ISSN (Print) : 0974-6846
Indian Journal of Science and Technology, *Vol 8(25), DOI: 10.17485/ijst/2015/v8i25/59160, October 2015* ISSN (Online) : 0974-5645

Effects of Chronic Ethanol Consumption on Biochemical Parameters and Oxidative Stress on Rat

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Abstract

Background: The biochemical effect of chronic alcohol consumption has been rarely studied although, most of the human population drinks moderate ethanol. The aims of this study was described some biochemical effects induced by moderate ethanol ingestion on rats in four and 12 weeks duration. **Methods**: Eighteen adult male albino Wistar rats (175 to 210 g) were fed with ethanol (1.6 ml/kg body weight/day) for 4 and 12 weeks. Animals randomly divided into 3 groups (1 to 3) comprised 6 animals each as following: Control group as group (1): Received orally drinking water. Group II: Treated orally with 1.6 g diluted ethanol/ kg body weight daily for 28 successive days. Group III:Treated orally with 1.6 g diluted ethanol/ kg body weight daily for 84 successive days. Blood samples were collected from retero-orbital plexus of animals before to start the ethanol administration (0 week), and at the end of 4 and 12 weeks of ethanol treatment. The serum was used for biochemical tests such as Blood Urea Nitrogen (BUN), Creatinine, Total protein, Albumin, Alanine Amino transferases (ALT), Aspartate Amino transferases (AST), Alkaline Phosphatases (ALP), Glutamyl transpeptidase (GGT) and Nitrite concentration. Reduced Glutathione (GSH), Mallondialdehyde (MDA) content, Catalase, Superoxide Dismutase (SOD) and Glutathione Peroxidase (GPH) activities were estimated on homogenized samples. For Analysis of data paired t-student test was used. **Results**: Ethanol exposure initially affects liver function followed by renal function. Chronic ethanol ingestion increased hepatocellular enzyme markers such as ALT, AST, ALP, GGT and MDA and nitrate concentration; while decreased total protein, albumin, GSH, ascorbic acid contents and SOD and catalase activities compare to control group. **Conclusion**: Long-term ethanol exposure induced toxicity which revealed by increase insystemic, local oxidative stress markers and hepatic enzyme indicators.

Keywords: Ethanol, Glutathione, Liver Function, Nitric Oxide, Oxidative Stress

1. Introduction

Alcoholic consumption has been familiar in human societies from many years ago. Alcohols beverages associate with some alterations and disturbs cell function and body antioxidant defense. Ethanol metabolism in body generate inflammatory mediators and result in may induce hepatocyte damages[1].

Ethanol is an amphipathic agent which soluble both in water and lipids consequently can absorb by the mucous membrane of the esophagus and stomach and excreted by expired air and urine. The ethanol not stored in the body but immediately after ingestion is oxidized. The Ethanol mostly metabolized in liver by alcohol dehydrogenase a liver cytosolic enzyme[2].

Oxidative stress is a state which associates with a decrease in antioxidants or over production of reactive oxygen species which may induce by ethanol consumption. This research work is managed for study of antioxidant status male wistar rats after moderate ethanol consumption for different time period

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2. Materials and Methods

Eighteen adult male albino Wistar rats (180 to 200 g) were obtained from our rat colony. Animals were kept in a controlled environment of 22 ± 2°C, 65 to 70% humidity, and 12 h light/dark cycle, and were fed a standard food pellets (Pars, Iran Ltd., Tehran, Iran). The animals were weighed and the doses suggested was 1.6 g diluted ethanol/kg body weigh daily[2].

The experiment was carried out according to the ethical guideline recommended by the animal ethical committee. For the present study animals randomly divided into 3 groups (1 to 3) comprised 6 animals each as follow:

Control group as Group (I): received normal diet and water orally. Group II: treated orally with 1.6 g diluted ethanol/kg body weight daily for 28 successive days. Group III: treated orally with 1.6 g diluted ethanol/kg body weigh daily for 84 successive days.

At the end of the experimental period blood samples were collected from retero-orbital plexus of animals before to start the ethanol administration (0 week), and at the end of 4 and12 weeks of ethanol treatment by heart puncture under light ether anesthesia. The serum was centrifuged at 2000 g for 15 min and was used for biochemical tests such as Blood Urea Nitrogen (BUN), Creatinine, Total protein, Albumin, Alanine Amino transferases (ALT), Aspartate Amino transferases (AST), Alkaline Phosphatases (ALP) and Glutamyl transpeptidase (GGT). All tests were carried out following manufacturer's instruction local pars Azmoon laboratory kits.

Plasma was prepared by EDTA as an anticoagulant for estimation of ascorbic acid[3] and ethanol according to sigma kit[2]. Nitrite level was measured on serum[4]. For stress evaluation, antioxidant enzyme markers were estimated in homogenized liver samples in 0.25 M sucrose solution. Protein of liver tissue was determined[5].

Reduced Glutathione (GSH)[6], Lipid Peroxidation Content (MDA)[7], Catalase[8], Superoxide Dismutase (SOD)[9] and Glutathione Peroxidase Activities (GPx)[10] were estimated on homogenized samples.

Values are expressed as mean ± Standard Deviation (SD). Statistical analysis was carried out by student's t-test using the SPSS statistics softwarever. 20. $p<0.05$ were considered significant.

3. Results

The concentration of ethanol in plasma stays unchanged in animal after consumption of alcohol.

In biochemical parameters, BUN and creatinine concentration in serum was increased non- significantly after 12 weeks of ethanol consumption when compared to control group (Table 1). Serum protein and albumin levels were decreased non-significantly following 4 and 12 weeks use of ethanol. However, globulin concentration was no changes compared to control group.

There was a significant ($P<0.01$) increased in the serumhepatic GPT, GOT, GGT and ALP enzyme activities following ethanol administration in duration of 4 and 12 weeks in rats compared with the control (Table 2).

Table 1. Effects of ethanol consumption on biochemical parameters in rats serum

Groups	Alcohol level	Protein (g%)	Albumin (g %)	Globulin (g %)	Urea (mg %)	Creatinine (mg %)
Control	0	7.33 ± 0.15	4.25 ± 0.21	3.08 ± 0.13	30.00 ± 1.51	0.43 ± 0.05
4weeks	39 ± 1.8	6.55 ± 0.31	3.53 ± 0.25	3.01 ± 0.14	30.08 ± 1.44	0.49 ± 0.02
12weeks	37.3 ± 2.1	6.18 ± 0.28	03.1 ± 0.12	3.08 ± 0.19	43.3 ± 4.08[ab]	0.51 ± 0.02[a]

Values are mean ± SD of 6 rats in each group

P values: a<0.05, compared to control group; b<0.05, compared to 4 weeks ethanol treated group

Table 2. Effects of ethanol consumption on liver enzyme marker activities (IU/L) in rats serum

Groups	AST	ALT	GGT	ALP
Control	029.63 ± 2.26	033.57 ± 1.88	07.67 ± 00.82	089.72 ± 06.49
4week	057.83 ± 5.64[a]	053.66 ± 5.27[a]	39.05 ± 07.69[d]	118.05 ± 07.34[c]
12weeks	143.66 ± 17.31[db]	111.00 ± 6.84[db]	91.83 ± 10.46[db]	139.17 ± 11.44[db]

Alanine Aminotransferases (ALT), Aspartate Aminotransferases (AST), Alkaline Phosphatases (ALP), Glutamyl transpeptidase (GGT); Values are mean ± SD of 6 rats in each group.

P values: a <0.05, compared to control group; b<0.05, compared to compared to 4 weeks ethanol treated group: c<0.01, d< 0.001 compared to control group

Intoxication of rats with ethanol in 4 and 12 weeks periods causes a non-significantly decrease in the GPX and catalase activities, whereas high level of lipid peroxidation (MDA) was reported in the homogenized compared with the control ($P \leq 0.05$) (Table 2). Consumption of ethanol in rats after 12 weeks causes a significantly decreased in glutathione concentrationin the homogenized compared to the control group.

Table 3. Effects of ethanol consumption on reduced glutathione (GSH) and Malondialdehyde (MDA) content in liver homogenized of rats

Groups	MDA (nmol/ml)	GSH (ug / ml
Control	**0.538 ± 0.012**	**40.95 ± 4.15**
4weeks	**0.926 ± 0.022**[a]	**37.57 ± 4.03**
12weeks	**1.016 ± 0.001**[a]	**31.18 ± 3.04**[a]

Values are mean ± SD of 6 rats in each group; values: a<0.05, compared to control group

Table 4. Effect of ethanol consumption on defense antioxidant activities in liver homogenized of rats

Groups	GPX	Catalase	SOD
Control	33.33 ± 6.25	38.76 ± 0.56	6.33 ± 0.15
4weeks	29.83 ± 3.43	32.63 ± 0.81	9.07 ± 0.21
12weeks	27.05 ± 3.78	30.22 ± 2.47[a]	9.15 ± 0.69

Glutathione Peroxidase (GPX)(U/g haemoglobin), Catalase (mmol H_2O_2 decomposed/mg protein/min, Superoxide Dismutase SOD (U/mg haemoglobin)

Values are mean ± SD of 6 rats in each group; Pvalues: a<0.05, compared to control group

Table 5. Effect of ethanol on ascorbic acid in plasma, and nitrite, levels in serum of rats for 4 and 12 weeks

Groups	Ascorbic acid (mg/dl)	Nitrite (micromole)
Control	2.2 ± 0.14	18.1 ± 1.26
4weeks	1.9 ± 0.18	37.1 ± 1.97[a]
12weeks	1.7 ± 0.14	67.6 ± 4.27[bc]

Values are mean ± SD of 6 rats in each group

P values: a<0.05, compared to control group; b<0.05, compared to compare to 4 weeks ethanol treated group: c<0. 01,d<0.001compared to control group

Animals that received ethanol for 4 and 12 weeks caused no changes in GPx activity in the homogenized; however reduced ascorbic acid and increased nitrite concentration in the blood were reported when compared to the control group (Table 4).

4. Discussion

Biochemical tests can be useful to determining of toxicity effects of the target organs and the common health status in living organisms. They also provide early warning of potentially harmful changes in stressed organisms[11].

In present study oxidative damage of ethanol on liver start after 4 and 12 weeks of ethanol use which evidenced by higher levels of hepatic enzyme markers and MDA; a product of lipid peroxidation and decreased in protein and albumin levels in serum. Alcohol toxicity was evidenced by significant (P<0.001) increases in the levels of AST, ALT, ALP and GGT and a decline in the total protein and albumin level in alcohol-treated animals as compared to controls. Generally hepatocellular damages are quantified by of serum activities of AST and ALT however; routinely in practice ethanol toxicity is evaluated by GGT enzyme[12].

Ethanol prevents the synthesis of protein in the liver therefore; hypo-albuminemia is one the most important para clinic test for diagnosis of chronic alcoholic liver disease[13]. The decrease in protein level may be due to the necrosis of cells and result in protein synthesis problem[14].

Oxidative stress may inform by increase in free radical or decreased of antioxidant activity. Ethanol-induced liver injure may be due to oxidative stress. These effects were observed by 1.6 g/kg ethanol at 4 and 12 weeks treatment that may due to presence of higher level of alcohol dehydrogenase in the liver that catalyzes alcohol to relative aldehyde compounds[15].

Kidney damage was diagnosed after 12 weeks of ethanol consumption according to increase in blood ureaand creatinine tests compared to normal control. Blood Urea Nitrogen (BUN) is the major excretory product of protein metabolism. It is synthesized in liver from amino groups of amino acids and free ammonia. Determination of urea is used to assess renal function and aid in the diagnosis of renal disease[16]. Increase of plasma urea level may accompanied by stress induced by alcohol concentration[17].

Red blood cells are sensitive to oxidative injure because of unsaturated fatty acids. Chronic ethanol consumption can be effect on erythrocyte metabolism[18]. Decreased GSH levels with the duration of ethanol exposure (Table 3.) indicated time-dependent elevation of oxidative stress in the blood.

Significant increase in levels of MDA in treatment groups was reported compared to control group which may reflect the dose dependent effect of ethanol toxicity. Elevated of MDA which evidenced by increase in lipid peroxidation is a primary mechanism of cell damage[19].

Decrease in GSH concentration, ascorbic acid and increase in MDA and nitrite levels prone and sustaining a pathogenic role for oxidative stress which present in chronic ethanol consumption[20].

Malondialdehyde (MDA) is a marker for estimation of lipid peroxidation, tissue damage and oxidative stress which widely induced in mammalians[21]. Super Oxide Dismutase (SOD) is an antioxidant enzyme that present in live organisms. It guards the cell and tissues from damages which induced by superoxide radicals.

SOD reduces superoxide radicals that generated by cell injuries via conversion of superoxide radicals into hydrogen peroxide. Superoxide radical is a very injurious species for body cellular and produced hydrogen peroxide through dismutation reaction.

Furthermore, the hydroxyl radical generation from hydrogen peroxide and lipid peroxidation are unfavorable effects of superoxide radicals in human body. Non-significant increase of SOD activity in present study may due to presence of free radicals that induced by ethanol. In ethanol-induced toxicity catalase activity was reduced due to increase in superoxide, lipid peroxidation and decrease in NADPH levels or combination of all[22].

Ethanol-induced toxicity is protected by ascorbic acid due to antioxidant and reducing properties.

According to some research results Nitric Oxide (NO·) in low concentration has protective effects in cells, although higher amounts inducecy to toxicity. Toxicity of NO· could be associated to the synthesis of oxidative substances free radicals such as peroxynitrite, a product of NO·and superoxide[23,24].

Reactive nitrogen species, derivatives of Nitric Oxide (NO), have also been associated inoxidative stress. In the present work, the nitrite concentration were significantly increased which reflecting increased NO production.

5. Conclusion

Oxidative damage of ethanol on liver evidenced by elevation of AST, ALT, ALP, and GGT and MDA markers and decline in the total protein and albumin level.

6. Acknowledgement

Authors are grateful to the director of medicinal plant research center for their financial supports.

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