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Project on: The Histological Effect of Alcohol on the Liver and kidney of Animals

A project paper submitted to Addis Ababa University, College of Health Sciences, School of Medicine, post Graduate studies, Department of Anatomy, in partial fulfillment for the degree of Master of Science in Human Anatomy.

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List of abbreviation

ACC	Acetyl-CoA carboxylase
ACR	Albumin creatinine ratio
ADH	Alcohol dehydrogenase
ALDH	Acetaldehyde deydrogenase
ALP	Alkaline phosphatases
ALT	Alanine aminotransferase
AST	Aspartate aminotransferase
BAC	Blood alcohol concentration
BMI	Body mass index
CAT	Catalase
CDNB	1-chloro-2,4-di-nitrobenene
CI	confidence interval
CKD	Chronic kidney disease
CYP2E1	Cytochrome P450 2E1
ESRD	End-stage renal disease
G6PD	Glucose-6-phosphate dehydrogenase
GFR	Glomerular filtration rate
GGT	Gamma glutamyltransferase
GPx	Glutathione peroxidase
GR	Glutathione reductase
GSH	Reduced glutathione
GSSG	Oxidized glutathione
GST	Glutathione s-transferase
H&E	Haematoxylin and eosin
HDL	High- density lipoprotein
IGF-I	Insulin-like growth factor
LSD	Least significant difference
MDA	Malondialdehyde
MDRD	Modification of Diet in Renal Disease
MEOS	Microsomal ethanol-oxidizing system
MnSOD	Manganese-superoxide dismutase
NADPH	reduced nicotinamide adenine dinucleotide phosphate
NEAFAs	Nonesterified fatty acids
OFIJ	*Opuntia ficus indica f. inermis* fruit juice
OR	odds ratio
RER	Rough endoplasmic reticulum
ROS	Reactive oxygen species
SD	Standard deviation
SOD	Superoxide dismutase
SPSS	Statistical Package for Social Sciences
TCA-TBA-HCl	Trichloroacetic acid-thiobarbituric acid-hydrochloric acid
TGF	Transforming growth factor
TNF-α	Tumor necrosis factor
VEGF	Vascular endothelial growth factor
x^2	Pearson’s Chi squared
XOD	Xanthine oxidase
ZT	Zeitgeber time

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Summary

Alcohol consumption represents the third largest risk factor for disease burden in most countries of the world. Alcohol can damage nearly every organ and system in the body such as liver and kidney. Alcohol is eliminated from the body by various metabolic mechanisms. The primary enzymes involved in these metabolic mechanisms are aldehyde dehydrogenase (ALDH), alcohol dehydrogenase (ADH), cytochrome P450 (CYP2E1), and catalase. Variations in the genes for these enzymes have been found to influence alcohol consumption, alcohol-related tissue damage, and alcohol dependence. The consequences of alcohol metabolism include oxygen deficits (i.e., hypoxia) in the liver; interaction between alcohol metabolism by products and other cell components, resulting in the formation of harmful compounds (i.e., adducts); formation of highly reactive oxygen-containing molecules (i.e., reactive oxygen species) that can damage other cell components; tissue damage; fetal damage; cancer; and medication interactions. The effects of alcohol on various tissues depend on its concentration in the blood i.e. blood alcohol concentration over time. Blood alcohol concentration (BAC) is determined by how quickly alcohol is absorbed, distributed, metabolized, and excreted. BAC is influenced by the rate of alcohol drinking, the presence of food in the stomach, and the type of alcoholic beverage, variations in the principal alcohol-metabolizing enzymes namely alcohol dehydrogenase and aldehyde dehydrogenase. Alcohol readily diffuses across membranes and distributes through all cells and tissues, and at these concentrations, it can acutely affect cell function by interacting with certain proteins and cell membranes. Alcohol metabolism also results in the generation of acetaldehyde that may contribute to tissue damage, the formation of damaging molecules known as ROS, and a change in the redox state of liver cells. Understanding the balance of alcohol's removal and the accumulation of potentially damaging metabolic byproducts, as well as how alcohol metabolism affects other metabolic pathways, is essential for appreciating both the short-term and long-term effects of the body's response to alcohol intake (Zakhari, 2006).

Key word: Alcohol, Liver, Kidney, Necrosis and Degenerative change

1. INTRODUCTION

1.1. Back ground

The consumption of alcohol causes a number of health related diseases (such as kidney and liver diseases) and other social costs. Alcohol consumption represents the third largest risk factor for disease burden in high-income countries, behind only smoking and hypertension, both of which are associated with alcohol misuse (WHO, 2004). Ethanol has varied effects on many systems of the body (Klassen and Persaud, 1978) and is toxic at higher dose level or at moderate dose level when used for long periods. The major toxic metabolites of ethanol are acetaldehyde and free radicals (Suzuki and Cherian, 2000). Acute alcohol abuse causes damage to and functional impairment of several organs affecting protein, carbohydrate, and fat metabolism and results in metabolic disturbance (Salvador and Alfredo, 2010).

Toxicity of alcohol consumption in the body involves the main organs of excretion and metabolism, especially the liver (Saalu *et al.,* 2012). Prolonged alcohol abuse has harmful effects on the liver and kidneys (Epstein, 1997).

Alcohol causes three types of liver injury: fatty liver (or steatosis) which is characterized by the accumulation of triglyceride in hepatocytes , alcoholic hepatitis which is characterized histologically by steatosis, the presence of hepatocyte injuryand pericellular fibrosis and cirrhosis which is characterized histologically by collagen deposition in a perisinusoidal distribution enveloping hepatocytes (Barclay *et al*., 2008). People who continue abusing alcohol may develop more serious liver damages like fibrosis, cirrhosis, and even liver failure (Albano, 2008).

Liver plays a major role in metabolism and has a number of functions in the body, including glycogen storage, plasma protein synthesis, and production of bile and detoxification of toxic substances (Gartner and Hiatt, 2000). The Kidney has many functions such as the removal of waste products and regulation of the amount of fluid and electrolytes balance in the body. Since the liver and kidneys are involved in the performance varied functions, they may be susceptible to injury particularly in situation of toxicity (Katzung, 1998).

Alcohol toxicity is most common in liver due to the fact that approximately 80% of ingested alcohol is metabolized in the liver by a process that generates many reactive oxygen species (Venkatraman *et al.*, 2004 and Tuma and Casey, 2003). Hepatic oxidative stress due to chronic alcohol consumption decreases anti- oxidant capacity of the liver which can damage the cell membranes and organelles with the release of reactive aldehydes (Chambers *et al.*, 1987).

For many countries the average level of alcohol consumption has exceeded a few drinks per day. Therefore, the negative effects may outweigh the beneficial effects and is usually regarded as the leading cause of liver cirrhosis (Gutjahr *et al*., 2001). Chronic ethanol ingestion induces changes in intestinal brush-border membrane (Bikle *et al*., 1986) and serum electrolytes (Bashir and Javed, 2005).

Chronic ethanol administration ameliorated and/or delayed the development of nephrotic syndrome in adriamycin nephropathy in rats (Tesar *et al*., 1995). Fatty liver, characterized by hyper accumulation of lipids in the liver, is often caused by excessive alcohol intake and patients with fatty liver are more susceptible to fibrotic liver diseases (Nanji and Zakim, 1996; Teli *et al.*, 1995).

Acetaldehyde, oxidative stress, hypoxia, immune response and membrane alterations are the factors that mediate the occurrence of ALD (Tuma and Casey, 2003; Diehl, 2002).

Alcohol is a diuretic, meaning that it causes water to be lost from the body through the kidneys, which can lead to dehydration (Noth and Swislocki, 2001). Alcohol can cause the loss of important minerals and salts from the body such as magnesium, calcium, phosphate, sodium and potassium (Fingerhood, 2007; Vonghia *et al*., 2008). Acute and chronic alcohol consumption does alter renal function, fluid flow and electrolyte balances (Epstein, 1997 and Chung, 2005). Oxidative stress may alter the structure and function of the glomerulus on mesangial and endothelial cells (Klahr, 1997). Long-term alcohol misuse is associated with water and salt retention, causing an expanded extracellular volume (Vamvakas *et al*., 1998).

1.2 General Overview of Alcohol

Alcohol (ethanol or ethyl alcohol) is the ingredient found in beer, wine and spirits which cause drunkenness and is formed when yeast ferments (breaks down without oxygen) the sugars in different foods **(**Centers for Disease Control and Prevention, 2010).

When alcohol is drunk faster than the liver can break it down, it caused higher BAC. The higher the BAC, the greater the effects on the body. The BAC level is influenced by**:** the ability of the liver to metabolize alcohol (Zakhari, 2006), the presence or absence of food in the stomach, the concentration of alcohol in the beverage, how quickly alcohol is drunk (Lohr, 2005, Diamond and Jay, 2000), body type, age and sex (Bennion and Li, 1976; Kopun and Propping, 1977)**.**
A constant amount of alcohol is eliminated per hour and this amount is not affected by the blood alcohol concentration (BAC) (Zeigler *et al.*, 2005).

1.3. Pharmacology of Alcohol

Alcohol can react with different medicines and drugs in different ways, such as increasing the sedating effect of sleeping tablets and opiate-based pain relief, increasing the potential for aspirin to irritate the stomach or increasing the potential of paracetamol to damage the liver.

Chronic and/or heavy episodic drinking activates the liver enzymes that are involved in breaking down prescription medicines, which can lead to these medicines being metabolized faster than usual and being less effective (Zakhari, 2006).

1.4. Mechanisms of action of Alcohol

Most of the alcohol a person drinks is eventually broken down by the liver. However, some products generated during alcohol metabolism (e.g., acetaldehyde) are more toxic than alcohol itself. In addition, a group of metabolic products called free radicals can damage liver cells and promote inflammation, impairing vital functions such as energy production. The body's natural defenses against free radicals (e.g., antioxidants) can be inhibited by alcohol consumption, leading to increased liver damage (Kurose, 1996). Acetaldehyde and reactive oxygen species (ROS) damages macromolecules in the cell, including lipids, proteins and DNA **(**Wu and Cederbaum, 2003). Chronic ethanol exposure increased ROS production which in turn decreased antioxidant defenses and enhanced the fatty acid oxidation by kidney peroxisome (Witek *et al*., 1999).

Oxidative stress and ROS-mediated toxicity have been implicated as the primary routes to alcohol-induced kidney injury (Rodrigo *et al.*, 2002 and Novitskiy *et al.*, 2006). Ethanol induces CYP2E1 which metabolizes and activates many toxicological substrates, such as ethanol, to more reactive toxic products (Lu and Cederbaum, 2008). CYP2E1 has been suggested to be a major contributor to ethanol-induced liver injury.

1.5. Distribution and metabolism of Alcohol

After alcohol is swallowed, it is absorbed primarily from the small intestine and stomach and from there it is carried to the liver, where it is exposed to enzymes and metabolized (Zakhari, 2006). Most (90 percent) of the metabolism of alcohol from a toxic substance to water and carbon dioxide is performed by the liver (Lohr, 2005) and the rest of alcohol excreted through the lungs, the kidneys and in sweat (Schuckit, 2005). The liver breaks down alcohol so that it can be eliminated from our body. However, when it is over consumed than the liver can process, the resulting imbalance can injure the liver by interfering its normal breakdown of proteins, fats, and carbohydrates (American Liver Foundation, 2012).

Alcohol is primarily metabolized by the successive oxidative activities of alcohol dehydrogenase (ADH) and ALDH (acetaldehyde dehydrogenase), (Lumeng and Crabb, 2001). The metabolization of ethanol mainly took place in the hepatocytes in three different sites: cytosol, endoplasmic reticulum and peroxisome while the acetaldehyde is metabolized in mitochondria (De Minicis and Brenner, 2006). Severely toxic manifestations are produced by the microsomal ethanol-oxidizing system (MEOS) in endoplasmic reticulum involving an ethanol-inducible CYP2E1 in which the oxidation of ethanol to acetaldehyde and acetate also leads to generation of ROS (Lieber, 1997).

In general, alcohol metabolism is achieved by both oxidative and nonoxidative pathways. Alcohol oxidation generates a highly reduced cytosolic environment in liver cells (i.e., hepatocytes) and these reactions leave the liver cells in a state that is particularly vulnerable to damage from the byproducts of ethanol metabolism, such as free radicals and acetaldehyde (Zakhari, 2006).

1.6. Efficacy of Alcohol

Moderate ethanol exposure can provide protection for kidneys against ischemia reperfusion-induced renal injury by enhancing antioxidant capacity characterized by higher activity of superoxide dismutase, which is a critical enzyme responsible for detoxifying ROS (Yuan *et al.*, 2011). Moderate drinking is usually defined as no more than two drinks in a given day for men and one drink per day for women who are not pregnant or trying to conceive.

According to Snow (2009) research, it was showed that healthy people who drink moderate amounts of alcohol may have a lower risk of developing coronary heart disease than nondrinkers. Drinking moderately can protect heart from clogged arteries; build up of fat in arteries and narrowing of the coronary arteries due to excess fat. It can raise the levels of HDL—or “good” cholesterol—in the blood. Drinking moderately also may help keep blood pressure levels in check. These benefits may not apply to people with existing medical conditions, or who regularly take certain medications. In addition, researchers discourage people from beginning to drink just for the health benefits (NIAAA, 2002).

1.7. Side effects of Alcohol

According to Marian (2012) alcohol affects the entire body, for the brain, liver, pancreas, lungs, kidneys, and every other organ and tissue system by invading within minutes after it passes into the blood stream. Effects of alcohol on the liver are: Impairs liver’s ability to filter waste and other toxins out of the bloodstream while it is processing the alcohol, can cause fatty liver, alcoholic hepatitis, cirrhosis of the liver, and increased risk of liver cancer and liver irritation which results in liver taking a longer time heal causing more liver damage (Julien, 2001).

One of the dangers of alcohol is that it can result in the breaking of capillaries and blood vessels. This can lead to hemorrhage, and even death. It can also cause anemia, decreased resistance to infection, and increase weight gain.

1.8. Histology of liver and kidney

1.8.1. Histology of Liver

The liver is our greatest chemical factory, it builds complex molecules from simple substances absorbed from the digestive tract, it neutralizes toxins, it manufactures bile which aids fat digestion and removes toxins through the bowels (Maton *et al.,* 1993).

The ability of the liver to perform its functions is often compromised by numerous substances such as alcohol. These substances include certain medicinal agents which when alcohol is taken in over doses and sometimes when introduced within therapeutic ranges injures the liver (Gagliano *et al*., 2007). Microscopic structure of the liver consists of various structures such as; lobules, sinusoids, portal area, bile ducts and central vein

1. 8.2. Histology of Kidney

Each kidney has a concave medial border, the hilum where nerves enter, blood and lymph vessels enter and exit, and the ureter exits and a convex lateral surface. The renal pelvis, the expanded upper end of the ureter, is divided into two or three major calyces. Each kidney is composed of nephrons. Each nephron consists of a dilated portion, the renal corpuscle; the proximal convoluted tubule; the thin and thick limbs of Henle's loop; the distal convoluted tubule; and the collecting tubules and ducts.

The Kidney is has many functions such as the removal of waste products from the blood and regulation of the amount of fluid and electrolytes balance in the body. The kidney is often involved in the development, maintenance and counter regulation of complex electrolyte disturbances (Heidland *et al*., 1985). Chronic ethanol ingestion itself is not nephrotoxic (Majumdar *et al*., 1982). regular alcohol consumption raises the blood pressure, which itself is a risk factor for renal damage (Heidland *et al.*, 1985).

Large amounts of ethanol have deleterious effects on the kidney. Structural and functional abnormalities of the kidney are reported with increasing exposure to ethanol in children who have been prenatally exposed(Cecchin and De Marchi, 1996).

2. REVIEW AND ANALYSIS OF PUBLISHED RESEARCH PAPERS ON THE HISTOLOGICAL EFFECT OF ALCOHOL ON LIVER AND KIDNEY

Different researches concerning the effect of alcohol on the histology of liver and kidney have been conducted by different researchers. All of the researches reviewed in this project paper were regarding the toxicological effect of alcohol on histology of liver and kidney.

In a study conducted by Das and Vasudevan (2005), the effect of ethanol on liver antioxidant defense systems was investigated. In this study, ten week old male albino rats of wistar strain, weighing 100-120g were used. The rats were housed in plastic cages under conventional conditions (inside a well-ventilated room). The room temperature was maintained at 22 ± 2°C with a 12-h natural light/dark cycle. All rats had free access to a standard diet containing proteins, carbohydrates, fat and adequate vitamin mixture. The rats were assigned to five groups where each group contains six rats. Group 1: control rats which were fed normal diet and water; Group 2: ethanol treated rats (0.8g ethanol/kg body weight/day for 4 weeks); Group 3: ethanol treated rats (1.2g ethanol/kg body weight/day for 4 weeks); Group 4: ethanol treated rats (1.6g ethanol/kg body weight/day for 4 weeks) and Group 5: ethanol treated rats (2g ethanol/kg body weight/day for 4 weeks). Ethanol was diluted with distilled water to get desired concentration and fed orally. The animals were weighed weekly and their general condition and behavior were recorded including their daily intake of liquid. At the end of the experimental period, the animals were sacrificed by cervical dislocation**.** The liver was dissected out and cleaned with ice-cold saline, blotted dry. The liver homogenates were used for the estimation of tissue protein, ascorbic acid content, lipid peroxidation, reduced glutathione content, catalase, glutathione reductase, glutathione S-transferase, glutathione peroxidase, superoxide dismutase and glucose-6-phosphate dehydrogenase.

Statistical significance was determined by Student's't' test for unpaired data. The level of significance was set at $p<0.05$.

The result of this study indicates that animals exposed to 0.8g ethanol/kg body weight/day for four weeks showed highest increase in body weight (69%), which is even greater than the control animals (54%). However, the liver weights of animals exposed to 1.6 and 2 g alcohol /kg body weight/day) for 4 weeks, were found to be reduced (23% and 18% respectively).Thus, rats treated with lower ethanol concentration showed gain in body and liver weight compared to those of control group while rats treated with higher concentration of ethanol showed lesser gain in body and liver weight. Significant decrease in ascorbic acid and GSH contents of liver was found in groups treated with 1.2, 1.6, 2g of ethanol when compared with the control group. However, the thiobarbituric acid reactive substances (TBARS) level of liver was increased in the groups consuming higher concentration of ethanol (i.e. groups treated with 1.2, 1.6 and 2g of ethanol) in comparison to control group as shown in table 1.

Table 1. Effect of alcohol on ascorbic acid content, thiobarbituric acid reactive substances (TBARS) and glutathione (GSH) levels in liver homogenate (Das and Vasudevan, 2005)

Group	Ascorbic acid (mg/g tissue)	TBARS [a]	GSH (µg/ mg tissue)
Group 1	2.87 ±0.18	0.652 ±0.015	3.82 ±0.23
Group 2	2.82 ±0.24	0.698 ±0.021	3.23 ±0.34
Group 3	2.69 ±0.62	0.783 ±0.013 *	2.65 ±0.13*
Group 4	2.53 ±0.43	0.872 ±0.023*	2.26 ±0.18*
Group 5	2.32 ±0.16*	0.883 ±0.034*	2.12 ±0.21*

a: *µmol MDA formed/min/100 mg tissue.*

** Indicates significant difference at $p < 0.05$ when compared to normal controls.*

In ethanol treated groups, the superoxide dismutase activity was found to be significantly higher compared to the control group. Interestingly, the activity of catalase was increased in lower concentration (groups treated with 0.8g and1.2g) of ethanol, but it was significantly decreased in higher concentration (groups treated with 1.6g and 2g) of ethanol compared to the control group.

The glucose-6-phosphate dehydrogenase activity was found to be unaltered in all the ethanol treated groups (table 2).

Table 2. Effect of alcohol on catalase, superoxide dismutase, and glucose 6- phosphate dehydrogenase activity in liver homogenate (Das and Vasudevan**,** 2005)

Group	Catalase[a]	Superoxide dismutase **[b]**	Glucose 6-phosphate dehydrogenase **[c]**
Group 1	38.76 ± 0.66	6.33± 0.15	8.42 ± 0.23
Group 2	43.24 ± 0.78 *	7.13 ± 0.34*	8.34 ± 0.46
Group 3	40.67 ±1.13	8.34± 0.45*	8.31± 0.32
Group 4	32.63± 0.83*	9.07± 0.21*	8.29± 0.36
Group 5	31.24± 0.72 *	9.80 ± 0.47*	8.30± 0.24

a*: µmole* $\mathbf{H_2 0_2}$ *decomposed / min/mg protein;*

b*: U/mg protein;*

c*: nmol/mg protein.*

******* *Indicates significant difference at* $p < 0.05$ *when compared to normal controls*

Ethanol exposure caused significant decreases in the glutathione reductase and glutathione peroxidase activities of liver while glutathione S-transferase activities of liver of all the ethanol exposed groups were found to be significantly increased compared to control group (Table 3).

Table 3. Effects of alcohol on glutathione reductase, glutathione peroxidase, and glutathione s-transferase activity in liver homogenate (Das and Vasudevan, 2005).

Group	Glutathione reductase^{a}	Glutathione peroxidase ^{b}	Glutathione S-transferase^{c}
Group 1	53.86 ± 1.34	80.46 ± 2.46	6.94 ± 0.11
Group 2	43.24 ± 0.46*	74.43 ± 1.21*	8.21 ± 0.38*
Group 3	34.45 ± 1.69*	68.73 ± 2.46*	10.94 ± 0.27*
Group 4	27.76 ± 0.84*	59.24± 1.58*	13.93 ± 0.31*
Group 5	24.45± 2.23*	53.93 ± 0.69*	14.59 ± 0.46*

a: *nmol NADPH breakdown/min/mg protein;*

b: *nmol NADPH breakdown/mini mg protein;*

c: *µmole CDNB conjugate formed / min/mg protein*

** Indicates $p < 0.05$ when compared with normal healthy control.*

Another study conducted by Das *et al*. (2009) investigated the effects of chronic ethanol consumption on liver and kidney of Wistar rat. In this study, six male Albino Wistar rats that were 16-18 weeks-old and weighing 200- 220 g were used. The animals were housed in plastic cages inside a well-ventilated room. The room was maintained under standard husbandry condition. All rats had free access of standard diet. The animals were weighed daily and its general condition was recorded including their daily intake of liquid. The treatment group received1.6 g ethanol/ kg body weight/ day. Ethanol was diluted with distilled water to get the desired concentration and was administered orally. Blood was collected from retero-orbital plexus of animals prior to start the ethanol feeding (0 week), and at the end of 4, 12 and 36 weeks of ethanol treatment. Serum was separated then total protein and creatinine in serum and ascorbic acid in plasma were estimated. Enzyme activities such as ALT, AST, ALP and GGT were also monitored. Reduced glutathione content, lipid peroxidation and activities of catalase, glutathione reductase, glutathione S-transferase, glutathione peroxidase and superoxide dismutase activity were estimated. All data were analyzed using the SPSS (version 11). Results are expressed as mean ± SD (standard deviation). The sources of variation for multiple comparisons were assessed by the analysis of variance (ANOVA), followed by Post Hoc test. The differences were considered significant at $P<0.05$.

The result indicates that urea and creatinine levels were elevated significantly after 12 weeks of ethanol exposure compared to the control group (Table 4). Serum protein level was decreased significantly in the treatment group compared to the control group (Table 4).

Table 4: Effects of ethanol on protein, urea and creatinine levels in serum of rats (Das *et al.*, 2009).

	Protein (g %)	Urea (mg %)	Creatinine (mg %)
Control	7.33 ± 0.15	30.0 ± 1.51	0.43 ± 0.05
4 weeks	6.55 ± 0.31a	30.8 ± 1.44	0.49 ± 0.02
12 weeks	6.18 ± 0.28a	39.3 ± 4.08bf	0.51 ± 0.02c
24 weeks	6.06 ± 0.17af	44.8 ± 5.2ad	0.57 ± 0.04af
36 weeks	5.91 ± 0.25ae	54.5 ± 6.0adgk	0.71 ± 0.05adgj
F value	32.59	37.27	36.584
Significance	<0.001	<0.001	<0.001

P values:

a* = *p *< 0.001 compared to control group*

b*= *p *< 0.01 compared to control group*

c* = *p *< 0.05 compared to control group*

d* =*p *< 0.001 compared to 4 weeks ethanol treated group*

e* =*p *< 0.01 compared to 4 weeks ethanol treated group*

f* =*p *< 0.05 compared to 4 weeks ethanol treated group*

g* = *p *< 0.001 compared to 12 weeks ethanol treated group*

j* =*p *< 0.001 compared to 24 weeks ethanol treated group*

k* = *p *< 0.01 compared to 24 weeks ethanol treated group*

Activities of liver specific enzymes such as AST, ALT, ALP and GGT increased significantly in response to duration of ethanol exposure (table 5). Compared to the control group, reduced glutathione content decreased significantly after 12 weeks of ethanol exposure, thiobarbituric acid reactive substances (TBARS) level elevated significantly after 4 weeks of ethanol exposure, GPx activity reduced significantly after 36 weeks of ethanol exposure, catalase activity reduced significantly due to ethanol exposure, superoxide dismutase (SOD) activity elevated significantly up to 12 weeks of ethanol exposure and then decreased after 36 weeks of ethanol exposure and 12 weeks of ethanol exposure caused significant change in GR and GST activities; (Table 6).

Table 5: Effect of ethanol on liver specific enzymes activities (IU/L) in serum of rats (Das *et al.*, 2009).

	AST	ALT	GGT	ALP
Control	29.63 ± 2.26	33.57 ± 1.88	7.67 ± 0.82	89.72 ± 6.49
4 weeks	57.83 ± 5.64b	53.66 ± 5.27c	39.5 ± 7.69a	118.5 ± 7.34a
12 weeks	143.66 ± 17.31ad	111 ± 6.84ad	91.83 ± 10.46ad	139.17± 11.44af
24 weeks	164.83 ± 34.36ad	138.66 ± 16.05adhl	105.17 ± 12.89ad	154.67± 14.26ad
36 weeks	231.83 ± 23.45adgj	209.17 ±19.29adgj	123.83 ±16.8adi	180.83± 8.82adgk
F value	97.759	208.282	113.718	71.052
Significance	<0.001	<0.001	<0.001	<0.001

IU/L: *international units per liter*

P values:

a*= *p *< 0.001 compared to control group*

b*= *p *< 0.01compared to control group*

c* =*p *< 0.05 compared to control group*

d* =*p *< 0.001 compared to 4 weeks ethanol treated group*

e* =*p *< 0.01 compared to 4 weeks ethanol treated group*

f* =*p *< 0.05 compared to 4 weeks ethanol treated group*

g*= *p *< 0.001, compared to 12 weeks ethanol treated group*

h*= *p *< 0.01, compared to 12 weeks ethanol treated group*

I* =*p *< 0.05 compared to 12 weeks ethanol treated group*

j *< 0.001 compared to 24 weeks ethanol treated group*

k *< 0.01 compared to 24 weeks ethanol treated group*

l *< 0.05 compared to 24 weeks ethanol treated group*

Table 6. Effect of ethanol on reduced glutathione (GSH) content, thiobarbituric acid reactive substance (TBARS) level, glutathione peroxidase (GPx) activity, glutathione reductase (GR) activity, glutathione s-transferase (GST) activity, catalase activity and superoxide dismutase (SOD) activity in hemolysate of rats **(**Das *et al.*, 2009).

Parameters	Control	Exposed rats				F value	Significance
		4 weeks	12 weeks	24 weeks	36 weeks		
TBARS (nmol/ ml)	0.638 ± 0.012	0.926 ± 0.022a	1.016 ± 0.1a	1.05 ± 0.094af	1.288 ± 0.066adgj	68.942	<0.001
GSH (mg / ml)	40.95 ± 4.15	37.57 ± 4.03	33.18 ± 3.4bf	30.05 ± 1.64	27.88 ± 2.77ah	15.525	<0.001
GPx (U/ g haemoglobin)	33.33 ± 6.25	29.83 ± 3.43	27.5 ± 3.78	25.5 ± 2.88	22.67 ± 2.16bf	6.384	0.001
GR (nmol NADPH oxidized/min/ mg protein)	1.7 ± 0.14	1.62 ± 0.12	1.47 ± 0.09c	1.37 ± 0.07	1.32 ± 0.1ae	13.22l	<0.001
GST (nmol CDNB conjugate formed/ mg protein/ min)	1.16 ± 0.07	1.22 ± 0.12	1.37 ± 0.12c i	1.38 ± 0.09	1.5 ± 0.14ae	8.746	<0.001
Catalase (mmol H2O2 decomposed/ mg protein / min)	38.76 ± 0.56	32.63 ± 0.81a	30.22 ± 2.47a	29.76 ± 1.28a	27.91 ± 3.52ae	25.158	<0.001
SOD (U/mg haemoglobin)	6.33 ± 0.15	9.07 ± 0.21a	9.15 ± 0.69a	7.47 ± 0.73bde	6.08 ± 0.19dgek	57.184	<0.001

P values:

a= p*< 0.001 compared to control group*

b= p*< 0.01compared to control group*

c =p*< 0.05 compared to control group*

d =p*< 0.001 compared to 4 weeks ethanol treated group*

e =p*< 0.01 compared to 4 weeks ethanol treated group*

f =p*< 0.05 compared to 4 weeks ethanol treated group*

g =p*< 0.001 compared to 12 weeks ethanol treated group*

h= p*< 0.01 compared to 12 weeks ethanol treated group*

i =p*< 0.05 compared to 12 weeks ethanol treated group*

j =p*< 0.001, compared to 24 weeks ethanol treated group*

k= p*< 0.01 compared to 24 weeks ethanol treated group*

l =p*< 0.05 compared to 24 weeks ethanol treated group*

Ethanol exposure decreased ascorbic acid content, significantly elevated serum TGF-β1 level, however, no significant change in VEGF-C level after 12 weeks of ethanol exposure compared to the control group in a time dependent manner (Table 7).

Table 7: Effect of ethanol on ascorbic acid in plasma, TGF-β1 and VEGF-C levels in serum of rats (Das *et al*., 2009).

	Ascorbic acid (mg/dl)	VEGF-C (pg/ ml)	TGF-β1 (pg/ ml)
Control	2.2 ± 0.14	22.9 ± 3.55	15.8 ± 3.32
4 weeks	1.9 ± 0.18c	25.8 ± 3.31	18.6 ± 2.16
12 weeks	1.7 ± 0.14a	26.6 ± 3.72	26.3 ± 3.5ae
24 weeks	1.7 ± 0.07a	28.8 ± 7.36	37.8 ± 4.91adg
36 weeks	1.6 ± 0.14af	29 ± 5.58	41.5 ± 2.88adg
F value	17.209	1.52	65.646
Significance	<0.001	0.227	<0.001

P values:

***a =p**< 0.001 compared to control group*

***c= p**< 0.05 compared to control group*

***d =p**< 0.001 compared to 4 weeks ethanol treated group*

***e= p**< 0.01 compared to 4 weeks ethanol treated group*

***f =p**< 0.05 compared to 4 weeks ethanol treated group*

***g= p**< 0.001 compared to 12 weeks ethanol treated group.*

Another study under taken by Fromenty *et al*. (2009) on the effects of chronic ethanol consumption reported a decrease in body Weight, liver triglycerides and diabetes in obese mice. In this study, sixteen 7-week-old male obese mice, weighing 35 to 43 g and sixteen 7-week-old male lean mice, weighing 21 to 25 g were purchased from Janvier (Le-Genest-St-Isle, France). Mice were accommodated in an animal care facility accredited by the French veterinary authorities. Mice were fed on a normal diet bringing 2820 kcal/kg food. After 1 week of acclimatization, the groups of lean and ob/ob mice were further split into two subgroups eight animals each as follow: group1: those lean mice treated with water. group2: those lean mice treated with ethanol solution. group3: those obese mice treated with water. group4: those obese mice treated with ethanol solution for 6 months. The daily ethanol consumption was 3 g/kg body weight for the first 10 days. Thereafter, the daily intake of ethanol was regularly incremented by 1 g/kg to finally reach 21 g/kg after 6 months. To achieve this progressive augmentation, the percentage of ethanol was adjusted in the drinking water every 10 days taking into account the body weight and the liquid intake of each mouse.

Consumption of food and liquid was evaluated twice a week, whereas body weight was measured every week. The liquid intake remained stable during the experiment in lean mice but not in obese mice. Thus, the percentage of ethanol in the drinking water was 1.5% for both genotypes at the beginning of the treatment (corresponding to 3 g/kg); it was 15 and 30% for lean and obese mice, respectively, to reach 21 g/kg in both genotypes.

At the end of the treatment, all mice were sacrificed by cervical dislocation. The liver was quickly removed and immediately frozen in liquid nitrogen. Blood was collected from the retro-orbitary sinus each month to monitor alanine aminotransferase (ALT) activity and for the measurement of plasma parameters before the sacrifice. Sample of blood was also taken one day before the initiation of the treatment to measure plasma glucose and insulin. Plasma ALT and aspartate aminotransferase (AST) activities, glucose, nonesterified fatty acids (NEFAs) and total antioxidant status were measured on an automatic analyzer. Liver specimens were fixed with 10% neutral formalin and embedded in paraffin. Sections were stained with hematoxylin and eosin. Steatosis was evaluated as the percentage of hepatocytes containing vacuoles of fat on 10 different fields at 200x magnification. Reduced glutathione levels and the activities of glutathione transferase, glutathione peroxidase, and glutathione reductase, CYP2E1and aconitase, an enzyme highly sensitive to oxidative damage and located both in cytosol and mitochondria were assessed in liver homogenates. Finally, hepatic oxidative stress and apoptosis were measured in lean and obese mice.

The values are expressed as means ± standard error of the mean (S.E.M). ANOVA was performed to assess statistical significances. When the ANOVA indicated a significant interaction between factors, individual means were compared with least significant difference (LSD) post hoc test. In experiments with only two sets of data, the Student's *t* test was used.

The result indicated that Plasma ALT of the liver was higher in obese mice compared with their lean counterparts. However, ethanol administration did not change ALT values in both groups of mice (Figure.1).

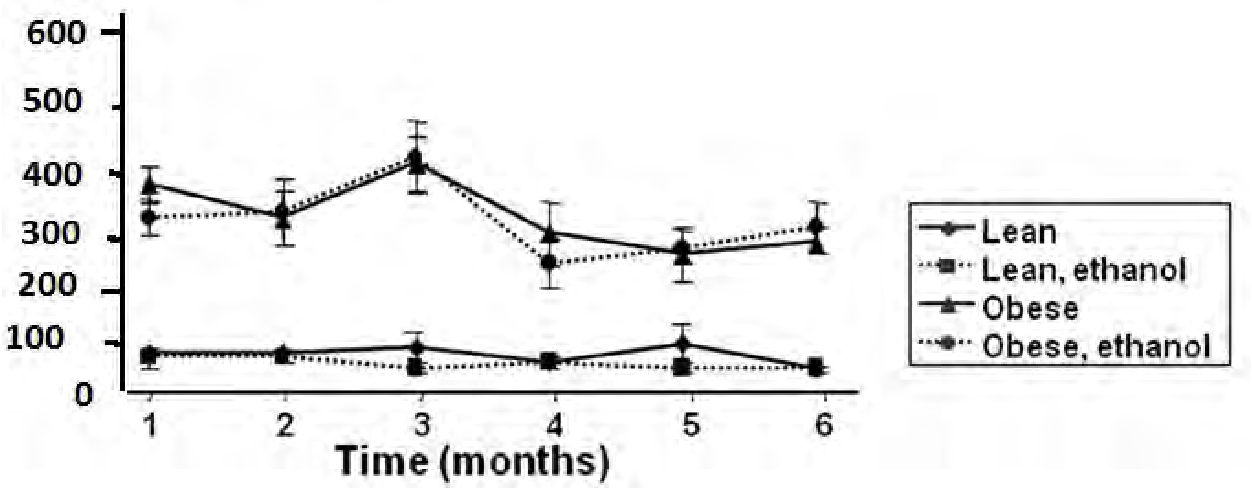

Figure 1. Monthly monitoring of plasma ALT, plasma ALT (mean ±S.E.M. for six to eight mice) (Fromenty *et al*. 2009).

Body weight was also monitored throughout the 6-month period of treatment. Ethanol consumption did not change body weight in lean mice. But, after 6 months body weight gain was 9.9 ± 0.70 and 9.3 ± 1.2 g in naive lean and ethanol-treated lean mice respectively. In contrast, ethanol intake significantly decreased by 43% the gain of body weight in obese mice as it was 26.2 ± 0.8 and 14.8 ± 0.5 g in naive and ethanol-treated obese mice respectively. Hence, at the end of the treatment, naive and ethanol-treated ob/ob mice weighted 65.5 ± 1.3 and 52.8 ± 1.0 g, respectively. The significant reduction of body weight gain in ethanol treated obese mice was observed after 6 weeks.

Before the initiation of ethanol administration, the initial levels of plasma glucose were significantly augmented in ob/ob mice compared with lean mice (Figure. 2); however, plasma levels of glucose after 6 months were lower in naïve obese mice. In contrast, plasma levels of glucose after 6 months were increased in the naive lean mice compared with the initial levels (Figure. 2). Consequently, plasma glucose after 6 months was significantly lower in ob/ob mice compared with their lean counterparts (Figure. 2).

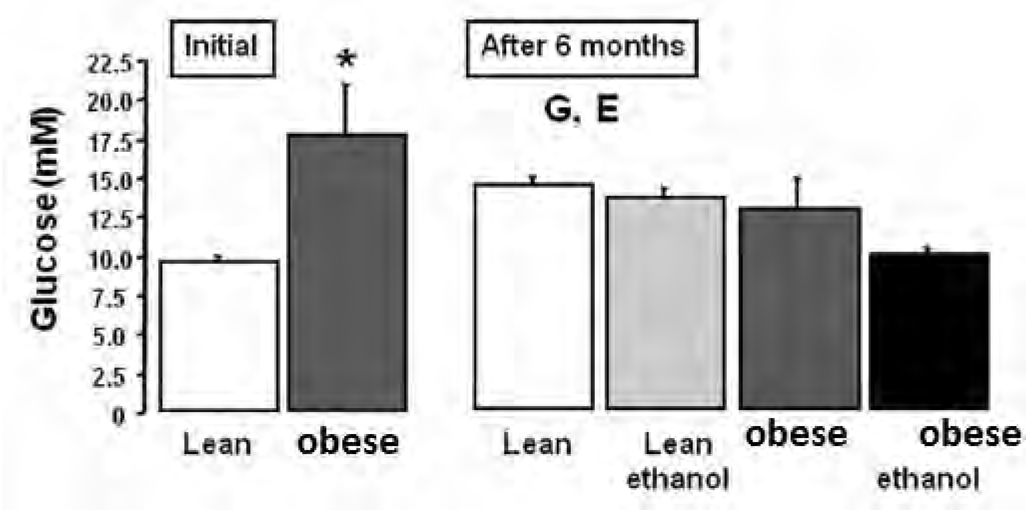

Figure 2. Plasma glucose before the onset of the experiment (initial) and after 6 months of water or ethanol drinking (Fromenty et al. 2009).

- *G = effect of the genotype*
- *E= effect of ethanol*
- *GxE = effect of interaction between genotype and ethanol*
- ** indicates significant difference at P < 0.05 when compared to lean mice*
- *† indicates significant difference at P < 0.05 when compared to naive mice*

Initial plasma levels of insulin were dramatically increased in obese mice, however, after 6 months, plasma insulin was less elevated in obese mice, but it was considerably reduced in ethanol-treated obese mice (Figure. 3).

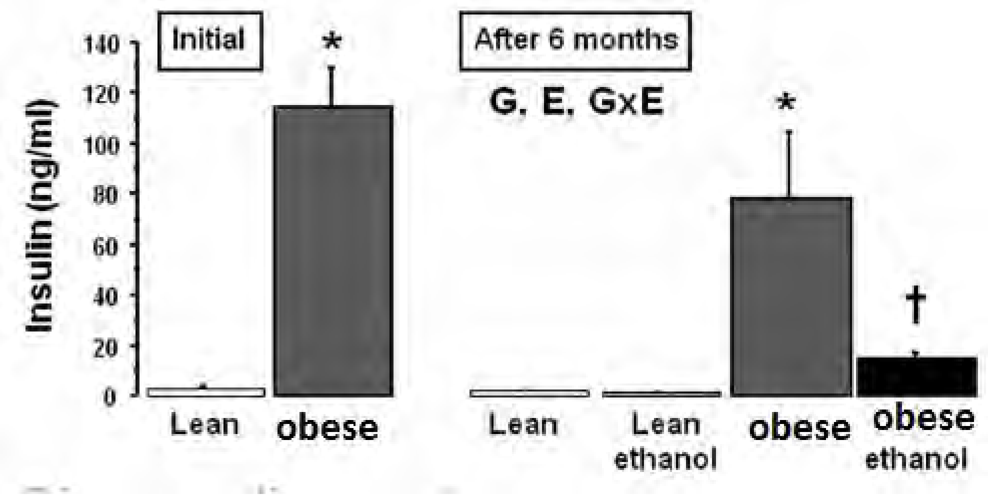

Figure 3. Plasma insulin before the onset of the experiment (initial) and after 6 months of water or ethanol drinking (Fromenty *et al*., 2009).

- ✓ *G = an effect of the genotype*
- ✓ *E = an effect of ethanol treatment or*
- ✓ *GxE= an interaction between genotype and ethanol*
- ✓ ** indicates significant difference at P < 0.05 when compared to lean mice*
- ✓ *† indicates significant difference at P < 0.05 when compared to naive mice*

Plasma TNF-α level was similar between obese and lean mice and ethanol administration did not modify TNF-α (table 8). Plasma ALT, AST and antioxidant status were significantly increased in obese mice compared with lean mice, but ethanol did not change these parameters (Table 8). Higher plasma antioxidant status in ob/ob mice compared with lean mice was observed (Table 8). Plasma levels of NEFAs were higher in ob/ob mice, and ethanol intake further increased NEFAs in obese animals (table 8), whereas ethanol intake only enhanced plasma glycerol in lean mice. Ethanol administration significantly lowered IGF-I in both lean and obese mice (Table 8).

Table 8: Plasma parameters in lean and ob/ob mice after 6 months of water or ethanol drinking (Fromenty *et al*. 2009).

	Lean	Lean, Ethanol	Obese	Obese, Ethanol	Factor***a***
Ethanol (g/l)	N.D	. 0.27 ± 0.06	N.D.	0.35 ± 0.04	
AST (UI/l)	94 ± 13	115 ± 15	234 ± 51	291 ± 33	G
ALT (UI/l)	40 ±8	39 ± 8	280 ± 24	303± 46	G
TNF-α(pg/ml)	11.1 ± 0.8	10.0 ± 0.9	9.6 ± 0.7	9.7 ± 0.7	
NEFAs (mM)	0.39 ± 0.05	0.36 ± 0. 04	0.45 ± 0.01	0.71± 0.08*†	G, E, GxE
Antioxidant status (mM)	0.93 ± 0.07	0.85 ± 0.08	1.36 ± 0.20	1.37 ± 0.16	G
IGF-I (ng/ml)	294 ± 28	213 ± 25†	395 ± 51*	167± 18†	E, GxE

N.D*. not determined*

a G*= effect of genotype*

a E*= effect of ethanol administration*

GxE*= interaction between genotype and ethanol administration.*

***** *= Significantly different from lean mice (P< 0.05).*

† = Significantly different from naïve mice (P < 0.05).

*†= Statistical differences between groups

Liver weight was dramatically increased in ob/ob mice compared with lean mice. Ethanol administration was associated with a significant reduction of liver weight only in obese mice. The liver/body weight ratio remained virtually unchanged in the obese animals because body weight was also reduced by ethanol in obese mice.

A histological examination of the livers was subsequently performed in 12 lean mice (including six naive and six ethanol-treated animals) and 14 obese mice (including six naive and eight ethanol-treated animals). In lean mice, ethanol administration did not induce significant histological changes such as necroinflammation while in both naive and ethanol-treated ob/ob mice, steatosis involved a majority (>80%) of the hepatocytes, reflecting massive fatty liver. In naive ob/ob mice, steatosis was both macrovacuolar and microvesicular but was predominantly microvesicular (Figure. 4). In contrast, in ethanol-treated obese animals, hepatic steatosis was both macrovacuolar and microvesicular but was predominantly macrovacuolar (Figure. 5).

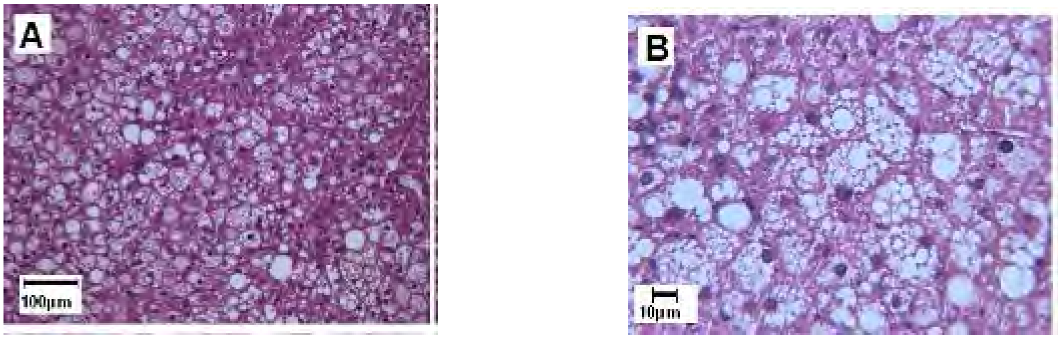

Figure 4. Evaluation of steatosis in naive obese mice (Fromenty *et al.*, 2009).

A. representative pictures of a naive obese mouse liver with predominant microvesicular steatosis at 100x magnifications.

B. representative pictures of a naive obese mouse liver with predominant microvesicular steatosis and 250x magnification. Scales are indicated on the pictures.

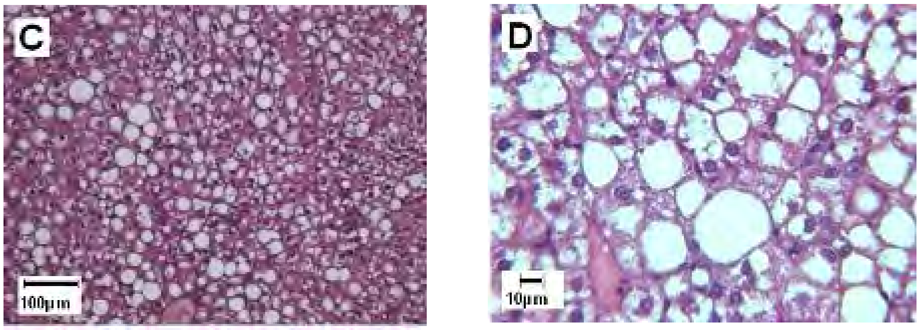

Figure 5. Evaluation of steatosis in intoxicated obese mice (Fromenty *et al.,* 2009).

C. representative picture of an ethanol-treated obese mouse liver with predominant macrovacuolar steatosis at 100 x magnification

D. representative picture of an ethanol-treated obese mouse liver with predominant macrovacuolar steatosis at 250x magnification.

Ethanol intake was associated with increased CYP2E1 activity, MnSOD protein, reduced the activities of glutathione peroxidase and aconitase but did not affect to glutathione reductase and glutathione transferase (Table 9). Both aconitase and glutathione peroxidase can be inactivated by reactive oxygen species and free radicals**.**

Table 9: Hepatic oxidative stress and antioxidant enzymes in lean and ob/ob mice after 6 months of water or ethanol drinking (Fromenty *et al.*, 2009).

	Lean	Lean, Ethanol	Obese	Obese, Ethanol	Factor*a*
CYP2E1	100.0 ± 6.9	155.6± 9.1	103.8 ± 11.5	146.3± 3.8	E
MnSOD	100.0 ± 6.0	175.8 ± 8.1	120.5 ± 4.7	166.0 ± 7.7	E
Aconitase (mU/mg protein)	8.55 ± 0.42	7.82± 0.56	7.2 ± 0.68	4.90± 0.17	G,E
Glutathione (nmol/mg protein)	57.3 ± 6.9	41.3± 3.4	78.3 ± 4.9	74.5 ± 4.5	G
Glutathione peroxidase (pmol/min/mg protein)	320 ± 16	287 ± 13	301 ± 13	215± 12	G,E
Glutathione reductase (pmol/min/mg protein)	57.1 ± 4.4	54.8 ± 2.6	59.3± 2.0	54.8 ± 2.0	
Glutathione transferase (µmol/min/mg protein)	1.74 ± 0.12	1.50± 0.09	1.62± 0.14	1.37± 0.22	

a G= effect of genotype

a E= effect of ethanol administration

In a study carried out by Shibata *et al*. (2009), the effect of chronic ethanol exposure on the liver of *Clock*-mutant mice was investigated. In this study, eight- to ten-week-old female wild-type and *Clock mutant* mice were used. The mice were grouped into two groups where each group contains 16 mice. During the ethanol treatment, wild-type and *Clock* mutant mice were given water or 15% ethanol for 8 weeks and then mice were sacrificed at ZT 0, 6, 12, or 18. Zeitgeber time (ZT) 0 and 12 were the lights on and lights-off times, respectively. The mice were maintained on a light-dark cycle and room temperature of 23 ± 1°C and provided with a standard diet.

Blood samples from each mouse were collected from the orbital sinus and centrifuged and sample of the serum from each mouse was used to obtain triglyceride content, cholesterol content, free fatty acid content, alanine aminotransferase, aspartate aminotransferase content, and ethanol content.

The accumulation of lipids was examined using the oil red O stain method in order to elucidate whether or not ethanol led to higher lipid accumulation in the liver of *Clock*-mutant mice compared to wild-type mice.

Mouse livers were fixed with 10% formalin and sliced in 10 μm sections with a cryostat. Next, the livers were washed in 60% isopropyl alcohol for 1 min, and stained by oil red O for 10 min at 37°C. Thereafter, slices were fractionated with 60% isopropyl alcohol for 2 min and stained with hematoxylin for 5 min. After the 2 min wash, slices were colored with lithium carbonate for 30 sec and cover slipped. The values are expressed as means ± standard error of the mean. For statistical analysis, one-way or two-way ANOVA were applied

The results of this research indicated that chronic ethanol consumption increased liver weight in Clock-mutant mice ($P < 0.05$) while wild-type mice did not exhibit a change in liver weight. Ethanol treatment did not alter levels of AST or ALT in either genotype (Table 10). These results demonstrate that the livers of *Clock*-mutant mice exhibit a weight increase under conditions of ethanol exposure that do not impact wild-type mice. Ethanol intake increased triglyceride content in the liver of both genotypes ($P< 0.01$). Moreover, the ethanol-induced increase in liver triglyceride content was significantly higher in *Clock*-mutant mice compared with wild-type ($P < 0.01$).

Table 10: Impact of ethanol exposure on wild-type and *Clock*-mutant mice (Shibata *et al.*, 2009).

	Control	Wild-type Ethanol	Control	*Clock*-mutant Ethanol
Ethanol Intake (g/kg/day)	na	30.5 ± 2.0	na	27.2 ± 1.6
Body Weight (g)	32.3 ± 0.6	32.3 ± 0.6	33.0 ± 0.5	31.9 ± 0.5
Serum TG (mg/dl)	68 ± 6.4	84 ± 8.5	62 ± 4.8	71 ± 4.9
Serum CH (mg/dl)	64.2 ± 4.7	60.2 ± 4.8	64.5 ± 3.0	62.2 ± 2.9
Serum FFA (mEq/l)	0.50 ± 0.03	0.53 ± 0.03	0.52 ± 0.03	0.46 ± 0.02
Serum ALT (Karmen)	28.0 ± 0.7	26.1 ± 0.5	30.1 ± 1.3	27.2 ± 0.3
Serum AST (Karmen)	75.0 ± 6.5	61.8 ± 2.9	62.8 ± 4.2	59.6 ± 2.3

TG*= triglycerides*

CH*= cholesterol*

FFA*= free fatty acids*

ALT*=alanine aminotransferase*

AST*= aspartate aminotransferase*

Na*= not available*

Histological analysis indicated that the liver tissue from the ethanol treated mice exhibited greater staining by oil red O. This increase in staining is consistent with lipid accumulation in ethanol treated mice and this lipid accumulation is greater in *Clock*-mutant mice than in wild-type mice. In contrast to the data from the liver, ethanol exposure did not alter measured levels of triglyceride or cholesterol in the serum of mice from either genotype (Table 10). Chronic ethanol altered the expression of genes involved in lipid metabolism in Clock-mutant mice.

A prospective study was conducted by Knight *et al.* (2003) to determine if there was an association between moderate alcohol consumption and rate of decline in renal function in 1990, 1994 and 1998. In this prospective study 1658 female nurses who were 30–55 years old, living in the USA were involved.

The creatinine measurements used to estimate renal function were initially obtained as part of a study designed to assess the impact of analgesic use on renal function. Participants were asked about alcohol consumption during the previous year, specifically, about average use of beer, wine and liquor over the past year. Total daily alcohol intake was calculated by adding the usual daily intake of alcohol. Daily alcohol intake was classified as none, 0.1–4.9, 5–14.9 and 15–59.9 g/day. Renal function was estimated using creatinine values from blood samples drawn and stored at –130ºC in 1989 and 2000. All samples were analysed at the same time in 2001 at Boston Children’s Hospital. Assessment of other factors also took place; such as Age, race, body mass index, diabetes, hypertension, smoking, analgesic medication use and antihypertensive medication use were initially included as potentially important confounders.

Statistical analyses were conducted using the mean, median and SD. All analyses were performed using SAS software (version 6.12).

The result of this study indicated in age adjusted analyses, average quantity of daily alcohol intake was not significantly associated with decline in renal function (Table 11). In multivariate analyses, with different cut points of estimated GFR, no statistically significant association between alcohol intake and decline in estimated renal function observed (Table 11) and the addition of potential confounders did not substantially affect the age adjusted point estimates over the 11 year period.

Table 11: Age-adjusted and multivariate ORs with 95% CIs for the association between daily alcohol intake and different cut points of decline in estimated GFR from 1989 to 2000 (Knight *et al.*, 2003).

Daily alcohol quantity	Decline in GFR≥ 20% (n=440/1658)		Decline in GFR ≥25% (n=287/1658)		Decline in GFR ≥30% (n=177/1658)	
	Age-adjusted OR	Multivariate ORa	Age-adjusted OR	Multivariate ORa	Age-adjusted OR	Multivariate ORa
None (n=659)	1.00 (referent)	1.00 (referent)	1.00 (referent)	1.00 (referent)	1.00 (referent)	1.00 (referent)
0.1–4.9 g (n=532)	0.80 (0.62–1.04)	0.83 (0.63–1.07)	0.94 (0.70–1.27)	0.98 (0.72–1.32)	0.92 (0.64–1.32)	0.94 (0.65–1.36)
5–14.9 g (n=303)	0.81 (0.60–1.11)	0.86 (0.63–1.19)	0.79 (0.54–1.14)	0.83 (0.56–1.21)	0.72 (0.45–1.15)	0.74 (0.45–1.19)
≥15 and <60g (n=164)	0.78 (0.53–1.16)	0.84 (0.56–1.26)	0.79 (0.49–1.26)	0.81 (0.50–1.31)	0.94 (0.55–1.62)	0.92 (0.52–1.62)

The protective effect of *Opuntia ficus indica f. inermis* fruit juice (OFIJ) against ethanol-induced renal dysfunction in rats was studied by Alimi *et al.* (2013). In this study, two months old male Wistar rats, weighing about 120 – 140 g were used. These rats were purchased from Pasteur institute (Tunisia) and maintained for two weeks adaptation period under the same conditions of temperature (22 ± 2 °C) and a 12 h light/dark cycle. After the adaptation period, animals were divided into controlled and experimental groups and treated for 12 weeks with distilled water and 3 g/kg b.w of ethanol respectively. At the end of experimental period animals were sacrificed by cervical decapitation under light ether anesthesia. Kidney samples were quickly removed, washed in ice-cold 1.15% KCl solution, homogenized into 2 ml ice-cold lyses buffer (pH 7.4) and centrifuged for 20 min at 3000 × *g*, 4 °C.

The serum creatinine, urea and GGT activities as well as urinary glucose level were assayed spectrophotometrically using BioMaghreb diagnostic kits. In serum and kidney homogenate the level of lipid peroxidation was measured as malondialdehyde content (MDA).

In kidney homogenate total GSH contents were measured. SOD activity was estimated. CAT activity was determined by measuring hydrogen peroxide decomposition at 240 nm. GSH-Px activity was assayed by the subsequent oxidation of NADPH at 240 nm. In serum and kidney homogenate the levels of ascorbic acid (vitamin C) concentration was measured and vitamin E level was determined. Serum, urinary and kidney homogenate protein contents were determined according to Lowry's method (Lowry OH *et al.*,1951).

Kidney tissues were cut into about 5-cm-thick slices and fixed with 10% phosphate-buffered formalin (pH 7.4). Tissue sections of 5–8 µm were made using microtome, and stained with hematoxylin-eosin solutions (H&E). All the values have been reported as means ± S D and were analyzed statistically by one way ANOVA and different group means were compared by Duncan's multiple range tests; $p < 0.05$ was considered significant in all cases. SPSS version 12.0 was used for data analysis.

The result of this study indicated that the exposure of Wistar rats to chronic ethanol intoxication (twelve weeks), significantly ($p < 0.01$) increased serum creatinine and urea levels as well as the activity of GGT when compared with control rats (Table 12).

Table 12. Effects of ethanol treatments on serum creatinin, urea and GGT contents of the control and experimental Wistar rats (Alimi *et al.*, 2013).

Groups	Serum		
	Creatinie^1	Urea^1	GGT^2
Control	0.42 ± 0.01^{aa}	33.61 ± 0.71^{aa}	2.08 ± 0.14^{aa}
Ethanol	0.76 ± 0.09^{bb}	51.82 ± 0.8^{bb}	6.32 ± 0.29^{bb}

Values are expressed as means ± SD, for eight rats in each group.^{1}mg/dl, ^{2}IU/l.

$^{aa=}$ *indicates significant difference at $p < 0.01$ when compared with ethanol group*

$^{bb=}$ *indicates significant difference at $p < 0.01$ when compared with control group*

^{1}= *mg/dl*

^{2}= *IU/l*

Chronic ethanol administration was found to cause a significant decrease ($p < 0.01$) in the levels of serum and kidney vitamin E, vitamin C and a significant increase ($p < 0.01$) in the level of malondialdehyde (MDA) content when compared with the control group(Table 13).

Table 13. Effects of ethanol treatments on vitamin E, vitamin C and MDA contents in serum and kidney of the control and experimental rats (Alimi *et al.,* 2013).

	Vitamin E		Vitamin C		MDA	
Groups	Serum[1]	Kidney[2]	Serum[1]	Kidney[2]	Serum[1]	Kidney[2]
Control	2.3 ± 0.42[a]	18.34 ± 0.83[a]	2.34±0.12[a]	163.41±0.18[a]	1.2±0.03[a]	0.6±0.02[a]
Ethanol	0.79 ± 0.18[bb]	11.3 ± 0.73[bb]	0.96±0.02[bb]	135.1±0.82[bb]	3.5±0.12[bb]	1.6±0.08[bb]

***a** = indicates significant difference at p < 0.01 when compared with ethanol group*

***b** = indicates significant difference at p < 0.05 when compared with control group*

***bb** = indicates significant difference at p < 0.01 when compared with control group.*

[1]= mg/dl

[2]= µg/g tissue

[3]= nmol/mg protein.

Chronic ethanol administration was found to cause a significant decrease (p < 0.01) in renal GSH level and the activities of antioxidant enzymes namely superoxide dismutase (SOD), catalase (CAT) and gluthatione peroxidase when compared with control group (Table 14).

Table 14. Effects of ethanol treatments on renal GSH content and the activities of antioxidant enzymes (Alimi et al., 2013).

Groups	GSH[1]	SOD [2]	CAT[3]	GSH-Px4
Control	0.21±0.04a	7 8.12 ± 0.43a	21.3±0.6a	2 2.4±0.8a
Ethanol	0.11±0.02bb	5 1.6 ± 0.91bb	15.61±0.2bb	1 4.09±1.2bb

a= indicates significant difference at p < 0.01 when compared to ethanol treated group

b= indicates significant difference at p < 0.05 when compared to controls

bb= indicates significant difference at p < 0.01 when compared to controls

[1]= mmol GSH/mg protein

[2]= Units/mg protein

[3]= µmol of H_2O_2consumed/min/mg protein

[4]= nmol of GSH oxidized/min/mg protein

SOD= superoxide dismutase

CAT= catalase

GSH-Px= glutathione peroxidase.

Ethanol treatment significantly ($p < 0.01$) increased urinary glucose and protein levels respectively by 61% and 176% (Figure 6).

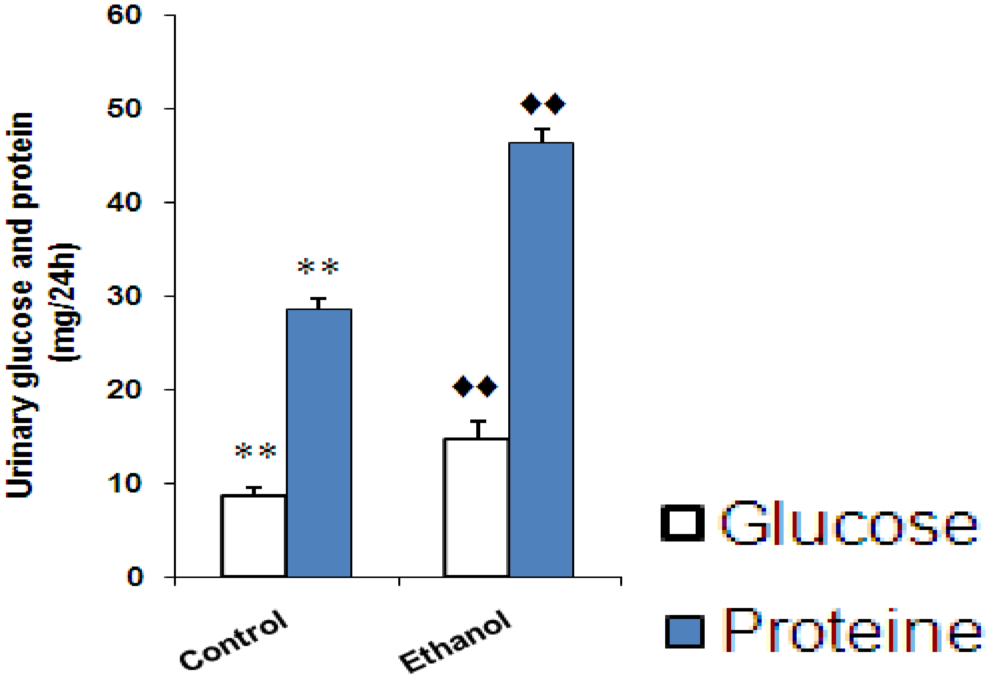

Figure 6. Effect of ethanol treatment on serum protein and glucose levels **(**Alimi *et al.*, 2013**)**.

*** indicates significant difference at p < 0.01versus ethanol group*

♦♦ *indicates significant difference at p < 0.01 versus control group*

.

Some of the histological observations in slides from ethanol-treated rats include severe congestion of blood vessels, Necrosis of the renal cell, severe degenerative changes in tubules and damaged glomeruli. Ethanol treated kidney showed that tubular and renal cells necrosis as well the congestion of blood vessels and damaged glomeruli.

Another study was conducted by Dinu *et al*. (2005) on the ethanol-induced alterations of the antioxidant defense system in rat kidney. This study was performed on thirty-two healthy male Wistar rats weighing between 140– 160 g. These rats were housed two per cage under controlled conditions of a 12 h light/dark cycle, 50% humidity and 24◦C.

Before the experiments, rats were monitored daily and had free access to water and standard pellet diet (10 g/100 g body weight/day).

After 1 week of adaptation, the animals were randomly divided into two groups of 16 each. Group 1, the control groups that receive water. Group 2, the ethanol-fed group, treated daily with 2 g/kg of ethanol as an aqueous solution, using an intragastric tube. After 10 weeks, eight rats of each group were killed by cervical decapitation under light ether anesthesia. The remained rats of each group were sacrificed in the same conditions after 30 weeks.

Both kidneys of each rat were quickly excised, cleared of adhering fat, rinsed with a cold 0.9% sodium chloride solution, and weighted. One kidney of each rat was immediately submerged in 7% perchloric acid and 2 mM phenanthroline and homogenized with a Potter–Elvejhem homogenizer at 0◦C until a uniform suspension was obtained. All deproteinized tissue homogenates were stored at −80◦C for assay of GSH. The other kidney was homogenized in demineralized water and stored at −80◦C until the analysis of enzymatic activities.

The activities of enzymes; CAT, SOD, total GPX, GST, GR, G6PD, GGT, and XOD were estimated. The ADH activity was assayed with a minor modification. The protein content was determined using the method of Lowry et al. (1951). The levels of lipid peroxidation were measured via the thiobarbituric acid color reaction. Level of MDA was measured. GSH level was determined and was expressed as nmol per mg protein. GSSG content was determined and was calculated as nmol per mg protein.

All values were expressed as means±SEM. The differences between control and ethanol-treated groups were compared by Student’s *t*-test using standard statistical packages. The results were considered significant only if the *P* value was less than 0.05.

This finding indicate that the ethanol treated animals exhibited an increase in the kidney weight as compare to the control group. Thus, the kidneys weight of the 30 weeks ethanol- treated rats was 40% greater ($P < 0.05$) than the corresponding control group (Table 15). However, the increase in the kidney weight cannot have a pathological meaning, as the body weight of the animals expanded during the experiment, whereas the relative kidney weight did not undergo significant modification (Table 15). The relative kidney weight is derived as the ratio between the kidney weight and body weight (Table 15). The protein content of the kidney, expressed as mg/g wet tissue, remained unmodified after exposure of the rats to ethanol (Table 15).

Table 15. Effect of chronic ethanol consumption on the weight of Rat Kidneys and their protein content (Dinu *et al.*, 2005).

	10 Weeks Experiment		30 Weeks Experiment	
	Control	Ethanol	Control	Ethanol
Kidneys' weight (g)	0.73±0.01	0.79±0.06	0.80±0.05	1.12±0.09*
Protein (mg/g wet tissue)	38.38±1.32	38.26±1.31	37.24±1.58	37.19±1.32
Relative kidney weight (g/100 g body weight)	0.35±0.01	0.31±0.02	0.32±0.02	0.36±0.03

** indicates significant difference at P <0.05 vs. controls.*

There were no changes observed in the electrophoretical protein patterns after 10 weeks of ethanol consumption. However, for prolonged treatment (30 Weeks-ethanol consumption) certain changes were observed. The activity of kidney ADH showed a significant increase after ethanol treatment, with increases of 31.5 and 70.6% after 10 and 30 weeks of ethanol treatment, respectively (Figure 7).

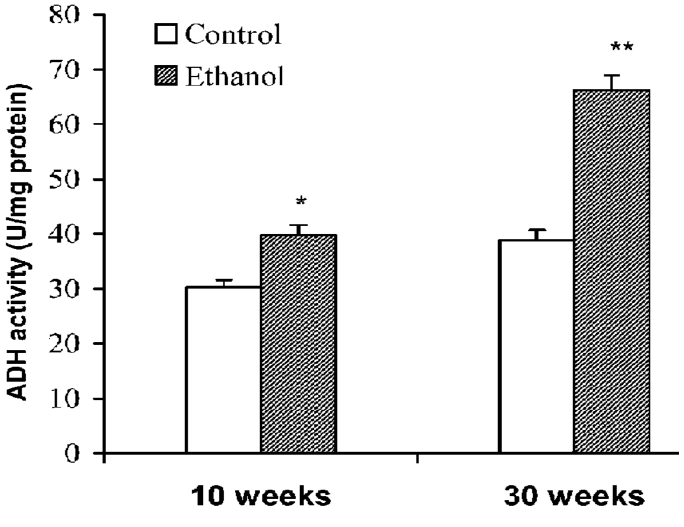

Figure 7. Effect of chronic ethanol consumption for 10 and 30 weeks on the activity of ADH in rat kidney (Dinu *et al.*, 2005).

** indicates significant difference at P<0.05 vs control*
*** indicates significant difference at P<0.01 vs ethanol*

The effects of ethanol treatments on XOD activity and the MDA levels were also investigated (Figure 8& 9). The results, which are illustrated in Figure 8, indicate that XOD activity remained unaltered in kidney after ethanol consumption compared to controls (*P* >0.05).

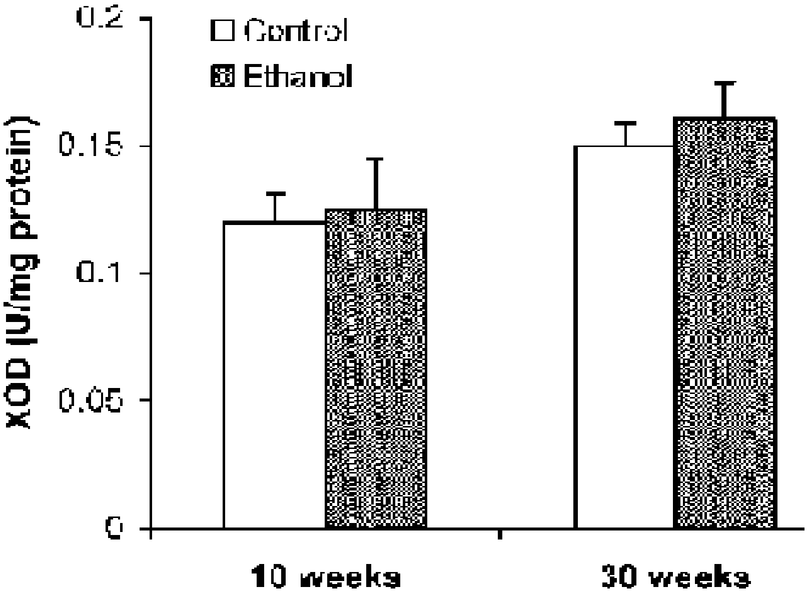

Figure 8. Effects of chronic ethanol consumption on the XOD activity in rat kidney (Dinu *et al*., 2005).

Figure 9 shows the MDA levels, a measure of lipid peroxidation, in controls and ethanol-treated rats. No important change in MDA level was observed in the rat kidney exposed to ethanol for 10 and 30 weeks, respectively versus controls (*P* >0.05).

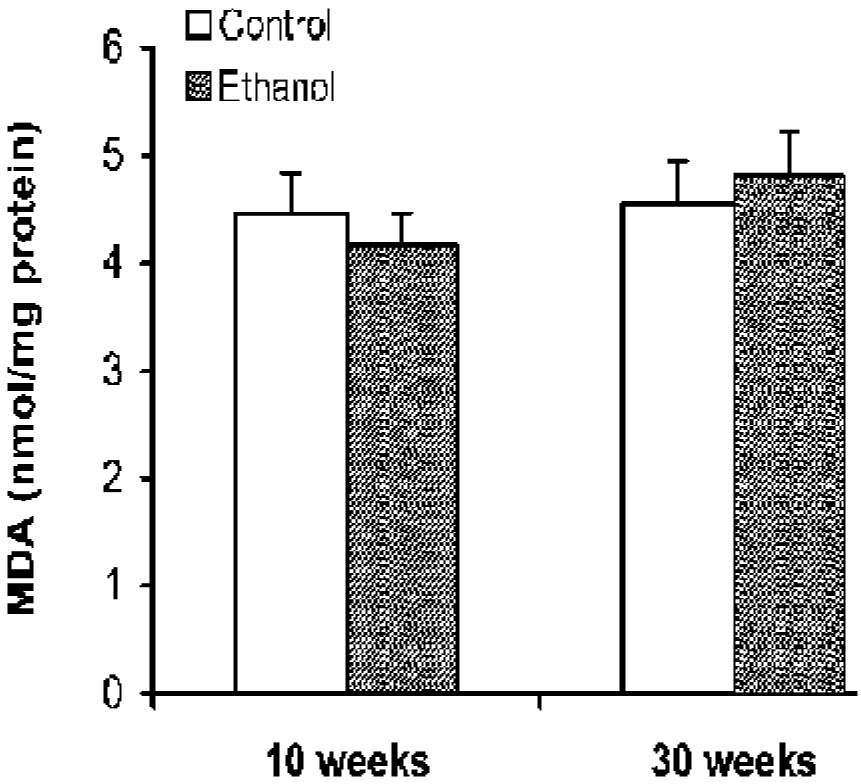

Figure 9.Effects of chronic ethanol consumption on the MDA levels (lipid peroxidation level) in rat kidney (Dinu *et al*., 2005).

The kidneys of ethanol-treated rats exhibited decreased levels of GSH and GSH/GSSG ratio. GSH was diminished by 32.7% after 10 weeks treatment and by 10.6% after 30 weeks treatment. The dramatic fall of the GSH/GSSG ratio recorded after 10 weeks of treatment may be correlated both with the decrease of the GSH level and the increase of the GSSG level (Table 16).

Table 16. Glutathione concentrations in the Rat Kidney after chronic ethanol treatment for 10 and 30 Weeks (Dinu *et al*., 2005),

	10 Weeks Experiment		30 Weeks Experiment	
	Control	Ethanol	Control	Ethanol
GSH (nmol/mg protein)	90.32±8.31	60.79±5.98**	76.32±4.35	68.23±7.31*
GSSG (nmol/mg protein)	16.23±0.98	20.31±1.93*	23.25±2.41	25.18±2.12
GSH/GSSG ratio	5.52±0.43	2.89±0.16*	3.21±0.29	2.65±0.18*

** indicates significant difference at P <0.05 vs. control.*

*** indicates significant difference at P <0.01 vs. control.*

Table 17 shows the effects of chronic ethanol administration on the activities of antioxidant enzymes in rat kidney. The CAT activity increased by 11.8% and 32.4% after 10 and 30 weeks of treatment, respectively, whereas SOD activity showed no change. The activity of GPX was up-regulated by 26% after 10 weeks, and GST was increased by 31.6% after 30 weeks of ethanol consumption. GGT activity remained unmodified after 10 weeks of ethanol treatment, but a 41.7% increase in the activity was recorded after 30 weeks of ethanol treatment. Most antioxidant enzymes display an age-dependent decline in their catalytic activities in different tissues. This observation has determined to observe the differences between the level of the antioxidant enzyme activities of control animals for 10 and 30 weeks. Thus, with the exception of GPX, it can be stated that the antioxidant enzyme activities remained unchanged or decreased in older-control group. The most striking modification was recorded for CAT and GGT, for which we found only 87.5 and 58.7% in the older-control groups, as compared to younger-control groups.

Table 17. Effects of chronic ethanol consumption for 10 and 30Weeks on the activities of antioxidant Enzymes in Rat Kidney[a] (Dinu *et al*., 2005).

	10 Weeks Experiment		30 Weeks Experiment	
Enzyme	Control	Ethanol	Control	Ethanol
SOD	2.71±0.11	2.66±0.24	2.52±0.13	2.55±0.17
CAT	210.30±7.27	235.18±17.79*	184.03±5.61$^{\Delta}$	243.60±7.38**
GPX	33.41±1.69	42.11±6.47*	40.25±1.75	44.36±1.94
GST	133.12±4.51	125.3±3.31	123.21±4.12	162.10±14.10*
GGT	1.43±0.37	1.39±0.28	0.84±0.03Δ	1.19±0.10**

a =activities are expressed as U/mg protein.

** indicates significant difference at P <0.05, ethanol-treated vs. control.*

*** indicates significant difference at P <0.01, ethanol-treated vs. control.*

Δ *indicates significant difference at P<0.05, 30 weeks control vs. 10 weeks control.*

The activities of GR increased substantially in the kidney of ethanol-treated rats, and this modification is more evident after the administration of ethanol for a long period of time (Figure 10). Thus, after 10 weeks of ethanol consumption, the GR activity has increased by 46.7%, and after 30 weeks of treatment the GR activity has increased by more than 2-fold.

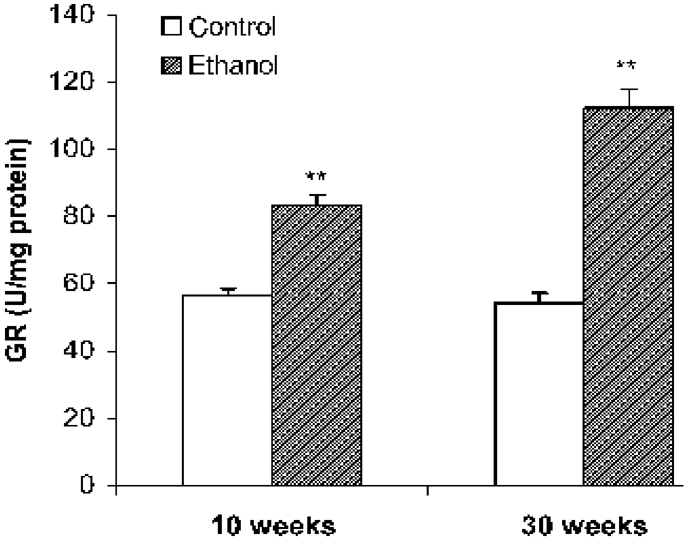

Figure 10. GR activity in kidneys of control and ethanol-fed rats for 10 and 30 weeks (Dinu *et al.*, 2005).

*** indicates significant difference at P <0.01 vs. control*

The activity of G6PD increased substantially in the kidney of ethanol-treated rats, and this modification is more evident after the administration of ethanol for a long period of time (Figure 11). Similarly, its activity level increased by 43.5% after 10 weeks of ethanol treatment, and the increase was 3-fold higher after 30 weeks of treatment, as compared to the control group of animals.

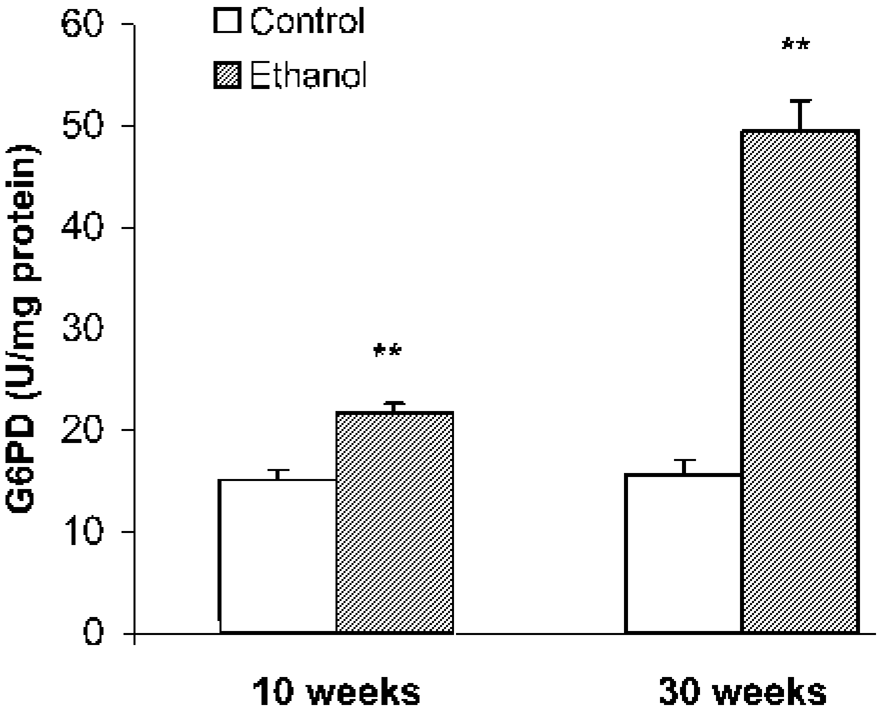

Figure 11. G6PD activity in kidneys of control and ethanol-fed rats for 10 and 30 weeks (Dinu *et al.*, 2005).

*** indicates significant difference at P <0.01 vs. control*

In a study conducted by Das *et al.* (2008) on the effects of chronic ethanol exposure on renal function tests and oxidative stress in kidney, sixteen to eighteen week old male albino rats of Wistar strain weighing 200- 220 g were used. The animals were housed in plastic cages inside a well-ventilated room. The room was maintained under standard husbandry condition. All rats had free access of standard diet. The animals were weighed daily and its general condition was recorded including their daily intake of liquid. The rats were divided into the 3 groups of 6 each. Group I: Control rats fed with normal diet and water; Group II treated with 1.6 g ethanol/ kg body weight/ day for 4 weeks; Group III treated with 1.6 g ethanol/ kg body weight/ day for 12 weeks.

Ethanol was diluted with distilled water to get desired concentration and was fed orally. At the end of the experimental period, the animals were sacrificed after over-night fast, by applying intra-peritoneal thiopentone (thiosol/Na+) (euthensia). The blood and the kidney were collected for investigation. Dissected kidney was cleaned with ice-cold saline, blotted dry, and immediately transferred to the ice chamber.

Various biochemical and oxidative stress related parameters were estimated. Serum was separated from blood and used for urea, creatinine and calcium estimation. Kidney was homogenized in 0.25 *M* sucrose solution, diluted with 0.9% saline, and the diluted samples were used for the estimation of tissue protein. The tissue (~100 mg) samples were homogenized in ice-cold 2 ml 0.1 M phosphate buffer (pH 7.4) and glutathione content was estimated. Lipid peroxidation of samples was measured using TCA-TBA-HCl. Activities of glutathione peroxidase (GPx), glutathione reductase (GR), glutathione s-transferase (GST), catalase (CAT) and superoxide dismutase (SOD) were determined. Histopathological examination was done from formaline fixed tissue samples using Haematoxylin and Eosin staining solutions.

All data were analyzed using the SPSS (version 11.0). The sources of variation for multiple comparisons were assessed by ANOVA, followed by Post Hoc test with Bonferroni's Multiple Comparisons test. The difference were considered significant at $P<0.05$.

The result of this study indicated that multiple effects of chronic ethanol consumption on the kidney function tests and its effects on oxidative stress related parameters in the kidney. No significant change was observed in relative weights of kidney (g/ 100g body weight) of different ethanol treated groups of rats compared to the control group and in serum calcium level in either of the group tested, however, urea and creatinine concentration in serum significantly elevated in rats exposed to ethanol (1.6 g / kg body wt/ day) for 12 weeks (Table 18).

Table 18: Effects of ethanol (1.6 g ethanol/ kg body weight/ day) on relative weight of kidney; and urea, creatinine and calcium concentration in serum (Das *et al.*, 2008).

Parameters	Control	4 weeks	12 weeks
Kidney wt. (g/ 100g body weight)	0.47 ± 0.003	0.46 ± 0.006	0.46 ± 0.006
Serum urea (mg/ dl)	32.55 ± 1.21	34.85±2.12	54.51 ± 2.45*#
Serum creatinine (mg/ dl)	0.52 ± 0.04	0.57 ± 0.04	0.72 ± 0.05*#
Serum calcium (mg/ dl)	8.45 ± 0.19	8.6 ± 0.1	8.63 ± 0.42

P value:

- *****indicates significant difference at p< 0.05 compared to Controls*
- *# indicates significant difference at p< 0.05 compared to 4 weeks treatment group.*

Reduced glutathione content, glutathione peroxidase level, activities of glutathione reductase, Catalase and Superoxide dismutase in the kidney homogenate significantly depleted after 12 weeks of exposure compared to the control group. However, 12 weeks of ethanol treatment significantly elevated thiobarbituric acid reactive substances, indicating that prolonged ethanol consumption increases lipid peroxidation (Table 19). No significant change in glutathione s-transferase level was observed in this study in ethanol treated rats compared to the control group (Table 19).

The histological changes in ethanol treated rats include, dilated tubules lined by thinner epithelium, widened capsular space, degeneration and necrosis of renal tubular epithelia. These damaging changes reflected toxic effects of ethanol with duration of exposure.

Table 19: Effect of ethanol (1.6 g ethanol/ kg body weight/ day) on reduced glutathione (GSH) content, thiobarbituric acid reactive substance (TBARS) content, glutathione peroxidase (GPx) activity, glutathione reductase (GR) activity, glutathione s-transferase (GST) activity, superoxide dismutase (SOD) activity and catalase activity (Das *et al.,* 2008).

Parameters	Control	4 weeks	12 weeks
GSH (mmol/ g)	2.24 ± 0.1	1.94 ± 0.07	1.68 ± 0.06*
TBARS (mmol MDA formed/ min/ 100 mg tissue)	0.427 ± 0.014	0.487 ± 0.014	0.582 ± 0.018*#
GPx (nmol NADPH breakdown/ min/ mg protein)	77.25 ± 2.39	72.51 ± 1.32	67.7 ± 1.31*
GR (nmol NADPH breakdown/ min/ mg protein)	48.33 ± 0.94	45.75 ± 0.83	39.62 ± 1.23*#
GST (mmol CDNB conjugate formed/ min/ mg protein)	6.44 ± 0.12	7.18 ± 0.52	7.58 ± 0.58
SOD (U/ mg protein)	6.22 ± 0.11	5.84 ± 0.38	5.19 ± 0.28*#
Catalase (mmol H2O2 utilized/ min/ mg protein)	42.67 ± 0.77	53.41 ± 1.95*	45.15 ± 4.92#

P value:

- **indicates significant difference at p< 0.05 compared to Controls*
- *# indicates significant difference at p< 0.05 compared to 4 weeks treatment group.*

In a study conducted by Van Thiel *et al.* (1977), the nephrotoxic effect of ethanol in the rat was investigated. In this study, forty male Wistar rats (24 days old) were used. The rats were obtained from Charles River Breeding Laboratories, North Wilmington. The rats were housed in individual metabolic cages and pair-fed a liquid diet containing alcohol or an identical liquid diet in which ethanol was isocaloricallv replaced with dextrose. The diet containing alcohol consisted of 5% ethanol by volume which accounted for 36% of the total caloric content. On average the animals ate 47±2.0 cm^3 diet/day. Animals were weighed and then sacrificed by exsanguination under light ether anesthesia.

The left kidney of each animal was weighed immediately after removal for determination of wet renal weight. The renal homogenates were extracted and total lipid, cholesterol, and phospholipid levels were analyzed. The right kidney of each animal was removed and bisected longitudinally in a frontal plane; half was then fixed in Bouin's solution for light microscopy and the other half was fixed in glutaraldehyde, osmiuim tetroxide for electron microscopic analysis. After fixation in Bouin's solution for 72 hours, the tissues were dehydrated in progressive ethanol solutions, embedded in paraffin, sectioned at 5µ and stained with hematoxylin and eosin for light microscopy. Blood alcohol content, serum electrolyte and urea nitrogen, renal protein and DNA content were determined utilizing the saline homogenates prepared from the freeze-dried left kidney. The glomeruli, proximal and distal tubules, loops of Henle, collecting ducts and the interstitial tissues for each animal were assessed.

Experimental results are expressed as the mean ± standard error. The P values were obtained by use of the Student t test for the difference between the two group means. Values were considered probably significant at $P < 0.05$ and significant at $P < 0.01$.

The result of this study indicated that the alcohol-fed animals consistently weighed less but had larger kidneys than the controls. In contrast to the greater wet renal weight, the dry renal weights did not differ between the two groups. When the dry renal weights were corrected for body weight, however, the alcohol-fed animals clearly had a greater renal mass per gram body weight than did the isocaloric controls. The serum creatinine levels of the alcohol-fed animals were 50% greater than those of the isocaloric controls. The alcohol-fed animals had lower blood glucose levels than did the isocaloric controls. However, no significant difference was observed for serum uric acid content or blood urea nitrogen between the alcohol-fed and the isocaloric control animals. Renal tissue protein, lipid, and DNA were increased significantly in the alcohol-fed animals compared to their isocaloric controls. In addition to the expected increase in absolute levels of triglyceride, the levels of the renal lipids were also markedly elevated.

Histological observed differences at the light microscopic level were the appearance of the renal arteries, renal veins and Proximal convoluted tubules of the alcohol-fed animals appeared normal. However, the distal tubules appeared dilated, with markedly flattened epithelial cells; a marked interstitial edema was present in the medulla, with wide separation of the individual renal tubules and a loose endematous appearance of the intervening connective tissue in alcohol-fed animals. Although the observed differences at the light microscopic level were not marked, it is apparent that the kidneys of the alcohol-fed animals have a different histologic appearance at the electron microscopic level compared to the appearance of kidneys from the isocaloric controls. These differences are especially striking in the renal cortex at the corticomedullarv junction; prominent differences involve the entire nephron, excluding the glomerulus, the epithelial cells of the proximal convoluted tubules exhibited marked cytoplasmic swelling with disruption of the brush border. Nuclei, mitochondria, and ribosomes were visualized pushing past the microvillus border filling the lumen as a consequence of cellular swelling. However the glomeruli, glomerular capillaries, basement membranes, the slit membrane between podocytes of kidneys obtained from the alcohol-fed animals appeared normal. Likewise, the fenestrated capillary endothelia appeared intact.

Kidneys of the alcohol-fed animals have also showed different histologic appearance including fragmentation and vesiculation of the cisternae of the rough endoplasmic reticulum (RER) and swelling of mitochondria (Figure 12).Thin segment of the loop of Henle showed more discrete cytoplasmic changes, thin epithelial cytoplasm detached from the basement membrane of the loop of Henle and sloughed into the lumen.

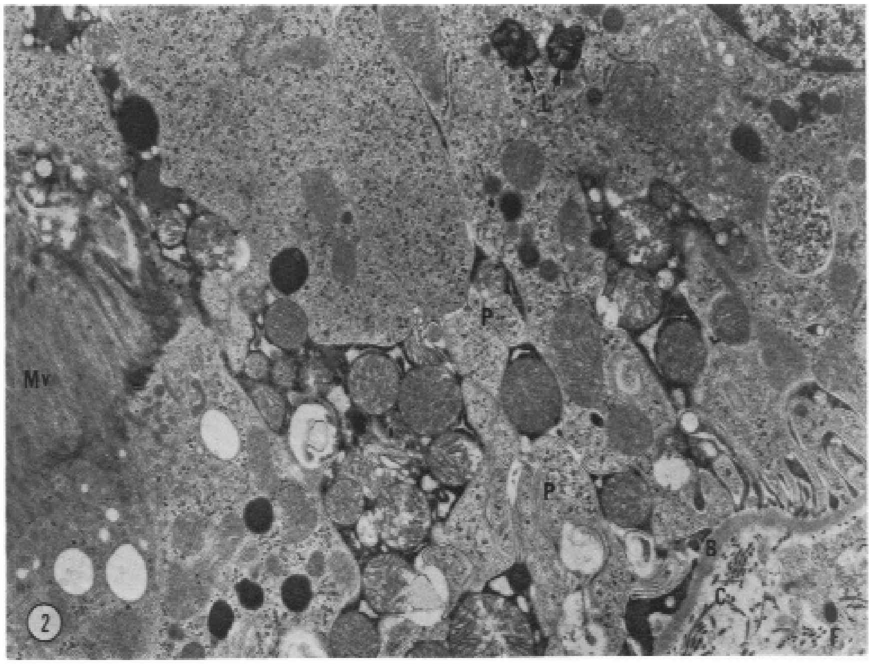

Figure 12-Proximal convoluted tubule with alcohol-induced changes.

Discontinuity of the brush border (Mv) is due to the swelling of the proximal cells which have pushed into the lumen. Numerous mitochondria are surrounded by the interdigitating plasma membranes (P) which extend down to the basement membrane (B). Abundant collagen fibers (C) are seen next to an interstitial fibroblast (F). Note the lysosomes (arrows, L) packed with membranes. (Fixation with glutaraldehyde, x 11,300) **(**Van Thiel *et al.*, 1977).

The distal convoluted tubule showed the most remarkable ultrastructural changes of the entire nephron in alcohol fed animals. The alcohol-induced changes detected in the distal convoluted tubule include; the distal epithelial cells demonstrated a progressive change or necrotic metamorphosis, lumen of distal convoluted tubule demonstrated dilated tubular lumen with flattened epithelial lining cells grossly, development of cytoplasmic epithelial cell protuberances which project into the lumen, the vesiculated rough and smooth endoplasmic reticulum and ballooning of the mitochondria and abundant collagen fibers could be seen underlying the basement membrane (Figure 13).

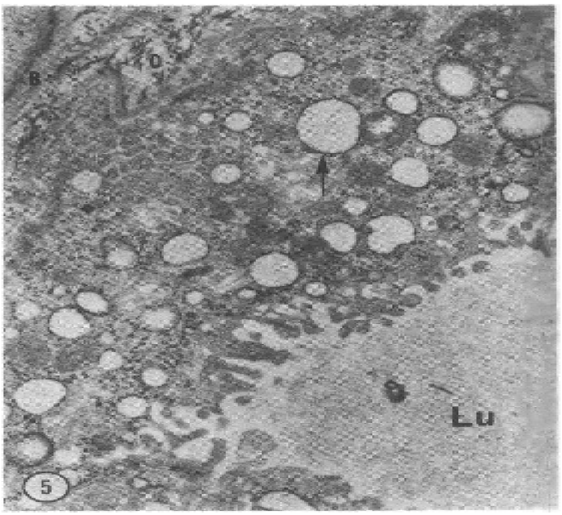

Figure 13-Distal tubule with alcohol-induced changes.

The rough endoplasmic reticulum (arrow) has vesiculated. Collagen (C) is seen deposited along the basement membrane (B). Note the appearance of delicate cytoplasmic projections into the lumen (Lu). (Fixation with glutaraldehyde, x 11,300) (Van Thiel *et al.*. 1977).

Interstitial and perivascular fibrosis were seen about the collecting ducts and subsequently, excess collagen accumulated around blood vessels and then spread into the interstitial tissue (Figure 14).

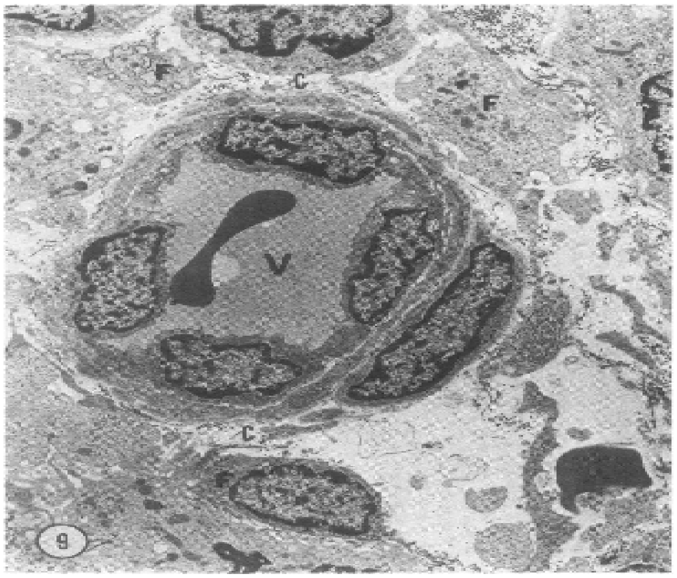

Figure 14-Perivascular accumulation of fibroblasts.

Collagen (c) deposition is seen around atypical vasa recta (V) with endothelial cell hyperplasia in the early alcohol-induced renal lesion. Three fibroblasts (F) can be identified around this blood vessel. (Fixation with glutaraldehyde, x 5000) (Van Thiel *et al.*, 1977).

3. DISCUSSION

A number of research papers have been conducted on the toxicological effect of ethanol on the liver and kidney (Javed *et al.*, 2008, Saalu*et al.*, 2012 and Knight *et al*., 2003). Some of these papers have been reviewed and presented in this project paper. The findings the papers reviewed are discussed as follow:

The results of the study conducted by Das and Vasudevan (2005), indicated that rats treated with ethanol in lower concentration (0.8g ethanol/kg body weight/day) for 4 weeks showed highest increase in body weight while those rats treated with ethanol in higher concentration (1.2g, 1.6g, 2g ethanol/kg body weight/day) for 4 weeks showed decrease in body and liver weight. The content of ascorbic acid significantly ($p<0.05$) decreased in liver in those consuming 2g ethanol per kg body weight per day. Thiobarbituric acid reactive substances (TBARS) level of liver was increased in those rats consuming higher concentration of ethanol (1.2g, 1.6g, 2g ethanol/kg body weight/day for 4 weeks). The reduced glutathione (GSH) content of liver was found to be decreased significantly($p<0.05$) in those rats consuming higher concentration of ethanol (1.2g, 1.6g, 2g ethanol/kg body weight/day for 4 weeks). The superoxide dismutase (SOD) and glutathione S-transferase (GST) activities of liver were found to be significantly higher ($p<0.05$) in ethanol consuming rats. Ethanol exposure caused significant ($p<0.05$) decreases in the glutathione reductase (GR) and glutathione peroxidase (GPx) activities of liver. The activity of catalase was increased in lower concentration (0.8g and 1.2g ethanol/kg body weight/day for 4 weeks) of ethanol, but in higher concentration (1.6g and 2g ethanol/kg body weight/day for 4 weeks) it was significantly decreased. The glucose-6-phosphate dehydrogenase activity was found to be unaltered in 0.8g, 1.2g, 1.6g, 2g ethanol/kg body weight/day for 4 weeks in all ethanol treated groups.

The result obtained by Husain et al. (2001) was consistent with the result of study of Das and Vasudevan (2005) with regard to glutathione (GSH) content in liver. In the study of Husain et al. (2001), ethanol treated rats showed a significant depletion of glutathione (GSH) content in liver. The dose applied in this study was 2 g/kg/day of ethanol for 6.5 weeks. This consistency in results obtained in the two studies may be due to the dose of alcohol used. Similarly, Scott *et al*. (2000) revealed that the hepatic GSH levels significantly decreased due to ethanol ingestion at 2 g/kg of ethanol.

However, a study done by Javed *et al.* (2008), which used 120 male and female quails (39 days old) was inconsistent with the study of Das and Vasudevan (2005) with regard to the relative weight of liver. In the study of Javed *et al.* (2008), 2, 4, 8 and 16% of alcohol treated quails (birds) for 4 weeks did not show statistically significance difference in body weight. This inconsistency in results obtained in the two studies may be due to the type of animal species used or dose of the administered alcohol.

In a study conducted by Oh *et al.* (1998), the effect of ethanol on glutathione status of liver in adult male rats (Sprague-Dawly strain) was investigated. The dose used in this study was 36% of total energy as alcohol for 6 wk. In that study the results indicated the hepatic concentration of GSH was significantly higher in the ethanol treated rats; activity of glucose-6-phosphate dehydrogenase was significantly lower in the ethanol group than in the controls. The level of hepatic thiobarbituric acid reactive substances did not significantly alter. These results are inconsistent with the results of Das and Vasudevan (2005). This inconsistency may be due to the type of animal species used, dose and duration of alcohol exposure.

Das *et al.* (2009) explained that administration of 1.6 g ethanol/ kg body weight/ day to male albino Wistar strain rats showed significant reduction ($p<0.001$) in GSH content after 12 weeks of ethanol exposure, significantly increased ($p<0.001$) thiobarbituric acid reactive substances (TBARS) level and the catalase activity reduced significantly ($p<0.001$) after 4 weeks of ethanol exposure. However, the superoxide dismutase (SOD) activity elevated significantly ($p<0.001$) up to 12 weeks of ethanol exposure and then decreased after 36 weeks. Ascorbic acid content also reduced ($p<0.001$) in ethanol exposed rats compared to the controls after 4 weeks.

Results obtained from Das *et al.* (2009) were consistent with those reported in Das and Vasudevan (2005) in that chronic ethanol intake increased in thiobarbituric acid reactive substances level and glutathione s-transferase activity in liver. In addition, chronic ethanol exposure decreased glutathione content, ascorbic acid content and catalase activity. This consistency of results may be due to usage of the same animal species and same dose of ethanol administration in both studies. However, these two studies were inconsistent with regard to the activity of superoxide dismutase (SOD) in liver, in that the superoxide dismutase (SOD) activity of liver was found to be significantly higher ($p<0.05$) in 1.6 g of ethanol consumption for 4 weeks (Das and Vasudevan, 2005). But the superoxide dismutase (SOD) activity of liver was found to be decreased ($p<0.001$) for 36 weeks when ethanol was administered (Das et al., 2009). This contradictory result may be explained by difference in the duration of ethanol exposure.

Another study done by Fromenty *et al*. (2009) suggested that ethanol consumption decreases the body weight and liver triglycerides in 7-week-old male obese and lean mice. The dose of ethanol applied in this study was 3 g/kg b.w for the first 10 days. Thereafter, the daily intake of ethanol was regularly incremented by 1 g/kg to finally reach 21 g/kg after 6 months. The result of this study indicated that ethanol administration did not change values of ALT of the liver in obese mice and lean mice. Activity of MnSOD increased. GSH was not lowered by ethanol intake. The activity of glutathione peroxidase was reduced. The activity glutathione reductase and glutathione transferase did not reduce.

In agreement with the finding of Fromenty *et al*. (2009), a study conducted by Shibata *et al*. (2009) that used 8- 10 week-old female wild-type and *Clock mutant* mice by giving 15% of ethanol for 8 weeks to wild-type and *Clock* mutant mice showed that ethanol intake did not alter levels of ALT in ethanol treated animals. This similar result may be due to the age of the animal species and usage of the same organ. However, Saalu *et al*. (2012) that used 10 to 12 weeks old male adult Wistar rats with administration of 5 mg /kg b. w of ethanol daily for 56 days was inconsistence with the results obtained from Fromenty *et al*. (2009) in that alcohol administration in rats showed a statistically significant reduction ($p < 0.001$) in GSH content, SOD activity and increased in serum alanine transaminase, indicating severe hepato-toxicity. This inconsistence of the two studies may be due to duration of alcohol exposure, age of species and dose of alcohol administration.

In a study carried out by Shibata *et al*. (2009) revealed that chronic ethanol consumption significantly increased *liver* weight ($P < 0.05$) and liver triglyceride content ($P < 0.01$) in the Clock *mutant* mice. However, ethanol treated Clock *mutant* mice did not show alteration in the levels of AST or ALT, which was consistent with the results of Fromenty *et al*. (2009). This similarity may be due to the age of the animal species and usage of the same organ.

In a prospective cohorts study conducted by the Knight *et al*. (2003) observed that there was no statistically significant association between moderate alcohol intake and decline renal function in women. Also the same finding was revealed by Schaeffer *et al*. (2005) who used prospective cohorts study on 11,023 healthy men over 14 years. The similarity may be due to the study design they used.

A population-based case-control study was conducted by Perneger *et al*. (1999) in Maryland in 1991 to assess the relation between alcohol consumption and risk of ESRD. In this study, 716 Participant patients who had started treatment for ESRD with age of 20-64 years were used. The result of this study indicated that consumption of more than two alcoholic drinks per day, on average was associated with an increased risk of kidney failure in the general population. This result was not similar with the result of Knight *et al*. (2003). The difference may be due to the study design, sample of population, race and gender. Results obtained from Das *et al*. (2008) was also inconsistent with the result of Knight *et al.* (2003) in which chronic exposure to moderate amount of ethanol (1.6 g/ kg body weight/ day) for 12 weeks caused deleterious effects on rats. This difference may be due to duration of exposure, type of species and method of study they used.

Another study demonstrated by Van Thiel *et al.* (1977) was disagreed with the result of Knight *et al*. (2003) in that chronic ethanol ingestion led to impairment of GFR associated with renal hypertrophy in alcohol-fed rats. In addition, Chung *et al*. (2005) that used a cross-sectional community-based study did not agree with the study of Knight *et al*. (2003) among 1466 Taiwan alcohol drinkers with the age of 40–95.The result of this study indicated that subjects with chronic alcohol consumption have significantly increased estimated GFR and CCr values in drinkers than non-drinkers. The difference may be due to race, age and type of methodology they used.

In the work of Alimi *et al*. (2013) they used two months old male Wistar rats, weighing about 120 – 140 g by administration of 3 g/kg b.w ethanol showed chronic ethanol intoxication significantly increased ($p < 0.01$) serum creatinine and urea levels as well as the activity of GGT which is similarly reported by Cigremis *et al*. (2004). Chronic ethanol administration was found to cause a significant decrease (*p* < 0.01) in the levels of serum and kidney vitamin E, vitamin C, renal GSH level, superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GSH-Px). However, the level of malondialdehyde (MDA) was significantly increased (*p* < 0.01) in exposed rats compared with the controls (Table 13). In addition, chronic ethanol intake induced the tubular and renal cells necrosis as well as the congestion of blood vessels and damaged glomeruli. This was supported by Omar (2013) who obtained similar results of renal tubules hypertrophy, degeneration of tubular mononuclear cells and dilation of renal glomeruli in 10% of ethanol treated male Wistar rats. Similarly, Saalu *et al*. (2012) observed that with administration of 5 mg /kg b. w of ethanol daily for 56 days can cause a significant decrease ($p < 0.01$) in superoxide dismutase (SOD), catalase (CAT) and gluthatione peroxidase (GSH-Px) activities in male adult Wistar rats. The similarity may be due to the type of species used.

Dinu *et al*. (2005) studied on male Wistar rats (weighing 140–160 g) who were treated with 2 g/kg body weight of ethanol and found out that significant increment (*P* <0.05) in the kidney weight in 30 weeks ethanol exposure . In addition, catalase, glutathione peroxidase, glutathione reductase were activated while superoxide dismutase, glutathione transferase, gamma glutamyl transferase (GGT) levels and malondialdehyde content were unchanged after 10 weeks of ethanol exposure. They had also reported that superoxide dismutase and glutathione peroxidase activities were unmodified, but catalase, glutathione transferase, G6PD and glutathione reductase activities were significantly increased after 30 weeks of ethanol exposure. These results showed specific changes in rat kidney antioxidant system and glutathione status as a consequence of long-term ethanol administration.

Inconsistent with the results of Dinu *et al*. (2005), Das *et al*. (2008) did not observe significant change in relative weights of kidney (g/ 100g body weight) in 1.6 g ethanol/ kg body weight/ day for 4 and 12 weeks of ethanol treated male albino Wistar rats of weighing 200- 220g.This difference may be due to the dose, weight and duration of exposure.

In addition, results of Alimi *et al*. (2013) was not similar with the findings of D. Dinu *et al*. (2005) who stated that 3 g/kg b.w ethanol administration during 12 weeks to Wistar rats, significantly increased (*p* < 0.01) the kidney malondialdehyde (MDA), gamma glutamyl transferase (GGT) and significantly decreased ($p < 0.01$) the glutathione content (GSH), activities of superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GSH-Px). Therefore, the difference of results in these two studies may be due to variation in dose and duration of alcohol administration to the treated animals.

Other study conducted by Scott *et al.*(2000) was not agree with Dinu *et al.* (2005) who demonstrated lipid peroxidation increased at different dose of ethanol administration (2,4,6 g/kg b.w) in the kidney of Male Fisher-344 rats. The difference of these results may be due to dose of ethanol administered to the treated rats. In addition, Oh *et al.* (1997) reported that 5 g/kg of ethanol intake for 6 hour had no effect on the activities of catalase, xanthine oxidase (XO), glutathione transferase (GST) and glucose-6-phosphate dehydrogenase (G6PDH) in liver of rats. This result was not agreed with the study of Dinu *et al.* (2005). The difference of these two results may be due to duration and dose of ethanol administration to the treated animals and also the type of organ used.

In a study conducted by Das *et al.* (2008) who treated male albino Wistar rats with 1.6 g ethanol/ kg body weight/ day for 4 and 12 weeks indicated that no significant change was observed in relative weights of kidney (g/ 100g body weight) and serum calcium level. Urea and creatinine concentration significantly elevated. Glutathione (GSH) content, Glutathione peroxidase (GPx) level and GR activity significantly reduced after 12 weeks of ethanol exposure. Furthermore, Thiobarbituric acid reactive substances (increase lipid peroxidation) were significantly elevated at 12 weeks of ethanol treatment. But, no significant change was observed in GR and GST level for 4 weeks of 1.6 g ethanol treated rats. However, catalase activity increased after 4 weeks of ethanol exposure and decreased after 12 weeks of ethanol exposure. Similarly, the activity of Superoxide dismutase decreased on chronic ethanol exposure for 12 weeks. The histological changes in ethanol treated rats in 12 weeks were: dilated tubules lined by thinner epithelium, widened capsular space, degeneration and necrosis of renal tubular epithelia and renal ultra structural abnormalities. Similar histological changes were also reported by Das and Vasudevan (2008) who detected multiple functional abnormalities of renal tubules may be associated with ethanol-induced changes in membrane composition and lipid peroxidation. In agreement with the findings of Das *et al.* (2008), the study done by Saravana and Nalini (2007) showed significant elevation in the levels of serum urea, creatinine and uric acid as well as kidney thiobarbituric acid reactive substances (TBARS) when applied 5 g/kg body weight/day dose of ethanol in male Wistar rats. In addition, level of GSH content significantly reduced **(**Saravana and Nalini, 2007). The similarity of these results may be due to usage of similar strain of rats with the same sex.

However, the study conducted by Chan-Yeung *et al.* (1981) that used group of 1,826 male workers was inconsistence with the above two studies in that alcohol consumption was significantly associated with lower blood urea nitrogen (BUN) and creatinine levels. This difference may be due to the type of species and sample of population used. Still another study conducted by Husain *et al.* (2001) that used Male Fisher-344 rats was not in line with the study of Das *et al.* (2008), in that no significant change of GSH content found in the kidney using same concentration of ethanol for 6.5 weeks. This difference of result may be due to duration of ethanol administration to the rats. Again another study done by Scott *et al.*(2000) that used male Fisher-344 rats at different dose of ethanol(2 , 4,6 g/kg b.w) did not agree with the study conducted by Das *et al.* (2008) in that acute exposure to ethanol increased GPx activity in the kidney. This difference of result may be due to dose of ethanol administration to the rats.

A dose-response study conducted by Money *et al.*(1989) was undertaken in adult mongrel dogs which was treated with ethanol(1.0, 2.0 g/kg) within 5 minutes was disagreed with the study conducted by Das *et al.* (2008). Results in this study (Money *et al.* 1989) showed that significant reductions in serum calcium level. The difference of these two results may be due to dose and type of species used.

The results obtained from study of Van Thiel *et al.* (1977) indicated that chronic alcohol exposed rats showed significant renal dysfunction and abnormalities of structures. Gross and microscopic changes of kidneys experienced in the alcohol exposed rats; namely, swelling of kidney, enlargement of cells, injured renal epithelial cells in the distal tubules and Henle's loops. Similar results were obtained from Assadi (2008) study which investigated that renal cell injury in the distal segment of nephron, impaired renal function in infants and swelling of mitochondrial in the epithelial cells of the distal nephron during gestational development in offspring of alcohol-exposed rats.

4. CONCLUSION

According to the present review, different researchers conducted researches of different variety in animals concerning the toxicological effect of alcohol on histology of liver and kidney, and almost all investigators confirmed that chronic consumption of alcohol causes biochemical, functional and morphological impairment of both human and animal tissues. Therefore, risk of adverse effects of an alcohol on the human liver and kidney is one of the most important safety issues that must be evaluated. Exposure of alcohol results in different major impairments of liver and kidney like liver cirrhosis, chronic alcoholic kidney disease. Some researchers found that there is gross architectural distortion such as steatosis and necrosis as well as reduction of the weight and abnormal liver function test of the liver.

However, some studies on toxic effects of ethanol on liver and kidney have demonstrated contradictory results. These studies found out that moderate alcohol consumption had no substantial adverse effect on renal function. These differences in effects of alcohol on liver and kidney parameters may have been due to sample size, method, dose, duration of exposure and type of animal species.

Therefore, researchers should continue further investigation regarding these contradictory findings of the above issue. In addition, avoidance of heavy alcohol consumption will be the best option for better function of liver and kidney. Otherwise people that consume alcohol should take moderate alcohol consumption in order to avoid at least the risk of heavy alcohol consumption.

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