Anatomical Study of the Effect of Ethanol on the kidney in Adult Male albino Rat and the Possible Protective Role of Vitamin E: Morphological and Ultrahistological study

Thesis

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بسم الله الرحمن الرحيم

(وعَلَّمَكَ مَا لَمْ تَكُن تَعْلَمُ
وكَانَ فَضْلُ اللَّهِ عَلَيْكَ
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ABTRACT

The electron microscopic study of the different experimental groups showed variable degrees of degeneration in the glomeruli and tubules with ballooning of mitochondria and fusion of foot processes of podocytes and thickened of the glomerular basement membrane.Furthermore, histological examination revealed that there was obvious improvement in group IV (Ethanol administration with vitamin E for six weeks) in the form of normal glomeruli and normal Bowman's space and normal thickness of Bowman's capsule. Few glomeruli still showed slight shrinkage and slight widening of Bowman's space.

KAY WORDS.

Anatomical _ effect _protective

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INTRODUCTION

Ethanol is a low molecular weight hydrocarbon, which is derived from the fermentation of sugars and cereals. It is widely available both as a beverage and as an ingredient in food extract, cough medications and mouth washers. Ethanol is rapidly absorbed across both the gastric mucosa and small intestine, reaching a peak concentration 20-60 minutes after ingestion. The amount of alcohol consumed and the frequency at which ethanol is consumed all affect the speed of metabolism .Chronic alcoholics and those with severe liver disease had increased rates of metabolism. Ethanol intoxication is common in modern society, largely because of its widespread availability. In some studies, more than half of all trauma patients were intoxicated with ethanol at time of arrival to the trauma center and it was considered a common co-ingestant in suicide attempts**.** Ethanol is widely consumed. It is regarded as the most commonly abused drug in the world with profound consequences, both societal and medical Up to 15% of population is considered at risk **(Masters**, **2004)**.

Acute alcohol intoxication and chronic alcoholism are common medical conditions that are difficult to treat**.** Oral administration of alcohol caused significant elevation of serum Potassium, sodium and creatinine as well as structural alterations in renal tubules and interstitial infiltration by chronic inflammatory cells **(Ligha et al., 2009).**

Around the world more and more people suffer from alcoholism, addiction problems and excessive use of drugs both medical and non-

medical are major causes of liver and kidney damage in adults **(Mona, 2007).**

Renal damage occurred as a result of acute intoxication or chronic alcoholism and this had been well established as much as sixty five percent of chronic alcoholics having nephropathy at autopsy **(Vamvakas et al., 1998).**

The mechanism by which ethanol induced renal damage was uncertain. Nevertheless, free radical induced lipid per oxidation played the main role in the renal damage. It also was noticed that there was a significant elevation of serum urea and creatinine in rats with ethanol intoxication **(Saravanan & Nalini, 2007).**

Rodrigo et al. (2002) reported that alcohol produced tissue damage by causing oxidative stress through depletion of glutathione, growth factor dysregulation and mitochondrial damage leading to renal damage**.**

Free radicals and oxygen spices could be present in situations of acute dose of ethanol but its deterious effects could be inhibited by presence of antioxidant substance as vitamin E **(Halliwell et al., 1998).**

It was noticed that there was significant reduction in the levels of vitamin E and C in ethanol intoxicated rats **(Pari & Karthikesan, 2007).**

Vitamin E is a major lipid soluble antioxidant present in all cell membranes which protected cells against lipid per oxidation by its reaction with the active free radicals producing tocopheroxyl radicals **(Jurczuk & Galazyn, 2004).**

The aim of the present work is to study the effect of ethanol administration on the kidneys of adult male albino rats and the possible protective role of vitamin E using light and electron microscopic means.

Review of Literature

Ethanol (ethyl alcohol) was a volatile, flammable, colorless liquid. It was a potent psychoactive drug. It had widespread use as a solvent of substances intended for human contact or consumption, including scents, flavorings, colorings, medicines and alcoholic beverages and in modern thermometers. It had a long history as a fuel for heat and light and more recently as a fuel for internal combustion engines. It was one of the oldest recreational drugs. Ethanol was a straight-chain alcohol and its molecular formula was C2H5OH. Its empirical formula was C2H6O. The fermentation of sugar into ethanol was one of the earliest organic reactions employed by humanity. The intoxicating effects of ethanol consumption had been known since ancient times. Ethanol was rabidly absorbed from the gastrointestinal tract within 30 to 60 minutes after ingestion. In chemistry, it was both an essential solvent and a feedstock for the synthesis of other products **(Richard et al., 2007).**

Ethanol induced intoxication was poorly understood and might be multi-factorial in etiology. More evidences suggested ethanol toxicity via the generation of free oxygen radicals. This was shown by demonstration of alcohol-induced free radical species directly via spectroscopic analysis and ethanol-induced alterations in the levels of endogenous tissue antioxidants **(Orellana et al., 1998).**

Effects of ethanol intoxication on different tissues

Alcohol caused a decrease in platelet count. Its withdrawal caused reticulocytosis, leucocytosis and falling in serum iron might

occur. It also depressed both humoral and cell-mediated immune responses **(Savage & Lindenbaum, 1996).**

Miller et al. (2001) found that osteopenia and fractures, especially of the spine and ribs, were associated with osteoporosis rather than osteomalacia in chronic alcoholics.

Martin et al. (2003) reported that chronic ethanol use was associated with polyneuropathy, Wernicke's encephalopathy, Korsakoff's psychosis, Cerebellar degeneration, dementia and central pontine myelinolysis. Polyneuropathy was the commonest neurological symptom in alcoholics including dysesthesia, anasthesia, weakness, decreased pain and temperture sensation, decreased touch and vibration sense more distal than proximal. The disease progression was gradual, bilateral and symmetric. Its withdrawal might result in slow incomplete recovery. Wernicke's encephalopathy was characterized by the clinical triad of occulomotor abnormalities, ataxia and global confusion. While, the main symptoms of Korsakoff's psychosis were amnesia and executive dysfunction. Cerebral atrophy was a common finding in alcoholic dementia and might be due to direct toxicity of alcohol or associated thiamine deficiency. A rare complication of chronic alcoholism was the central pontine myelinolysis and was due to demyelination of the pontine corticobulbar white fibers. Such condition was characterized by dysarthria, dysphagia, facial and neck weakness, dysfunctional tongue movement and mental confusion.

Ethanol altered the activity of the hypothalamic –pituitary-adrenal axis, leading to increased plasma adrenocortical trophic hormone (ACTH) and corticosteroid levels primarily by stimulating the release of corticotrophin releasing factor (CRF). Increased CRF gene transcription

in the hypothalamus might also be important. Alcohol inhibited suckling – induced prolactin release in the lactating rat**.** Chronic ethanol administration decreased the response of the thyrotropes to thyroxin releasing hormone (TRH) while left its hypothalamic content unchanged or somewhat increased **(Fentiman et al., 2006).**

Lawler et al. (2007) added that ethanol had a damaging effect on muscarinic receptors in pancreas, duodenum and the sphincter of Oddi. It increased acetylcholine sensitivity, stimulating protein rich pancreatic juice and hypertonicity of the duodenum leading to duodenopancreatic reflux which explained acute alcoholic pancreatitis. Also, the most common pancreatic disease that caused diabetes mellitus was chronic pancreatitis resulting from alcoholabuse.

Barve et al. (2008) found that alcoholic liver disease had a known etiology but an incompletely known pathogenesis. It was an extremely common disease with significant morbidity and mortality and only a relatively small proportion of heavy alcohol drinkers progress to advanced disease**.** Alcoholic liver disease including acute liver hepatitis and cirrhosis was a major cause of morbidity and mortality in the world. Ethanol liver toxicity was related to duration of alcoholism, amount of daily intake and patient's nutrition. The threshold of alcohol toxicity on liver was about 40 g of ethanol daily .However, liver cirrhosis developed in no more than 20% of patients exceeding these values. Ethanol was oxidized in liver to acetaldehyde, a compound more toxic than ethanol itself.

Alcohol caused a modest fall in blood pressure in acute intoxication, but chronic abuse resulted in a dose dependant rise in blood pressure **(Catena et al., 2003).**

The Holiday heart syndrome was a supraventricular arrhythmia induced by drinking beers. Atrial fibrillation was the most common arrhythmia but atrial flutter, atrial tachycardia, junctional tachycardia and multiple atrial premature beats had also been observed. About 10 to 20 years of high alcohol use might elapse before cardiac decompensation. However, sudden cardiac death might occur. In addition, other ECG changes might be recorded including left ventricular hypertrophy, abnormal T-waves and non-specific ST-T wave changes. Alcoholic cardiomyopathy with beriberi presented by cardiac dilatation, tachycardia, elevated venous pressure and peripheral edema. However, beriberi patient exhibited a high cardiac output state and warm extremities, while the cardiomyopathic patient had depressed cardiac output and ventricular hypo contractility **(Connor et al., 2009).**

Ethanol toxic effects on Kidney

Van thiel et al. (1979) found that the kidney of alcoholics had a different histological appearance at the electron microscope level. These differences were especially striking in the renal cortex and at the corticomedullary junction. 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 the microvillus border filling the lumen. The thin segment of the loop of Henle showed more discrete cytoplasmic changes. the thin cytoplasm forms numerous excrescences which projected into lumen. In some cases, the nuclei of the tubular epithelial cells were flattened and fibroblasts infiltrated the interstitium and deposited excess collagen diffusely along the basement membranes. The distal tubules showed cell flattening, mitochondrial swelling, proliferation and

dilatation of the rough and smooth endoplasmic reticulum and formation of multi locular vacuolated inclusions. The distal tubular epithelial cells were completely necrosed in the end stage lesion. The basement membrane appeared to be irreversibly damaged.

Sakamura (1998) added that ethanol exerts its effects on the renal Bruch membrane by causing structural change in the phospholipid layer which activated sodium intake. Moreover, **Keller et al. (1994)** reported that chronic ethanol consumption caused toxic effects in the kidney as well as the liver. A possible link between alcoholism and glomerulonephritis is observed. Alcohol consumption might also cause acute tubular necrosis and renal tubular dysfunction **(De Marchi, 1996).**

Furthermore, **Epstein (1997)** found that chronic alcoholic patients might experience low blood concentrations of key electrolytes as well as potentially severe alterations in the body acid-base balance. In addition, alcohol could disrupt the hormonal control mechanisms that governed kidney function.

Ethanol metabolites as hyaline accumulated in tubular epithelial cells were observed after ten weeks of ethanol administration. However, long administration showed atrophy of tubular epithelial cells, urinary casts, and cell infiltration to interstitial tissue. In addition, thickening of basement membrane of the glomerulus, PAS positive deposits in glomeruli and proliferation of mesangial cell were observed in the kidney**.** Oral administration of ethanol in rats for a week caused swelling of glomeruli and tubules, proliferation of mesangial cells, and hyaline drop in tubular epithelial cells were seen in the kidney **(Omoto et al., 1997).**

Orellena et al. (1998) reported a link between alcoholism and nephritis. In addition, nephrotoxic effects of acetaldehyde should not be ruled out due to increased activities of alcohol dehydrogenase and catalase in the kidney. Moreover, **Saravanan & Nalini (2007)** found that ethanol-fed rats had larger kidneys with a significant increase in protein and lipid contents with reduction in creatinine and osmotic clearance. Furthermore, varying degrees of cellular injury were found in renal epithelial cells, particularly in the distal tubules and Henle's loops**.**

Non-traumatic rhabdomyolysis was an important but unrecognized cause of acute renal failure. In alcoholics, rhabdomyolysis developed following muscle necrosis during alcohol induced coma. Renal biopsy showed acute tubular necrosis with pigment casts **(Muthukmar et al., 1999).**

A number of reports supported the hypothesis that habitual consumption of large amounts of alcohol had a variety of deleterious effects on the kidney. Ethanol might cause functional abnormalities in the kidney depending on the quantity ingested and the duration of drinking. Thus, consumption of more than two standard drinks per day was associated with an increased risk of kidney failure in the general population **(Parekh & Klag, 2001).**

Sonal et al. (2001) found that the histopathology of the kidneys in the ethanol treated rats and ethanol and cadmium treated rats showed vacuolation in glomeruli, degeneration of the basement membrane of Bowman's capsule and renal tubular epithelial degeneration in the form of the syncytial appearance of nuclei of the epithelium**.**

Rodrigo et al. (2002) added that multiple functional abnormalities of the renal tubules might be associated with ethanol-induced changes in membrane composition and lipid peroxidation of epithelial cells. Ethanol interfered with the carrier function by decreasing Na+K+-ATPase activity. Alcohol concentration in the tubular fluid approached that of the peri-tubular fluid due to its high permeability.

Trujillo et al. (2002) using electron microscopic study of the glomeruli in ethanol treated rats found endothelial cell swelling, apoptotic podocytes, increased mesangial secretion of extracellular matrix and basement membrane thickening. The distal convoluted tubule wall showed abundant mitochondria, absence of transport vacuoles and active fibroblast secretion of extracellular matrix, as shown by the polymerization of tropocollagen molecules into microfibrils.

Ethanol exposure affected glomeruli in the form of swelling of basement membrane of, PAS positive deposits, proliferation of mesangial cell, proliferation of juxtaglomenular cell, dilation of tubular lumen, swelling of tubular epithelial cell, falling of hyaline droplets in the tubular epithelial cell, cell infiltration of interstitial tissue and basophilic tubule in the kidney **(Tsukamoto et al., 2002).**

Mokutima & Eluwa (2002) reported that teratogenic effects of ethanol on the developing kidneys. Histological result showed marked distortion of the kidney architecture in the treated groups. The results suggested that consumption of beer might be nephrotoxic to the developing kidneys.

Sawicki et al. (2003) exposing animals to cadmium and ethanol separately as well as in combination. Alcohol-fed animals had larger kidneys than controls. Reduction in creatinine clearance and osmolar