Research Article

# Effects of Acute Toxicity of Ethanol on NF-κB, IL-1 beta, TNF-alpha, and IL10 in Rats

**Abdulghaffar A. Abdulameer*, Nada N. Al-Shawi, Hayder Adnan Fawzi, Ammar A. Fadhil**

College of Pharmacy, University of Baghdad, Iraq.
***Corresponding author's E-mail:** haider_bahaa@yahoo.com

**Received:** 07-11-2017; **Revised:** 28-11-2017; **Accepted:** 13-12-2017.

**ABSTRACT**

Alcohol consumption had been linked to the -reduction in monocytes antigen presenting capacity and -alteration in inflammatory cytokine production, and thus it may increase the susceptibility for infection. This study was designed to emphasize the effect of acute toxicity of ethanol on IL-1 beta, TNF-alpha, IL10, and NF-κB in rats**.** Fourteen (14) adults Wistar Albino male rats (the available sex) weighing 200-250 gm with the age range (3-5) months were utilized in this study. Rats were randomly allocated into two groups (7 animals each) as follows: negative control group [(rats injected a single intraperitoneal (IP) volume (0.5ml) normal saline (NS)] and then the animals were euthanized by anesthetic ether 24 hours after NS administration; and ethanol-treated animals (rats were injected with single toxic I.P. dose (2.78g/kg) ethanol and then the animals were euthanized by anesthetic diethyl ether 24 hours after ethanol administration). In group of rats received acute toxic dose (2.78g/kg) ethanol, serum levels of NF-κB, IL1β and TNF-α were significantly lower; while, serum IL10 level was significantly higher ($P$<0.05) compared to negative control rats. In addition, there was an inverse correlation between IL10 and TNF-α level. Acute ingestion of ethanol led to suppression of proinflammatory cytokines and induction of anti-inflammatory cytokine thus it exert immune modulatory effect in rats.

**Keywords:** NF-κB, IL1 β, TNF-α, IL10, ethanol, rats.

## INTRODUCTION

The intensity of a toxic effect depends primarily on the concentration and persistence of the ultimate toxicant at its site of action. It may react with the endogenous target molecule or critically alters the biological environment, initiating structural and/or functional alterations that result in toxicity. Often the ultimate toxicant is the original chemical to which the organism is exposed (parent compound). The concentration of the ultimate toxicant at the target molecule has been reported to be dependent on the relative effectiveness of the processes that increase or decrease it's concentration at the target site. Various mechanisms of harmful effects of the drugs and chemicals were demonstrated; it may includ generation of reactive oxygen species (ROS), reactive nitrogen species (RNS) that may cause destruction of target molecules; dysregulation of gene expression and transcription; dysregulation of electrically excitable cells such as neurons, skeletal, cardiac, and smooth muscle cells; alteration in immune response; and others [1, 2]. Ethanol is therapeutically used as antiseptic, antitussive, antidote to methanol, and at high concentration it can be used as a solvent to dissolve many water-insoluble medications and related compounds. Additionally, it acts as a CNS depressant and has significant psychoactive effects in sub-lethal doses. Based on its abilities to alter human consciousness, ethanol is commonly consumed as a recreational drug, due to its psychoactive effects. Death from ethanol consumption is possible when blood alcohol levels reach 0.4%. A blood level of 0.5% or more is commonly fatal. Levels of even less than 0.1% can cause intoxication, with unconsciousness often occurring at 0.3–0.4%. Acute consumption of alcohol can cause significant damage to the brain and other organs [3]. Moreover, it has been reported that acute alcohol intoxicated rodents may modulate the levels of some proinflammatory and inflammatory factors following hemorrhagic shock [4]. This study was designed to emphasize the effect of acute toxicity of ethanol on IL-1 beta, TNF-alpha, IL10, and NF-κB in rats.

## MATERIALS AND METHODS

### Animals

Fourteen (14) adults Wistar Albino male rats (the available sex) with age range (3-5) months weighing 200-250 gm were utilized in this study. They were obtained from the Animal House of the College of Pharmacy/Baghdad University. They were kept in a polypropylene-plastic cages (three animals/cage), under controlled conditions [temperature, humidity and light/dark cycles]. The animals had free access to commercial pellet diet and water *ad libitum*. Rats were randomly allocated into two groups (7 animals each) as follows: negative control group (rats intraperitoneally injected with a single volume [(0.5ml normal saline (NS)]; and ethanol-treated animals (rats were injected with a single toxic I.P. dose (2.78g/kg) ethanol. After 24 hr of either treatment, the animals were euthanized by anesthetic diethyl ether

### Collection and preparation of serum samples

After euthanization of rats by anesthetic diethyl ether after 24hrs of either treatment, whole blood was

withdrawn from each rat by intracardiac puncture and divided into two parts: First, was put into anticoagulant tube which was used for the measurement of ethanol concentration. Second, blood sample put into plane tube and left to clot then centrifuged at 3500 rpm for 15 minute to obtain serum, which was separated and stored at -40 Cº until the time of analysis. Serum was utilized for the estimation of TNF-α, NF-κB, IL10, and IL1β.

**Measurement of serum nuclear factor kappa-light-chain-enhancer of activated B cells (NF-κB) level**

Determination of serum NF-κB level was based on Sandwich-ELISA technique; the micro-elisa strip plate was provided in this kit and has been pre-coated with an antibody specific to NF-κB. Standards or samples were added to the appropriate micro-ELISA strip plate wells and combined to the specific antibody. Then a Horseradish Peroxidase (HRP)-conjugated antibody specific for NF-κB was added to each micro-ELISA strip plate well and incubated. Free components are washed away. The TMB substrate solution was added to each well. Only those wells that contain NF- κB and HRP conjugated NF-κB antibody will appear blue in color and then turn yellow after the addition of $H_2SO_4$ solution. The optical density (OD) was spectrophotometrically measured at 450 nm. The OD value is proportional to the concentration of NF-κB. Calculation of the NF- κB concentration in samples was brought about by comparing the OD of the samples to the standard curve. Serum NF-κB level was expressed as pg/ml.

**Determination of serum interleukin-1beta (IL-1β) level**

The principle of IL-1β level determination in serum was based on ELISA. This assay employed an antibody specific for IL-1β coated on well plate. Standards and samples were pipetted into the wells and IL-1β present in a sample is bound to the wells by the immobilized antibody. The wells were washed and biotin conjugated antibody was added. Then incubated and washed to remove any unbound substance. Horseradish peroxidase (HRP)-conjugated streptavidin was pipetted to the wells, which were again incubated and washed; then TMB substrate solution was added to the wells after incubation. The color develops in proportion to the amount of IL-1 beta bound. The stop solution (acidic solution) changed the color from blue to yellow. The intensity of the color was measured at 450 nm. Serum IL1β level was expressed as pg/ml.

**Measurement of serum tumor necrosis factor-alpha (TNF-α) level**

Determination of serum TNF-α level was based on sandwich enzyme-linked immune-sorbent assay (ELISA) technique, Anti-TNF-α polyclonal antibody was pre-coated onto well plates. The biotin conjugated antibody was used as detection antibodies. Then incubate and wash to remove unbound substance then avidin-biotin-peroxidase complex (ABC) working solution added into each well; after incubation and washing, tetramethylbenzidine (TMB) substrate was added; then a stop solution (acidic solution) was added to each well. The intensity of color was spectrophotometrically measured at 450nm by means of a microplate reader. Serum TNF-α level was expressed as pg/ml.

**Determination of serum interleukin-10 (IL10) level**

Serum IL-10 level was determined by utilization of the quantitative sandwich ELISA technique; this assay employed an antibody specific for IL10 coated on well plate. Standards and samples were pipetted into the wells and IL10 present in a sample was bound to the wells by the immobilized antibody. Horseradish peroxidase (HRP)-conjugated reagent was pipetted to the wells, which are incubated and then washed; after that, chromogen solution A and chromogen solution B was successively added to each well. Then incubated and washed; lastly, a stop solution (acidic solution) was added to each well; this can change the color from blue to yellow. The intensity of the color was measured at 450 nm. Serum IL10 level was expressed as pg/ml.

**Statistical Analysis**

Anderson darling test was done to asses if continuous variables follow normal distribution. Independent Student t test was used to analyze the differences in means between two groups (if both follow normal distribution with no significant outlier). SPSS 20.0.0, GraphPad Prism 7.0 software package used to make the statistical analysis, *P* value of less than 0,05 is considered significant in all the results of this study.

**Results and Discussion**

Table 1 showed that, serum IL-1β level was significantly lower ($P<0.05$) in the ethanol-treated rats compared to control (1.44 vs. 6.01pg/ml; $P=0.008$); it is 4 folds lower in alcoholic-treated rat compared to control. Authors reported that IL-1β is considered as a proinflammatory marker and it has been found to be elevated in brains of alcoholic humans [7]; furthermore, IL-1β was found to be a significant proinflammatory marker that correlated with neurodegeneration processes [8]. The finding of this study was consistent with the work of Mandrekar *et al* in which, they found that an acute intake of alcohol led to inhibition of IL-1β [9]; moreover, this study were in agreement with those of Szabo*et al* who reported that ethanol suppression occurred at both mRNA and protein levels and ethanol lowered the stability of IL-1β mRNA [10].

Moreover, table 1 showed that serum level of IL-10 was significantly higher ($P<0.05$) in ethanol-treated rats compared to control (51.530 ± 7.680 vs. 36.265 ± 9.506 pg/ml). The findings obtained from this study was in agreement with Mandrekar *et al* at 1996 [11] in which, authors found that after acute treatment with ethanol, IL-10 levels was elevated after 10 hours and reach its peak after 18 hours. This effect was not only observed in the serum but also at the level of protein and mRNA [11]. Moreover, consistent results were observed concerning

the elevation of serum level of IL-10 after ethanol administration in this study with the results of Drechsler*et al* study by utilization of mice model [12]. In the current study serum level of TNF-α was significantly reduced (*P*<0.05) by acute IP dose (2.78g/kg) of ethanol compared to control (7.849 ± 2.234 vs. 17.961 ± 2.816 pg/ml). Table 1.

**Table 1:** Effects of acute toxic dose of ethanol (2.78g/kg) on serum levels of NF-κB, IL-1 β, TNF-α, and IL-10 compared to negative control rats

| Variables | Ethanol-treated | Negative control | *P* value |
|---|---|---|---|
| NF-κB (pg/ml) | 229.271 ± 26.452 | 396.314 ± 20.372 | <0.001 |
| IL-10 (pg/ml) | 51.530 ± 7.680 | 36.265 ± 9.506 | 0.006 |
| IL-1 β (pg/ml) | 1.222 ± 0.361 | 6.008 ± 3.083 | 0.008 |
| TNF-α (pg/ml) | 7.849 ± 2.234 | 17.961 ± 2.816 | <0.001 |
| Independent Student t test was used | | | |

The finding of this study was in agreement with Mandrekar *et al* at 2002 [9] in which TNF-α was reduced in ethanol treatment mice. Moreover, the results of this study were also consistent with those of Drechsler*et al* [12] results.

Table 1 also showed that, serum level of NF-kB was significantly lowered in ethanol treated rats compared to control (229.271 vs 396.314 pg/ml). The results of this study were similar to those of Mandrekar *et al* at 2002 [9] and Drechsler*et al* [12] studies. Investigators reported that NF-kB is a central regulator of gene transcription for proinflammatory (TNF-α, IL-1, IL-6) and anti-inflammatory cytokines (IL-10), and the inhibition of NF-kB by ethanol can lead to reduction of TNF-α and IL-1β as seen by the results of this study.

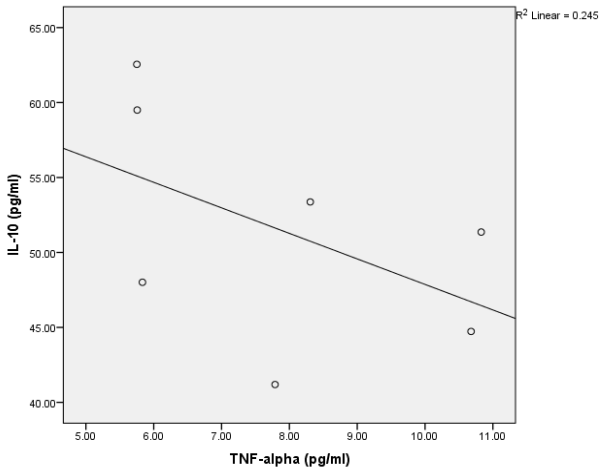


**Figure 1:** Scatter-plot of the relationship between IL10 with TNF-α showing inverse relationship between them (r=-0.495, *P*=0.258)

Figure 1 showed that, there was an inverse correlation between serum level of TNF-α with IL10; but this study could not determine which cause the effect (i.e. if ethanol induce IL10 and thus inhibiting TNF-α or ethanol suppress TNF-α thus leading to elevated level of IL10). The finding of this study was in agreement with those of Mandrekar *et al* at 1996 [11]; where, authors suggested that elevated monocyte IL-10 induction be the probable cause of TNF-α inhibition.

## CONCLUSION

Acute ingestion of ethanol led to suppression of proinflammatory cytokines and induction of anti-inflammatory cytokine thus it may exert an immuno modulatory effects in rats

## REFERENCES

1. Curtis, D. Klaassen. Casarett and Doull's Toxicology. The basic Science of Poisons. 2008; 7th edition.Unit one; PP 11-45.
2. Swift, Robert. "Direct measurement of alcohol and its metabolites". *Addiction* 98,2003, 73–80.
3. Becker, CE. "The Clinical Pharmacology of Alcohol".*California Medicine* 113 (3), 2013, 37–45.
4. Urbano-Marquez, A and Fernandez-Sola, J. Effects of alcohol on skeletal and cardiac muscle. *Muscle Nerve* 30, 2004, 689–707.
5. Karinch, A. M; Martin, J. H and Vary, T. C. Acute and chronic ethanol consumption differentially impact pathways limiting hepatic protein synthesis. American Journal of Physiology 295 (1), 2008, E3–E9.
6. Tsung-Ming, Hu Ru-Ping, Lee; Chung-Jen, Lee *et al*. Heavy Ethanol Intoxication Increases Proinflammatory Cytokines and Aggravates Hemorrhagic Shock-Induced Organ Damage in Rats. *Mediators of Inflammation* 2013; 2013: 9 pages. Article ID 121786, http://dx.doi.org/10.1155/2013/121786.
7. He, J and Crews, FT: Increased MCP-1 and microglia in various regions of the human alcoholic brain. *Exp Neurol* 210(2), 2008, 349–358.
8. Simi, A; Tsakiri, N; Wang, P and Rothwell, NJ: Interleukin-1 and inflammatory neurodegeneration. *Biochem Soc Trans* 35(Pt 5), 2007, 1122-1126.
9. Mandrekar, P; Dolganiuc, A; Bellerose, G *et al*. Acute Alcohol Inhibits the Induction of Nuclear Regulatory Factor κB Activation Through CD14/Toll-Like Receptor 4, Interleukin-1, and Tumor Necrosis Factor Receptors: A Common Mechanism Independent of Inhibitory κBα Degradation?. Alcoholism: *Clinical and Experimental Research*. 26(11), 2002, 1609-14.

10. Szabo, G; Mandrekar, P and Catalano, D. Inhibition of superantigen-induced T cell proliferation and monocyte IL-1 beta, TNF-alpha, and IL-6 production by acute ethanol treatment. *Journal of leukocyte biology* 58(3), 1995, 342-50.

11. Mandrekar, P; Catalano, D; Girouard, L and Szabo, G. Human monocyte IL-10 production is increased by acute ethanol treatment. *Cytokine* 8(7), 1996, 567-77.

12. Drechsler, Y; Dolganiuc, A; Norkina, O *et al*. Heme oxygenase-1 mediates the anti-inflammatory effects of acute alcohol on IL-10 induction involving p38 MAPK activation in monocytes. *The Journal of Immunology* 177(4), 2006, 2592-600.

**Source of Support:** Nil, **Conflict of Interest:** None.