitochondria to oxidize acetaldehyde. Acetaldehyde is implicated as a mediator of many of the biochemical and pharmacological toxic effects of chronic ethanol abuse (Browning and Horton, 2004).

Cogent suggestions of involvement of acetaldehyde in cellular liver damage are partly based on impairment of mitochondria (Day *et al.*, 1993). Acetaldehyde has the capacity to, and actually causes functional disturbance of the mitochondria by reducing the activity of various shuttles involved in the disposition of reducing equivalents and by inhibiting oxidative phosphorylation (Caro and Cederbaum, 2004).

The rate of ethanol clearance in alcoholics is either equal to or faster than that of non-alcoholics. So chronic and excessive consumption of alcohol invariably leads to greater production of toxic metabolites of alcohol (acetaldehyde) resulting in a vicious cycle.

acetaldehyde leading to mitochondrial dysfunction which in turn promotes higher acetaldehyde accumulation which generates further damage to liver mitochondria and other structures of the hepatocyte (Testino, 2008).

Chronic and excessive consumption of alcohol often leads to increased concentration in blood acetaldehyde, due in part, to increased ethanol metabolism (Tsukamoto *et al.*, 1995). The microsomal ethanol oxidizing system (MEOS) has a crucial role to play in this adaptive acceleration in the rate of alcohol metabolism. Cytochrome P450, NADPH, Cytochrome- C-reductase, phospholipids, and components of the microsomal drug metabolizing system constitute the MEOS (Lieber, 1987). Since the increased concentration of acetaldehyde produced by the increased ethanol metabolism is generated primarily in the endoplasmic reticulum and not by the alcohol dehydrogenase pathway in the cytosol (Scott and Taylor, 2007), it then follows that the local acetaldehyde level in the endoplasmic reticulum is consequentially raised after chronic and excessive alcohol consumption. At this sub cellular level, acetaldehyde is capable of rising to a dangerously toxic level which can engender hepatotoxicity. This underscores the devastating consequences of local production of acetaldehyde (Day *et al.*, 1993).

Furthermore, liver microtubules are another site for acetaldehyde toxicity. Hepatic microtubular alterations, lipid accumulation and protein retention accounts for the hepatomegaly and disorganization of sub cellular architecture in acetaldehyde mediated liver dysfunctions (Perlmutter, 2002).

1.11 Alcoholic Liver Diseases

Hepatic injuries could be induced by long-term consumption of large amounts of alcohol. The injury stages progress from fatty infiltration to fibrosis and if the hepatocellular necrosis and liver insult continue, advance to cirrhosis and even death. The fibrosis and cirrhosis are irreversible (Barreto *et al.*, 2014).

Indeed, excessive and consistent consumption of alcohol is the leading cause of liver diseases. The three distinctive but overlapping forms of alcohol induced liver diseases are:

1. Hepatic steatosis
2. Alcoholic hepatitis
3. Cirrhosis

They are collectively referred to as alcoholic liver disease.

1.11.1 Hepatic Steatosis (Fatty Liver)

Following even a moderate intake of alcohol, small (microvesicular) fat droplets accumulate in hepatocytes (You and Crabb, 2004). With chronic intake of alcohol, lipid accumulates to the point of creating large, clear, macrovesicular globules, compressing and displacing the nucleus to the periphery of the hepatocyte. With continued intake of alcohol, fibrous tissues develop around the terminal hepatic veins and extend into the adjacent sinusoids (Deleve *et al.*, 2002). The fatty liver is due to accelerated hepatic fatty acid synthesis as well as ethanol-induced impairment in hepatic lipid output. If the hepatocyte accumulates too much lipid, the cell will burst and die. Areas of dead tissue within the liver are known as cirrhosis. When too many tissues die, the liver may cease to function and the alcoholic dies.

1.11.2 Alcohol Hepatitis

Alcoholic hepatitis is characterized by hepatocyte swelling and necrosis. The swelling results from the accumulation of fat and water, as well as proteins that are normally exported (Ishak, 2000). In some cases, there is cholestasis in surviving hepatocytes and mild deposition hemosiderin (Iron) in hepatocyte and kupffer cells (Testino, 2008).

Alcoholic hepatitis is almost always accompanied by prominent activation of sinusoidal stellate cells and portal tract fibroblast, giving rise to fibrosis (Cuthbert, 2007). Although steatotic hepatocytes are present, they are interspersed with the inflammatory cells and activated stellate cells. In macroscopic appearance, the liver is mottled red with bile-stained areas (Patrick, 1983; Testino, 2008). Although, the liver may be of normal or increased size, it often contains visible nodules and fibrosis, indicative of evolution to cirrhosis.

1.11.3 Alcoholic Cirrhosis

The final and irreversible form of alcoholic liver disease usually evolves slowly and insidiously. At first, the cirrhotic liver is yellow, fatty and enlarged (Butterworth, 2000). Latter, it is transformed into brown, shrunken and non fatty organ. Initially, the developing fibrous septae are delicate and extend through sinusoids from central to portal regions as well as from portal tract to portal tract (Crawford, 2001). Regenerative activity of entrapped parenchymal hepatocytes generates fairly uniformly sized "micronodules". With time, the nodularity becomes more prominent and scattered. As fibrous septae dissect and nodules, the liver becomes more fibrotic, loses fat, and shrinks progressively in size. Ischemic necrosis

and fibrous obliteration of nodules eventually create broad expanses of tough, pale scar tissue (Kita *et al.*, 2003). Bile stasis often develops; it is salient to point out the fact that cirrhosis may develop without antecedent evidence of steatosis or alcoholic hepatitis.

Alcoholic liver disease, thus, is a chronic disorder featuring steasis, hepatitis, progressive fibrosis and cirrhosis. In essence, alcoholic liver can be regarded as a maldaptive state in which cells of the liver respond in an increasingly pathologic manner to alcohol stimulus (Leon and McCambridge, 2006).

1.12 Alcohol Induced Liver Degeneration and Intracellular Accumulation

Damage due to toxic insult and assault resulting from excessive and chronic ingestion of alcohol could cause swelling of hepatocytes. Moderate cell swelling due to mild alcohol, abuse is reversible. However, with more severe damage (ballooning degeneration), swollen hepatocytes usually develop irregularly clumped cytoplasmic organelles and large clear spaces (Lieber, 1995).

In cholestatic liver injury resulting from alcohol abuse, retained biliary materials impart a diffuse foamy appearance to the swollen hepatocyte (feathery degeneration). Other instances of intracellular accumulation with grave consequences include the accumulation of triglyceride fat droplets within hepatocytes known as steatosis. Intracellular accumulation of multiple tiny droplets that do not displace the nucleus is known as microvesicular steatosis. A single large droplet that displaces the nucleus is known as macrovesicular steatosis. Both microvesicular and macrovesicular steatosis are present in alcoholic fatty liver. It is painful to point out that degeneration and intracellular accumulation resulting from alcoholic liver diseases constitute a major cause of death among those who consistently abuse alcohol (Kamath and Kim, 2007).

1.13 Bilirubin Metabolism and Alcohol-Induced Liver Dysfunction

Accurate monitoring of plasma bilirubin is pivotal in the differential diagnosis of alcohol induced liver disease and in the assessment of the structural and functional integrity of the liver (Room *et al.*, 2005).

Essentially, bilirubin is derived from the breakdown of haemoglobin when senescent erythrocytes are phagocytosed. The erythrocytes are loaded with haemoglobin, in a complex molecule containing four heme group (Ferroprotoporphyrin) attached to a protein globin (Crawford, 2002). The erythrocytes survive in the body for about 120 days before the aged cells are engulfed and digested by the phagocytic cells. These scavenger cells of the

reticuloendothelial system that destroy the old red blood cells are located primarily in the spleen, liver and bone marrow. The spleen is the main graveyard of the old erythrocytes (Weiss *et al.*, 1983).

The majority of the bilirubin formed daily is derived from the breakdown of the senescent erythrocytes by the mononuclear phagocytic system. Then most of the remainder of bilirubin is derived from the turnover of hepatic heme or hemoproteins such as the P450 cytochrome and from premature destruction of newly formed erythrocytes in the bone marrow. The latter pathway is important in haematologic disorders associated with excessive intramedullary hemolysis of defective erythrocytes (ineffective erythropoiesis) (Zhu *et al.*, 2008).

The mechanism for the production of bilirubin involves the splitting of the protein globin from hemoglobin. The globin may be reused or hydrolyzed to amino acids that join the amino acid pool for recycling. The protoporphyrin ring of heme molecule is broken open, with loss of one of the methine groups connecting the four pyrrole rings (William *et al.*, 1996). The resulting open-chain tetrapyrrole loses its iron, which becomes bound to a protein (ferritin) where it is stored until it is used again for the synthesis of new heme compounds in the bone marrow. The tetrapyrrole is reduced to form bilirubin (Zucker *et al.*, 2004).

Bilirubin leaves the reticuloendothelial cell and is solubilized in plasma by firmly binding to the protein albumin. Upon reaching the liver sinusoids, the bilirubin-albumin complex to the hepatocyte membrane. The bilirubin is detached from its albumin carrier and is transported inside the hepatic cell network to microsomes in rough endoplasmic reticulum by a transport protein, ligandin (Shapiro *et al.*, 2006).

Hepatic processing of bilirubin involves carrier-mediated uptake at the sinusoidal membrane. Conjugation of bilirubin takes place in the endoplasmic reticulum. An enzyme Uridyldiphosphate glucuronyl (UDPG) transferase, transfers a glucuronic acid molecule to one or both of the two propionic acid side chains in bilirubin, thereby converting bilirubin into mono or diglucuronide ester. The glucuronide are frequently referred to as conjugated bilirubin (Bosma *et al.*, 1994).

The water-soluble, non toxic bilirubin glucuronides are secreted from the hepatic cell into the bile canaliculi. Most bilirubin glucuronides are deconjugated by bacterial β -glucuronidase and degraded to colourless Urobilinogens. The Urobilinogens are largely excreted in faeces. Part of the Urobilinogens formed are reabsorbed in the ileum and colon, returned to the liver and promptly re-excreted into bile. The small amount that escapes this enterohepatic circulation is excreted in urine. Unconjugated bilirubin and bilirubin

glucuronides can accumulate systematically and deposit in tissues as a result of hepatic dysfunction (Rambaldi *et al.*, 2005).

Alcohol toxicity can cause obstruction or stagnation of biliary flow leading to cholestasis. In cholestasis, the elevated concentration of serum bilirubin consists of both the conjugated and the unconjugated fractions. Diffuse hepatocellular damage resulting from excessive and chronic abuse of alcohol could lead to impairment in the excretory transport system or obstruction in biliary tree that impedes the excretion of bile into the intestine. These defects resulting from chronic alcohol abuse can lead to hyperbilirubinamia (Laker, 1990).

1.14 Diagnosis of Alcohol-Induced Liver Problems

1.14.1 Enzymes of Diagnostic Utility in Alcohol-Induced Liver Dysfunction

Essentially, enzymes are proteins with catalytic properties due to their powers of specific activation of their substrates (Moss and Henderson, 1994). Some enzymes are valuable indicators of cellular damage and can serve prognostic and diagnostic functions. The level of enzyme in the blood depends on the factors that affect the entrance of the enzymes into circulation from their cells of origin. Normally, most enzymes are localized within their cells of origin by plasma membrane surrounding the cell. Any factor or chemical that promotes cellular injury or membrane deterioration can lead to leakage of the cell membrane and enzymes could leak out of their cells of origin. When the intracellular enzymes are transferred to the extracellular fluid, there often occurs a rapid rise in enzyme activity (Longstreth *et al.*, 2010).

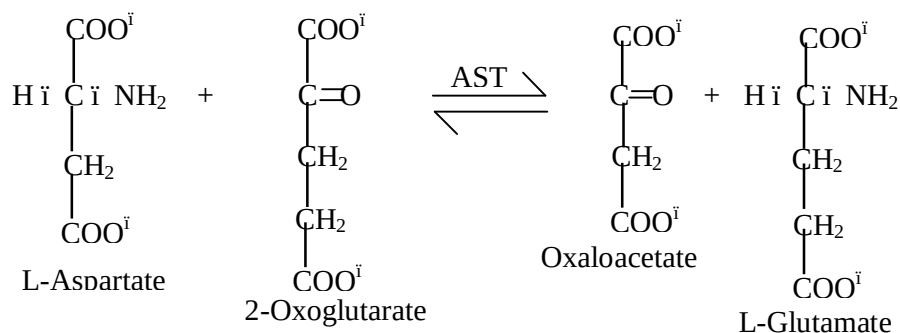
In normal health, the activities of a vast majority of diagnostic enzymes in plasma are fairly constant. Some of the enzymes may exhibit temporary increases in their activity but in general, plasma enzyme activity almost always represents a steady state in which the rate of release of the enzymes from the cells into plasma is equal to the rate of removal from the plasma. Founded on this fundamental ground therefore, alterations in plasma enzyme activity could often be attributed to increases in the rate of release of enzymes into plasma.

Pathological deterioration of a tissue could lead to elevation of enzyme activities in blood as a result of increased permeability of cell membrane which allows cytoplasmic enzymes to leak out into the blood. Enzymes induction is another contributing factor that accounts for increase activity of enzymes in plasma. Chemical assault on the liver due to chronic and excessive abuse of alcohol very often leads to changes in the activities of hepatocellular enzymes.

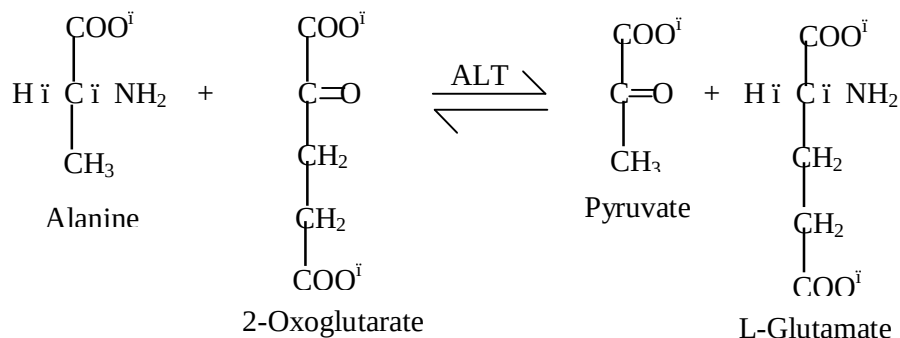
1.14.1.1 Aminotransferases

Aminotransferases of diagnostic utility comprise aspartate aminotransferase and alanine aminotransferase (Abraham and Grieze, 2004). The aminotransferase otherwise called transaminase are a group of enzymes that catalyze the interconversion of amino acids and 2-oxoacids by transfer of amino groups. It is pertinent to mention that these aminotransferases have different isoenzymes. The existence of these multiple molecular forms of the enzymes has significant implications for the analysis of tissue damage and disease processes. Furthermore, these isoenzymes of aminotranferases are compartmentalized. There are some isoenzymes that are cytosolic while some are mitochondrial. The type of isoenzyme detected gives information about the severity of the injury. Severe alcohol-induced hepatic damage leads to the release of mitochondrial isoenzyme (Clark *et al.*, 2003).

The 2-oxoglutarate ÷ L-glutamate couple serves as one amino acid group acceptor and donor pair in all amino transfer reactions, the specificity of the individual enzymes derives from the particular amino acid that serves as the other donor of an amino group. Thus, Aspartate Aminotranferase (AST) E.C.2.6.1.1, also known as glutamate oxaloacetate transminase (GOT) catalyzes the reaction (Klatsky *et al.*, 2006).



Also Alanine Aminotranferase (ALT) E.C.2.6.1.2, formerly called glutamate pyruvate transaminase (GPT) catalyse the reaction.



Pyridoxal-5'-phosphate acts as a prosthetic group in the amino transfer reaction. The pyridoxal-5'-phosphate bound to the apoenzyme accepts the amino acid group from the first substrate, aspartate or alanine, to generate enzyme-bound pyridoxamine-5'-phosphate and the first product of the reaction, oxaloacetate or pyruvate, respectively. The coenzymes, in amino form, then transfers its amino form to second substrate, 2-oxoglutarate, to form second product, glutamate pyridoxal-5'-phosphate is regenerated (Niemela, 2007).

Alcohol induced liver dysfunction almost always leads to variations in the levels serum AST and ALT even before the clinical signs and symptoms of the disease become obvious. Actually increased activities of AST in plasma are of considerable diagnostic importance in the recognition and monitoring of liver damage resulting from excessive and consistent alcohol consumption. Remarkable increase in AST activity is indicative of continuing hepatocellular damage (Conigrave *et al.*, 2003).

Although the activities of both AST and ALT change whenever hepatotoxic agents like alcohol adversely affect cell integrity, ALT appears to be more liver specific and can persist for a longer duration (Klatsky *et al.*, 2006). Plasma ALT activity is vastly utilized as a powerful indicator of Hepatocellular damage (Clark *et al.*, 2003).

1.14.1.2 Alkaline Phosphatase (ALP)

Alkaline phosphatases (E.C. 3.1.3.1) are a group of enzymes with low substrate specificity that catalyzes the hydrolysis of a variety of phosphate esters at alkaline pH. The isoenzymes of ALP manifest optimal activity in vitro at a pH of 10 (Moss, 1982). Alkaline phosphatase occurs at high levels in the intestinal epithelium, bone and liver.

Laboratory assay of the activity of ALP in plasma is pivotal in the investigation of hepatobiliary disease (Clifton, 2003). The hepatocytes near the biliary canaliculi are specific sites for the production of ALP in the liver. Based on the concept of enzyme induction, the production of ALP can be elicited as a response to alcohol induced liver dysfunctions. Elevation in the activities of ALP is characteristically associated with both intrahepatic and extrahepatic obstruction resulting from excessive and consistent consumption of alcohol. Nevertheless, elevation in the activities of ALP is remarkably higher in extrahepatic obstruction (Sherman, 1991).

1.15 Justification of the Study

There is scarcity of information about the insidious and debilitating effects of excessive and consistent consumption of alcohol in form of palmwine, beer and distilled spirit.

1.16 Aim and Objectives of the Study

1.16.1 Aim of the Study

The major objective of this study was to investigate the impact of alcohol abuse on some biochemical parameters of alcoholics.

1.16.2 Objectives of the Study

This study was designed to achieve the following specific objectives:-

1. To determine the impact of chronic and excessive ingestion of alcohol on the kidney.
2. To determine the impact of alcohol abuse on some biochemical parameters associated with the liver.
3. To investigate how variations in the concentrations of some biochemical parameters could help in the detection of organ dysfunction especially when early clinical or visible manifestation of such malfunction is masked by high reserve capacity of the organs involved.

CHAPTER TWO

MATERIALS AND METHODS

2.1 Materials

2.1.1 Subjects Used

A total of one hundred and eighty (180) male alcoholics consisting of sixty (60) alcoholics who showed preference for palmwine; sixty (60) alcoholics who showed preference for beer; and sixty (60) alcoholics who showed preference for distilled spirit (local gin) were used. Also, forty non-consumers of alcohol who served as controls were used.

2.1.2 Location/Zone of Study

The zone of research where the alcoholic subjects were located covered, Enugu-Ezike in Igbo Eze North Local Government Area, Orba in Udenu Local Government Area; Nru Nsukka in Nsukka Local Government Area of Enugu State.

2.1.3 Chemicals/Reagents/Samples

All chemicals used in this study were of analytical grade and products of May and Baker, England; BDH, England and Merck, Darmstadt, Germany. Reagents used for all the assays were commercial kits and products of Randox, USA; Teco (TC), USA.

2.1.4 Instruments/Equipment

Water Bath (Gallenkamp, England), sensitive Balance (Metler instrument, England), Conical Flasks (Pyrex, England), Hotbox (Gallenkamp, England), Centrifuge (PIC, England), Digital Colorimeter (SP-6 400 Pye Unicam, England), Adjustable Micropipette (Perfect, U.S.A.), Refrigerator (Kelvinator, Germany), pH Meter (Pye, Unicam 293, England).

2.2 Methods

2.2.1 Experimental Design

The health status, patterns, type and amounts of ethanol intake of one hundred and eighty (180) male alcoholics who cut across different social strata and forty (40) non-drinkers of alcohol were assessed with the use of specifically designed and validated questionnaires. Individuals who had clinical or laboratory evidence of recent illnesses, infections, hypertension, treated kidney or liver problems or had used any hard drug were excluded. None of those used as controls (non consumers of alcohol) was a former alcoholic or had any social or medical records of drinking or associated medical disorders. After interviews and administration of carefully structured questionnaires containing general questions on age, sex, type and quantity of alcohol, duration of drinking, attitudes to social, family, occupational and health issues, habits, the alcoholics were categorized into three groups namely:

Group 1: Sixty male alcoholics who showed preference for palm wine (The subjects in this group were in the habit of drinking an average of four or more cups of palm wine per day for at least three months (the volume of one who send youô cup was 60 cl and the alcoholic content varied according to the time of the day but on the average ranges from 4- 5 % v/v alcohol).

Group 2: Sixty male alcoholics who showed preference for beer. (The subjects in this group were in the habit of drinking an average of 4 or more bottles of beer per day for at least 3 months (The volume of a bottle of beer was 60 cl and the alcoholic content was 5% v/v).

Group 3: Sixty male alcoholics who showed preference for distilled spirit (ogogoro). (The subjects in this group were in the habit of drinking four or more ôet cupsô of distilled spirit per day for at least 3 months (the volume of a ôet cupsô was 16 cl and the alcoholic content was 45 ï 85% v/v).

Group 4: Forty non- consumers of alcohol (teetotallers). This group served as the control.

After expressed consent, blood and 24- hour urine samples were obtained from the subjects and used for biochemical analysis.

2.2.2 Collection of 24-Hour Urine Sample

At the start of the collection, the subjects emptied their bladders as completely as possible. These urine specimens were discarded. Then, all urine specimens voided from 8.00 am on the day of collection till 8.00 am on the next day were collected and used for the tests.

2.2.3 Collection of Blood Specimens

Blood samples of the alcoholic and non-alcoholic subjects were collected by venipuncture. The median cubital vein in the antecubital fossa was used as the site for the collection of venous blood. The skin was well cleaned with methylated spirit over the area selected for collecting the blood specimen. Tourniquet was applied 12cm above the puncture site to effect temporary venous stasis in order to distend and reveal vein prior to venipuncture. Prolonged venous stasis was, however, avoided as this could markedly raise the concentration of plasma protein for instance. After inserting the needle into the vein, blood specimens were collected with only moderate suction to avoid haemolysis. The shaking of the needle was removed from the syringe before transferring blood to plain containers. The containers were also performed slowly, in order to avoid haemolysis. The containers were correctly and sufficiently labelled with names of the subjects, date, time and location of collection. When the needle was removed from the vein, the subjects were advised to hold cotton wool over the puncture site and the arm raised to avoid leakage of blood. The blood samples without anticoagulant were centrifuged for 5minutes at 1000rpm in order to separate the clotted blood cells from the serum. The serum samples were then collected and used within 24h.

2.2.4 Experimental Analyses

2.2.4.1 Determination of Urine Total Protein Concentration

Principle

The determination of urine total protein concentration was carried out according to the method of Slater (1986). This method is based on the principle that cupric ions, in

an alkaline medium, interact with peptide bonds of proteins resulting in the formation of a coloured complex.

Reagents

20% Trichloroacetic acid, 1M sodium hydroxide (40 g per 1 litre of distilled water).

Standard protein solution: 5 g/l in 0.90% NaCl.

Stock Biuret reagent 45g of sodium potassium tartrate was dissolved in 400ml of 0.2M NaOH. A known weight, 15 g, of Copper sulphate ($\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$), 15 g was added with constant stirring. KI (5 g) was added and the solution diluted to 1 litre with 0.2 M NaOH.

Working Biuret solution: 200ml of stock solution was diluted to 1 litre with 0.2 mol of NaOH containing 5.00 g KI per litre.

Procedure

To 1ml of urine in a test tube labelled sample was added an equal volume of trichloroacetic acid. The contents of the test tube were properly mixed and allowed to stand for 5 minutes. The tube was then centrifuged for 5 minutes at 1000 rpm. The supernatant fluid was decanted without disturbing the deposit. The precipitated protein was dissolved in 1ml of 1M NaOH and 2 ml of distilled water added. Distilled water (3 ml) was added to another test tubes labeled blank. Standard protein solution (3 ml) was added to another. To each of the three tubes was added 5 ml of working biuret reagent, mixed thoroughly and placed in 37°C water bath for 10 minutes for colour development. The tubes were allowed to cool and the absorbance read at 540nm (Baker and Silvertson, 1985).

$$\text{Total protein concentration} = \frac{\text{Absorbance of Sample}}{\text{Absorbance of Standard}} \times \text{Concentration of Standard}$$

2.2.4.2 Measurement of Urine Specific Gravity

Specific gravity of the urine samples were measured using specific gravity bottle (Baker and Silvertson, 1985).

Procedure

The empty, clean and dry stoppered specific gravity bottle was weighed. The bottle was filled with the test urine. The ground glass stopper was carefully inserted to ensure that no air was entrapped and excess urine ejected from the central hole in the stopper. All traces of the urine outside the bottle were wiped. The specific gravity bottle together with the urine was weighed. The specific gravity bottle was emptied and rinsed several times with distilled water. The bottle was then filtered with distilled water, the stopper replaced as before, the outside wiped and then weighed. The weight of the empty stoppered bottle was subtracted from the total weight of the bottle and urine. Also, the weight of the empty stoppered bottle was subtracted from the total weight of the bottle and distilled water. The weight of the urine was divided by the weight of the distilled water in order to get the specific gravity of the urine (Baker and Silverton, 1985).

Specific gravity =

$$\frac{\text{Weight of stoppered specific gravity bottle with urine}}{\text{Weight of stoppered specific gravity bottle with distilled water}} - \frac{\text{Weight of empty stoppered specific gravity bottle without urine}}{\text{Weight of empty stoppered specific gravity bottle without distilled water}}$$

$$= \frac{\text{Weight of urine}}{\text{Weight of same volume of distilled water}}$$

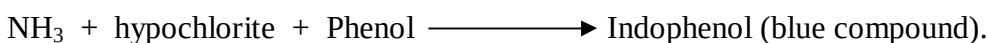
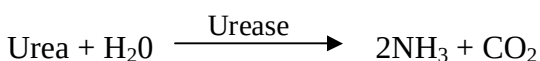
Normal range = 1.016 to 1.025

2.2.4.3 Determination of Urea Concentration

Urease Berthelot method (Weatherburn, 1967) for the *in vitro* determination of urea in serum using Randox enzymatic urea test Kit was employed.

Principle

Urea in serum is hydrolyzed to ammonia in the presence of urease. The ammonia is measured photometrically by Berthelot's reaction.



Reagents

R1 i 116 mmol/L EDTA, 6 mmol/L sodium nitroprusside, 1g/L urease.

R2 i 120mmol phenol (diluted).

R3 i Alkaline hypochlorite which contains 27mmol/L sodium hypochlorite and 0.14N sodium hydroxide.

CAL i Standard containing aqueous solution of urea equivalent to 80mg/dl.

Procedure

R1, the sodium nitroprusside and urease solution was prepared by transferring the contents of vial R/a (urease enzyme) into bottle R/b (sodium nitroprusside). The bottle was mixed gently. R2 (phenol) was prepared by diluting the contents of bottle R2 (phenol concentrate) with 660ml of distilled water and mixing properly. R3 (Sodium hypochlorite) was prepared by diluting the contents of bottle R3 with 750ml of distilled water. To each of the 3 test tubes labeled blank, standard and sample respectively was added 100 μ l of R1 (sodium nitroprusside, urease) Then 10 μ l of serum sample was added to the test tube labeled sample, 10 μ l of the standard was added to the test tube labeled standard and 10ml of distilled water was added to the test tube labeled blank. Each of the tubes was mixed thoroughly and incubated at 37⁰C. Then 2.50ml of reagent 2 (R2) was added to each of the test tubes. Also, 2.50ml of reagent 3 (R3) was added to each of the tubes. Each of the tubes was again incubated for 15 minutes at 37⁰C. The absorbance was read against the blank at 546nm.

The concentration of urea in mg/dl was obtained using the formula:

$$= \frac{\text{Absorbance of Sample}}{\text{Absorbance of Standard}} \times \frac{80}{1} = \text{mg Urea/dl}$$

Where 80 = concentration of standard aqueous solution of urea.

2.2.4.4 Determination of Creatinine Concentration

The modified Jaffe method (Blass *et al.*, 1974) for the *in vitro* determination of creatinine concentration in serum using the Randox test kit was employed in the determination of creatinine concentration.

Principle

Creatinine in alkaline solution reacts with picric acid to form a coloured complex. The amount of the complex formed is directly proportional to the creatinine concentration.

Reagents

R/a = 35 mmol/L Picric acid

R/b = 0.32 mol/L Sodium hydroxide

Cal = 177 μ mol/L (2 mg/dl) of creatinine standard

Procedure

A working reagent composed of equal volumes of solutions R/a and R/b (NaOH and picric acid solution) was prepared. For each determination, 0.5ml of solution R/a mixed with 0.5ml of Reagent B gave 1ml of working reagent. For each serum/urine sample, 0.1ml of sample was mixed with 1.0 ml of the working reagent in a clean test tube. It was mixed properly and 30 seconds after the absorbance A_1 of the standard and sample were read. Exactly 2 minutes latter, the absorbance A_2 of the standard and sample was read at 492nm.

The following formula was used to calculate the serum creatinine concentration of each sample.

Serum creatinine concentration (mg/dl)

$$= \frac{\Delta A_{\text{Sample}}}{\Delta A_{\text{Standard}}} \times \text{Standard Concentration (mg/dl)}$$

Where ΔA = Change of Absorbance

$$\Delta A_{\text{Sample}} \text{ or } \Delta A_{\text{Standard}} = A_2 - A_1$$

$$= \frac{\Delta A_{\text{Sample}}}{\Delta A_{\text{Standard}}} \times 100$$

$$\text{Creatinine clearance } (\mu\text{l/min}) = \frac{\text{Urine creatinine concentration (mg/dl} \times \text{volume of urine (ml/24hrs))}}{\text{Serum creatinine concentration (mg/dl)} \times 1440}.$$

2.2.4.5 Determination of Serum Potassium Ion Concentration

The determination of potassium concentration was carried out using the method of Tietz (1983). The amount of potassium is determined by using sodium tetraphenylboron in a specifically prepared mixture to produce a colloidal suspension. The turbidity of which is proportional to potassium concentration in the range of 2 ÷ 7 mEq/L.

The test tubes were well labeled as standard, control, test, etc. Also, a blank was provided. A volume of 1 ml of potassium reagent was pipette into all tubes. Also, 0.01ml (10 μ l) of samples was added to the respective test tubes and mixed thoroughly. The mixtures were allowed to stay under room temperature for 3 minutes. After 3 minutes, the absorbances of the solutions in the different test tubes were read at a wavelength of 500.00 nm against the reagent blank.

2.2.4.6 Determination of Serum Sodium Ion Concentration

The determination of sodium ion was carried out using the methods described by Maruna (1958) and Tietz (1983). The present method is used based on modifications of those first described by Maruna (1958) in which sodium is precipitated as the triple salt, sodium magnesium uranyl acetate, with the excess uranium then being reacted with ferrocyanate, producing a chromophore whose absorbance varies inversely as the test specimen.

Different test tubes were labeled as standard (S), Control (C), test (T) and blank (B). The filtrate reagent (1 ml) was pipette into all test tubes. The sample (50 μ l) was added to all test tubes and distilled water was then added to blank. All the test tubes were shaken vigorously and mixed continuously for 3 minutes. All the test tubes were

centrifuged at $1,500 \times g$ for 10 minutes. A volume of 1 ml of acid reagent was pipetted into all test tubes at the colour development stage. The supernatant (50 μ l) was added to respective tubes and mixed thoroughly. The colour reagent (50 μ l) was added to all the test tubes and mixed thoroughly. The spectrophotometer was zeroed with distilled water, after which the absorbance readings were taken at a wavelength of 550 nm.

$$\text{Conc of Sample (mEq/L)} = \frac{\text{Abs of Blank} - \text{Abs of S}}{\text{Abs of Blank} - \text{Abs of Std}} \times \text{Conc of Std}$$

Where

Abs = Absorbance

S = Sample

Std = Standard

Expected value = 135 $\pm$ 155 mEq/L

2.2.4.7 Determination of Direct Bilirubin Concentration

A colorimetric method with a kit supplied by Randox (R) was used to determine the concentration of direct bilirubin according to the method of Jendrassik and Grof (1938) and Sherlock (1951).

Principle:

Direct (conjugated) bilirubin reacts with diazotized sulphanilic acid in alkaline medium to form a blue coloured complex. Total bilirubin is determined in the presence of caffeine, which releases albumin bound bilirubin, by the reaction with diazotized sulphanilic acid.

Reagent

Content	Initial Concentration of Solution
R1: Sulphanilic acid	29mmol/L
Hydrochloric acid	0.17N
R2: Sodium nitrite	38.5 mmol/L
R3: Caffeine	0.26mol/L
Sodium benzoate	0.52mol/L

R4 Tartrate	0.93mol/L
Sodium hydroxide	1.9N

Procedure

Reagent 1 (0.20 ml) was pipetted into two test tubes labeled sample Blank (B) and sample (A). A drop (0.05 ml) of reagent 2 was pipetted into the test tube containing sample (A) only. This was followed by the addition of 2 ml sodium chloride (9 g/l) into both test tubes containing sample blank (B) and sample A respectively (for direct) and 1ml caffeine for total bilirubin. Serum (0.20 ml) was finally pipetted into both test tubes, sample blank (B) and sample (A). The contents of the test tubes were mixed thoroughly and allowed to stand for exactly 5 minutes at 25⁰C (10 minutes for total and 1 ml R4 (tartrate) added for total bilirubin and allowed for 5 minutes. The absorbance of the sample against the sample blank (A_{DB}) was read at 550 nm.

Direct bilirubin values were obtained from the following calculation:

$$\text{Direct bilirubin (mmol/L)} = 246 \times A_{DB} (550\text{nm})$$

$$\text{Direct bilirubin (mg/dl)} = 14.4 \times A_{DB} (550\text{nm})$$

Normal Value in Serum:

Total bilirubin:	Up to 17 μ mol/L
	Up to 1 mg/dl.
Direct bilirubin:	Up to 4.3 μ mol/L
	Up to 0.25 mg/dl.

2.2.4.8 Assay of Alkaline Phosphatase (ALP) Activity

Optimized standard method according to the recommendation of Deutsche Gesellschaft Fur Klinische Chemie (Rec, 1972) for the *in vitro* determination of alkaline phosphatase in serum using Randox test kit was employed.

Principle

Alkaline phosphatase hydrolyzes p-nitrophenylphosphate to generate phosphate and p-Nitrophenol according to the equation.



Reagents

R/a (Buffer) contained diethanolamine buffer (1 mol/l, pH 9.8) and 0.5 mmol/l of MgCl_2 . R/b (substrate) reconstituted contained 1 vial of substrate R/b (p-nitrophenylphosphate and 10 ml of R/a (buffer). This reconstituted R/b became the reagent R1.

Ra=Buffer

Rb=Substrate

Procedure

After reconstituting R/b to get the reagent R1, 1.0ml of the reagent was pipetted into each test tube and 0.02 ml of the serum sample added to each tube. The reagent (R1) was mixed with the serum sample and the initial absorbance read. A stopwatch was simultaneously started. The absorbance was read again after 1, 2 and 3 minutes.

To calculate the ALP activity, the following formula was used:

$$\text{ALP activity (U/L)} = 2760 \times \Delta A_{405\text{nm}/\text{min}}$$

Where ΔA = Change in absorbance

2.2.4.9 Assay of Aspartate Aminotransferase (AST) Activity

The activity of aspartate aminotransferase (AST) was determined according to the Reitman-Frankel colorimetric method (1957) for *in vitro* determination of GOT/AST in serum using Randox test kit.

Principle

Aspartate aminotransferase (AST) also called glutamate-oxaloacetate transaminase (GOT) is measured by monitoring the concentration of oxaloacetate hydrazone formed with 2, 4-dinitrophenylhydrazine. The enzyme catalyzes the transfer of the α -amino group from aspartate to α -ketoglutarate with the release of oxaloacetate and glutamate.



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

Reagents

AST substrate solution (Reagent 1), containing phosphate buffer pH 7.4, α -oxoglutarate and L-aspartate. Colour developer (Reagent 2), containing 2, 4-dinitrophenylhydrazine (DNPH), sodium hydroxide (0.4mol/l).

Procedure

Half milliliter (0.5ml) of reagent 1 (AST substrate solution) was put in a test tube labeled 'sample' and 0.1ml of serum added. The tube was incubated at 37°C for 30 minutes. Then 0.5ml of Reagent 2 (2, 4-dinitrophenylhydrazine) was added and allowed to stand for exactly 20 minutes at 25°C. Sodium Hydroxide (5.0ml) was also added. In another test tube labeled 'sample blank' was added 0.5ml of reagent 1 and as well incubated for 30 minutes at 37°C. Then 0.5ml of reagent 2 and 0.1ml of serum were added and left to stand for exactly 20 minutes at 25°C. Sodium hydroxide 5.0ml was added and the absorbance of the samples (A sample) read against the sample blank after 5 minutes at 546nm.

AST activity in IU/L was extrapolated from the AST standard curve as shown in the Appendix.

2.2.4.10 Assay of Alanine Aminotransferase (ALT) Activity

The activity of alanine aminotransferase (ALT) was determined by the Reitman and Frankel colorimetric method (1957) for *in vitro* determination of ALT in serum using Randox test kit.

Principle:

Alanine aminotransferase also called glutamic-pyruvate transaminase (GPT) catalyses the transfer of α -amino group from alanine to α -ketoglutarate with the release of pyruvate and glutamate.



ALT activity was measured by monitoring the concentration of pyruvate hydrazone formed with 2, 4, -dinitrophenylhydrazine which is proportional to its concentration

Reagents

ALT substrate solution (solution 1) containing phosphate buffer pH 7.4, α -oxoglutarate and L-alanine. Colour developer (solution 2), containing 2, 4-dinitrophenylhydrazine (DNPH) sodium hydroxide (0.4 mol/l NaOH) which needed no refrigeration.

Procedure

Solution 1 {ALT substrate solution} (0.5ml) was put in a test tube and 0.1ml of serum added. The tube was incubated at 37°C for 30 minutes. After incubation 0.5ml of solution 2 (2, 4-dinitrophenylhydrazine) was added and allowed to stand for exactly 20 minutes at 25°C. Then, 5.0ml of sodium hydroxide (0.4 mol/l) was added. Solution 1 (0.5ml) was also added to another test tube labeled blank and incubated for 30 minutes at 37°C. The 0.5ml of solution 2 and 0.1ml of the serum sample were added to the tube and left to stand for exactly 20 minutes at 25°C. Finally, 5.0ml of sodium hydroxide was added to the test tube (blank). The contents of the test tubes were mixed and the absorbance of the samples (A sample) read against the sample blank after 5 minutes at 546nm.

ALT activity in IU/L was extrapolated from the ALT standard curve as shown in the Appendix.

2.2.4.11 Determination of Gamma-Glutamyltransferase Activity

Gamma-glutamyltransferase assays are based on the use of L-γ-glutamyl-p-nitroanilide as the donor substrate and glycylglycine as the glutamyl acceptor (Moss and Henderson, 1996).

Preparation of Reagents

1. Buffer-Glycylglycine Reagent

A total of 6.96 g of Tris (hydroxymethyl) aminomethane (base) and 9.11g of glycylglycine was dissolved in 400 ml water. The pH was adjusted to 8.5 at 30°C by the addition of 0.5 molar NaOH. The solution was diluted to 500 ml with water and stored in the refrigerator.

2. L-Gamma-Glutamyl-p-Nitroanilide Substrate

Gamma-glutamyl-p-nitroanilide (104mmol/L) in 0.5 molar HCl was prepared by dissolving 29.5mg of L-gamma-glutamyl-p-nitroanilide monohydrate per ml of 0.5molar HCl. This was prepared and used within two hours of preparation.

Procedure

Buffer-glycylglycine (2.00 ml) and 100 µl of serum was pipetted into a test tube. A known volume, 0.20 µl, of L-gamma-glutamyl-p-nitroanilide substrate was added to the test tube and mixed rapidly. The blank was also prepared using 100µl of water in place of the serum. Changes in absorbance were observed at 405 nm. γ-GT activity was extrapolated from the γ-GT standard curve.

2.2.4.12 Measurement of Body Mass Index

The body mass index (BMI) of the subjects was derived by measuring their weights in kilogrammes (kg) using a balance and heights in metres (m) using a measuring tape on long wood. BMI was calculated as weight in kilogramme divided by height in metre squared.

$$\text{BMI (Kg/m}^2\text{)} = \frac{\text{Body weight (in Kg)}}{\text{Height (in metre) squared}}$$

2.2.4.13 Measurement of Blood Pressure

Blood pressure was measured after the subject had rested for 5 minutes in a sitting position. The arterial blood pressure was measured by means of a sphygmomanometer containing a column of mercury to which is attached a millimeter scale. A cuff was placed around the upper arm of the subject which was inflated by means of a rubber bulb attached to the manometer by a length of rubber tubing. A stethoscope was placed over the brachial artery in the region of the elbow. Air was pumped into the armlet till no sounds were audible. The pressure was then allowed to fall slowly by opening the valve. At the point when regular sound became audible, a reading was taken i this was the systolic pressure. The pressure was still allowed to fall, and the sounds changed in character. Ultimately, the sound became practically inaudible, when another reading was taken i this was the diastolic pressure.

Systolic pressure is the pressure corresponding to systole, or contraction of the ventricle of the heart. Diastolic pressure is the pressure corresponding to diastole, or relaxation of the ventricle.

Normal systolic blood pressure varies from 100 to 140 mmHg while normal diastolic pressure varies from 60 to 90 mmHg.

2.3 Statistical Analysis

Data were reported as means $\pm$ SD, where appropriate. One-way analysis of variance (ANOVA) was used to analyse the experimental data and Duncan multiple range test was used to compare the group means obtained after each treatment with control measurements. Differences were considered significant when $p \leq 0.05$ (Appendices).

CHAPTER THREE

RESULTS

3.1 Urine Total Protein Concentration in Human Subjects

The results of the urine total protein concentrations of the three categories of alcoholics who showed preference for palmwine, beer and distilled spirit together with the controls are shown in Fig. 3. The results reveal that the urine total protein concentrations of the alcoholics were significantly ($p < 0.05$) higher compared with the urine total protein concentration of the control subjects. Fig. 3 also shows that alcoholics who showed preference for distilled spirit (ogogoro) had the highest urine total protein concentration of 153.96 ± 0.43 mg/dl followed by alcoholics who showed preference for beer and palmwine who had urine total protein concentration of 152.74 ± 0.42 and 151.34 ± 0.60 mg/dl respectively. Furthermore, the urine total protein of alcoholic subjects who showed preference for beer was significantly ($p < 0.05$) higher than those who showed preference for palmwine. The urine total protein concentration of each of the three categories of alcoholics who showed preference for palmwine, beer and distilled spirit was significantly higher ($p < 0.05$) compared with the urine total protein concentration of the control (teetotallers).

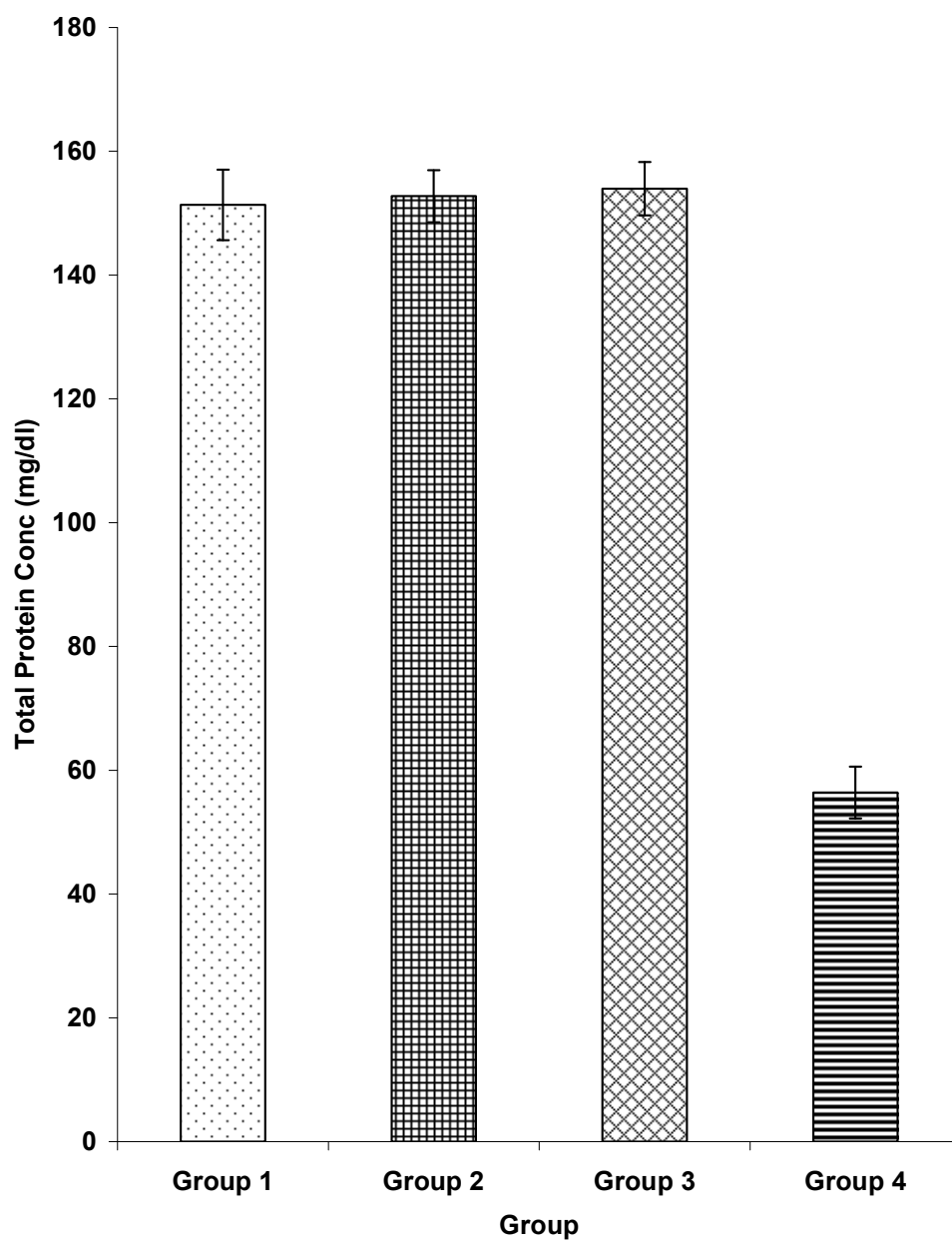


Fig. 3: Urine total protein concentration (mean $\pm$ SD) of alcoholic and control subjects

Group 1=Alcoholics who showed preference for Palmwine
Group 2=Alcoholics who showed preference for Beer
Group 3=Alcoholics who showed preference for Distilled Spirit
Group 4=Control (Non-Consumers of Alcohol)

($p < 0.05$)

3.2 Urine Specific Gravity in Human Subjects

The results show that the urine specific gravity in the control was 1.021 ± 0.001 and 1.036 ± 0.004 ; 1.037 ± 0.004 ; 1.039 ± 0.001 in the alcoholic subjects who showed preference for palmwine, beer and distilled spirit respectively. The urine specific gravity of the alcoholics was significantly ($p < 0.05$) higher compared with the urine specific gravity of subjects in the control group. Comparing the different alcoholic groups, the highest ($p < 0.05$) urine specific gravity was got in alcoholics who showed preference for distilled spirit followed by those who showed preference for beer. Alcoholics who showed preference for palmwine had the lowest urine specific gravity. Fig. 4 shows the urine specific gravity of the three groups of alcoholics who showed preference for palmwine, beer and distilled spirit together with non-consumers of alcohol that served as control. The urine specific gravity of each of the three groups of alcoholics who showed preference for palmwine, beer and distilled spirit was significantly higher ($p < 0.05$) compared with the urine total protein concentration of the control group.

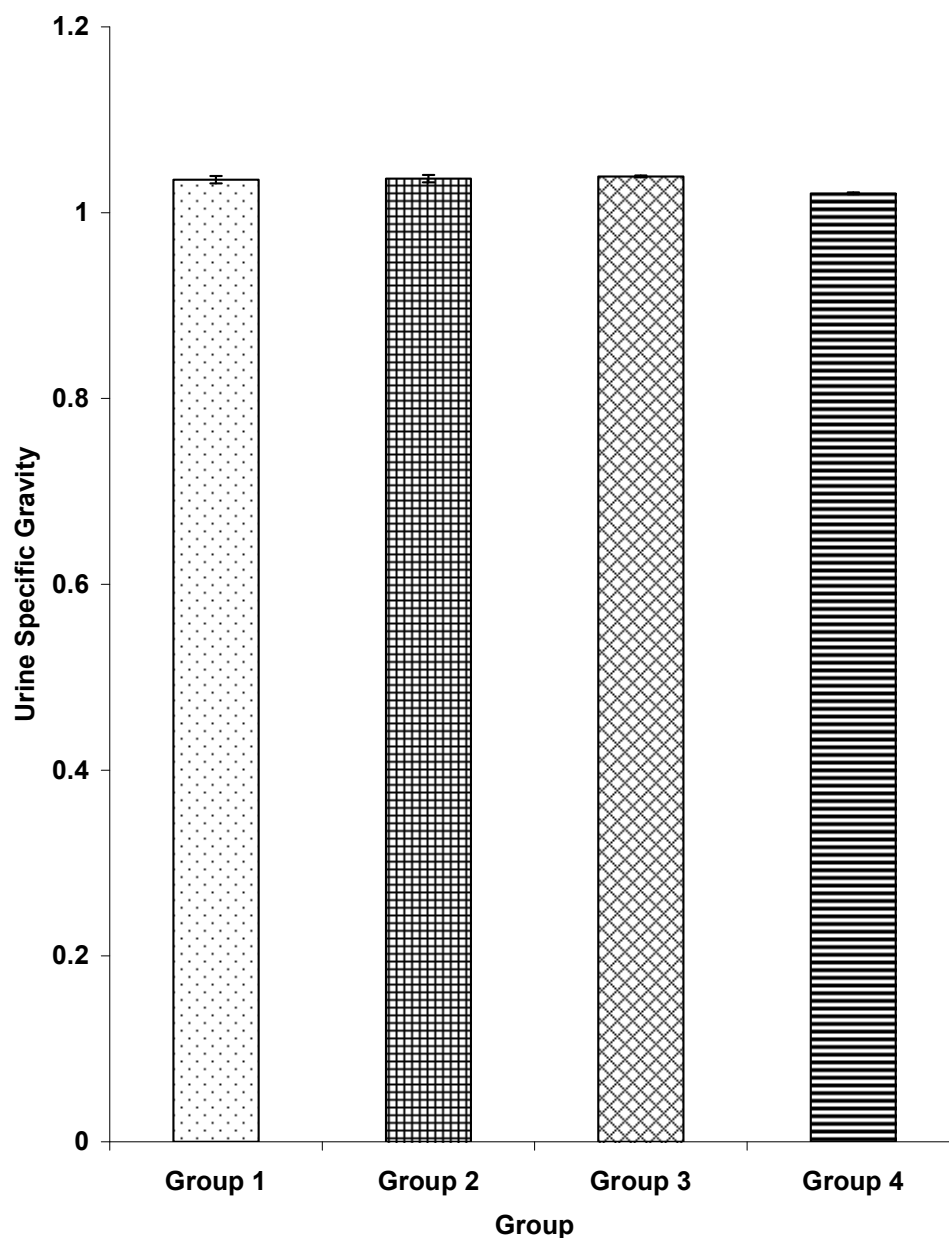


Fig. 4: Urine specific gravity of the control and alcoholic subjects

Group 1=Alcoholics who showed preference for Palmwine
Group 2=Alcoholics who showed preference for Beer
Group 3=Alcoholics who showed preference for Distilled Spirit
Group 4=Control (Non-Consumers of Alcohol)

($p < 0.05$)

3.3 Serum Urea Concentration in Human Subjects

The results of the serum urea concentrations of the different groups of alcoholics who showed preference for palmwine, beer and distilled spirit together with the control are shown in Fig. 5. The serum urea concentrations of the alcoholics were significantly ($p < 0.05$) higher compared with the serum urea concentrations of the control subjects. The serum urea concentration was 18.88 ± 0.31 mg/dl in teetotaler (control) and 57.30 ± 2.80 mg/dl; 62.49 ± 1.50 mg/dl; 64.00 ± 1.70 mg/dl in alcoholics who showed preference for palmwine, beer and distilled spirit respectively. From the results, the alcoholic subjects who showed preference for distilled spirit had the highest serum urea concentration ($p < 0.05$) followed by the alcoholics who showed preference for beer and palmwine.

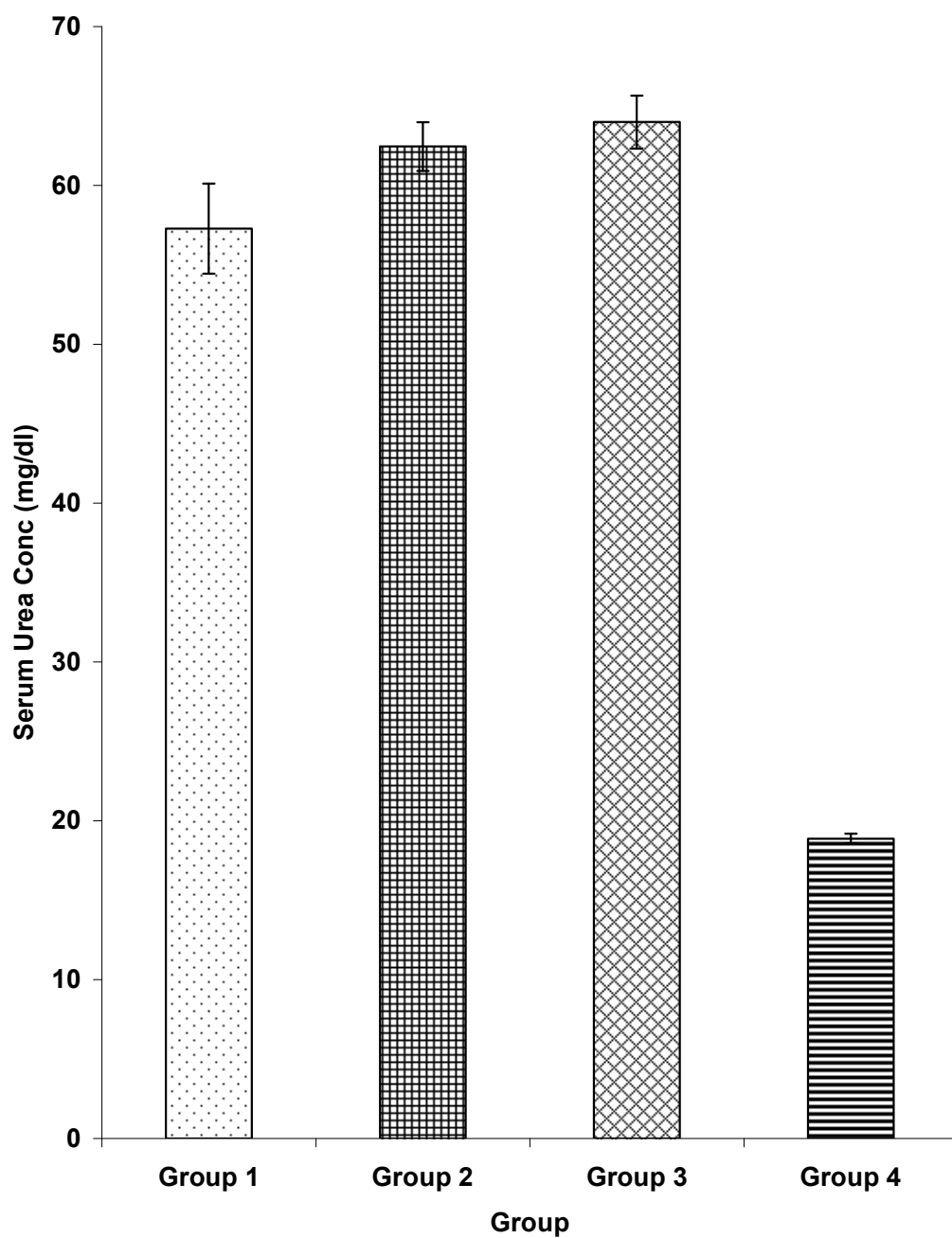


Fig. 5: Serum urea concentration (mean $\pm$ SD) of control and alcoholic subjects

Group 1=Alcoholics who showed preference for Palmwine

Group 2=Alcoholics who showed preference for Beer

Group 3=Alcoholics who showed preference for Distilled Spirit

Group 4=Control (Non-Consumers of Alcohol)

(p < 0.05)

3.4 Serum Creatinine Concentration in Human Subjects

The serum creatinine concentration of the alcoholics was significantly ($p < 0.05$) higher in the alcoholics compared with non-consumers of alcohol that served as controls (Fig. 6). The serum creatinine concentrations of alcoholics who showed preference for distilled spirit was the highest ($p < 0.05$) followed by alcoholics who showed preference for beer ($p < 0.05$). The serum creatinine concentration of the control was 0.86 ± 0.04 mg/dl and 3.14 ± 0.21 mg/dl; 3.46 ± 0.29 mg/dl; 3.71 ± 0.17 mg/dl in the alcoholic subjects who showed preference for palmwine, beer and distilled spirit respectively.

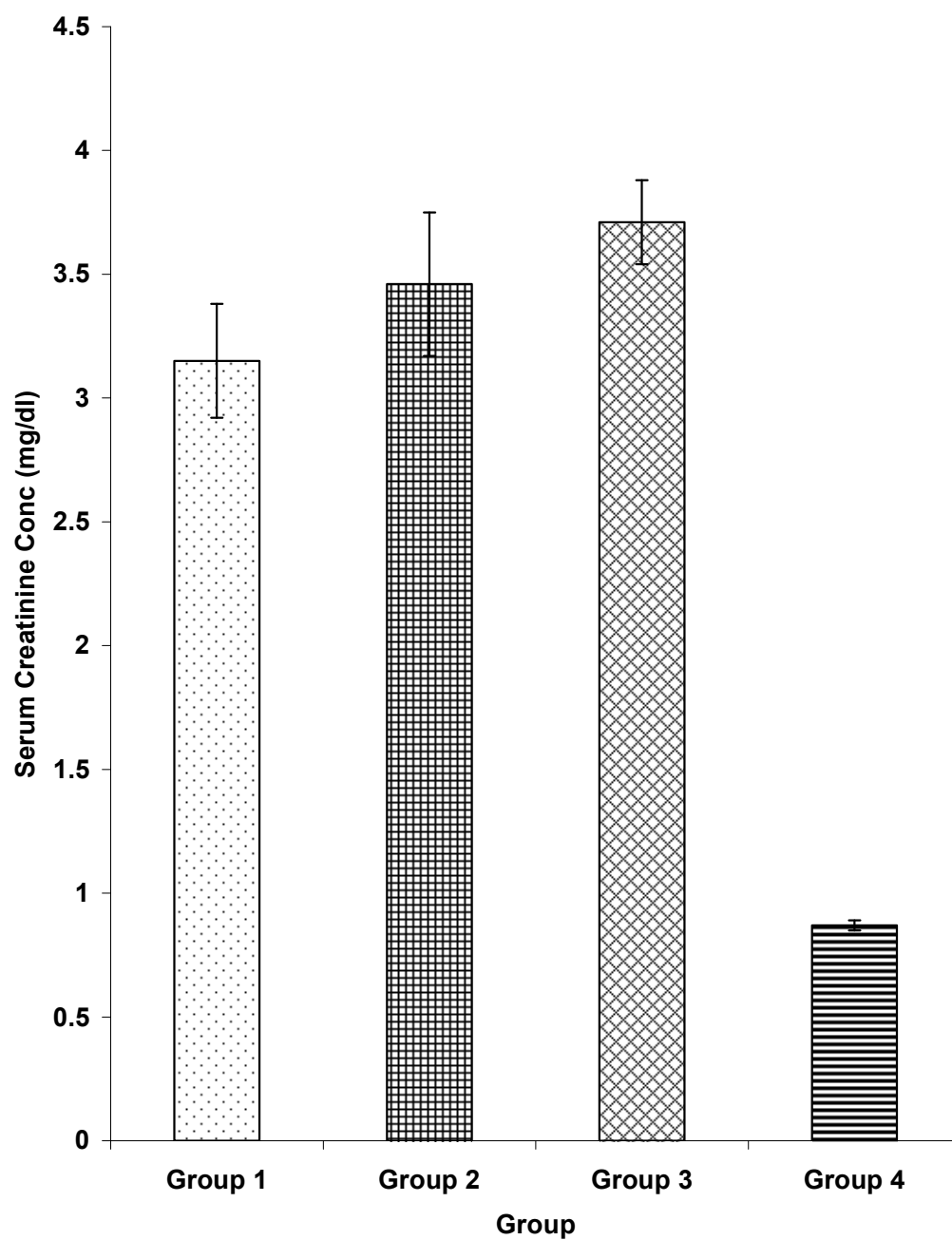


Fig. 6: Serum creatinine concentration (mean $\pm$ SD) of control and alcoholic subjects

Group 1=Alcoholics who showed preference for Palmwine

Group 2=Alcoholics who showed preference for Beer

Group 3=Alcoholics who showed preference for Distilled Spirit

Group 4=Control (Non-Consumers of Alcohol)

($p < 0.05$)

3.5 Urine Creatinine Concentration in Human Subjects

The concentrations of urine creatinine of the three groups of alcoholics who showed preference for palmwine, beer and distilled spirit together with the control are shown in Fig 7. The urine creatinine concentration of the alcoholics was significantly lower ($p < 0.05$) compared with the urine concentrations of subjects in the control group. The urine creatinine concentration in alcoholics who showed preference for distilled spirit was the lowest ($p < 0.05$) compared with the alcoholics who showed preference for beer and palmwine respectively. Furthermore, the concentration of urine creatinine was 13.17 ± 1.28 mg/dl in the control and 85.94 ± 5.34 mg/dl; 83.59 ± 14.24 mg/dl; 80.44 ± 14.20 mg/dl in the alcoholic subjects who showed preference for palmwine, beer and distilled spirit respectively.

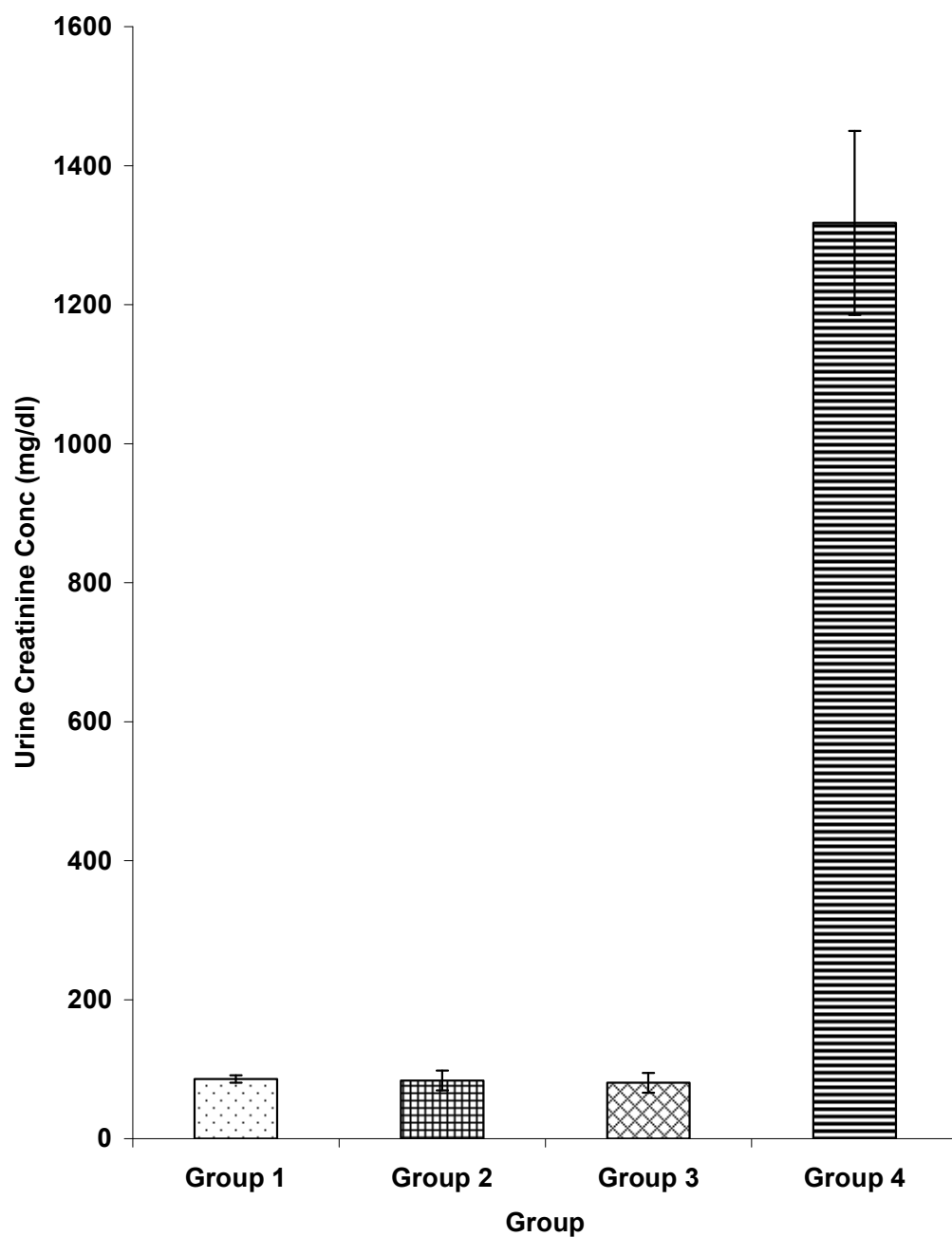


Fig. 7: Urine creatinine concentration (means $\pm$ SD) of control and alcoholic subjects

Group 1=Alcoholics who showed preference for Palmwine

Group 2=Alcoholics who showed preference for Beer

Group 3=Alcoholics who showed preference for Distilled Spirit

Group 4=Control (Non-Consumers of Alcohol)

($p < 0.05$)

3.6 Creatinine Clearance in Human Subjects

Fig. 8 shows the creatinine clearance of the three groups of alcoholics who showed preference for palmwine, beer and distilled spirit together with non-consumers of alcohol that served as controls. The creatinine clearance of the alcoholics was significantly lower ($p < 0.05$) compared with non-consumers of alcohol (controls). Alcoholics who showed preference for distilled spirit with clearance of 31.28 ± 3.80 ml/min showed the lowest creatinine clearance ($p < 0.05$). This was followed by alcoholics who showed preference for beer whose creatinine clearance was 37.46 ± 1.50 ml/min and then alcoholics who showed preference for palmwine whose creatinine clearance was 44.23 ± 4.10 ml/min. However, the creatinine clearance of the control group was 129.23 ± 0.66 ml/min. The creatinine clearance of each of the three groups of alcoholics who showed preference for palmwine, beer and distilled spirit was significantly lower ($p < 0.05$) compared with the control group.

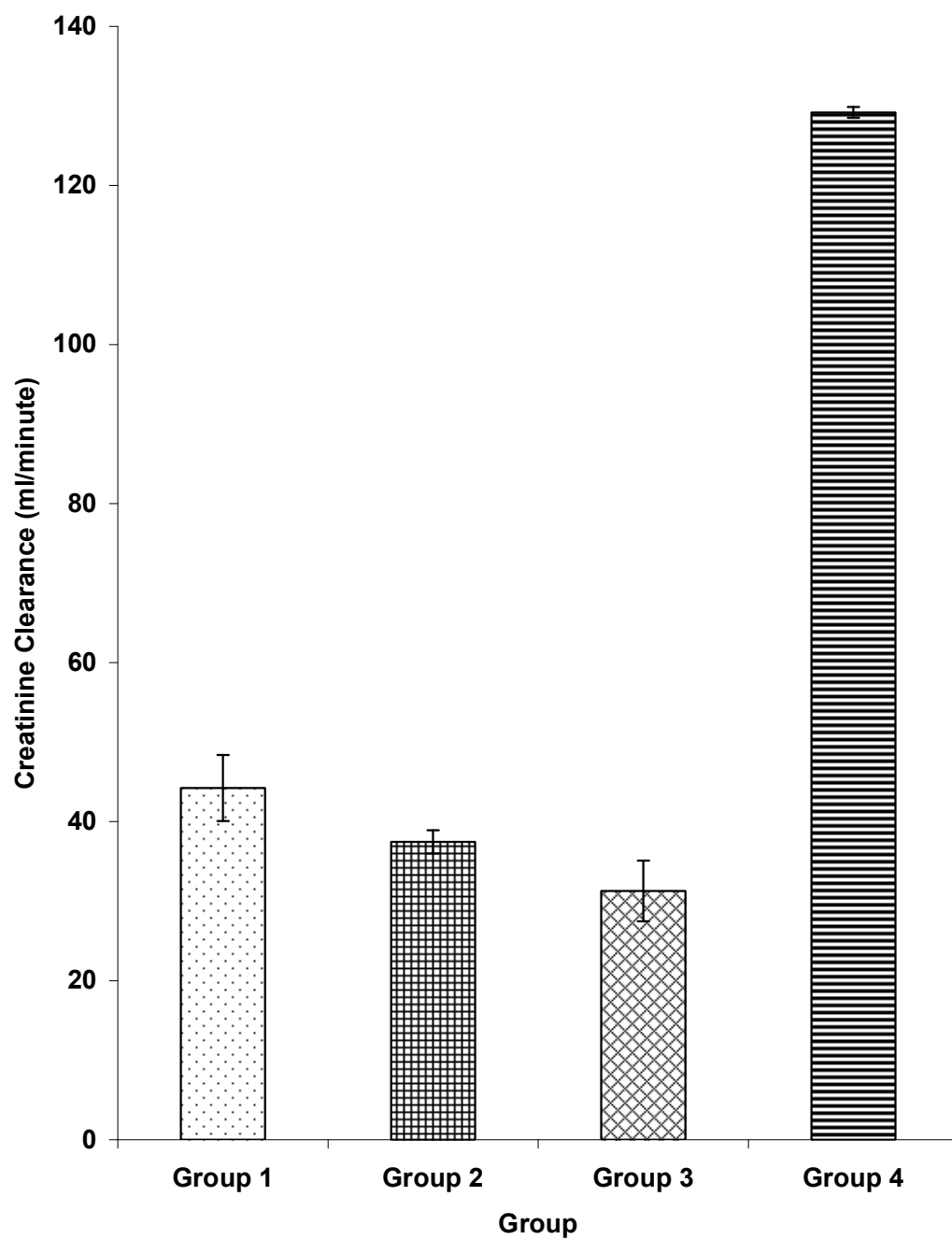


Fig. 8: Creatinine clearance (mean $\pm$ SD) of control and alcoholic subjects

Group 1=Alcoholics who showed preference for Palmwine
Group 2=Alcoholics who showed preference for Beer
Group 3=Alcoholics who showed preference for Distilled Spirit
Group 4=Control (Non-Consumers of Alcohol)

($p < 0.05$)

3.7 Serum Potassium Ion Concentration in Human Subjects

The concentration of serum potassium of the alcoholics was significantly lower ($p < 0.05$) compared with the serum potassium ion concentration of control subjects. The plasma potassium ion concentration was 4.20 ± 0.30 mEq/L in the control and 3.30 ± 0.10 mEq/L; 3.10 ± 0.20 mEq/L and 2.00 ± 0.10 mEq/L in alcoholics who showed preference for palmwine, beer and distilled spirit respectively. The lowest potassium ion concentration ($p < 0.05$) was observed in alcoholics who showed preference for distilled spirit. This was followed by alcoholics who showed preference for beer ($p < 0.05$).

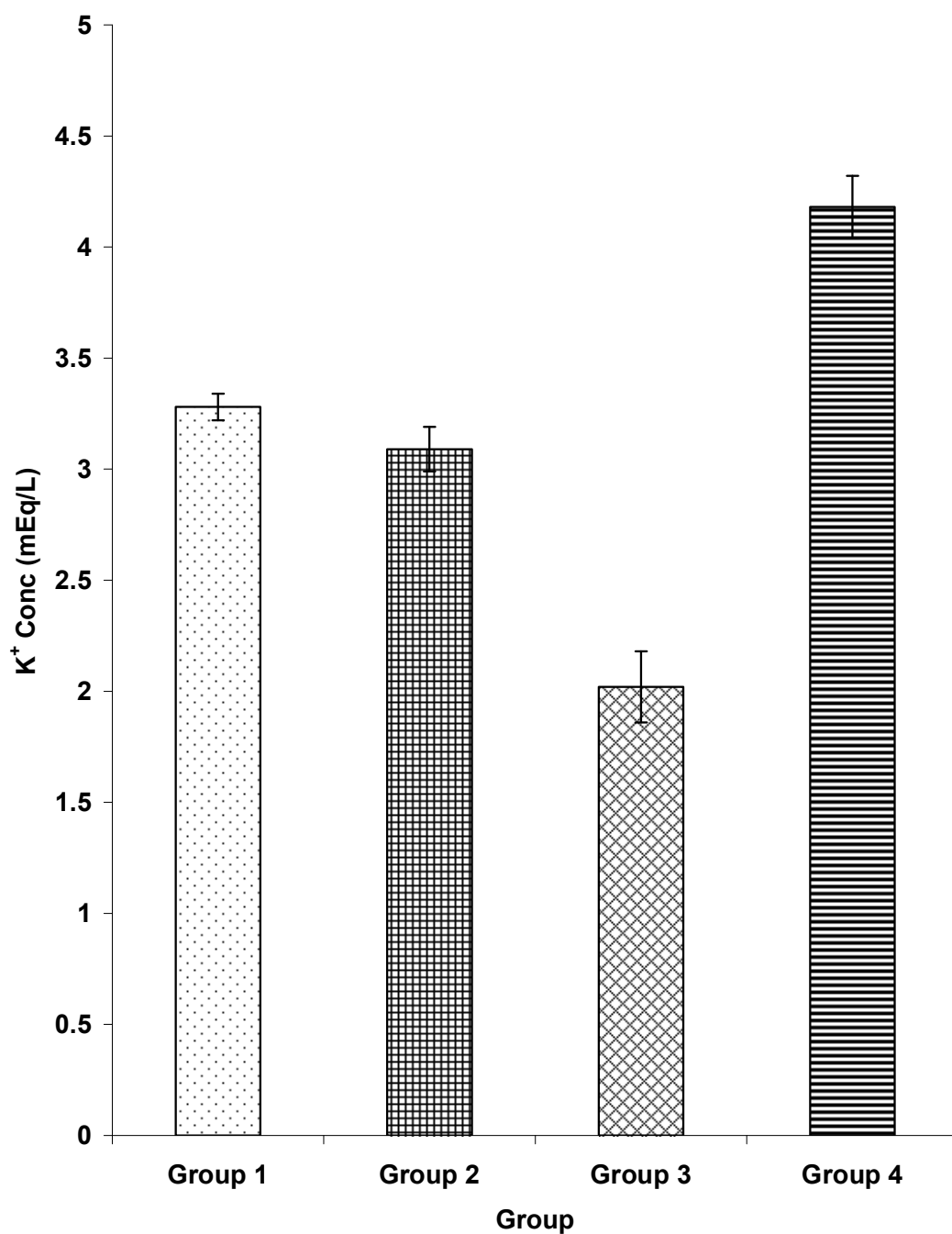


Fig. 7: Potassium ion concentration (means $\pm$ SD) of control and alcoholic subjects

Group 1=Alcoholics who showed preference for Palmwine
Group 2=Alcoholics who showed preference for Beer
Group 3=Alcoholics who showed preference for Distilled Spirit
Group 4=Control (Non-Consumers of Alcohol)

(p < 0.05)

3.8 Serum Sodium Concentration in Human Subjects

Fig. 10 shows the serum sodium concentrations of the alcoholics who showed preference for palmwine, beer and distilled spirit together with non consumers of alcohol that served as controls. The serum sodium concentrations of the alcoholics in the three test groups were significantly lower ($p < 0.05$) compared with the control. The serum sodium concentration was 137.00 ± 2.00 mEq/L in the control and 131.00 ± 1.00 mEq/L; 129.00 ± 1.50 mEq/L; 118.00 ± 2.00 mEq/L in alcoholics who showed preference for palmwine, beer and distilled spirit respectively. Alcoholics who showed preference for distilled spirit had the lowest level of reduction in serum sodium concentration. This was followed by alcoholics who showed preference for beer and palmwine ($p < 0.05$).

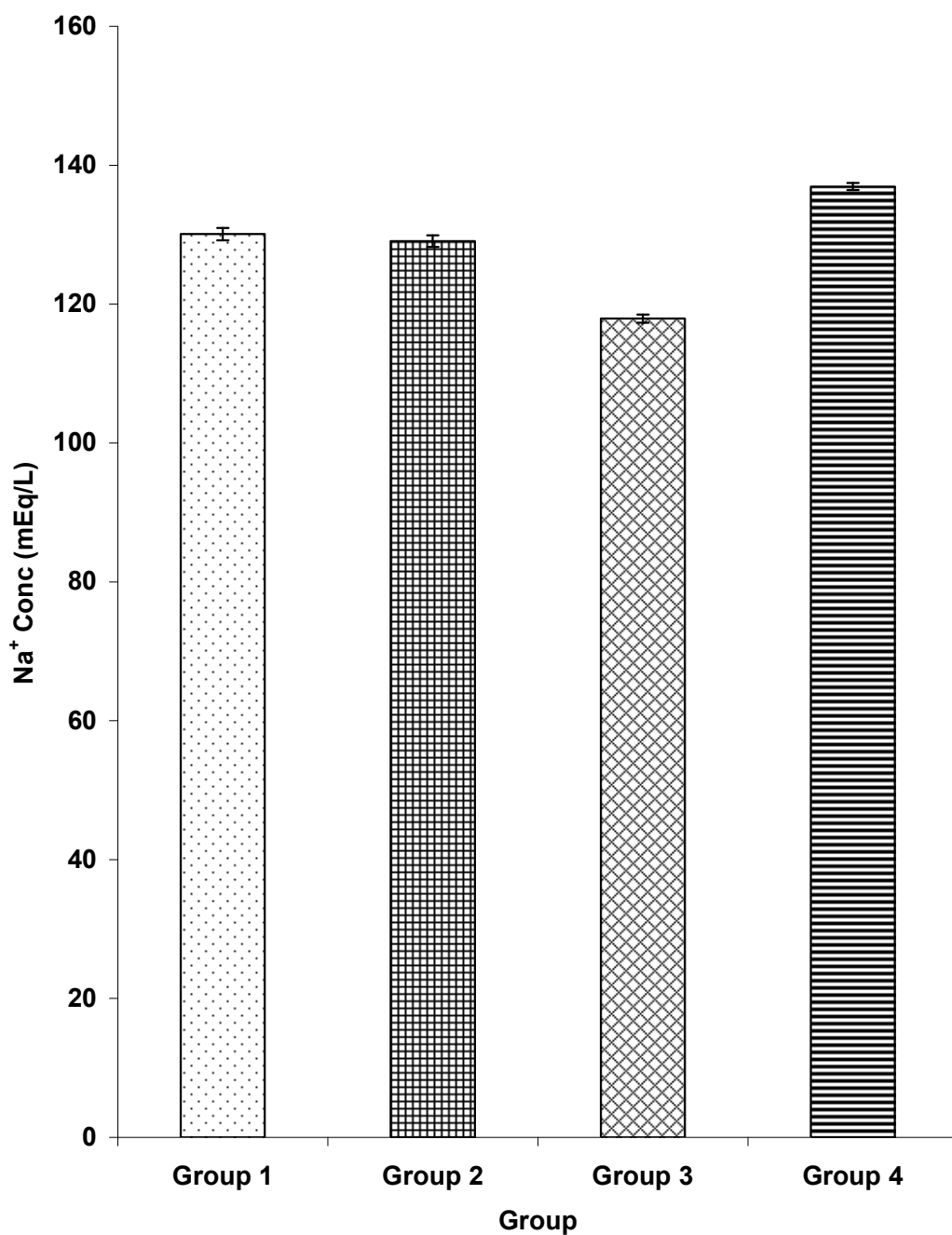


Fig. 10: Sodium ion concentration (means $\pm$ SD) of control and alcoholic subjects

Group 1=Alcoholics who showed preference for Palmwine

Group 2=Alcoholics who showed preference for Beer

Group 3=Alcoholics who showed preference for Distilled Spirit

Group 4=Control (Non-Consumers of Alcohol)

($p < 0.05$)

3.9 Serum Total Bilirubin Concentration in Human Subjects

The concentrations of serum total bilirubin of the three groups of alcoholics who showed preference for palmwine, beer and distilled spirit respectively along side that of non-consumers of alcohol who served as controls are shown in Fig. 11. The serum total bilirubin concentration was $8.30 \pm 2.00 \text{ } \mu\text{mol/l}$ in the controls and $18.65 \pm 1.50 \text{ } \mu\text{mol/l}$; $19.40 \pm 1.50 \text{ } \mu\text{mol/l}$ and $22.75 \pm 1.60 \text{ } \mu\text{mol/l}$ in the alcoholics who showed preference for palmwine, beer and distilled spirit respectively. The serum total bilirubin concentrations of the alcoholics was significantly higher ($p < 0.05$) compared with the serum total bilirubin concentrations of subjects in the control group. Alcoholics who showed preference for distilled spirit had the highest ($p < 0.05$) concentrations of serum total bilirubin followed closely by alcoholics who showed preference for beer. Alcoholics who showed preference for palmwine had the least serum total bilirubin concentrations ($p < 0.05$).

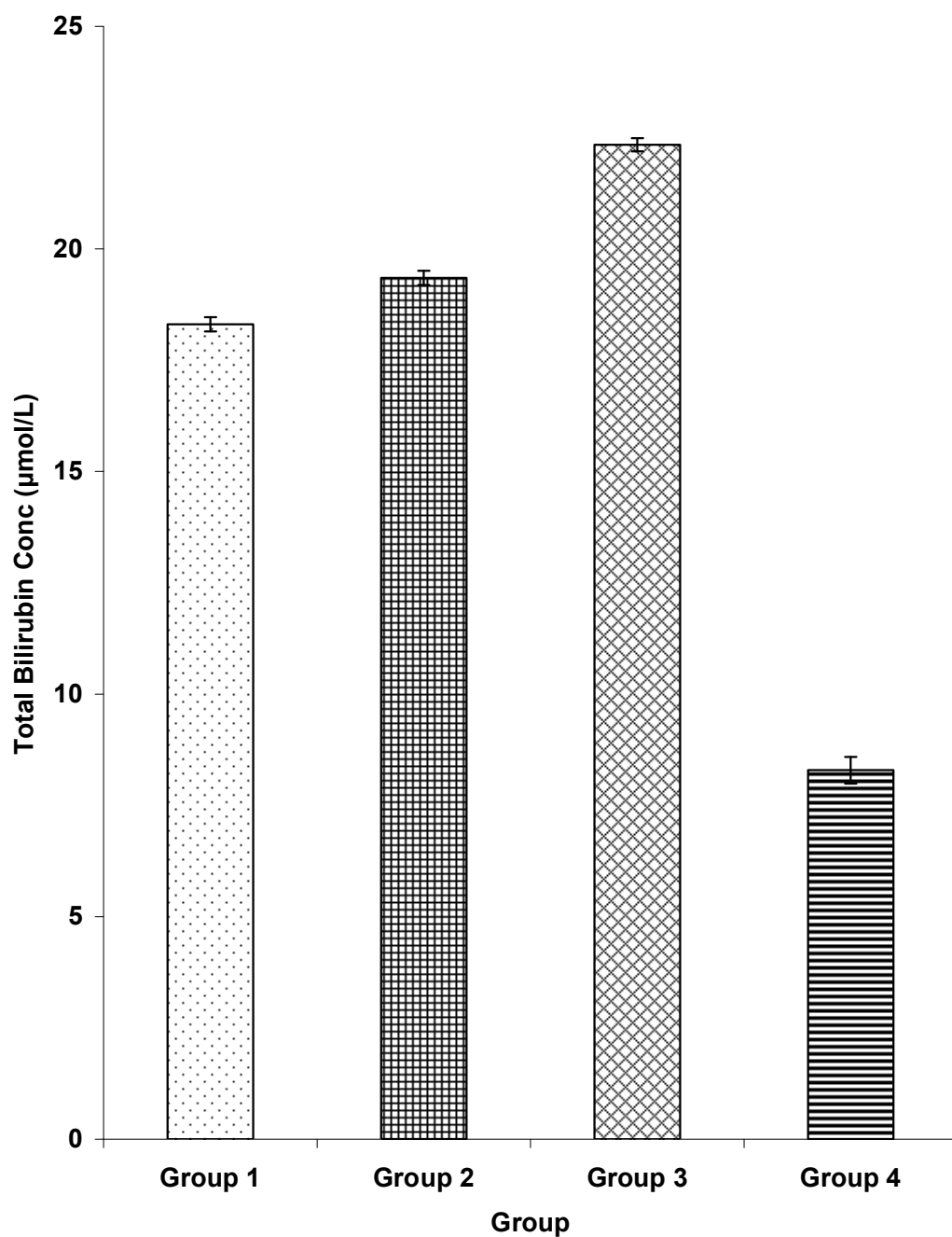


Fig. 11: Serum total bilirubin concentration (means $\pm$ SD) of control and alcoholic subjects

Group 1=Alcoholics who showed preference for Palmwine
Group 2=Alcoholics who showed preference for Beer
Group 3=Alcoholics who showed preference for Distilled Spirit
Group 4=Control (Non-Consumers of Alcohol)

($p < 0.05$)

3.10 Serum Alkaline Phosphatase Activity in Human Subjects

There was significantly higher ($p < 0.05$) activities in alkaline phosphatase of the alcoholics compared with the alkaline phosphatase activities of the subjects in control groups. The alkaline phosphatase activities of the control subjects was 61.10 ± 1.90 IU/L. The value was 173.80 ± 11.00 IU/L in alcoholics who showed preference for palmwine; 175.00 ± 10.30 IU/L in alcoholics who showed preference for beer and 177.00 ± 11.00 IU/L in alcoholics who showed preference for distilled spirit ($p < 0.05$). Comparatively, the highest activity of alkaline phosphatase was observed in alcoholics who showed preference for distilled spirit. This was followed by alcoholics who showed preference for beer and then palmwine ($p < 0.05$).

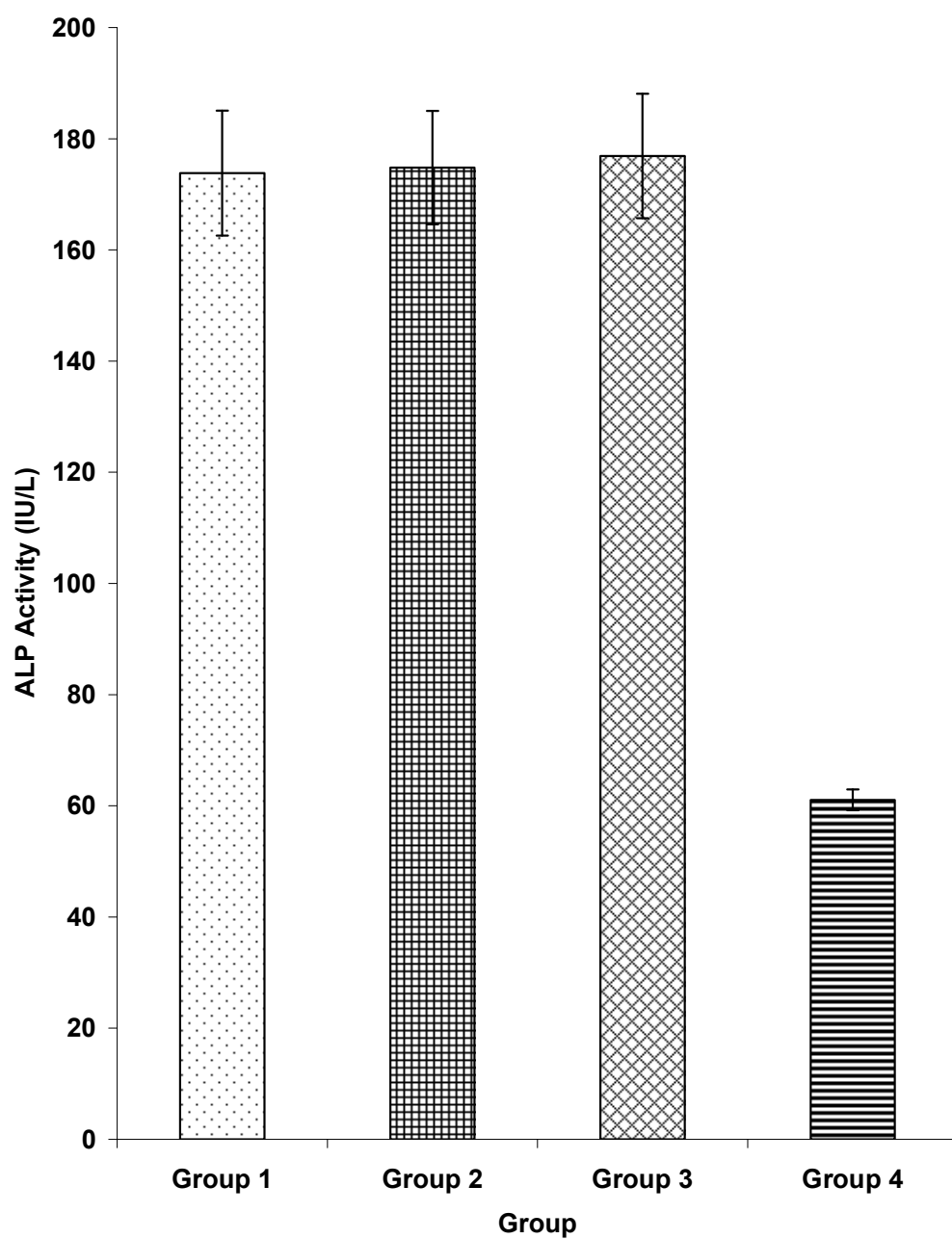


Fig. 10: Alkaline phosphatase activity (means $\pm$ SD) of control and alcoholic subjects

Group 1=Alcoholics who showed preference for Palmwine

Group 2=Alcoholics who showed preference for Beer

Group 3=Alcoholics who showed preference for Distilled Spirit

Group 4=Control (Non-Consumers of Alcohol)

($p < 0.05$)

3.11 Serum Aspartate Aminotransferase Activity in Human Subjects

Fig. 13 depicts the aspartate aminotransferase (AST) activity of alcoholics who showed preference for palmwine, beer and distilled spirit. The AST activity of non-consumers of alcohol that served as control are also shown. The activities of AST was 9.51 ± 0.35 IU/L in the control and 18.44 ± 0.40 IU/L, 19.21 ± 0.19 IU/L; 20.32 ± 0.64 IU/L in alcoholics who showed preference for palmwine, beer and distilled spirit respectively. The AST activity of the alcoholics was significantly higher ($p < 0.05$) compared with the AST activities of control subjects. Alcoholics who showed preference for distilled spirit had the highest in activity ($p < 0.05$) compared with the AST activities of alcoholics who showed preference for palmwine or beer. Again, there was a higher AST activity in the alcoholics who showed preference for beer than in the alcoholics who showed preference for palmwine ($p < 0.05$).

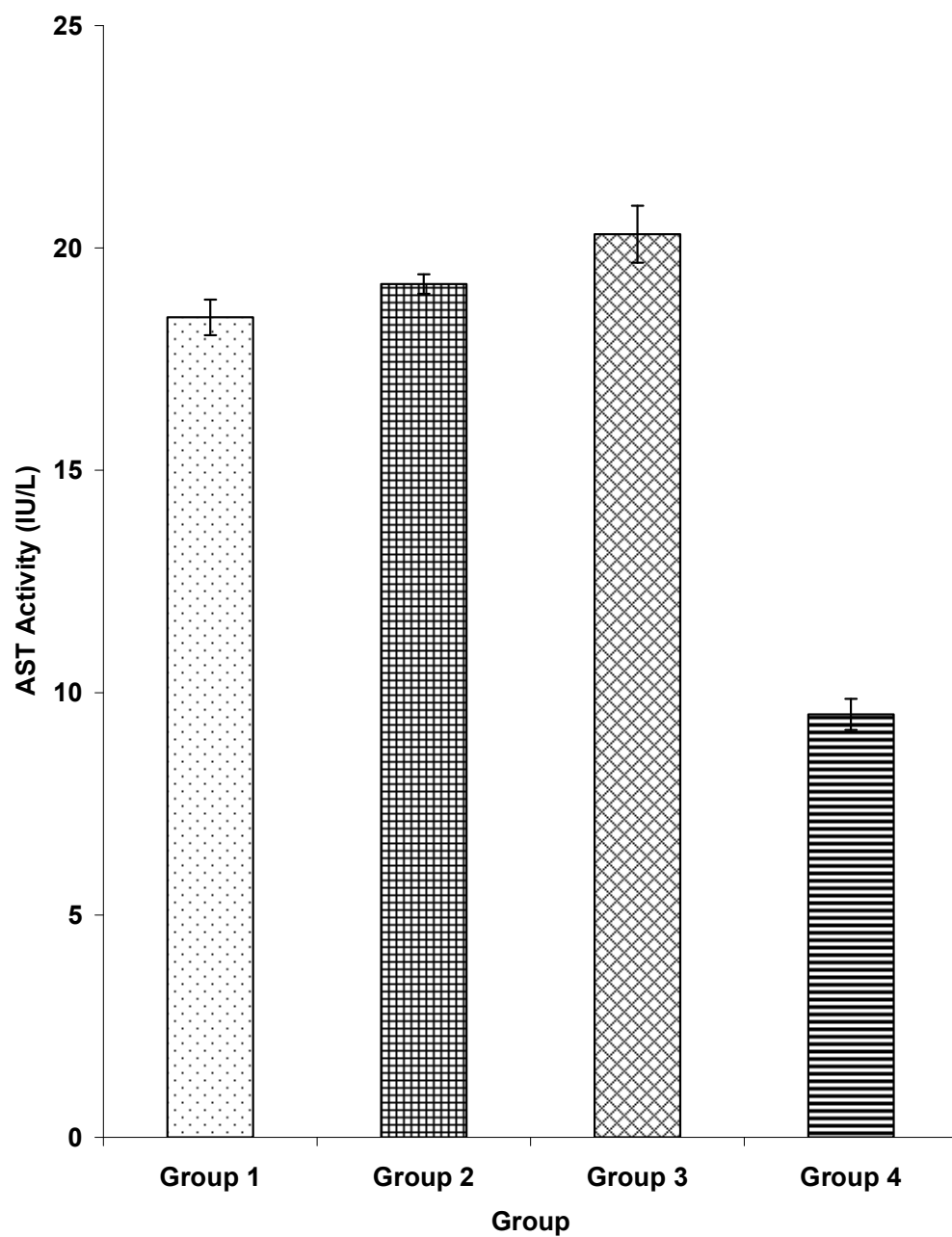


Fig. 12: Aspartate aminotransferase activity (means $\pm$ SD) of control and alcoholic subjects

Group 1=Alcoholics who showed preference for Palmwine
 Group 2=Alcoholics who showed preference for Beer
 Group 3=Alcoholics who showed preference for Distilled Spirit
 Group 4=Control (Non-Consumers of Alcohol)

($p < 0.05$)

3.12 Serum Alanine Aminotransferase Activity in Human Subjects

The activities of the alanine aminotransferase (ALT) of the alcoholics were significantly ($p < 0.05$) higher compared with the alanine aminotransferase activities of the subjects in the control group. The result shows that the ALT activity of the control was 10.50 ± 2.00 IU/L. This value was 16.50 ± 1.50 IU/L in alcoholics who showed preference to palmwine; 17.50 ± 1.00 IU/L in alcoholics who showed preference for beer and 18.31 ± 2.00 IU/L in alcoholics who showed preference for distilled spirit. The highest activity of the ALT ($p < 0.05$) was observed in alcoholics who showed preference for distilled spirit which was closely followed by the group of alcoholics who showed preference for beer and then palmwine ($p < 0.05$).

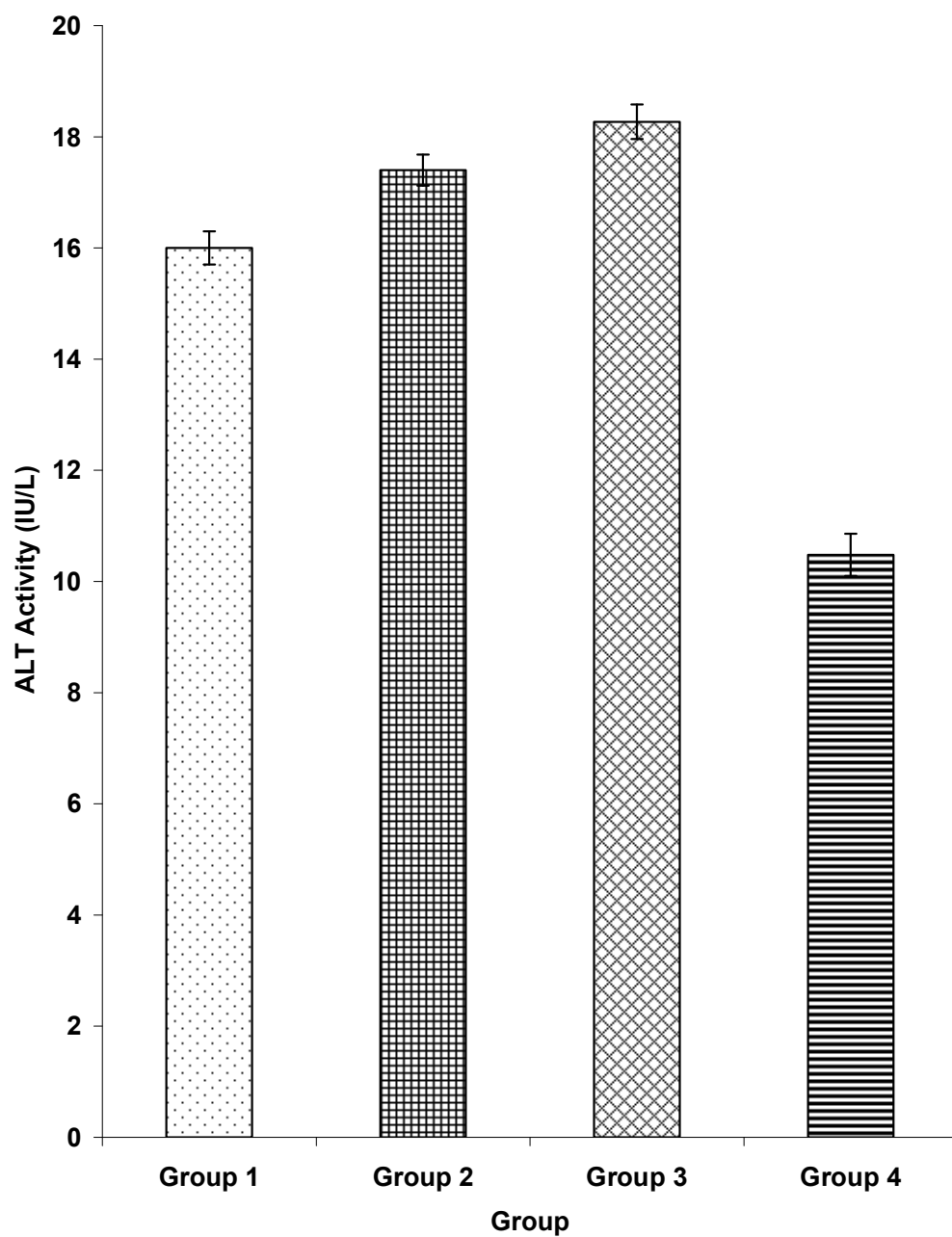


Fig. 11: Alanine aminotransferase activity (means $\pm$ SD) of control and alcoholic subjects

Group 1=Alcoholics who showed preference for Palmwine
 Group 2=Alcoholics who showed preference for Beer
 Group 3=Alcoholics who showed preference for Distilled Spirit
 Group 4=Control (Non-Consumers of Alcohol)

($p < 0.05$)

3.13 Gamma Glutamyltransferase (γ -GT) Activities in Human Subjects

Shown in Fig. 15 are the activities of the alcoholic subjects who exhibited preference for palmwine, beer and palmwine. The activity of gamma glutamyltransferase (γ -GT) of the control was also depicted. The activities of γ -GT of the subjects was 20.50 ± 0.30 IU/L in the controls and 53.00 ± 0.40 IU/L; 55.50 ± 0.30 IU/L and 58.00 ± 0.50 IU/L in alcoholics who showed preference to palmwine, beer and distilled spirit respectively. The γ -GT activities of the alcoholics was significantly higher ($p < 0.05$) compared with the γ -GT activities of the control subjects. Alcoholics who showed preference for distilled spirit had the highest activities ($p < 0.05$) compared with the γ -GT activities of alcoholics who showed preference for palmwine or beer. Furthermore, there were significantly higher ($p < 0.05$) γ -GT activities in the alcoholics who showed preference for beer than in alcoholics who showed preference for palmwine.

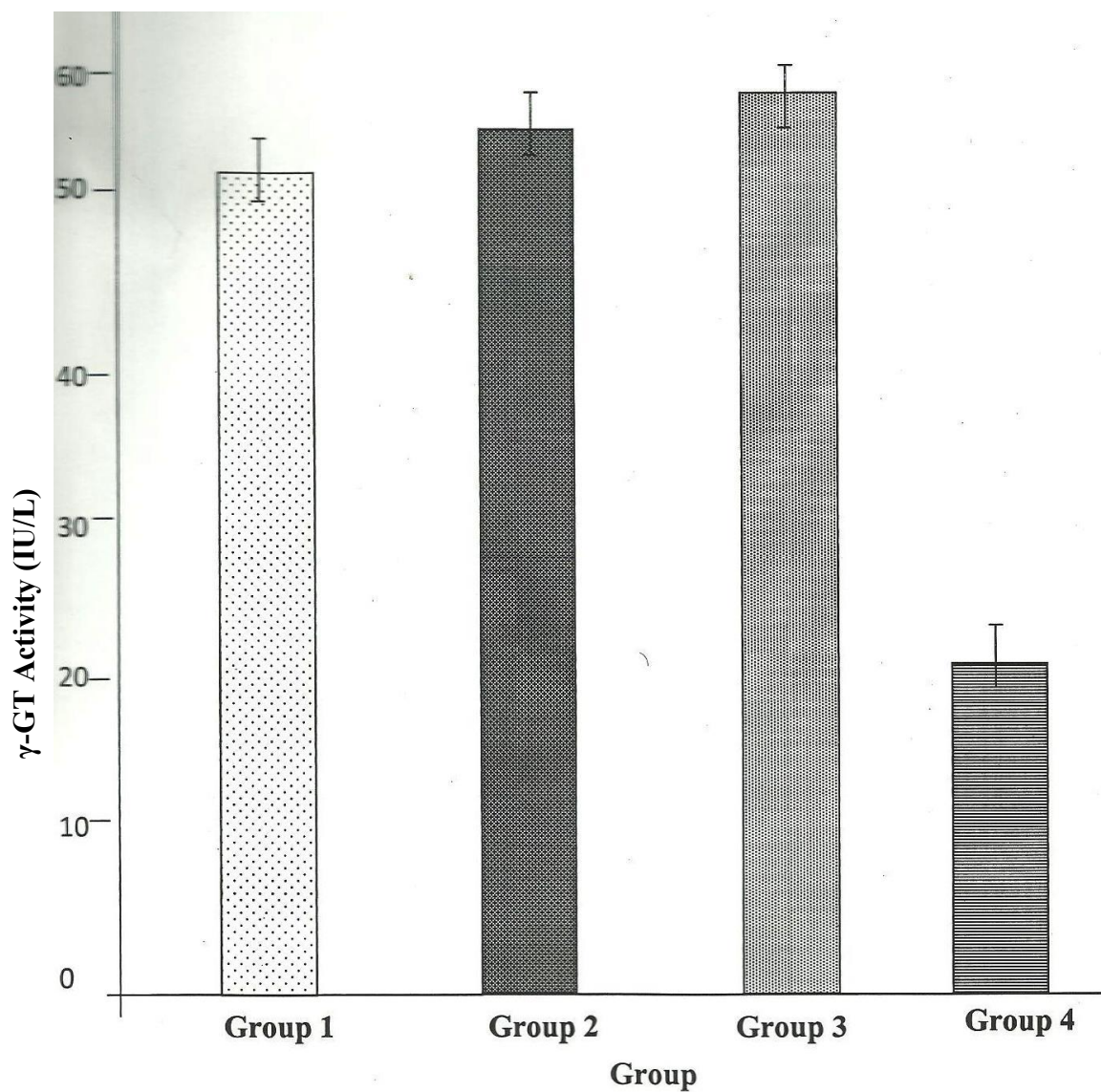


Fig. 15: Gamma glutamyltransferase activity (means $\pm$ SD) of control and alcoholic subjects

Group 1=Alcoholics who showed preference for Palmwine
Group 2=Alcoholics who showed preference for Beer
Group 3=Alcoholics who showed preference for Distilled Spirit
Group 4=Control (Non-Consumers of Alcohol)

($p < 0.05$)

3.14 Body Mass Index in Human Subjects

The body mass index (BMI) of the three groups of alcoholics who showed preference for palmwine, beer and distilled spirit together with non consumers of alcohols that served as controls are shown in Fig. 16. The BMI of the different categories of alcoholics was 24.00 ± 2.00 Kg/m² in the control and 23.00 ± 1.50 Kg/m²; 21.00 ± 2.00 Kg/m²; 20.00 ± 2.00 Kg/m² in alcoholics who showed preference for palmwine, beer and distilled spirit respectively. The BMI in the alcoholics was significantly lower ($p < 0.05$) compared with the control. The lowest BMI ($p < 0.05$) was got in alcoholics who showed preference for distilled spirit. Furthermore, the BMI of the alcoholic subjects who showed preference for beer was significantly lower ($p < 0.05$) compared with alcoholics who showed preference for palmwine. Also, the BMI value of alcoholics who showed preference for beer (group 2) was significantly higher ($p < 0.05$) compared with the BMI of alcoholics who showed preference for distilled spirit (group 3).

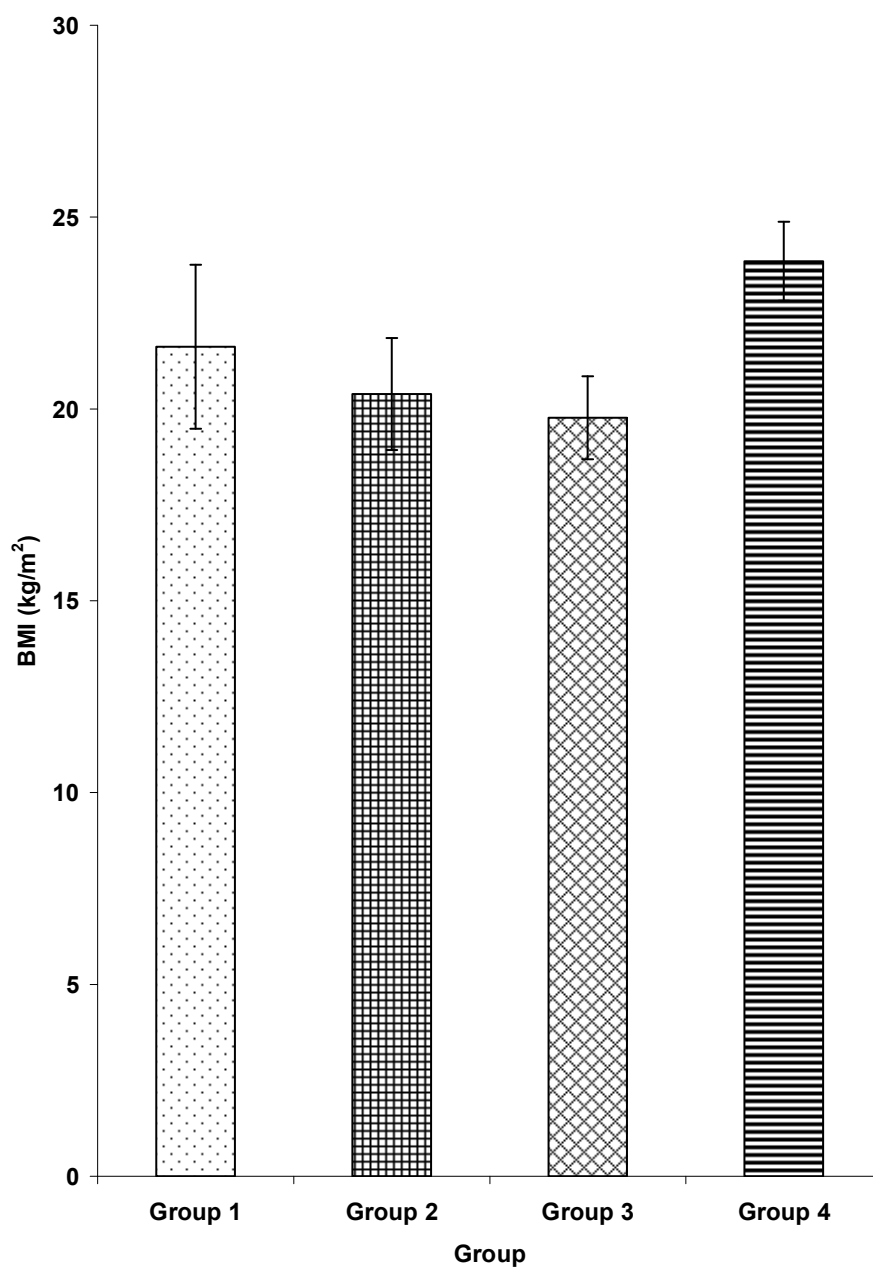


Fig. 13: Body mass index (BMI) (means $\pm$ SD) of control and alcoholic subjects

Group 1=Alcoholics who showed preference for Palmwine
 Group 2=Alcoholics who showed preference for Beer
 Group 3=Alcoholics who showed preference for Distilled Spirit
 Group 4=Control (Non-Consumers of Alcohol)

($p < 0.05$)

3.15 Blood Pressure in Human Subjects

The blood pressure of the different groups of alcoholics who showed preference for palmwine, beer distilled spirit together with non consumers of alcohol who served as controls are shown in Fig 17. The ratio of the systolic and diastolic blood pressures of the alcoholics were $124.00/83.00 \pm 4.00$, $123.00/82.00 \pm 3.00$ and $125.00/82.00 \pm 5.00$ mmHg for alcoholics who showed preference for palmwine, beer and distilled spirit respectively. Also, the ratio of the systolic and diastolic blood pressure for the control was $122.00/80.00 \pm 3.00$ mmHg. The change in blood pressure in the alcoholics was not significant ($p > 0.05$) compared with the control.

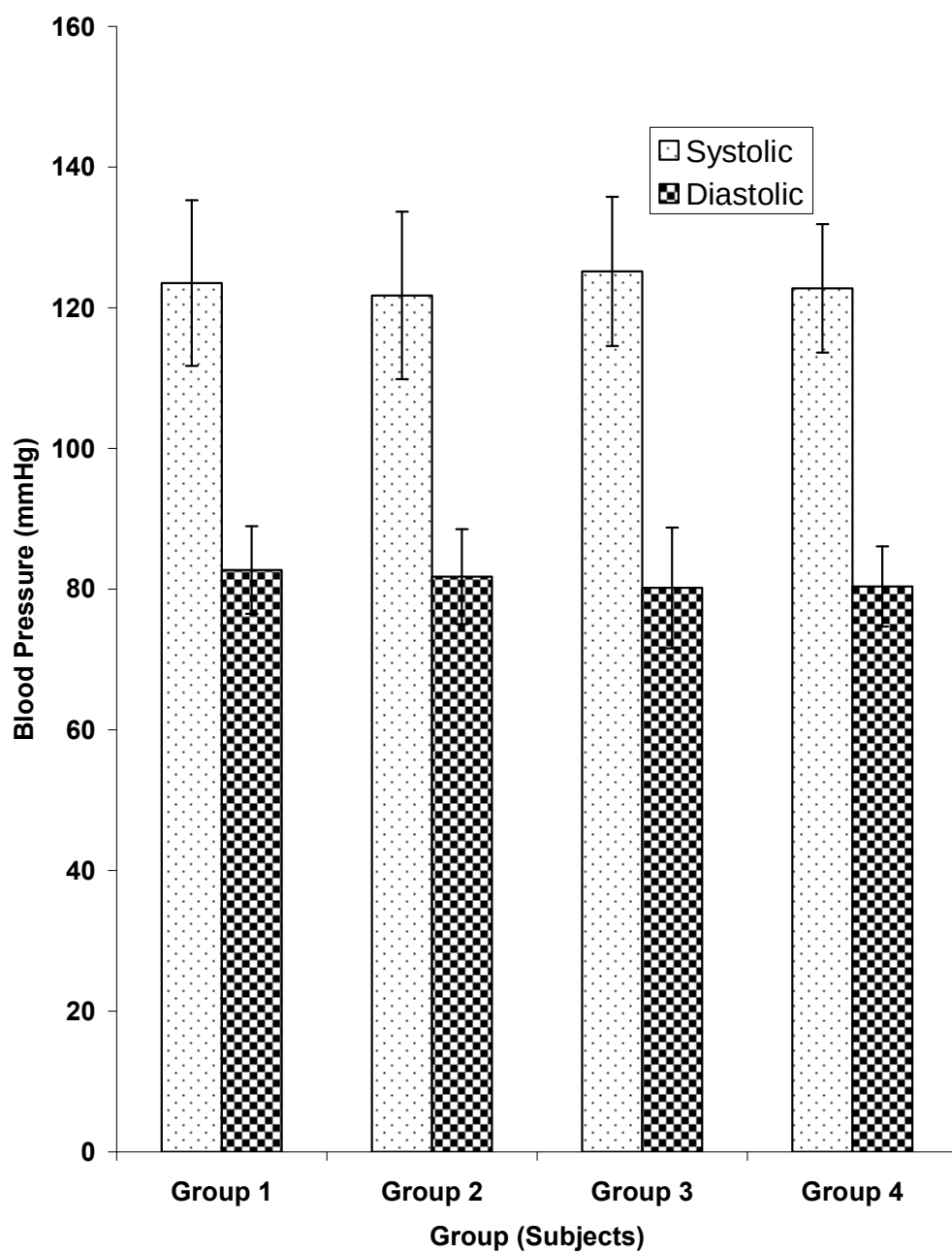


Fig. 12: Blood pressure (systolic and diastolic) (means $\pm$ SD) of control and alcoholic subjects

Group 1=Alcoholics who showed preference for Palmwine
 Group 2=Alcoholics who showed preference for Beer
 Group 3=Alcoholics who showed preference for Distilled Spirit
 Group 4=Control (Non-Consumers of Alcohol)

($p > 0.05$)

CHAPTER FOUR

DISCUSSION

The findings of this study show that frequent intake of alcohol in large quantities has deleterious effects on the liver and the kidney. These findings are in tandem with the report of Carey (2003) who reported that both quantity of alcohol consumed and pattern of drinking profoundly affect the functions of several organs in the body particularly the liver and kidney. Furthermore, another finding of this study shows that the type of alcohol consumed determines the level of damage unleashed on such vital organs such as the liver and the kidney. Alcohol in form of distilled spirit caused the severest damage to the liver and the kidney which was followed by beer and palmwine.

In this study, chronic intake of large quantities of alcohol in form of palmwine, beer or distilled spirit by the alcoholics adversely affected the functioning of their livers and kidneys. The alcohol-induced hepatic and renal dysfunctions manifest in form of significant variations in many biochemical parameters of immense prognostic and diagnostic utility.

Principally, the kidneys are responsible for the excretion of waste products of metabolism which is achieved through glomerular filtration. The unique structure of the glomerular capillary walls enables them to behave like high capacity ultrafiltration membranes which allows a high rate of fluid filtration without significant filtration of larger plasma proteins. The findings of this study showed significant elevation in the concentration of urine total protein in the alcoholics compared with non drinkers. This finding is in agreement with previous reports which showed that alcohol abuse remarkably increased urine total protein concentrations in a cohort of alcoholics as shown by Sun *et al.* (2001).

The glomerular filter operating as both size-selective and charge-selective barrier normally prevents the passage of relatively large molecules into the urine. However, if the structure of the glomerular basement membrane is altered, as a result of alcohol-induced nephropathies, those molecules that ordinarily may not be able to pass through the well

ordered arrangement of the outer and inner laminae rarae and the tightly structured central lamina densa now pass easily. Chronic alcohol abuse enhances glomerular dysfunction by increasing the permeability of the glomerular walls to relatively large plasma proteins. The increase in urine total protein was as a result of the destabilization of the glomerular basement membrane which resulted in the alteration of the intrinsic barrier properties of the glomerular walls. Proteinuria is a useful indicator of renal damage (Tryggyason and Wartiovaara, 2001).

The urine specific gravity of the alcoholics was slightly elevated when compared with non consumers of alcohol. This finding is in harmony with the reports of other workers who observed that excessive and consistent alcohol ingestion increased the urine specific gravity in humans (Sun *et al.*, 2001). The specific gravity of urine varies directly with the quantity of solutes excreted into the urine (Gausch *et al.*, 1993). So the urine specific gravity gives a measure of the number of particles in urine.

The serum urea concentration of the alcoholics was significantly elevated when compared with the non consumers of alcohol. These findings indicate that chronic alcohol abuse adversely affects the ability of the kidney to excrete metabolic waste. The findings of this study concur with previous reports of Glodny *et al.* (2009). Ammonia generated from the deamination of amino acids is usually converted to urea in the liver. The synthesized urea is freely filtered from the glomeruli and then excreted out of the body. In contrast to this observation, results of the serum urea concentrations of the different categories of alcoholics studied indicate that the ability of the kidneys to excrete urea was adversely affected by chronic alcohol abuse giving rise to toxic accumulation of urea in the body. In other words, the kidneys of the alcoholics appear to be compromising their vital roles of getting rid of some of the excretory wastes of metabolism as a result of alcohol-mediated renal damage.

Furthermore, creatinine a waste product of muscle metabolism formed by the non-enzymatic dehydration of muscle creatine is freely filtered by normal kidney (Shikata *et al.*, 1990). However, the findings of this study showed that the serum creatinine concentration of the alcoholics was significantly higher compared with non consumers of alcohol. This

finding is in agreement with the report of Hallan *et al.* (2004) who showed that chronic abuse of alcohol caused a significant increase ($p < 0.05$) in the serum creatinine concentration of a cohort of alcoholic subjects. The elevation in the serum creatinine concentration in the alcoholics was as a result of alcohol induced deterioration in the kidney's ability to perform its excretory functions of getting rid of creatinine from the body consequent upon the reduction in clearance of creatinine from the blood of the alcoholics. The finding is in tandem with previous reports of Knight *et al.* (2003) who showed that alcohol-induced nephropathies reduce creatinine clearance. Actually, renal clearance expresses the relative ability of the kidney to completely clear a given volume of plasma of a particular substance in a given time period (Hallan *et al.*, 2004). Thus, as the plasma creatinine concentration increases, the creatinine clearance decreases. The accumulation of creatinine in the plasma is indicative of the inability of the kidneys to rid the plasma of the waste. This invariably provides a useful insight into the adverse effects of chronic alcohol abuse on the kidneys.

The kidney is a vital organ in the body that is highly vulnerable to the devastating effects of chronic alcohol abuse. The kidneys primarily filter and remove the metabolic wastes that build up in body's blood stream while at the same time controlling the body's water and electrolyte balance. The kidneys also regulate the concentrations of ions such as sodium and potassium. These ions have to be in balance in the blood stream to maintain healthy metabolic process. However chronic alcohol abuse destroys the delicate balance of ions and water in the body by altering the filtering, and reabsorption abilities of the kidneys (Costello-Boerrigter *et al.*, 2003).

The findings of this study show a decrease in the serum concentration of both sodium and potassium ions in the different groups of alcoholics studied. This is in harmony with the findings of other workers that showed that chronic alcohol abuse caused reduction in serum electrolyte concentrations (Sun *et al.*, 2004). Useful insight into the cause of the decrease in the serum electrolyte levels could be gained from considering the fact that chronic and excessive consumption of alcohol is capable of causing renal tubular dysfunction which could in turn manifest in form of low tubular reabsorption of the

electrolytes. Also, chronic alcohol ingestion is capable of inhibiting antidiuretic hormone (ADH) (Hays, 2006). Alcohol induced inhibition of antidiuretic hormone makes the tubules of the nephrons to be less permeable to water. Excess water leaving the body affects the concentrations of the electrolytes in the body. The suppression of ADH by alcohol ingestion could lead to diuresis; thereby, causing more water to leave the body. Consequently more sodium ions also leave the body.

However, sodium and water excretion is normally balanced by potassium reabsorption. There is a relationship between serum sodium and potassium concentration and blood pressure (BP). Serum sodium/potassium ratio is directly related to blood pressure reported by Marmot *et al.* (1994). Also, there is an inverse relation between serum potassium concentration and blood pressure (Dyer *et al.*, 1994). Parekh and Klag (2001) considered the relationship between alcohol abuse, renal function and blood pressure and showed that ethanol ingestion can alter serum sodium/potassium ratio, thereby affecting blood pressure. In this study, the systolic blood pressure of most of the alcoholics was less than 140 mmHg and the diastolic blood pressure was less than 90 mmHg. These values are within the normal range for systolic and diastolic blood pressures. Ethanol is known to alter the activity of a number of neurotransmitters and hormonal systems (Snyder, 1992). Thus, ethanol intake can stimulate the activity of substances having antihypertensive effects (Criscione *et al.*, 1989). Therefore alcohol could as well affect blood pressure by regulating aldosterone production and sodium reabsorption.

Alcohol is not effectively stored in tissues and the body rids itself of alcohol by oxidizing it in the liver. The complexity of the liver and its unique role in alcohol oxidation to acetaldehyde contribute in no small measures to many deleterious and toxic effects of alcohol particularly in the liver itself (Latvala *et al.*, 2005). The findings of this study show that prolonged and excessive consumption of alcohol induced significant elevation in serum total bilirubin in the various categories of alcoholics studied. The findings are in tandem with the report of Heathcote (2000). The hepatocytes near the biliary canaliculi are specific sites for the production of alkaline phosphatase (ALP) in the liver. Elevation in the activities of ALP without bone dysfunction is almost always characteristically associated

with intrahepatic and extrahepatic obstructions (Stranges *et al.*, 2004). Therefore, the increase in the concentration of serum total bilirubin coupled with the rise in the activities of ALP in the subjects studied serve as a pointing indicator of alcohol induced pathologic response of the liver to damage in the hepatocytes and obstructions in the biliary tree or cholestasis (Wardlaw and Kessel, 2002).

In this study, the activities of aspartate aminotransferase (AST), alanine aminotransferase (ALT), gamma glutamyl transferase (GGT) and alkaline phosphatase (ALP) which are serum liver-derived enzymes were significantly elevated in the alcoholics when compared with non drinkers of alcohol. This finding suggests the possibility of alcohol induced deterioration of liver cells leading to the leakage of the intracellular enzymes to extracellular fluid. As a result of this leakage into the extracellular fluid, the activities of the enzymes in serum become elevated. The findings of this study substantially concur with previous report that showed that chronic alcohol abuse increases the activities of serum liver derived enzymes such as aspartate aminotransferase alanine aminotransferase, alkaline phosphatase, etc (Steffenson *et al.*, 1997; Sun *et al.*, 2001). Furthermore, the activities of the liver enzymes were higher in alcoholics who showed preference for distilled spirit (ogogoro) than in other alcoholics who showed preference for palmwine or beer.

The body mass index (BMI) of the alcoholics was less than 25, which indicates that they were in no risk for body weight-related health disorders such as obesity or atherosclerosis. The BMI corresponded with the acceptable range of 18.50-24.90 (Wardlaw and Kessel, 2002). Although alcohol contains calories, chronic drinking did not lead to increase in the body mass index (BMI) in the alcoholics because the calories in alcohol are empty. There is no other associated nutritive value. Thus, the use of alcohol as a caloric source results in eventual undernutrition. Ingestion of alcohol tends to satisfy some of the caloric requirements needed by the alcoholics and as consumption of large quantity of alcohol diminishes appetite and induces vomiting, alcoholics are put on a trajectory towards gross undernutrition and decrease in BMI as observed in this study. Other reports corroborated this finding and also showed that chronic alcohol abuse could reduce BMI by

inducing adverse changes in the intestinal absorption mechanism (Marway *et al.*, 1993; Carey, 2003).

4.2 Conclusion

This work shows that chronic alcohol abuse could induce both hepatic and renal dysfunctions in the alcoholics which manifest in form of negative variations in some biochemical parameters of immense prognostic and diagnostic utilities. An important beneficial contribution of this work to knowledge is the observation that the type of alcoholic beverage abuse could lead to different levels of nephrotoxic and hepatotoxic effects.

4.3 Suggestions for Further Studies

1. Studies on the effects of alcohol on female alcoholics should be carried out as well.
2. Studies to find out whether diet has any ameliorating roles on the negative effects of alcohol on different organs of the body could also be investigated.

These areas were not covered due to logistic constraints.

REFERENCES

- Abraham, K. and Grieze, E. (2007). Effects and dangers of alcohol. *American Journal of Clinical Chemistry*, **92**: 105-120.
- Abuelo, J.G. (1983). Proteinuria: Diagnostic principles and procedures. *Annals of Internal Medicine*, **98**: 186-191.
- Agarwal, K.K. and Agarwal, D.P. (2000). Genetic predisposition for alcoholism. *Therumsch*, **57**(4): 179-184.
- Agarwal, A.S., Carolyb, E., Lynsky, M.T., Grant, J.D. and Nellson, E.C. (2009). Evidence for an interaction between age at first drink and genetic influences on DSM-IV alcohol dependence symptoms: Alcoholism. *Clinical and Experimental Research*, **33**(12): 17-25.
- Andreasson, S. and Allebeck, P. (2005) Alcohol as medication is no good more risks than benefits according to a survey of current knowledge. *Lakartidningen*, **102** (9): 632-637.
- Antkiewicz, M.L, Romaska, I. and Papla, I. (2000). Neurochemical Changes induced by acute and chronic administration of 1,2,3,4-tetrahydro-isoquinoline and salsolinol in dopaminergic structures of rat brain. *Newuroscience*, **5**: 10-20.
- Arias, I. M., Che, M. and Gatmaitan, Z. (1993). The biology of the bile canaliculus. *Hepatology*, **17**: 318-320.
- Baker, F.J and Silvertown R.E. (1985) Renal Function Test. In: Introduction to Medical Laboratory Technology. 6th Ed. Butterworth and Co Ltd United Kingdom. pp 143-158.
- Ballerman, B.J. and Marsden, P.A. (1991). Endothelium-derived vasoactive mediators and renal glomerular function. *Clinical and Investigative Medicine*, **249**: 508-510.
- Bard, J., Vize, P., Peter, D. and Woolf, S. (2003). The Kidney from Normal Development to Congenital Disease. Academic Press, Boston. pp 154-156.
- Barreto, R., Faguendes, C. and Guevara, M. (2014). Type-1 hepatorenal syndrome associated with infections in cirrhosis: Natural history, outcome of kidney function and survival. *Hepatology*, **59**: 1505-1507.
- Bartels, H. and Bomer, M. (1972). Serum Creatinine Assay. *Acta*, **37**: 193-194.
- Bath, R.D. (1989). Absorption, Distribution, Elimination of Alcohol. Human Metabolism of Alcohol. CRC Press, USA. pp 3-8.

- Batsford, S.R., Rohrbach, R. and Vogt, A. (1987). Size restriction in the glomerular capillary wall, importance of Lamina densa. *Kidney International*, **31**: 710-711.
- Belleville, J. (2002). The French paradox: Possible involvement of ethanol in the protective effect against cardiovascular diseases. *Nutrition*, **18**: 173i 177.
- Berggren, U., Fahike, C. and Aronsson, E. (2006). The taq1DRD2 A1 allele is associated with alcohol dependence although its effect size is small. *Alcohol and Alcoholism*, **41**(5): 478-485.
- Bertolatus, J.A. and Hunsicker, L.G. (1985). Glomerular sieving of anionic and neutral bovine albumins in proteinuric rats. *Kidney International*, **28**: 467-476.
- Bertorella, A.M. and Katz, A.I. (1993). Short-term regulation of renal Na-K-ATPase activity: Physiological relevance and cellular mechanism. *American Journal of Physiology*, **265**: 743-755.
- Bierut, Y, Schuckit, M.A. Hesselbrock, V. and Reich, T (2000). Co-occurring risk factors for alcohol dependence and habitual smoking. *Alcohol Research and Health*, **24**(4):233-241.
- Billman, J. and McDevitt, S. (1980). Convergence of parent and observer ratings of temperament with observations of peer interaction in nursery school. *Child Development*, **51**:395-400.
- Bingham, S.A. and Cummings, J.H. (1985). Urine nitrogen as an independent validity measure of dietary intake: A study of nitrogen balance in individuals consuming their normal diet. *American Journal of Clinical Nutrition*, **42**: 1276i 1289.
- Blackson, T., Tartar, R., Martin, C. and Moss, H. (1994). Temperament mediates the effects of family history of substance abuse on externalizing and internalizing child behaviour. *American Journal of Addiction*, **3**: 158i 166.
- Blass, K. G., Thiebert, R. J. and Lam, L. K. (1974). A study of the mechanism of the Jaffe reaction. *Journal of Clinical Chemistry and Clinical Biochemistry*, **12**: 336-343.
- Blanco, G. and Mercer, R.W. (1998). Isozymes of the Na-K-ATPase: Heterogeneity in structure, diversity in function. *American Journal of Physiology and Renal Physiology*, **275**: 633i 650.
- Bosnia, P.J., Seppen, J. and Goldhoom, B. (1994). Bilirubin UDP-Glucuronosyltransferase I is the only relevant bilirubin glucuronidating isoform in man. *Journal of Biological Chemistry*, **269**: 17960i 17965.

- Brenner, B.M., Dworkin, L.D. and Ichikawa, I. (1986). Glomerular Filtration: The kidney. Saunders, Philadelphia. pp. 124-144.
- Brown, S.I. Tapert, S.F. and Granholm, E. (2000) Neurocognitive functioning of adolescents: effects of protracted alcohol use. *Alcohol Clinical and Experimental Research*, **24**(2): 164-171.
- Browning, J. D. and Horton, J.D. (2004). Molecular mediators of hepatic steatosis and liver injury. *Journal of Clinical Investigation*, **114**: 147 ï 152.
- Busse, R., Edwards, G., Fleming, I. and Weston, A. H. (2002). Endothelium-dependent hyperpolarisation bringing the concepts together. *Trends in Pharmacological Science*, **23**: 374 ï 380.
- Butterworth, R.F (2000). Complications of cirrhosis: Hepatic encephalopathy. *Journal of Hepatology*, **32**: 171-180.
- Burger, M., Mensink, G., Bronstrup, A., Thierfelder, W. and Pietzlik, K. (2004). Alcohol consumption and its relation to cardiovascular risk factors in Germany. *European Journal of Clinical Nutrition*, **58**: 605ï 614.
- Caan, W. and Bellerocche, J. (2002). Drinks, Drugs and Dependence: Science to Clinical Practice. W.B. Saunders, Philadelphia. pp 19 -20.
- Canaan, K.S., Venkatraman, E.S. and Ernst, S.I.B. (1993). Relationships among protein and albumin concentrations and oncotic pressure in nephritic plasma. *American Journal of Physiology*, **246**: 1052ï 1059.
- Carey, W.D. (2003). Disease management project: A guide to commonly used liver tests. *The Cleveland Clinical Foundation*, **99**: 45ï 89.
- Cargiulo, T. (2007) Understanding the health impact of alcohol dependence. *American Journal of Health System and Pharmacology*, **64**(3): 55-511.
- Caro, A.A. and Cederbaum, A.L. (2004). Oxidative stress, toxicology and pharmacology of CYP2E1. *Annual Review of Pharmacological Toxicology*, **44**: 27ï 42.
- Cecchin, E. and DeMarchi, S. (1996). Alcohol misuse and renal damage. *Addiction Biology*, **1**(1): 7ï 17.
- Chastain, G. (2006) Alcohol, neurotransmitter systems and behaviour. *The Journal of general Psychology*, **133**(4): 329-335.

- Chen, C.Y., Storr, C.L. and Anthony, J.C. (2009). Early onset drug use and risk for drug dependence problems. *Addictive Behaviours*, **34**(3): 319-322.
- Chiaochicy, C. and Shijium, Y. (2008). Alcohol abuse and related factors in Asia. *International Review of Psychiatry*, **20**(5): 425-433.
- Chung, F.M., Yang, Y.H., Shin, S.J. and Tsai, J.C. (2005). Effect of alcohol consumption on estimated glomerular filtration rate and creatinine clearance rate. *Nephrology, Dialysis and Transplant*, **20**: 1610 ï 1616.
- Clark, J. N., Brancati, F. L. and Diehl, A. M. (2003). The prevalence and etiology of elevated aminotransferase le Levels in the United States. *American Journal of Gastroenterology*, **98**: 960 ï 967.
- Clifton, P.T. (2003). Liver function tests: What is the risk? *Journal of Insurance Medicine*, **35**: 26ï 35.
- Colditz, G. (1991). Alcohol intake in relation to diet and obesity in women and men. *American Journal of Clinical Nutrition*, **54**: 49-55.
- Comell, A.G., Bardawill, C.O. and David, M.M. (1949). Protein estimation. *Journal of Biochemistry*, **177**: 751-752.
- Conigrave, K.M., Davies, P., Haber, P. and Whitefield, J.B. (2003). Traditional markers of excessive alcohol use. *Addiction*, **98**: 31ï 43.
- Cornelius, J.R., Bukstein, O., Salloum, I. and Clark, D. (2003). Alcohol and psychiatric comorbidity. *Recent Developments in Alcohol*, **16**: 361-374.
- Cosci, F., Schruers, K.R. Abrahams, K. and Griez, E. (2007). Alcohol use disorders and panic disorder: a review of the evidence of a direct relationship. *Journal of Clinical Psychiatry*, **68**(6): 874-880.
- Costello-Boerrigter, L.C., Boerrigter, G. and Burnett, J.C. (2003). Revisiting salt and water retention: New diuretics, aquaretics and natriuretics. *Alcohol Clinical and Experimental Research*, **28**: 1550ï 1568.
- Crabb, D.W., Bosron, W.F. and Li, J.K. (1987). Ethanol metabolism. *Pharmacological Therapy*, **34**:59-73.
- Crawford, J.M. (2002). Bilirubin Metabolism and the Pathophysiology of Jaundice. Diseases of the Liver. W.B. Sanders, Philadelphia. pp 167-220.

- Crawford, J.M. (2001). *Cirrhosis Pathology of the Liver*. W.B. Saunders, Philadelphia. pp. 575-619.
- Crawford J.M. (2002) *Bilirubin Metabolism Diseases of the Liver*. W.B. Saunders, Philadelphia. pp 167-220.
- Crews, F.T. and Buettiger, C.A. (2009). Impulsivity frontal lobes and risk for addiction. *Pharmacological Biochemical Behaviours*, **93**(3):237-247.
- Crews, F.T., He, J. and Hodge, C. (2007). Adolescent and cortical development: A Critical period of vulnerability for addiction. *Pharmacology and Biochemistry of Behaviour*, **86**(2): 189-199.
- Criscione, L., Powell, J.R., Burdet, R., Engesser, S. and Schoepfer, A. (1989). Alcohol suppresses endothelium-dependent relaxation in rat mesenteric vascular beds. *Hypertension*, **13**: 964-967.
- Cushman, W. C. (2001). Alcohol consumption and hypertension. *Journal of Clinical Hypertension*, **3**(3): 166 - 170.
- Cuthbert, J.A. (2007). Hepatitis: Old and new. *Clinical Microbiological Review*, **14**: 38-58.
- Daniels, B.S., Deen, W.M. and Mayer, G. (1993). Glomerular permeability barrier in the rats: Functional assessment by *in vitro* methods. *Journal of Clinical Investigation*, **92**: 929-936.
- Das, S.K., Dhanya, T. and Vasudevan, D.M. (2008). Biomarkers of alcoholism an updated review. *Scandinavian Journal of Clinical Laboratory Investigation*, **68**(2): 81 - 92.
- Davis, J.M, Haberle, D.A, Kawata T (1988) Function of the Juxtaglomerular apparatus: Local control of glomerular hemodynamic. *Journal of Physiology*, **402**: 472 - 425.
- Dawson D.A, Goldstein, R.B. and Grant B,F. (2007) Rates and Correlates of relapse among individuals in remission DSM W alcohol dependence: A 3 - year follow-up. *Alcoholism, Clinical and Experimental Research*, **31**(12): 2036 - 2045.
- Dawson, D.A., Grant, B.F., Stinson, F.S. and Chou, P.S. (2005). Recovery DSM-IV alcohol dependence United States. *Addiction*, **100**(3): 281-292.
- Day, C.P., James, O.F., Bassendine, M.F. and Li, T.K. (1993). Alcohol dehydrogenase polymorphisms and predisposition to alcoholic cirrhosis. *Hepatology*, **18**: 230-231.

- Deen, W.M., Bridges, C.R. and Brenner, B.M. (1985). Heteroporous model of glomerular size selectivity: Application to normal and nephritic humans. *American Journal of Physiology*, **249**: 374-389.
- Deen, W.M. and Satrat, B. (1981). Determinants of the glomerular filtration of proteins. *American Journal of Physiology*, **241**: 162-170.
- Deleve, L.D., Shulmem, H.M. and Medonald, G.B. (2002). Toxic Injury to hepatic sinusoids: Sinusoidal obstruction syndrome (Veno-occlusive disease). *Seminars on Liver Diseases*, **22**: 22-41.
- Deoballa, R., Rarsons, O. and Yohman, R. (1983). Minimal brain dysfunction symptoms claimed by primary and secondary alcoholics: Relation to cognitive Functioning. *International Journal of Neuroscience*, **20**: 173-181.
- Devor, E.J., Reich, T. and Cloninger, C.R. (1988). Genetics of alcoholism and related end-organ damage. *Seminars on Liver Diseases*, **8**: 1-11.
- Diagnostic and Statistical Manual of Mental Disorders (D.S.M. III) (1980). Alcoholism American Psychiatric Association. Washington DC 2-25.
- Dick, D.M. and Bierut, L.J. (2006). The genetics of alcohol dependence current psychiatry reports. **8**(2): 151-157.
- Diehl, A.M. (2004). Obesity and alcoholic liver disease. *Alcohol*, **34**: 81-87.
- Doll, R., Peto, R. and Boreham J. (2005). Mortality in relation to alcohol consumption: A prospective study among male British doctors. *International Journal of Epidemiology*, **34**(1): 199-204.
- Dunn, W., Angulo, P. and Sanderson, S. (2006). Utility of a new model to diagnose an alcohol basis for steatohepatitis. *Gastroenterology*, **131**: 1057-1063.
- Dyer, A.R., Shipley, M. and Elliott, P. (1994). Urinary electrolyte excretion in 24 hours and blood pressure in the INTERSALT Study. 1: Estimates of reliability. *American Journal of Epidemiology*, **139**: 927-939.
- Ehlers, C.L. (2007). Variations in ADH and ALDH in Southwest California Indians. *Alcohol Research and Health*, **30**(1): 14-17.
- Eng, M.Y., Luccak, S.E. and Wall, T.L. (2007) ALDH₂, ADH 18, ADH1 (genotypes) in Asians: a literature review. *Alcohol Research and Health*, **30**(1): 22-27.

- Enoch, M.A, (2006) Genetic and environmental influences on the development of alcoholism, resilience versus risk. *Annals of New York Academy of Science*, **1094**: 193 ï 201.
- Esterbrooks, A. and Ende, R. (1988). Marital and Parentï Child Relationships: The Role of Affection in the Family System. In: Relationships within Families: Mutual Influences. Oxford University Press, Oxford. pp 104 ï 141.
- Fergusson, D.M., Boden, J.M. and Horwood, C. (2009). Tests of casual links between alcohol abuse or dependence and major depression. *Archieve of General Psychiatry*, **66**(3): 260ï 266.
- FerenzBiro, K. and Pietruszko, R. (1984). Human aldehyde dehydrogenase: Catalytic activity in oriental liver. *Biochemical, Biophysical Research Communication*, **118**: 97 -102.
- Fowkes, A. and Steven, F. (2012). Living with alcohol. *Smart Drug News*, **5**: 2-3.
- French, L. (2008). Psychoactive agents and native American spirituality: Past and present. *Contemporary Justice Review*, **11**(2): 155ï 163.
- Gabrielli, W.F. and Plomin, R. (1985). Individual differences in anticipation of alcohol sensitivity. *Journal of Nerve and Mental Disorder*, **173**: 111-14.
- Gansevoort, R.T., Dezeeuw, D. and Dejong, P.E. (1994). Is the antiproteinuria effect of ACE inhibition mediated by interference in the renninï angiotensin system. *Kidney International*, **45**:861ï 867.
- Gausch, A., Deen, W.M. and Myers, B.D. (1993). Charge selectivity of the glomerular filtration barrier in healthy and nephritic humans. *Journal of Clinical investigation*, **92**: 2274ï 2282.
- Gibson, I.W., Downie, I. and Downie, T.T. (1992). The parietal podocyte: A study of the vascular pole of the human glomerulus. *Kidney International*, **41**: 211ï 212.
- Giebisch, G, Malnic, G. and Berliuer, R.W. (1991). Renal Transport and Control of Potassium Excretion. The Kidney Saunders, Philadelphia. pp 283-317.
- Giebisch, G. and Wang, W. (1996). Potassium transport: From clearance to channels and pumps. *Kidney International*, **49**: 1624ï 1631.
- Gitlow, S. (2006). Substance Use Disorders: A Practical Guide. Lippincott Williams and Wilkins Press, U.S.A. pp 52 -121.

- Glavas, M.M. and Weinberg, V.T. (2006). Stress, Alcohol Consumption and the Hypothalamic-Pituitary-Adrenal Axis. *Nutrients, Stress and Medical Disorder*. Totowa Humana Press, pp 165-183.
- Glodny, B., Unterholzner, V. and Tafeerner, B. (2009). Normal kidney size and its influencing factors. *Urology*, **9**: 19-20.
- Goedde, H.W. and Agarwal, D.P. (1987). Polymorphism of aldehyde dehydrogenase and alcohol sensitivity. *Enzyme*, **37**: 29-44.
- Goldstein, D.B. (1986). Ethanol-induced adaptation in biological membranes. *Annals of New York Academy of Science*, **492**: 103-111.
- Good, D.W. and Knepper, M.A. (1990). Mechanisms of ammonium excretion: Role of the renal medulla. *Seminar in Nephrology*, **10**: 166-173.
- Gosling, J.A., Dixon, J.S. and Humpherson, J. (1982). *The Microscopic Anatomy of the Ureter, Renal Calyces and Pelvis: Functional Anatomy of the Urinary Tract. An Integrated Text and Colour Atlas*. University Park Press, Baltimore. pp 2-8.
- Greger, R. (1985). Ion transport mechanisms in thick ascending limb of Henle's loop of mammalian nephron. *Physiological Review*, **65**: 760-797.
- Greger, R. and Velazquez, H. (1987). The cortical thick ascending unit and early distal convoluted tubule in the urinary concentrating mechanism. *Kidney International*, **31**: 590-596.
- Hallan, S., Asberg, A., Lindberg, M. and Johnsen, H. (2004). Validation of the modification of diet in renal disease formula for estimating GFR with special emphasis on calibration of the serum creatinine assay. *American Journal of Kidney Diseases*, **44**: 84-93.
- Halperin, M.I., Kamel, K.S. and Ethier, J.H. (1992). Biochemistry and Physiology of Ammonium Excretion. In: *The Kidney: Physiology and Pathophysiology*. Seldin, D.W. and Giebisch, G. (Eds). Raven Press, New York. pp 2645-2679.
- Harada, S., Agarwal, D.P., Goedde, H.W. and Ishikawa, B. (1983). Aldehyde dehydrogenase isozyme variation and alcoholism in Japan. *Pharmacological Biochemical Behaviours*, **18**: 151-153.
- Hasin, D.C. (2007). Prevalence, correlates, disability and comorbidity of DSM IV alcohol abuse and dependence in the United States. *Archives of General Psychiatry*, **64**(7): 830-842.

- Hayashi, S.I., Watanabe, J. and Kawajiri, K. (1991). Genetic polymorphism in 5- flanking region change in transcriptional regulation of the human cytochrome P450 II E1 gene. *Journal of Biochemistry*, **110**: 559i 565.
- Hays, R.M. (2006). Vasopressin antagonists: Progress and promise. *North England Journal of Medicine*, **355**: 146i 148.
- Heathcote, E.J. (2000). Management of primary biliary cirrhosis. The American Association for the Study of Liver Diseases Practice Guidelines. *Hepatology*, **31**: 1005i 1013.
- Hoog, J.O, VonBahrllindstrom, H., Hedem, L.O., Holmquist, B, Larsson, K, Hempel, J.D., Vallie, B.L. and Jornvall, H. (1987). Structure of the class II enzyme of human liver alcohol dehydrogenase: Combined cDNA and protein sequence determination of the P_i subunit. *Biochemistry*, **26**: 1926-1932.
- Howie, A.J. and Rollason, T.P. (1993). Reconsideration of the development of the distal tubule of the human kidney. *Journal of Anatomy*., **183**: 141i 142.
- Hyman, S.E. (2005). Addiction: A disease of learning and memory. *American Journal of Psychiatry*, **162**: 1414i 1424.
- Ishak, K.G. (2000). Pathologic features of chronic hepatitis: A review and update. *American Journal of Clinical Pathology*, **113**: 40i 55.
- Jamieson, R.L. and Kriz, W. (1982). Urinary Concentrating Mechanism: Structure and Function. Oxford University Press, New York. pp 54 i 55.
- Jendrassik . L and Grof, P. (1938). Colorimetric measurement of bilirubin. *Biochemistry Z*, **297**: 81i 82.
- Johansson, B.A., Berylund, M. and Hanson, M. (2003). Dependence on legal psychotropic drugs among alcoholics. *Alcohol Alcohol*, **38**(6): 613i 618.
- Kamath, P.S. and Kim, W.R. (2007). The model for end-stage liver disease. *Hepatology*, **45** (3): 797 i 805.
- Kandel, D.B., Huang, F.Y. and Davies, M. (2001). Comorbidity between patterns of substance use dependence and psychiatric syndromes. *Drug Alcohol Dependence*, **64**(2): 233i 241.
- Kaufman, J.A., Waltman, A.C., Rivits, S.M. and Geller, S.C. (1995). Anatomical observations on the renal veins and inferior vena Cava at magnetic resonance angiography. *Cardiovascular Intervention Radiology*, **18**: 153 i 154.

- Kaysen, G.A., Don, B., Schambelarm, M. (1991). Proteinuria, albumin synthesis and hyperlipidemia in the nephritic syndrome. *Nephrology, Dialysis and Transplant*, **6**: 141 ï 149.
- Kita, H., Nalbandian, G., Keefe, E.B. and Coppel, R.L. (2003). Pathogenesis of primary biliary cirrhosis. *Clinical Liver Disease*, **7**: 821ï 823.
- Klatsky, A.L., Morton, C., Udaltsova, N. and Friedman, G.D. (2006). Coffee, cirrhosis and transaminase enzymes. *Archives of Internal Medicine*, **166**(11): 1190ï 1195.
- Klesges, R.C. (1994). Effects of alcohol intake on resting energy expenditure in young women social drinkers. *American Journal of Clinical Nutrition*, **59**: 805ï 809.
- Kloth, S., Aigner, J. and Brandt, E. (1993). Histochemical markers reveal an unexpected heterogenous composition of the renal collecting duct epithelium. *Kidney international*, **44**: 527 ï 528.
- Kluth, D.C. (1999). Anti-glomerular basement membrane disease. *Journal of American Society of Nephrologists*. **10**: 2446 ï 2447.
- Knight, E.C., Stampfer, N.J., Rimm, E.B., Hankinson, S.E. and Curhan, G.C. (2003). Moderate alcohol intake and renal function decline in women: A prospective study. *Nephrology, Dialysis and Transplant*, **18**: 1549ï 1554.
- Kreisberg, J., Venkatachalam, M and Troyer, D. (1985). Contractile properties of cultured glomerular mesangial cells. *American Journal of Physiology*, **249**: 999ï 1000.
- Kushner, M.G., Abrahams, K. and Borchardt, C. (2000). The relationship between anxiety disorders and alcohol use disorders: a review of major perspectives and findings. *Clinical Psychology Review*, **20** (2): 149ï 171.
- Laker, M.F. (1990). Liver function test. *Journal of Biological Medicine*, **301**: 250ï 251.
- Latvala, J., Hietala, J., Koivisto, H., Jarvi, K., Anttila, P. and Niemela, O. (2005). Immune responses to ethanol metabolites and cytokine profiles differentiate alcoholics with or without liver diseases. *American Journal of Gastroenterology*, **100**: 1303ï 1310.
- Leon, D. A. and McCambridge, J. (2006). Liver Cirrhosis Mortality rates in Britain from 1950 to 2002: An analysis of routine data. *Lancet*, **367**: 52ï 56.
- Lerner, J and Vicary, J. (1984). Difficult temperament and drug use: Analysis from the New York longitudinal study. *Journal of Drug Education*, **14**: 1-8.

- Levey, A.S., Perrone, R.D. and Media, N.E. (1988). Serum creatinine and renal function. *Annual Review of Medicine*, **39**: 465ï 490.
- Lieber, C.S. (1980). Alcohol, protein metabolosm and liver injury. *Gastroenterology*, **79**: 373ï 390.
- Lieber, C.S. (1987). Microsomal ethanol-oxidizing system. *Enzyme*, **37**: 45ï 56.
- Lieber, C.S. (1995). Medical disorders of alcoholism. *North England Journal of Medicine*, 333: 1058ï 1065.
- Lieber, C.S. (2004). Alcoholic fatty liver: Its pathogenesis and mechanism of progress to inflammation and fibrosis. *Alcohol*, **34**: 9ï 19.
- Longstreth, T., George, F. and Zieve, D. (2010). Alcoholic liver disease. *MedLine Plus*, **3**: 10ï 30.
- Mannisto, S. (1997). Alcohol beverage drinking, diet and body mass index in a cross-national Survey, European. *Journal of Clinical Nutrition*, **151**: 326ï 332.
- Maruna, R. F. L. (1958). Determination of sodium concentration. *Clinica Chemica Acta*. **2**:581.
- Markman, H. and Jonesleonard, D. (1985). Marital Discord and Children at Risk: Implications for Research and Prevention. In: Identification of the Child at Risk. An International Perspective. Plenum, New York. pp 59 ï 77.
- Marmot, M.G., Elliot, P. and Shipley, M.J. (1994). Alcohol and blood pressure: The intersalt study. *British Medical Journal*, **308**: 1263ï 1267.
- Martin, C.S. Chung, T. and Langenhucher, S. (2008). How should we revise diagnostic criteria for substance use disorder in the DSM V? *Abnormal Psychology*, **117**(3): 561ï 575.
- Marway, J.S., Keating, J.W., Reeves, J., Salisbury, J.R. and Preedy, V.R. (1993). Seromuscular and mucosal protein synthesis in various anatomical regions of the rat gastrointestinal tract and their response to acute ethanol toxicity. *European Journal of Gastroenterology and Hepatology*, **5**: 27ï 34.
- Maziade, M., Caron, C., Cofe, R., Boutin, P. and Thiverge, J. (1990). Extreme temperament and diagnosis: A study in a psychiatric sample of consecutive children. *Archive of General Psychiatry*, **47**: 477 ï 484.

- Maziade, M, Cote, R, Boudreault, M., Caperaa, P. and Thiverge, J. (1984). The NYLS model temperament: Gender differences and demographic correlates in a French Speaking population. *Journal of American Academy of Child Psychiatry*, **23**: 582 ï 587.
- McGue, M., Pickens, R.W. and Svikis, D.S. (1992). Sex and age effects on the inheritance of alcohol problems: A twin study. *Journal of Abnormal Psychology*, **102**: 3 -8.
- Menon, K.V., Gores, G.J. and Shah, V.H. (2001). ñPathogenesis, diagnosis and treatment of alcoholic liver diseaseò. *Mayo Clinical Procedure*, **76** (10): 1021ï 1029.
- Michael, G., Sandra, E. F. and Gian, L. G. (1998). Effects of moderate alcohol consumption on the central nervous system. *Clinical and Experimental Research*, **22**: 998 ï 1040.
- Michielson, P. and VeinRenterghen, Y. (1983). Proteinuria and non-steroidal anti-inflammatory drugs. *Advances in Nephrology*, **12**: 139ï 150.
- Moore, S., Montemejaime, L.K., Carr, L.G. and Ehlers, C.L. (2007). Variations in alcohol ï metabolizing enzymes in people of East Indian and African descent from Trinidad and Tobago. *Alcohol Research and Health*, **30**(1): 28ï 30.
- Moreno, A. and Pares, X. (1991). Purification and characterization of a new alcohol dehydrogenase from human stomach. *Journal of Biological Chemistry*, **266**: 1128ï 1133.
- Moss, D.W. (1982). Alkaline phosphatase isoenzymes. *Clinical Chemistry*, **28**: 2007ï 2016.
- Moss, D.W. and Henderson, A.R (1994). Enzymes. In: Tietz, Fundamentals of Clinical Chemistry. 4th Edn. C.A. Burtis, E.R. Ashwood (Eds). W.B. Saunders, Philadelphia London, pp. 283ï 334.
- Moss, D.W. and Henderson, A.R. (1996). Enzymes. In: Tietz Textbook of Clinical Chemistry. 4th Ed. C.A Burtis, E.R. Ashwood, Eds. W.B. Saunders, Philadelphia. pp 317-318.
- Mueller, G.C, Fleming, M.F., Lemahieu, M.A, Lybrand, G.S., and Barry, K.J (1988). Synthesis of Phosphatidyl ethanol ï a potential marker for adult males at risk for alcoholism. *Proceedings of National Academic Science (USA)*, **85**: 9778 ï 9782.
- Nagoshi, C.T. and Wilson, J.R. (1989). Longï term repeatability of human alcohol metabolism, sensitivity and acute tolerance. *Journal of Studies on Alcohol*, **50**: 162ï 169.
- Nappi, A. and Vass, E. (1999). Contrasting effects of Catecholic and Oï methylated tetrahydroisoquinlines on hydroxyl radical production. *Biophysica Acta*, **12**: 10ï 109.

- Nielson, S., Rehling, M., Schmitz, A. and Mogensen, C.E. (1999). Validity of rapid estimation of glomerular filtration rate in type diabetic patients with normal renal function. *Nephrology, Dialysis and Transport*, **14**: 615ï 619.
- Niemela, O. (2007). Biomarkers in alcoholism. *Clinical Chemical Acta*, **377**: 39ï 49.
- Niemela, O., Parkkila, S., Juvonen, R.O. and Vitala, K. (2000). Cytochromes P450, 2A6, 2E1 and 3^a and production of protein-aldehyde adducts in the liver of patients with alcoholic and non-alcoholic liver diseases. *Journal of Hepatology*, **33**: 893ï 901.
- Nurnberger, J., John, I. and Bierut, L.J. (2007). Seeking the connections: Alcoholism and our genes. *Scientific American*, **269**(4): 7ï 25.
- Obarzanek, E., Velletri, P.A. and Cutler, J.A. (1996). Dietary protein and blood pressure. *JAMA*, **274**: 1598ï 1603.
- Oscarberman, M. and Marinkovit, K. (2003). Alcoholism and the brain: An overview. *Alcohol Research and Health*, **27**(2): 125ï 133.
- Panza, F., Capurso, C., Dintrono, A., Colaizzo, A.M. and Frisardi, V. (2009). Alcohol drinking, cognitive functions in older age, predementia and dementia syndromes. *Journal on Alzheimers Disease*, **17**(1): 7ï 31.
- Patrick, R.S. (1983). Hepatitis: Colour Atlas of Liver Pathology. Oxford University Press, New York. pp 42 ï 43.
- Patsenka, A. (2004). Inhibition of rodent brain monoamine oxidase and tyrosine hydroxylase by endogenous compounds ï 1, 2, 3, 4, - tetrahydroisoquinoline alkaloids. *Pharmacology*, **1**: 2 ï 25.
- Parekh, R.S. and Klag, M.J. (2001). Alcohol: Role in the development of hypertension and end stage renal diseases. *Current Opinion on Nephrology and Hypertension*, **10**: 385ï 390.
- Perlmutter, D.H. (2002). Liver injury in alpha 1- antitrypsin deficiency: An aggregated proteinï induces mitochondrial injury. *Journal of Clinical Invesigation*, **110**: 1579ï 1580.
- Peters, R., Peters, J., Warner, J., Beckett, N. and Bulpitt, C. (2008). Alcohol, dermentia and cognitive decline in the elderly: A systematic review age. *Ageing*, **37**(5): 505ï 512.
- Pieruccilagha, A.D. (2003). Alcoholism and aging. Alcoholic dementia or alcoholic cognitive impairment. *Psychological Neuropsychiatric*, **1**(4): 237 ï 249.

- Popper, H. and Mandel, E. (1937). Filtrations and reabsorption. *Nierenpathologic Ergebnisse der Inneren Medizin und Kinderheilkunde*, **53**: 685ï 694.
- Poupon, R.E., Nalpas, B., Coutelle, C., Fleury, B., Couzigou, P. and Higuieret, D. (1992). Polymorphism of the alcohol dehydnoenase, alcohol and aldehyde dehydrogenase activities: Implication in alcoholic cirrhosis in white patients. *Hepatology*, **15**: 1017ï 1022.
- Pusey, C.D., Dash, A. and Kershaw, M.J. (1987). A single autoantigen in good pastures syndrome identified by a monoclonal antibody to human glomerular basement memberane. *Laboratory Investigations*, **56**: 23 ï 24.
- Raddatz, R. and Parini, A. (1995). Imidazoline/quanidine binding domains on monoamine oxidases. *Journal of Biological Chemistry*, **4**: 12 ï 34.
- Rambaldi, A., Jacobs, B.P., Laquinto, G. and Gluud, C. (2005). Milk thistle for alcoholic and/or hepatitis B or C liver diseases ï a systematic Cochrane hepato ï biliary group review with meta-analyses of randomized clinical trials. *American Journal of Gastroenterology*, **100**(11): 2583ï 2591.
- Rec, G.S. (1972). Alkaline phosphatase assay. *Journal of Clinical Chemistry and Clinical Biochemisty*, **10**: 182ï 194.
- Reitman, S. and Frankel, S. (1952). Alanine aminotransferase measurement. *American Journal of Clinical Pathology*, **28**: 56ï 58.
- Remuzzi, G. and Bertani, T. (1990). Is glomerulo-sclerosis a consequence of altered glomerular permeability to macromolecules? *Kidney International*, **38**: 384ï 394.
- Remuzzi, G., Ruggenenti, P. and Benigni, A. (1997). Understanding the nature of renal disease progression. *Kidney International*, **51**: 2 -3.
- Rex, D.K., Bosron, W.F., Smialek, J.E. and Li, T.K. (1985). Alcohol and aldehyde dehydrogenase Isoenzymes in North Indians. *Alcohol Clinical Experimental Research*, **9**: 147ï 152.
- Reynolds, J.N., Wu, P.H. and Khanna, J.M. (1990). Ethanol tolerance in hippocampal neurons: Adaptive changes in cellular responses to ethanol measure *in vitro*. *Journal of Pharmacological Experimental Therapy*, **252**: 265 ï 271.
- Romero, J.C. and Knox, G.G. (1988). Mechanisms underlying pressure related natriuresis: The role of the renninï angiotensin and prostaglandin systems. *Hypertension*, **11**: 724ï 738.

- Rottenberg, H. (1986). Membrane solubility of ethanol in chronic alcoholism: The effect of ethanol feeding and its withdrawal on the protection by alcohol of rat red blood cells from hypotonic haemolysis. *Biochem. Biophysics Acta*, **855**: 211-222.
- Room, R., Babor, T. and Rehm, J. (2005). Alcohol and public health. *Lancet*, **365**: 519-530.
- Sakmann, B. (1992). Elementary steps in synaptic transmission revealed by currents through single ion channels. *Science*, **256**: 503 - 512.
- Saxena, R., Theise, N.D. and Crawford, J.M. (1999). Microanatomy of the human liver: exploring the hidden interfaces. *Hepatology*, **30**: 1339-1346.
- Schnermann, J. and Briggs, J. (1985). Function of the Juxtaglomerular Apparatus: Local Control of Glomerular Haemodynamics. The Kidney Raven Press, New York. pp 669 - 698.
- Schuckit, M.A., Smith, T.L. and Danko, G.P. (2007). A comparison of factors associated with substance induced versus independent depressions. *Studies on Alcohol Drugs*, **68**(6): 805-812.
- Schwitters, S.Y., Johnson, R.C., Johnson, S.B. and Ahern, F.M. (1982). Familial resemblances in flushing following alcohol use. *Behavioural Genetics*, **12**: 349-352.
- Scott, D.M. and Taylor, R.E. (2007). Health related effects of genetic variations of alcohol metabolizing enzymes in African Americans. *Alcohol Research and Health*, **30**(1): 18-21.
- Seltman, C. (1980) Fermentation: Wine in the Ancient World. Routledge and Kegan Paul Publishers, London. pp 10.
- Shankar, A., Klein, R. and Klein, B.E. (2006). The association among smoking, heavy drinking and chronic kidney disease. *American Journal of Epidemiology*, **164**: 263-271.
- Shapiro, S.M., Bhutani, V.K. and Johnson, L. (2006). Hyperbilirubinemia and kernicterus. *Clinical Perinatology*, **33**: 387-390.
- Sherlock, S. (1951). *Liver Disease*. Churchill: London. pp 204.
- Shermann, K. E. (1991). Alanine aminotransferase in clinical practice: A review. *Archives of International Medicine*, **151**: 260 - 265.
- Shibuya, A. and Yoshida, A. (1989). Genotypes of alcohol metabolizing enzymes in Japanese with alcoholic Liver diseases: A strong correlation of the usual Caucasian type aldehyde

- dehydrogenase gene (ALDH 2i1) with the disease. *American Journal of Human Genetics*, **43**: 744i 748.
- Shikata, K., Ichiyasu, A., Makino, H. and Ota, Z. (1990). Three dimensional ultrastructure of the GBM revealed by Chemical treatment. XIth International Congress of Nephrology. pp 365i 366.
- Slater, R. J. (1986). *Experiments in Molecular Biology*. Clifton, New Jersey: Humana Press. Pp 269.
- Sorensen, H.T., Thulstrup, A.M. and Mellemkjar, L. (2003). Longi term survival and cause i specific mortality in patients with cirrhosis of the liver: A Nationwide Cohort Study in Denmark. *Journal of Clinical Epidemiology*, **56**(1): 88i 93.
- Steffensen, F.H., Sorensen, H.T., Brock, A., Vilstrup, H. and Lauritzer, T. (1997). Alcohol consumption and serum liver derived enzymes in a Danish population aged 30 i 50 years. *International Journal on Epidemiology*, **26**: 92i 99.
- Steinhausen, M., Holz, F.G. and Parekh, N. (1988). Regulation of Prei and Posti Glomerular Resistances Visualized in the Split Hydronephrotic Kidney, *Nephrology*, Balliere Tindall, London. pp 37i 45.
- Stewart, M. and Dubloiz, C. (1981). Wife abuse among families attending a child Psychiatry Clinic. *Journal of American Academy of Child Psychiatry*, **20**: 845i 862.
- Stewart, R.B., McBride, W.J., Lumeng, L., Li, T.K. and Murphy, J.M. (1991). Chronic alcohol Consumption in the alcoholi preferring rats attenuates subsequent conditioned taste aversion produced by ethanol Injections. *Psychopharmacology*, **105**: 530 i 534.
- Stoke, J.B. (1982). Consequences of potassium recycling in the renal medulla: Effects of ion transport by the medullary thick ascending limb of Henle's loop. *Journal of Clincial Investigation*, **70**: 219i 229.
- Stranges, S., Dorn, J.M. and Muti, P. (2004). Body fat distribution, relative weight and liver enzyme levels: A population-based study. *Hepatology*, **39**: 754i 763.
- Sun, H., Fang, Q. and Mayhan, W.G. (2004). Inward rectifier potassium chemicals in the basilar artery during chronic alcohol consumption. *Alcohol Clinical and Experimental Research*, **28**: 1557i 1561.
- Sun, A.Y., Ingelmansunderg, M., Neve, E., Matsuniolo, H. and Nishitani, Y. (2001). Ethanol and Oxidative stress in alcoholism. *Clinical and Experimental Research*, **25**: 2375i 2435.

- Suzuki, A., Lymp, J., Sauver, J.S., Angulo, P. and Lindor, K. (2006). Values and limitations of serum aminotransferases in clinical trials of nonalcoholic steatohepatitis. *Liver International*, **28**: 1209i 1216.
- Snyder, S.H. (1992). Nitric oxide: First in a new class of neurotransmitters: *Science*, **257**: 494i 496.
- Tarter, R., Blackson, T., Martin, C., Seilhamer, R., Pelham, W. and Loeber, R. (1993). Mutual dissatisfaction between mother and son in substance abuse normal families. *American Journal of Addiction*, **2**: 1 i 10.
- Tannen, R.L. (1991). Disorders of Potassium Balance. The Kidney. 4th Edn. W.B. Saunders. Philadelphia. pp 805i 840.
- Tarter, R., Hegedus, A. and Gavaler, J. (1985). Hyperactivity in sons of alcoholics. *Journal of Studies Alcohol*, **46**: 259i 261.
- Tarter, R., Kirisci, L., Hegedus, A., Mezzich, and Vanyukov, M. (1994) Heterogeneity of adolescent alcoholism. *Annals of New York Academy of Science*, **708**: 172 -180.
- Terri, A.E. and Sesin, P.G. (1958) Potassium assay using sodium tetraphenylboron. *American Journal of Clinical Pathology*, **29**: 86 i 87.
- Testino, G. (2008). Alcoholic diseases in hepatogastroenterology: A point of view. *Hepatogastroenterology*, **55**(82): 371i 377.
- Tietz, N.W. (1983). Liver functions tests and the objective evaluation of the patient with liver disease. *Journal of Clinical Chemistry and Clinical Biochemistry*. **21**: 731-748.
- Thacker, S.B., Veech R.L., Vernon, A.A. and Rutstein, D.D. (1984). Genetic and biochemical factors relevant to alcoholism. *Clinical Experimental Research*, **8**: 375i 383.
- Thomasson, H.R., Li, T.K. and Crabb, D.W. (1990). Correlations between alcohol-induced flushing, genotypes for alcohol and aldehyde dehydrogenases and alcohol elimination rates. *Hepatology*, **12**: 903.
- Tisher, C.C. and Madsen, K.M. (1996). Anatomy of the Kidney. 5th Edn. W.B Saunders, Philadelphia. pp. 3 i 71
- Trinder, P. (1951). Soduim magnesuim uranyl acetate method of soduim measurement. *Analyst*, **76**: 596 i 597.

- Tryggvason, K. and Wartio-Vaara, I. (2001). Molecular basis of glomerular permselectivity. *Current Opinion on Nephrological Hypertension*, **10**: 543.
- Tsukamoto, H., Horne, W. and Kamimura, A. (1995). Experimental liver cirrhosis-induced by alcohol and iron. *Journal of Clinical Investigation*, **96**: 620i 630.
- Tsutsuni, M., Lasker, J.M., Shimizu, M., Rosman, A.S. and Lieber, C.S. (1989). The intralobular distribution of ethanolⁱ inducible P450 IIE1 in rat and human liver. *Hepatology*, **10**: 437i 446.
- Tu, G.C. and Weiner, H. (1987). Aldehyde dehydrogenase: A multifunctional enzyme. *Progress in Clinical Biological Research*, **232**: 67i 75.
- Tuma, D.J., Jenneth, R.B. and Somell, M.F. (1981). Effect of ethanol on the synthesis and secretion of hepatic secretory glycoproteins and albumin. *Hepatology*, **1**: 590i 598.
- Verbalis, J.G. (1990). Clinical Aspects of Body Fluid Homeostasis in Humans. Handbook of Behavioural Neurobiology. Plenum Press, New York. pp 421i 462.
- Verbalis, J.B. (1993). Hyponatremia: Epidemiology, pathophysiology and therapy. *Current Opinion on Nephrology and Hypertension*, **2**: 636i 652.
- Verbaten, M.N. (2009). Chronic effects of low to moderate alcohol consumption on structural and functional properties of the brain beneficial or not? *Human Psychopharmacology*, **24**(3): 199 i 205.
- Wagner, F.W., Pares, X., Holmquist, B. and Vallee, B.L. (1984). Physical and enzymatic properties of a class III Isozyme of human liver alcohol dehydrogenase: Chiⁱ ADH. *Biochemistry*, **23**: 2193 i 2199.
- Wardlaw, G.M. and Kessel, M. (2002). Perspectives: Alcohol Involvement in Liver Dysfunction. Perspectives in Nutrition. 5th Edn. McGraw Hill, New York. pp 7i 45.
- Weatherburn, M.W. (1967). Ureaseⁱ Berthelot Method. *Analytical Chemistry*, **39**: 971i 972.
- Weiss, J.S., Gautam, A. and Lauff, J.J. (1983). The clinical importance of a protein-bound fraction of serum bilirubin in patients with hyperbilirubinemia. *English Journal of Medicine*, **309**: 147i 150.
- Welterling, T. and Junghanns, K. (2000). Psychopathology of alcoholics during withdrawal and early abstinence. *European Psychiatry*, **15**(8): 483 i 488.

- Whitfield, J.B., Starmer, G.A. and Martin, M.G. (1990). Alcohol metabolism in men and women. *Clinical Experimental Research*, **14**: 785 ï 786.
- William, F, Balisteri, M.D. and Robert, R.E. (1996). Liver Function. In: Tietz, Fundamentals of Clinical Chemistry. 4th Edn. C.A. Burtis, E.R Ashwood, W.B Saunders Company Philadelphia London. pp 544-545.
- Wilson, J.R., Erwin, V.G., and Meelean, G.E (1984). Effects of ethanol: Acute metabolic tolerance and ethnic differences. *Clinical Experimental Research*, **8**: 226ï 232.
- Windle, M. (1992). Temperament and Social Support in adolescence: Interrelations with depressive symptoms and delinquent behaviours. *Journal of Youth Adolescence*, **21**:1ï 21.
- Wingo, C.S. and Smolka, A. (1995). Function and Structure of H, K_i ATPase in the Kidney. *American Journal of Physiology*, **269**:1ï 16.
- World Health Organisation (1978). ñAlcoholò: Alcohol Policy in the WHO European Region: Current Status and the Way Forward. *World Health Organisation*, **1**: 4- 5.
- Yin, S.J, Wang, M.F., Liao, C.S., Chen, C.M. and Will, C.W (1990). Identification of a Stomach alcohol dehydrogenase with distinctive kinetic properties. *Biochemistry International*, **22**: 829 ï 835.
- Yoshida, A. (1983). A possible structural variant of aldehyde dehydrogenase with diminished enzyme activity. *American Journal of Human Genetics*, **35**: 115 ï 116.
- Yoshida, A., Dave, V., Ward, R.J. and Peters, T.J. (1989). Cytosolic aldehyde (ALDH1) variants found in alcohol flushers. *Annals of Human Genetics London*, **53**: 1 ï 7.
- You, M. and Crabb, D.W. (2004) Recent advances in alcoholic liver disease II. Miniview molecular mechanisms of alcoholic fatty liver. *American Journal of Physiology: Gastro – Intestinal and Liver Physiology*, **287**: 1 -3.
- Zhu, Z., Wilson, A.T. and Nathahs, M.M. (2008). Heme oxygenase-1 suppresses hepatitis C virus replication and increases resistance of hepatocytes to oxidant injury. *Hepatology*, **48**: 1430 ï 1435.
- Zucker, S.D., Horn, P.S. and Sherman, K.E. (2004). Serum bilirubin levels in the US population: Gender effect and inverse correlation with colorectal cancer. *Hepatology*, **40**: 827ï 830.
- Zuskin, E., Juki, V., Liozenci, J., Matosi, A., Mustajbegovi, J. and Turci, N. (2006). Alcoholism ï how it affects health and working capacity. *Arh HigRada Toksikol*, **57**(4): 413 ï 426.

APPENDICES

Table 1: Shows the results (mean $\pm$ standard deviation) of liver function tests of the control group and the three groups of alcoholic subjects who showed preference for palmwine, beer and distilled spirit (ogogoro)

	No of Subjects	Serum total bilirubin concentration ($\mu\text{mol/l}$)	Alkaline phosphatase activity (IU/L)	Aspartate aminotransferase (AST) activity (IU/L)	Alanine aminotransferase (ALT) activity (IU/L)	Gamma glutamyl transferase (GGT) activity (IU/L)	p-Value
Control (Non-Consumers of alcohol)	40.00	8.30 ± 2.00	61.50 ± 3.00	9.51 ± 0.35	10.50 ± 2.00	20.50 ± 0.30	$p < 0.05$
Alcoholics who showed preference for palmwine	60.00	18.65 ± 2.10	174.20 ± 2.50	18.44 ± 0.40	16.50 ± 1.50	53.00 ± 0.40	$p < 0.05$
Alcoholics who showed preference for beer	60.00	19.40 ± 1.50	175.10 ± 1.50	19.21 ± 0.19	17.50 ± 2.00	55.50 ± 0.30	$p < 0.05$
Alcoholics who showed preference for distilled spirit (ogogoro)	60.00	22.75 ± 1.60	177.40 ± 1.00	20.32 ± 0.64	18.31 ± 2.00	58.00 ± 0.50	$p < 0.05$

Serum total bilirubin concentration and the activity of ALP, AST, ALT, GGT of the alcoholic subjects were significantly higher ($p < 0.05$) compared with the control. Furthermore, the parameters were significantly higher ($p < 0.05$) in alcoholics who showed preference for distilled spirit compared with those who showed preference for beer and palmwine. Also the parameters were significantly higher ($p < 0.05$) in alcoholics who showed preference for beer compared with those who showed preference for palmwine.

Table 2: Shows the results (mean $\pm$ standard deviation) of kidney function tests of the control group and the three groups of alcoholic subjects who showed preference for palmwine, beer and distilled spirit (ogogoro)

	No of Subjects	Urine total concentration (mg/dl)	Urine specific gravity	Serum urea (mg/dl)	Serum creatinine (mg/dl)	Urine creatinine (mg/dl)	Creatinine clearance (ml/min)	Serum Electrolytes		p < Value
								K ⁺ (mEq/l)	Na ⁺ (mEq/l)	
Control (Non-Consumers of alcohol)	40.00	56.40 $\pm$ 0.40	1.021 $\pm$ 0.001	18.88 $\pm$ 0.31	0.86 $\pm$ 0.04	13.17 $\pm$ 1.28	129.23 $\pm$ 0.66	4.20 $\pm$ 0.30	137.00 $\pm$ 2.00	p < 0.05
Alcoholics who showed preference for palmwine	60.00	151.34 $\pm$ 0.60	1.036 $\pm$ 0.004	57.30 $\pm$ 2.80	3.14 $\pm$ 0.21	85.94 $\pm$ 5.34	44.23 $\pm$ 4.10	3.30 $\pm$ 0.10	131.00 $\pm$ 1.00	p < 0.05
Alcoholics who showed preference for beer	60.00	152.74 $\pm$ 0.42	1.037 $\pm$ 0.004	62.49 $\pm$ 1.50	3.46 $\pm$ 0.29	83.59 $\pm$ 14.24	37.46 $\pm$ 1.50	3.10 $\pm$ 0.20	129.00 $\pm$ 1.50	p < 0.05
Alcoholics who showed preference for distilled spirit (ogogoro)	60.00	153.96 $\pm$ 0.43	1.039 $\pm$ 0.001	64.00 $\pm$ 1.70	3.71 $\pm$ 0.17	80.44 $\pm$ 14.20	31.28 $\pm$ 3.80	2.00 $\pm$ 0.10	118.00 $\pm$ 2.00	p < 0.05

Table 3: Summary table showing the mean and standard deviation of the body mass index (BMI) and the blood pressure of the control and alcoholic subjects who showed preference for palmwine, beer and distilled spirit

	No of Subjects	Body Mass Index (BMI) (Kg/m ²)	Systolic blood pressure (mmHg)	Diastolic blood pressure (mmHg)	Ratio of systolic and diastolic blood pressure (mm/Hg)
Control (Non-Consumers of alcohol)	40.00	24.00 ± 2.00	122.00 ± 9.00	80.50 ± 6.00	$\frac{122}{80}$
Alcoholics who showed preference for palmwine	60.00	23.00 ± 1.50	124.00 ± 12.00	83.00 ± 6.00	$\frac{124}{83}$
Alcoholics who showed preference for beer	60.00	21.00 ± 2.00	123.00 ± 12.00	82.00 ± 7.00	$\frac{123}{82}$
Alcoholics who showed preference for distilled spirit (ogogoro)	60.00	20.00 ± 2.00	125.00 ± 11.00	82.00 ± 9.00	$\frac{125}{82}$

Table 3 shows that the blood pressure of both the control and the alcoholic subjects were normal ($< \frac{140}{90}$ mmHg). The body mass index (BMI) of the alcoholic subjects was significantly lower ($p < 0.05$) compared with the control. The BMI of alcoholics who showed preference for beer was significantly lower ($p < 0.05$) compared with those who showed preference for palmwine. Also, the BMI of alcoholics who showed preference for distilled spirit was the lowest ($p < 0.05$).

APPENDICES

Appendix I

		Descriptives							
		N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Minimum	Maximum
						Lower Bound	Upper Bound		
Urine Total Protein	Group 1=Alcohol + Palm Wine	60	151.3433	.57412	.07412	151.1950	151.4916	150.00	152.30
	Group 2=Alcohol + Beer	60	152.7400	.42394	.05473	152.6305	152.8495	151.70	153.40
	Group 3=Alcohol + Distilled Spirit	60	153.9567	.42600	.05500	153.8466	154.0667	153.00	154.60
	Group 4=Control	40	56.3975	.41540	.06568	56.2646	56.5304	55.60	57.20
	Total	220	135.1741	37.23583	2.51044	130.2264	140.1218	55.60	154.60
Urine Specific Gravity	Group 1=Alcohol + Palm Wine	60	1.0355	.00391	.00051	1.0345	1.0365	1.03	1.04
	Group 2=Alcohol + Beer	60	1.0366	.00389	.00050	1.0356	1.0376	1.03	1.04
	Group 3=Alcohol + Distilled Spirit	60	1.0390	.00088	.00011	1.0387	1.0392	1.04	1.04
	Group 4=Control	40	1.0207	.00118	.00019	1.0203	1.0211	1.02	1.02
	Total	220	1.0340	.00708	.00048	1.0331	1.0350	1.02	1.04
Creatinine Clearance	Group 1=Alcohol + Palm Wine	60	44.2292	4.14342	.53491	43.1588	45.2995	36.85	54.30
	Group 2=Alcohol + Beer	60	37.4550	1.47351	.19023	37.0744	37.8356	35.00	42.40
	Group 3=Alcohol + Distilled Spirit	60	31.2762	3.81907	.49304	30.2896	32.2627	25.00	38.42
	Group 4=Control	40	129.1880	.66723	.10550	128.9746	129.4014	127.50	130.50
	Total	220	54.2961	35.83726	2.41615	49.5342	59.0580	25.00	130.50
Urine Creatinine	Group 1=Alcohol + Palm Wine	60	85.9448	5.34349	.68984	84.5645	87.3252	72.33	93.43
	Group 2=Alcohol + Beer	60	83.5913	14.24263	1.83872	79.9121	87.2706	61.00	121.00
	Group 3=Alcohol + Distilled Spirit	60	80.4377	14.19810	1.83297	76.7699	84.1054	58.00	118.00
	Group 4=Control	40	1317.6100	1.27677	.20188	1317.2017	1318.0183	1314.50	1319.50
	Total	220	307.7401	477.26978	32.17752	244.3229	371.1574	58.00	1319.50
Serum Creatinine	Group 1=Alcohol + Palm Wine	60	3.1543	.22901	.02956	3.0952	3.2135	2.50	3.80
	Group 2=Alcohol + Beer	60	3.4575	.28757	.03713	3.3832	3.5318	2.50	3.90

	Group 3=Alcohol + Distilled Spirit	60	3.7108	.17101	.02208	3.6667	3.7550	3.00	3.90
	Group 4=Control	40	.8653	.02050	.00324	.8587	.8718	.83	.91
	Total	220	2.9726	1.03840	.07001	2.8346	3.1106	.83	3.90
Serum Urea	Group 1=Alcohol + Palm Wine	60	57.2792	2.82741	.36502	56.5488	58.0096	47.90	63.10
	Group 2=Alcohol + Beer	60	62.4450	1.52831	.19730	62.0502	62.8398	59.00	64.75
	Group 3=Alcohol + Distilled Spirit	60	63.9892	1.66934	.21551	63.5579	64.4204	60.00	66.30
	Group 4=Control	40	18.8835	.31448	.04972	18.7829	18.9841	17.75	19.50
	Total	220	53.5370	16.68518	1.12491	51.3200	55.7540	17.75	66.30
Sodium Ion	Group 1=Alcohol + Palm Wine	60	130.0900	.89380	.11539	129.8591	130.3209	128.50	132.00
	Group 2=Alcohol + Beer	60	129.0733	.82212	.10614	128.8610	129.2857	127.00	130.20
	Group 3=Alcohol + Distilled Spirit	60	117.9150	.59227	.07646	117.7620	118.0680	117.00	119.00
	Group 4=Control	40	136.9200	.51251	.08103	136.7561	137.0839	135.00	138.10
	Total	220	127.7341	6.66165	.44913	126.8489	128.6193	117.00	138.10
Potassium Ion	Group 1=Alcohol + Palm Wine	60	3.2813	.06094	.00787	3.2656	3.2971	3.15	3.45
	Group 2=Alcohol + Beer	60	3.0868	.09900	.01278	3.0613	3.1124	2.90	3.30
	Group 3=Alcohol + Distilled Spirit	60	2.0192	.15652	.02021	1.9787	2.0596	1.50	2.50
	Group 4=Control	40	4.1768	.14134	.02235	4.1315	4.2220	3.90	4.50
	Total	220	3.0469	.74286	.05008	2.9482	3.1456	1.50	4.50
Total Bilirubin	Group 1=Alcohol + Palm Wine	60	18.3100	1.21484	.15683	17.9962	18.6238	16.00	20.40
	Group 2=Alcohol + Beer	60	19.3483	1.24431	.16064	19.0269	19.6698	17.00	21.40
	Group 3=Alcohol + Distilled Spirit	60	22.3433	1.17666	.15191	22.0394	22.6473	20.30	24.60
	Group 4=Control	40	8.2888	.18553	.02933	8.2294	8.3481	7.50	8.55
	Total	220	17.8711	4.90869	.33094	17.2189	18.5234	7.50	24.60
ALP	Group 1=Alcohol + Palm Wine	60	173.8000	11.24730	1.45202	170.8945	176.7055	155.00	205.00
	Group 2=Alcohol + Beer	60	174.8333	10.20496	1.31745	172.1971	177.4696	156.00	197.00
	Group 3=Alcohol + Distilled Spirit	60	176.8833	11.19121	1.44478	173.9923	179.7743	158.00	207.50
	Group 4=Control	40	61.0650	1.88955	.29876	60.4607	61.6693	57.90	70.00

	Total	220	154.4255	45.20659	3.04783	148.4186	160.4323	57.90	207.50
AST	Group 1=Alcohol + Palm Wine	60	18.4442	.39586	.05110	18.3419	18.5464	17.50	19.50
	Group 2=Alcohol + Beer	60	19.1932	.21922	.02830	19.1365	19.2498	18.30	19.75
	Group 3=Alcohol + Distilled Spirit	60	20.3137	.64321	.08304	20.1475	20.4798	19.50	23.00
	Group 4=Control	40	9.5143	.34703	.05487	9.4033	9.6252	8.50	10.30
	Total	220	17.5347	3.87729	.26141	17.0195	18.0499	8.50	23.00
ALT	Group 1=Alcohol + Palm Wine	60	16.0000	.30042	.03878	15.9224	16.0776	15.00	16.45
	Group 2=Alcohol + Beer	60	17.3992	.27563	.03558	17.3280	17.4704	16.50	18.10
	Group 3=Alcohol + Distilled Spirit	60	18.2672	.30583	.03948	18.1882	18.3462	17.50	19.20
	Group 4=Control	40	10.4812	.37871	.05988	10.3601	10.6024	9.50	11.50
	Total	220	15.9965	2.75734	.18590	15.6301	16.3629	9.50	19.20

ANOVA

		Sum of Squares	df	Mean Square
Urine Total Protein	Between Groups	303597.494	3	101199.165
	Within Groups	47.488	216	.220
	Total	303644.982	219	
Urine Specific Gravity	Between Groups	.009	3	.003
	Within Groups	.002	216	.000
	Total	.011	219	
Creatinine Clearance	Between Groups	279244.855	3	93081.618
	Within Groups	2018.906	216	9.347
	Total	281263.761	219	
Urine Creatinine	Between Groups	4.986E7	3	1.662E7
	Within Groups	25610.070	216	118.565

	Total	4.989E7	219	
Serum Creatinine	Between Groups	226.426	3	75.475
	Within Groups	9.715	216	.045
	Total	236.141	219	
Serum Urea	Between Groups	60190.846	3	20063.615
	Within Groups	777.742	216	3.601
	Total	60968.589	219	
Sodium Ion	Between Groups	9600.742	3	3200.247
	Within Groups	117.952	216	.546
	Total	9718.694	219	
Potassium Ion	Between Groups	117.830	3	39.277
	Within Groups	3.022	216	.014
	Total	120.852	219	
Total Bilirubin	Between Groups	5015.401	3	1671.800
	Within Groups	261.454	216	1.210
	Total	5276.854	219	
ALP	Between Groups	426419.630	3	142139.877
	Within Groups	21136.523	216	97.854
	Total	447556.152	219	
AST	Between Groups	3251.123	3	1083.708
	Within Groups	41.187	216	.191
	Total	3292.309	219	
ALT	Between Groups	1644.124	3	548.041
	Within Groups	20.919	216	.097
	Total	1665.043	219	

ANOVA

		F	Sig.
Urine Total Protein	Between Groups	460302.135	.000
	Within Groups		
	Total		
Urine Specific Gravity	Between Groups	344.882	.000
	Within Groups		
	Total		
Creatinine Clearance	Between Groups	9958.676	.000
	Within Groups		
	Total		
Urine Creatinine	Between Groups	140175.042	.000
	Within Groups		
	Total		
Serum Creatinine	Between Groups	1678.046	.000
	Within Groups		
	Total		
Serum Urea	Between Groups	5572.207	.000
	Within Groups		
	Total		
Sodium Ion	Between Groups	5860.472	.000
	Within Groups		
	Total		
Potassium Ion	Between Groups	2807.396	.000
	Within Groups		
	Total		
Total Bilirubin	Between Groups	1381.158	.000

	Within Groups		
	Total		
ALP	Between Groups	1452.567	.000
	Within Groups		
	Total		
AST	Between Groups	5683.377	.000
	Within Groups		
	Total		
ALT	Between Groups	5658.739	.000
	Within Groups		
	Total		

Post Hoc Tests

Multiple Comparisons

Dependent Variable	(I) Group	(J) Group	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval		
						Lower Bound	Upper Bound	
Urine Total Protein	LSD	Group 1=Alcohol + Palm Wine	Group 2=Alcohol + Beer	-1.39667 [*]	.08561	.000	-1.5654	-1.2279
			Group 3=Alcohol + Distilled Spirit	-2.61333 [*]	.08561	.000	-2.7821	-2.4446
			Group 4=Control	94.94583 [*]	.09571	.000	94.7572	95.1345
		Group 2=Alcohol + Beer	Group 1=Alcohol + Palm Wine	1.39667 [*]	.08561	.000	1.2279	1.5654
			Group 3=Alcohol + Distilled Spirit	-1.21667 [*]	.08561	.000	-1.3854	-1.0479
			Group 4=Control	96.34250 [*]	.09571	.000	96.1539	96.5311
		Group 3=Alcohol + Distilled Spirit	Group 1=Alcohol + Palm Wine	2.61333 [*]	.08561	.000	2.4446	2.7821
			Group 2=Alcohol + Beer	1.21667 [*]	.08561	.000	1.0479	1.3854

				Group 4=Control	97.55917 [*]	.09571	.000	97.3705	97.7478		
				Group 4=Control	Group 1=Alcohol + Palm Wine	-94.94583 [*]	.09571	.000	-95.1345	-94.7572	
					Group 2=Alcohol + Beer	-96.34250 [*]	.09571	.000	-96.5311	-96.1539	
					Group 3=Alcohol + Distilled Spirit	-97.55917 [*]	.09571	.000	-97.7478	-97.3705	
Urine Specific Gravity	LSD	Group 1=Alcohol + Palm Wine	Group 2=Alcohol + Beer		-0.00113 [*]	.00054	.037	-0.0022	-0.0001		
			Group 3=Alcohol + Distilled Spirit		-0.00350 [*]	.00054	.000	-0.0046	-0.0024		
			Group 4=Control		.01477 [*]	.00061	.000	.0136	.0160		
		Group 2=Alcohol + Beer	Group 1=Alcohol + Palm Wine		.00113 [*]	.00054	.037	.0001	.0022		
			Group 3=Alcohol + Distilled Spirit		-0.00237 [*]	.00054	.000	-0.0034	-0.0013		
			Group 4=Control		.01590 [*]	.00061	.000	.0147	.0171		
		Group 3=Alcohol + Distilled Spirit	Group 1=Alcohol + Palm Wine		.00350 [*]	.00054	.000	.0024	.0046		
			Group 2=Alcohol + Beer		.00237 [*]	.00054	.000	.0013	.0034		
			Group 4=Control		.01827 [*]	.00061	.000	.0171	.0195		
		Group 4=Control	Group 1=Alcohol + Palm Wine		-0.01477 [*]	.00061	.000	-0.0160	-0.0136		
			Group 2=Alcohol + Beer		-0.01590 [*]	.00061	.000	-0.0171	-0.0147		
			Group 3=Alcohol + Distilled Spirit		-0.01827 [*]	.00061	.000	-0.0195	-0.0171		
		Creatinine Clearance	LSD	Group 1=Alcohol + Palm Wine	Group 2=Alcohol + Beer		6.77417 [*]	.55818	.000	5.6740	7.8743
					Group 3=Alcohol + Distilled Spirit		12.95300 [*]	.55818	.000	11.8528	14.0532
					Group 4=Control		-84.95883 [*]	.62406	.000	-86.1889	-83.7288
				Group 2=Alcohol + Beer	Group 1=Alcohol + Palm Wine		-6.77417 [*]	.55818	.000	-7.8743	-5.6740
Group 3=Alcohol + Distilled Spirit					6.17883 [*]	.55818	.000	5.0787	7.2790		
Group 4=Control					-91.73300 [*]	.62406	.000	-92.9630	-90.5030		
Group 3=Alcohol + Distilled Spirit	Group 1=Alcohol + Palm Wine			-12.95300 [*]	.55818	.000	-14.0532	-11.8528			
	Group 2=Alcohol + Beer			-6.17883 [*]	.55818	.000	-7.2790	-5.0787			
	Group 4=Control			-97.91183 [*]	.62406	.000	-99.1419	-96.6818			
Group 4=Control	Group 1=Alcohol + Palm Wine			84.95883 [*]	.62406	.000	83.7288	86.1889			
	Group 2=Alcohol + Beer			91.73300 [*]	.62406	.000	90.5030	92.9630			

				Group 3=Alcohol + Distilled Spirit	97.91183*	.62406	.000	96.6818	99.1419
Urine Creatinine	LSD	Group 1=Alcohol + Palm Wine	Group 2=Alcohol + Beer		2.35350	1.98801	.238	-1.5649	6.2719
			Group 3=Alcohol + Distilled Spirit		5.50717*	1.98801	.006	1.5888	9.4255
			Group 4=Control		-1231.66517*	2.22266	.000	-1236.0460	-1227.2843
		Group 2=Alcohol + Beer	Group 1=Alcohol + Palm Wine		-2.35350	1.98801	.238	-6.2719	1.5649
			Group 3=Alcohol + Distilled Spirit		3.15367	1.98801	.114	-.7647	7.0720
			Group 4=Control		-1234.01867*	2.22266	.000	-1238.3995	-1229.6378
		Group 3=Alcohol + Distilled Spirit	Group 1=Alcohol + Palm Wine		-5.50717*	1.98801	.006	-9.4255	-1.5888
			Group 2=Alcohol + Beer		-3.15367	1.98801	.114	-7.0720	.7647
			Group 4=Control		-1237.17233*	2.22266	.000	-1241.5532	-1232.7915
		Group 4=Control	Group 1=Alcohol + Palm Wine		1231.66517*	2.22266	.000	1227.2843	1236.0460
			Group 2=Alcohol + Beer		1234.01867*	2.22266	.000	1229.6378	1238.3995
			Group 3=Alcohol + Distilled Spirit		1237.17233*	2.22266	.000	1232.7915	1241.5532
Serum Creatinine	LSD	Group 1=Alcohol + Palm Wine	Group 2=Alcohol + Beer		-.30317*	.03872	.000	-.3795	-.2268
			Group 3=Alcohol + Distilled Spirit		-.55650*	.03872	.000	-.6328	-.4802
			Group 4=Control		2.28908*	.04329	.000	2.2038	2.3744
		Group 2=Alcohol + Beer	Group 1=Alcohol + Palm Wine		.30317*	.03872	.000	.2268	.3795
			Group 3=Alcohol + Distilled Spirit		-.25333*	.03872	.000	-.3297	-.1770
			Group 4=Control		2.59225*	.04329	.000	2.5069	2.6776
		Group 3=Alcohol + Distilled Spirit	Group 1=Alcohol + Palm Wine		.55650*	.03872	.000	.4802	.6328
			Group 2=Alcohol + Beer		.25333*	.03872	.000	.1770	.3297
			Group 4=Control		2.84558*	.04329	.000	2.7603	2.9309
		Group 4=Control	Group 1=Alcohol + Palm Wine		-2.28908*	.04329	.000	-2.3744	-2.2038
			Group 2=Alcohol + Beer		-2.59225*	.04329	.000	-2.6776	-2.5069
			Group 3=Alcohol + Distilled Spirit		-2.84558*	.04329	.000	-2.9309	-2.7603
Serum Urea	LSD	Group 1=Alcohol + Palm Wine	Group 2=Alcohol + Beer		-5.16583*	.34644	.000	-5.8487	-4.4830
			Group 3=Alcohol + Distilled Spirit		-6.71000*	.34644	.000	-7.3928	-6.0272

				Group 4=Control	38.39567 [*]	.38733	.000	37.6322	39.1591	
				Group 2=Alcohol + Beer	Group 1=Alcohol + Palm Wine	5.16583 [*]	.34644	.000	4.4830	5.8487
					Group 3=Alcohol + Distilled Spirit	-1.54417 [*]	.34644	.000	-2.2270	-.8613
					Group 4=Control	43.56150 [*]	.38733	.000	42.7981	44.3249
				Group 3=Alcohol + Distilled Spirit	Group 1=Alcohol + Palm Wine	6.71000 [*]	.34644	.000	6.0272	7.3928
					Group 2=Alcohol + Beer	1.54417 [*]	.34644	.000	.8613	2.2270
					Group 4=Control	45.10567 [*]	.38733	.000	44.3422	45.8691
				Group 4=Control	Group 1=Alcohol + Palm Wine	-38.39567 [*]	.38733	.000	-39.1591	-37.6322
					Group 2=Alcohol + Beer	-43.56150 [*]	.38733	.000	-44.3249	-42.7981
					Group 3=Alcohol + Distilled Spirit	-45.10567 [*]	.38733	.000	-45.8691	-44.3422
Sodium Ion	LSD	Group 1=Alcohol + Palm Wine	Group 2=Alcohol + Beer	1.01667 [*]	.13492	.000	.7507	1.2826		
			Group 3=Alcohol + Distilled Spirit	12.17500 [*]	.13492	.000	11.9091	12.4409		
			Group 4=Control	-6.83000 [*]	.15084	.000	-7.1273	-6.5327		
		Group 2=Alcohol + Beer	Group 1=Alcohol + Palm Wine	-1.01667 [*]	.13492	.000	-1.2826	-.7507		
			Group 3=Alcohol + Distilled Spirit	11.15833 [*]	.13492	.000	10.8924	11.4243		
			Group 4=Control	-7.84667 [*]	.15084	.000	-8.1440	-7.5494		
		Group 3=Alcohol + Distilled Spirit	Group 1=Alcohol + Palm Wine	-12.17500 [*]	.13492	.000	-12.4409	-11.9091		
			Group 2=Alcohol + Beer	-11.15833 [*]	.13492	.000	-11.4243	-10.8924		
			Group 4=Control	-19.00500 [*]	.15084	.000	-19.3023	-18.7077		
		Group 4=Control	Group 1=Alcohol + Palm Wine	6.83000 [*]	.15084	.000	6.5327	7.1273		
			Group 2=Alcohol + Beer	7.84667 [*]	.15084	.000	7.5494	8.1440		
			Group 3=Alcohol + Distilled Spirit	19.00500 [*]	.15084	.000	18.7077	19.3023		
Potassium Ion	LSD	Group 1=Alcohol + Palm Wine	Group 2=Alcohol + Beer	.19450 [*]	.02160	.000	.1519	.2371		
			Group 3=Alcohol + Distilled Spirit	1.26217 [*]	.02160	.000	1.2196	1.3047		
			Group 4=Control	-.89542 [*]	.02414	.000	-.9430	-.8478		
		Group 2=Alcohol + Beer	Group 1=Alcohol + Palm Wine	-.19450 [*]	.02160	.000	-.2371	-.1519		
			Group 3=Alcohol + Distilled Spirit	1.06767 [*]	.02160	.000	1.0251	1.1102		

				Group 4=Control	-1.08992 [*]	.02414	.000	-1.1375	-1.0423	
				Group 3=Alcohol + Distilled Spirit	Group 1=Alcohol + Palm Wine	-1.26217 [*]	.02160	.000	-1.3047	-1.2196
					Group 2=Alcohol + Beer	-1.06767 [*]	.02160	.000	-1.1102	-1.0251
				Group 4=Control		-2.15758 [*]	.02414	.000	-2.2052	-2.1100
				Group 4=Control	Group 1=Alcohol + Palm Wine	.89542 [*]	.02414	.000	.8478	.9430
					Group 2=Alcohol + Beer	1.08992 [*]	.02414	.000	1.0423	1.1375
					Group 3=Alcohol + Distilled Spirit	2.15758 [*]	.02414	.000	2.1100	2.2052
Total Bilirubin	LSD	Group 1=Alcohol + Palm Wine	Group 2=Alcohol + Beer		-1.03833 [*]	.20087	.000	-1.4342	-.6424	
			Group 3=Alcohol + Distilled Spirit		-4.03333 [*]	.20087	.000	-4.4292	-3.6374	
			Group 4=Control		10.02125 [*]	.22458	.000	9.5786	10.4639	
		Group 2=Alcohol + Beer	Group 1=Alcohol + Palm Wine		1.03833 [*]	.20087	.000	.6424	1.4342	
			Group 3=Alcohol + Distilled Spirit		-2.99500 [*]	.20087	.000	-3.3909	-2.5991	
			Group 4=Control		11.05958 [*]	.22458	.000	10.6169	11.5022	
		Group 3=Alcohol + Distilled Spirit	Group 1=Alcohol + Palm Wine		4.03333 [*]	.20087	.000	3.6374	4.4292	
			Group 2=Alcohol + Beer		2.99500 [*]	.20087	.000	2.5991	3.3909	
			Group 4=Control		14.05458 [*]	.22458	.000	13.6119	14.4972	
		Group 4=Control	Group 1=Alcohol + Palm Wine		-10.02125 [*]	.22458	.000	-10.4639	-9.5786	
			Group 2=Alcohol + Beer		-11.05958 [*]	.22458	.000	-11.5022	-10.6169	
			Group 3=Alcohol + Distilled Spirit		-14.05458 [*]	.22458	.000	-14.4972	-13.6119	
ALP	LSD	Group 1=Alcohol + Palm Wine	Group 2=Alcohol + Beer		-1.03333	1.80605	.568	-4.5931	2.5264	
			Group 3=Alcohol + Distilled Spirit		-3.08333	1.80605	.089	-6.6431	.4764	
			Group 4=Control		112.73500 [*]	2.01922	.000	108.7551	116.7149	
		Group 2=Alcohol + Beer	Group 1=Alcohol + Palm Wine		1.03333	1.80605	.568	-2.5264	4.5931	
			Group 3=Alcohol + Distilled Spirit		-2.05000	1.80605	.258	-5.6097	1.5097	
			Group 4=Control		113.76833 [*]	2.01922	.000	109.7884	117.7482	
		Group 3=Alcohol + Distilled Spirit	Group 1=Alcohol + Palm Wine		3.08333	1.80605	.089	-.4764	6.6431	
			Group 2=Alcohol + Beer		2.05000	1.80605	.258	-1.5097	5.6097	

				Group 4=Control	115.81833 [*]	2.01922	.000	111.8384	119.7982	
				Group 4=Control	Group 1=Alcohol + Palm Wine	-112.73500 [*]	2.01922	.000	-116.7149	-108.7551
					Group 2=Alcohol + Beer	-113.76833 [*]	2.01922	.000	-117.7482	-109.7884
					Group 3=Alcohol + Distilled Spirit	-115.81833 [*]	2.01922	.000	-119.7982	-111.8384
AST	LSD	Group 1=Alcohol + Palm Wine	Group 2=Alcohol + Beer		-.74900 [*]	.07972	.000	-.9061	-.5919	
			Group 3=Alcohol + Distilled Spirit		-1.86950 [*]	.07972	.000	-2.0266	-1.7124	
			Group 4=Control		8.92992 [*]	.08913	.000	8.7542	9.1056	
		Group 2=Alcohol + Beer	Group 1=Alcohol + Palm Wine		.74900 [*]	.07972	.000	.5919	.9061	
			Group 3=Alcohol + Distilled Spirit		-1.12050 [*]	.07972	.000	-1.2776	-.9634	
			Group 4=Control		9.67892 [*]	.08913	.000	9.5032	9.8546	
		Group 3=Alcohol + Distilled Spirit	Group 1=Alcohol + Palm Wine		1.86950 [*]	.07972	.000	1.7124	2.0266	
			Group 2=Alcohol + Beer		1.12050 [*]	.07972	.000	.9634	1.2776	
			Group 4=Control		10.79942 [*]	.08913	.000	10.6237	10.9751	
		Group 4=Control	Group 1=Alcohol + Palm Wine		-8.92992 [*]	.08913	.000	-9.1056	-8.7542	
			Group 2=Alcohol + Beer		-9.67892 [*]	.08913	.000	-9.8546	-9.5032	
			Group 3=Alcohol + Distilled Spirit		-10.79942 [*]	.08913	.000	-10.9751	-10.6237	
ALT	LSD	Group 1=Alcohol + Palm Wine	Group 2=Alcohol + Beer		-1.39917 [*]	.05682	.000	-1.5112	-1.2872	
			Group 3=Alcohol + Distilled Spirit		-2.26717 [*]	.05682	.000	-2.3792	-2.1552	
			Group 4=Control		5.51875 [*]	.06352	.000	5.3935	5.6440	
		Group 2=Alcohol + Beer	Group 1=Alcohol + Palm Wine		1.39917 [*]	.05682	.000	1.2872	1.5112	
			Group 3=Alcohol + Distilled Spirit		-.86800 [*]	.05682	.000	-.9800	-.7560	
			Group 4=Control		6.91792 [*]	.06352	.000	6.7927	7.0431	
		Group 3=Alcohol + Distilled Spirit	Group 1=Alcohol + Palm Wine		2.26717 [*]	.05682	.000	2.1552	2.3792	
			Group 2=Alcohol + Beer		.86800 [*]	.05682	.000	.7560	.9800	
			Group 4=Control		7.78592 [*]	.06352	.000	7.6607	7.9111	
		Group 4=Control								
		Group 4=Control		Group 1=Alcohol + Palm Wine	-5.51875 [*]	.06352	.000	-5.6440	-5.3935	

	Group 2=Alcohol + Beer	-6.91792*	.06352	.000	-7.0431	-6.7927
	Group 3=Alcohol + Distilled Spirit	-7.78592*	.06352	.000	-7.9111	-7.6607

*. The mean difference is significant at the 0.05 level.

Appendix II: Questionnaire

UNIVERSITY OF NIGERIA NSUKKA
DEPARTMENT OF BIOCHEMISTRY

Sir,

I am a post-graduate student of the Department of Biochemistry, University of Nigeria Nsukka. I am currently carrying out a research titled: The Effect of Alcohol on some Biochemical parameters.

The major objective of this study is to investigate the impact of alcohol abuse on some principal biochemical parameters of diagnostic utility. The findings of this study will help alcoholics to know the health implications of their lifestyles and possibly adjust positively. Indeed this questionnaire is strictly for research purposes. Your sincere response is therefore highly solicited. Be assured that your responses will be treated with utmost respect and confidentiality.

PERSONAL DATA

1. Name -----
 2. Date of Birth ----- (3) Age ----- (4) Sex -----
 5. Occupation ----- (6) Marital Status ----- Town -----

1. Do you drink alcohol

(a) Yes (b) No

2. What type of alcoholic beverage do you normally prefer.

(a) Palm wine (b) Beer (c) Distilled spirit (ogogoro, root or Kai kai) (d) none of the above (e) all

3. Have you suffered from liver problems before

(a) Yes (b) No

4. Have you suffered from kidney problem before

(a) Yes (b) No

5. Are you suffering from high blood pressure (hypertension) currently

(a) Yes (b) No

6. How often do you drink alcohol.

(a) Daily (b) 2 days (c) 3 days in a week (d) 4 days in a week
(e) 5 days in a week (f) 6 days in a week

7. Do you feel you can not do without drinking alcohol

(a) Yes (b) No

8. How many bottles do you normally take in a day.

(a) one bottle. (b) two bottles (c) three bottles (d) four bottles
(e) five bottles (f) six bottles

9. When you started drinking really how many bottles do you drink before you become intoxicated (a) 1 bottle (b) 2 bottles (c) 3 bottles (d) 4 bottles (e) 5 bottles and above

10. How many bottles of drink do you take now

(a) 1 bottle (b) 2 bottles (c) 3 bottles (d) 4 bottles (e) 5 bottles and above.

11. Have you ever had an automobile accident as a result of your drinking habit

(a) Yes (b) No

12. Have you ever had any occupational problems as a result of alcohol consumption.

(a) Yes (b) No

13. Do you beat or abuse spouse or children when you are intoxicated

(a) Yes (b) No

14. Have you ever been attacked by criminals when drunk

(a) Yes (b) No

15. Do you normally run into financial constraints as a result of your drinking habit

(a) Yes (b) No

16. Do you quarrel with your friends and relatives after drinking

(a) Yes (b) No

17. Are you suffering from stomach ulcer

(a) Yes (b) No

18. Do you find it difficult to eat food after drinking

(a) Yes (b) No

19. Do you feel depressed and dull if you don't drink alcohol

(a) Yes (b) No

20. Do you develop fear or phobia when you abstain from drink

(a) Yes (b) No

21. Do you feel that you can not live without drinking alcohol

(a) Yes (b) No

22. Do you develop anxiety when intoxicated with alcohol

(a) Yes (b) No

23. Would you like to stop alcohol consumption if you can

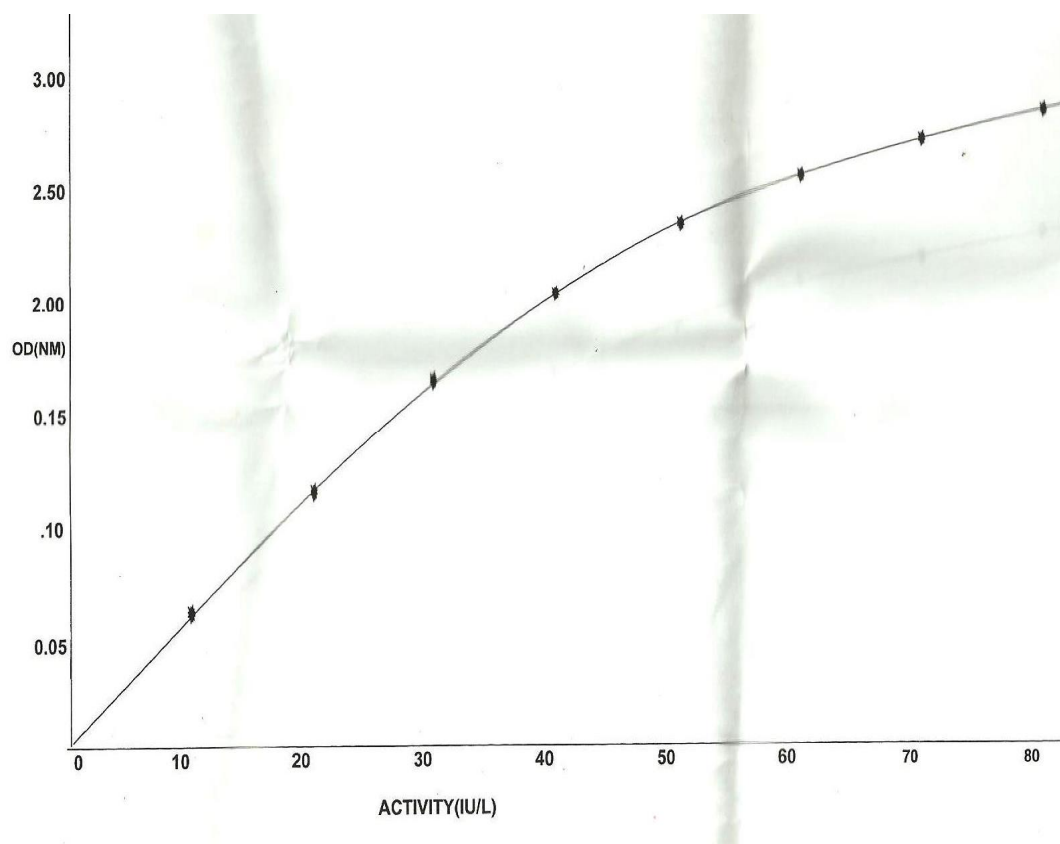
(a) Yes (b) No

24. Did you parent(s) drink alcohol

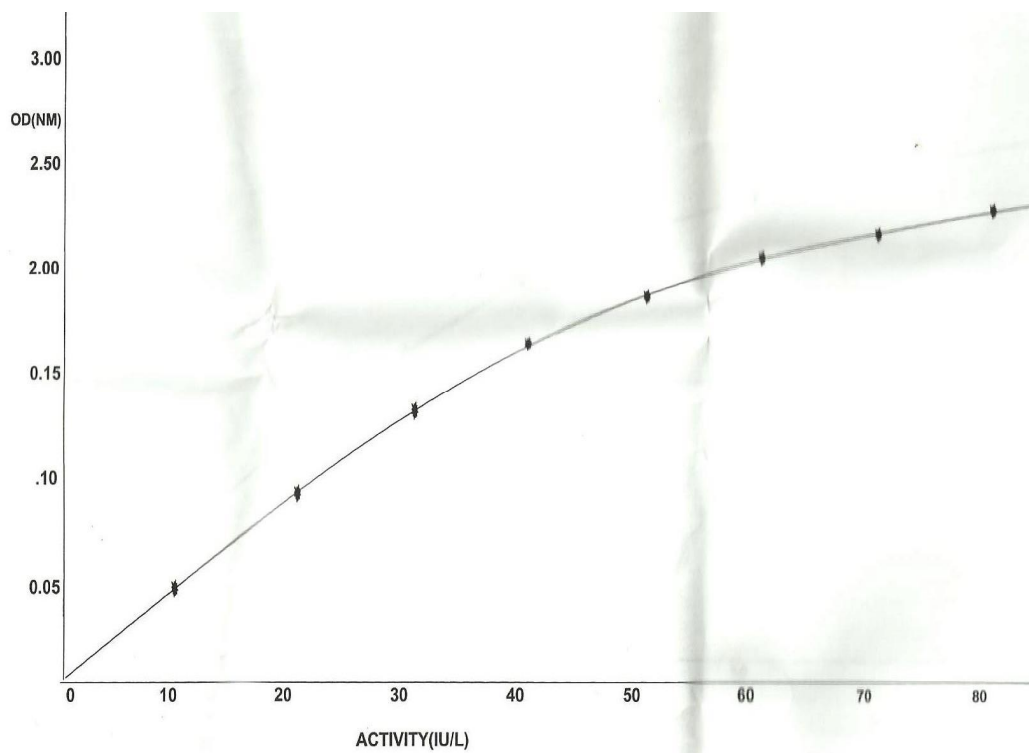
(a) Yes (b) No

25. Any other useful information, please specify -----

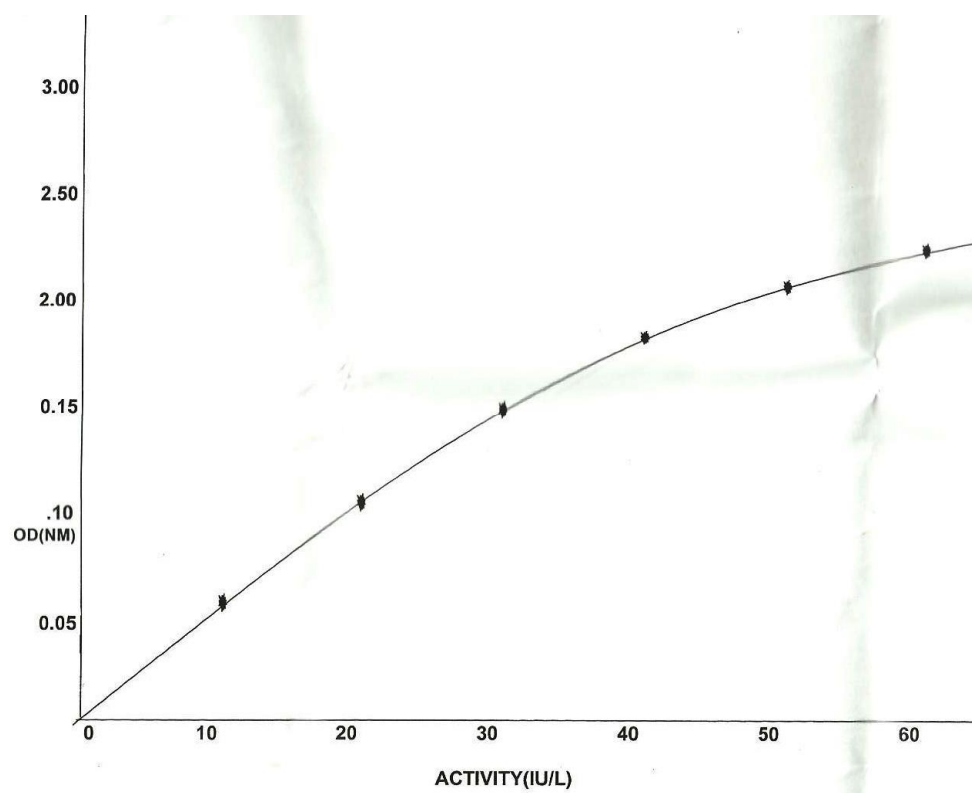
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Appendix III: AST standard curve



Appendix IV: GGT standard curve



Appendix V: ALT standard curve

Appendix VI: Shows the results (mean $\pm$ standard deviation) of liver function tests of the control group and the three groups of alcoholic subjects who showed preference for palmwine, beer and distilled spirit (ogogoro)

	No of Subjects	Serum total bilirubin concentration ($\mu\text{mol/l}$)	Alkaline phosphatase activity (IU/L)	Aspartate aminotransferase (AST) activity (IU/L)	Alanine aminotransferase (ALT) activity (IU/L)	Gamma glutamyl transferase (GGT) activity (IU/L)	p-Value
Control (Non-Consumers of alcohol)	40.00	8.30 ± 2.00	61.50 ± 3.00	9.51 ± 0.35	10.50 ± 2.00	20.50 ± 0.30	$p < 0.05$
Alcoholics who showed preference for palmwine	60.00	18.65 ± 2.10	174.20 ± 2.50	18.44 ± 0.40	16.50 ± 1.50	53.00 ± 0.40	$p < 0.05$
Alcoholics who showed preference for beer	60.00	19.40 ± 1.50	175.10 ± 1.50	19.21 ± 0.19	17.50 ± 2.00	55.50 ± 0.30	$p < 0.05$
Alcoholics who showed preference for distilled spirit (ogogoro)	60.00	22.75 ± 1.60	177.40 ± 1.00	20.32 ± 0.64	18.31 ± 2.00	58.00 ± 0.50	$p < 0.05$

Serum total bilirubin concentration and the activity of ALP, AST, ALT, GGT of the alcoholic subjects were significantly higher ($p < 0.05$) compared with the control. Furthermore, the parameters were significantly higher ($p < 0.05$) in alcoholics who showed preference for distilled spirit compared with those who showed preference for beer and palmwine. Also the parameters were significantly higher ($p < 0.05$) in alcoholics who showed preference for beer compared with those who showed preference for palmwine.

Appendix VII: Shows the results (mean $\pm$ standard deviation) of kidney function tests of the control group and the three groups of alcoholic subjects who showed preference for palmwine, beer and distilled spirit (ogogoro)

	No of Subjects	Urine total concentration (mg/dl)	Urine specific gravity	Serum urea (mg/dl)	Serum creatinine (mg/dl)	Urine creatinine (mg/dl)	Creatinine clearance (ml/min)	Serum Electrolytes		p < Value
								K ⁺ (mEq/l)	Na ⁺ (mEq/l)	
Control (Non-Consumers of alcohol)	40.00	56.40 $\pm$ 0.40	1.021 $\pm$ 0.001	18.88 $\pm$ 0.31	0.86 $\pm$ 0.04	13.17 $\pm$ 1.28	129.23 $\pm$ 0.66	4.20 $\pm$ 0.30	137.00 $\pm$ 2.00	p < 0.05
Alcoholics who showed preference for palmwine	60.00	151.34 $\pm$ 0.60	1.036 $\pm$ 0.004	57.30 $\pm$ 2.80	3.14 $\pm$ 0.21	85.94 $\pm$ 5.34	44.23 $\pm$ 4.10	3.30 $\pm$ 0.10	131.00 $\pm$ 1.00	p < 0.05
Alcoholics who showed preference for beer	60.00	152.74 $\pm$ 0.42	1.037 $\pm$ 0.004	62.49 $\pm$ 1.50	3.46 $\pm$ 0.29	83.59 $\pm$ 14.24	37.46 $\pm$ 1.50	3.10 $\pm$ 0.20	129.00 $\pm$ 1.50	p < 0.05
Alcoholics who showed preference for distilled spirit (ogogoro)	60.00	153.96 $\pm$ 0.43	1.039 $\pm$ 0.001	64.00 $\pm$ 1.70	3.71 $\pm$ 0.17	80.44 $\pm$ 14.20	31.28 $\pm$ 3.80	2.00 $\pm$ 0.10	118.00 $\pm$ 2.00	p < 0.05

Appendix VIII: Summary table showing the mean and standard deviation of the body mass index (BMI) and the blood pressure of the control and alcoholic subjects who showed preference for palmwine, beer and distilled spirit

	No of Subjects	Body Mass Index (BMI) (Kg/m ²)	Systolic blood pressure (mmHg)	Diastolic blood pressure (mmHg)	Ratio of systolic and diastolic blood pressure (mm/Hg)
Control (Non-Consumers of alcohol)	40.00	24.00 ± 2.00	122.00 ± 9.00	80.50 ± 6.00	$\frac{122}{80}$
Alcoholics who showed preference for palmwine	60.00	23.00 ± 1.50	124.00 ± 12.00	83.00 ± 6.00	$\frac{124}{83}$
Alcoholics who showed preference for beer	60.00	21.00 ± 2.00	123.00 ± 12.00	82.00 ± 7.00	$\frac{123}{82}$
Alcoholics who showed preference for distilled spirit (ogogoro)	60.00	20.00 ± 2.00	125.00 ± 11.00	82.00 ± 9.00	$\frac{125}{82}$

Table 3 shows that the blood pressure of both the control and the alcoholic subjects were normal ($< \frac{140}{90}$ mmHg). The body mass index (BMI) of the alcoholic subjects was significantly lower ($p < 0.05$) compared with the control. The BMI of alcoholics who showed preference for beer was significantly lower ($p < 0.05$) compared with those who showed preference for palmwine. Also, the BMI of alcoholics who showed preference for distilled spirit was the lowest ($p < 0.05$).