Indian Journal of Clinical Biochemistry, 2008 / 23 (4) 341-344

EFFECTS OF CHRONIC ETHANOL EXPOSURE ON RENAL FUNCTION TESTS AND OXIDATIVE STRESS IN KIDNEY

Subir Kumar Das, Sowmya Varadhan, Dhanya L, Sukhes Mukherjee and D M Vasudevan

Department of Biochemistry, Amrita Institute of Medical Sciences, Elamakkara P.O. Cochin 682 026, India

ABSTRACT

After administration, ethanol and its metabolites go through the kidneys and are excreted into urine. The kidney seems to be the only vital organ generally spared in chronic alcoholics. Therefore, we investigated the multiple effects of chronic ethanol exposure on renal function tests and on oxidative stress related parameters in the kidney. Chronic ethanol (1.6 g ethanol/ kg body weight/ day) exposure did not show any significant change in relative weight (g/ 100g body weight) of kidneys, serum calcium level or glutathione s-transferase activity. However, urea and creatinine concentration in serum, and TBARS level in kidney elevated significantly, while reduced glutathione content and activities of glutathione peroxidase, glutathione reductase and superoxide dismutase diminished significantly after 12 weeks of ethanol exposure. Catalase activity showed increased activity after 4 weeks of ethanol exposure and decreased activity after 12 weeks of ethanol exposure. Genesis of renal ultrastructural abnormalities after 12 weeks of ethanol exposure may be important for the development of functional disturbances. This study revealed that chronic ethanol exposure for longer duration is associated with deleterious effects in the kidney.

KEY WORDS

Ethanol, Glutathione, Kidney, Oxidative stress, Renal function.

INTRODUCTION

The kidney is an important organ having not only excretory function but also other functions such as production of the substances that activates a living body, enzymatic reaction, immunization etc. After ethanol administration, ethanol and its metabolites go through kidneys and are excreted into urine, and its content in the urine is higher than that of the blood and the liver. The kidney is often involved in the development, maintenance and counter regulation of complex electrolyte disturbances (1). Some studies suggest that chronic ethanol ingestion per se is not nephrotoxic (2). The kidney seems to be the only vital organ generally spared in chronic alcoholics without advanced alcoholic liver disease or hepato-renal syndrome. But, regular alcohol consumption raises the blood pressure, which per se is a risk factor for renal damage (1). Large amounts of ethanol have deleterious effects on the kidney (3). Structural and functional abnormalities of the kidney are reported with increasing frequency in the fetal alcohol syndrome seen in children who have been prenatally exposed to ethanol (3). The basic objective of this study was to investigate the effects of chronic ethanol exposure on the oxidative stress related parameters and histopathological changes in the kidneys in a time dependent manner.

Address for Correspondence :

Dr. Subir Kumar Das
Department of Biochemistry,
Agartala Govt. Medical College,
Kunjaban 799006, Agartala
Phone: 91-381-2356072 • E-mail: drsubirkdas@yahoo.co.in

MATERIALS AND METHODS

Ethanol was purchased from Bengal Chemicals, Kolkata. Chemicals were purchased from SISCO Research Laboratory (SRL), India; Sigma Chemical Co., St. Louis, USA; and E. Merck, India.

Animal Selection: 16-18 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 (4) and water *ad libitum*. The animals were weighed daily and its general

condition was recorded including their daily intake of liquid. The Animal Ethics Committee of the Institution approved the procedures in accordance with the CPCSEA guideline.

The rats were divided into the 3 groups of 6 each i. e. Group I: Control rats- the rats were fed normal diet and water; Group II: 1.6 g ethanol/ kg body weight/ day for 4 weeks; Group III: 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.

Experimental procedure: 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 (5), creatinine (6) and calcium (7) 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 (8). The tissue (~100 mg) samples were homogenized in ice-cold 2 ml 0.1 *M* phosphate buffer (*p*H 7.4) and glutathione content was estimated (9). Lipid peroxidation of samples was measured using TCA-TBA-HCl (10). Activities of glutathione peroxidase (GPx; EC 1.11.1.9) (11), glutathione reductase (GR; EC 1.6.4.2) (12), glutathione S-transferase (GST; EC 2.5.1.18) (13), catalase (EC 1.11.1.6) (4) and superoxide dismutase (SOD, EC 1.15.1.1) (14) were determined. Histopathological examination was done from formaline fixed tissue samples using Haematoxylin and Eosin staining solutions.

All data were analyzed using the statistical package SPSS (version 11.0, SPSS Inc., Chicago, IL). Results are expressed as mean ± SE (standard error). The sources of variation for multiple comparisons were assessed by the analysis of variance (ANOVA), followed by Post Hoc test with Bonferroni's Multiple Comparisons test. The difference were considered significant at $P<0.05$.

RESULTS AND DISCUSSION

The present work focused on the 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 (Table I.). When rats were exposed to ethanol (1.6 g / kg body wt/ day) for 12 weeks, urea and creatinine concentration in serum significantly elevated compared to other two groups (Table I). Though one study showed that alcohol consumption was significantly associated with lower blood urea nitrogen (BUN) and creatinine (15), another study reported elevation of serum urea and creatinine levels after administering 5 g/ kg body weight/day of ethanol for 60 days to male Wistar rats (16). Though a direct effect of alcohol in reducing calcium level was reported in one experimental study (17); but no significant change was observed in serum calcium level in either of the group tested in this study (Table I).

Table I: Effects of ethanol (1.6 g ethanol/ kg body weight/ day) for different time period on relative weight of kidney; and urea, creatinine and calcium concentration in serum [Values are mean ± SE of 6 rats in each group]

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 $p< 0.05$ compared to Control Group, # indicates $p< 0.05$ compared to 4 weeks treatment group.

Glutathione (GSH) is a major non-protein thiol in living organisms, which plays a central role in coordinating the body's antioxidant defence processes. It is critical in preserving the proper cellular redox balance and for its role as a cellular protectant. In the present study, reduced glutathione content significantly depleted only after 12 weeks of exposure compared to the control group (Table II). Interestingly, while one research group also did not find any significant change of GSH content in the kidney using same concentration of ethanol for 6.5 weeks (18). However, after administering 5 g/ kg body weight/ day of ethanol for 60 days to male Wistar rats GSH level significantly reduced (16).

12 weeks of ethanol treatment significantly elevated thiobarbituric acid reactive substances compared to other two groups, indicating that prolonged ethanol consumption increases lipid peroxidation (Table II). Though in one study, 10 weeks of ethanol treatment (2 g/ kg body weight/ 24 h) did not show any alteration in lipid peroxidation in the kidney (19), another study showed that lipid peroxidation increased with increasing dose of ethanol in the kidney (20). The vulnerability

of the kidney to oxidative damage has been partly attributed to its high content of long-chain polyunsaturated fatty acids (21).

Glutathione peroxidase (GPx) level depleted significantly after 12 weeks of ethanol exposure compared to the control group (Table II). Decrease in the activity of GPx in the present investigation may be due to exhaustion or inactivation of the enzyme by reactive oxygen species after chronic ethanol treatment for longer duration. Glutathione reductase (GR) is concerned with the maintenance of cellular level of GSH by effecting fast reduction of oxidized glutathione to reduced form. In this study, GR activity in the kidney homogenate significantly reduced after 12 weeks of ethanol treated rats compared to other two groups (Table II). But no significant change was observed in GR level for 4 weeks of ethanol treated rats compared to the control group (Table II). While one study showed that chronic ethanol treatment caused GPx depletion (18), another study showed that acute exposure to ethanol increased GPx activity and decreased GR activity compared to the control group in the kidney (20). Decreased GR activity may be a predominant cause for GSH depletion. Glutathione S-transferase (GST) plays an essential role in liver by eliminating toxic compounds by conjugating them with glutathione, but no significant change in GST level in ethanol treated rats compared to the control group was observed in the present study (Table II).

Catalase catalyses the dismutation of hydrogen peroxides. Its activity increased after 4 weeks of ethanol exposure and decreased after 12 weeks of ethanol exposure (Table II), suggesting that catalase activity in the kidney is changeable. Decreased catalase activity may be due to loss of NADPH, or generation of superoxide, or increased activity of lipid peroxidation or combination of all. Decreased activity of

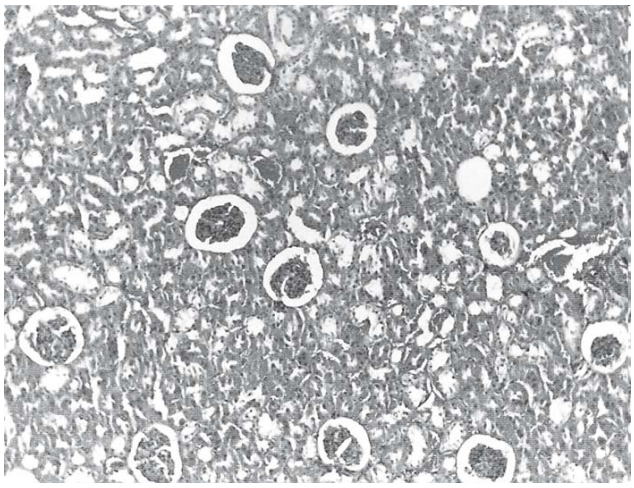

Fig. I: Normal texture of kidney observed in Control group as well after 4 wks of ethanol (1.6 g/ kg bw/ day) exposure

superoxide dismutase on chronic ethanol exposure for 12 weeks (Table II) may be due to excessive generation of $O_2^{\cdot -}$ leading to inactivation of the enzyme.

Dilated tubules lined by thinner epithelium were seen in ethanol treated rats. However, widened capsular space, degeneration and necrosis of renal tubular epithelia were noticed in 12 weeks ethanol exposed animals (Fig. II). These damaging changes reflected toxic effects of ethanol with duration of exposure. Renal ultra structural abnormalities due to ethanol exposure may be important in the genesis of functional disturbances (21).

The antioxidant enzymatic defense system showed a different response during the two periods of ethanol administration in this study. Remarkable changes have been registered after 12 weeks of ethanol administration. Long-term alcohol abuse has also been associated with many renal alterations in

Table II : Effect of ethanol (1.6 g ethanol/ kg body weight/ day) for different time period 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 [Values are mean ± SE of 6 rats in each group]

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 H_2O_2 utilised/ min/ mg protein)	42.67 ± 0.77	53.41 ± 1.95*	45.15 ± 4.92#

P value: * indicates $p < 0.05$ compared to Control Group, # indicates $p < 0.05$ compared to 4 weeks treatment group.

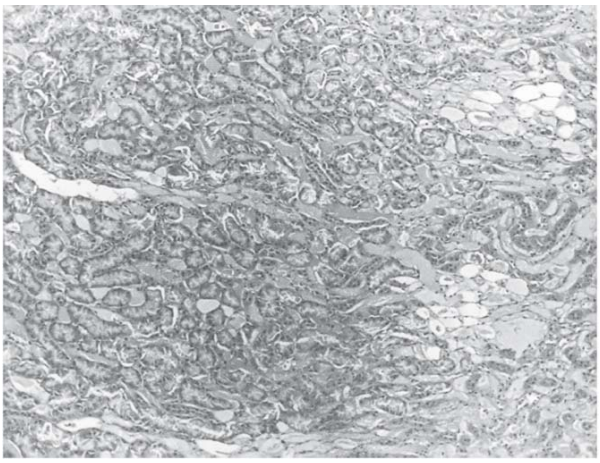

Fig. II: Dilated tubules lined by thinner epithelium, widened capsular space, degeneration and necrosis of renal tubular epithelia were noticed in 12 weeks ethanol exposure.

humans (22). Studies revealed that kidney alcohol dehydrogenase activities increased significantly after ethanol administration (19). It was also reported that ethanol oxidation by the kidney is favored in chronic ethanol-treated rats, thereby suggesting a pathogenic role for acetaldehyde in the nephrotoxic effect of ethanol ingestion. Also, increased reactive oxygen species, partly generated from acetaldehyde oxidation, may contribute to the occurrence of oxidative stress (23).

In conclusion, this study revealed that chronic exposure to moderate amount of ethanol (1.6 g/ kg body weight/ day) for long duration (12 weeks) caused deleterious effects on rats by producing oxidative stress and also was evidenced in histological changes.

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