Original Article

# Green Tea Extract Ameliorates Ethanol Induced Liver Injury in Albino Rats

**Poonam Lodhi*[1], Neeraj Tandan[2], Neera Singh[1], Divyansh Kumar[3]**

[1]Environmental Endocrinology and Biomedical Research Unit, Department of Zoology, Meerut College, Meerut 250003, India.
[2]Scientific and Applied Research Center, Meerut, U.P., 250001, India.
[3]D.A.V. PG College, Bulandshahr, Uttar Pradesh, Pin-203001, India

**Address for Correspondence**

Environmental Endocrinology and Biomedical Research Unit, Department of Zoology, Meerut College, Meerut 250003, India.
Tel:+ 917409827767.
**E-mail:** poonamlodhi@gmail.com

## ABSTRACT

In the present study, it was try to investigate the effects of green tea on some biochemical parameters of male albino rats with ethanol toxicity. All rats were divided into 4groups, Group 1 (controls) and group 2 (ethanol control) remaining 2 groups were treated with ethanol and green tea extract for 30 days. The results showed significant elevation in the level of ACP, sugar ($P<0.001$), and Bilirubin ($P<0.005$) where as liver weight Showed no significant elevation with ethanol as compared to normal. These changes in all parameters indicated liver injury and hepatomegaly due to the enhancement in the ROS which causes decrease in antioxidants of body defense system. The green tea extract with ethanol treatment was able to restore these changes at various levels of significance comparing with the control group of rats. These data suggested that the green tea exerts improvement in liver function by preventing the production of reactive oxygen species (ROS) and enhancing the antioxidant defense system capacity. Thus green tea extract has protective effects against ethanol toxicity.

**Keywords**: Ethanol, Green Tea Extract (GTE), Liver, ACP, Bilirubin, sugar.

## INTRODUCTION

Ethanol is used for avoiding stress and in milder respiratory infections in winter season. Ethanol consumption causes various diseases of liver, other organ and affects many systems of the body[10]. The liver is the target organ of ethanol toxicity and causes fatty change, hepatitis and cirrhosis in liver. The major toxic metabolites of ethanol are acetaldehyde and free radicals[23]. Chronic ethanol ingestion induces changes in intestinal brush-border membrane, serum electrolytes and haematology[4]. Deficiency in enzymes and non-enzyme activity is associated with alcoholic liver disease[7,17].

Oxidative stress is a key step in the pathogenesis of tissue injury by ethanol generating reactive oxygen species (ROS) in many tissues and decreasing in the endogenous antioxidants[20].

Medicinal plants play an important role in pharmacology and medicine for many years. Today, it is estimated that about 80% of the world population relies on botanical preparations as medicine to meet their health needs[15]. The green tea belongs to family Theaceae. The green tea extract and its main catechin polyphenols have medicinal value for prevention and therapeutics in several diseases[12,16]. Antioxidants play an important role in the protection of cells and tissues against free radical mediated tissue injury[18,22]. In the present study, it was tried to find out the effects of green tea on biochemical parameters against ethanol toxicity.

## MATERIALS AND METHODS

### Preparation of extract

Green tea was procured from Tea State of Tata Group of Company, TALAT, Assam. The aqueous extract of green tea was prepared by following the method described by Dahiru *et al*[6]. Two doses of extract were given orally to treat rats. One was 5mg/kg bw another 10mg/kg bw.

### Experimental animals

All animals were (7-8 weeks old, Wistar strain albino rats) purchased from Animal Division of IVRI, Izatnagar, were acclimatized for laboratory conditions at room temperature with standard food pellets and tap water *ad libitum.* All animals were cared for according to guidelines of the Institutional Animal Ethics registered by IAEC (384/PO/a/01/CPCSEA 28-03-2001).

### Experimental design

After 2 weeks, each rat received a dose of 0.25ml/100 g body weight ethanol by oral route for 30 days. All experimental animals were divided into following 4 groups. Each group had 6 animals:

Group I: Normal rats.

Group II: Ethanol control group.

Group III: Ethanol+ Green tea aqueous extract (5mg/kg bw) dose.

Group IV: Ethanol+ Green tea aqueous extract (10mg/kg bw) dose.

### Haematological studies

After 30 days, all rats were sacrificed under light ether anesthesia. The blood was collected by retro-orbital pluxes in EDTA vials for biochemical analysis. All the chemicals used were of analytical grade. The kits for all biochemical estimations were purchased from Nice Pvt, Ltd. Banglore, India.

### Statistical analysis

The significance value of all the data was analyzed using student's t-test from Sigma Plot software (version 11). Results were presented as Mean ± SD and *P* value less than 0.05 was considered significant.

## RESULTS

In the present study, a no significant increase was observed in the liver weight with 0.25 ml dose of alcohol whereas both doses of green tea extract reduced the liver weight after 30 days (Table 1). The levels of acid phosphatase, sugar, total and direct bilirubin showed significant increase with ethanol dose at different significance level (Figure 1). No significant decrease was observed in these all parameters with 5 mg dose of green tea extract whereas with 10 mg dose of extract was in normal range as compared to normal groups of rats (Table 2). The level of urea showed no change.

## DISCUSSION

The increase in the weight of liver may be due to hepatomegaly or hepatotrophy. It is attributed to the fact that chronic alcohol consumption causes accumulation of lipids and proteins in hepatocytes with an impaired protein secretion by hepatocytes[3,24]. Water is retained in the cytoplasm of hepatocytes leading to enlargement of liver cells resulting in increased total liver mass and volume[5]. This alcohol induced increase in total liver weight was prevented by treatment with green tea extract. *Tylophora indica* leaf extract and *Solanum nigrum* fruit extract (SNFEt) indicating a hepatoprotective effect against ethanol[9,1]. Chronic ethanol administration is known to enhance the protein and fat accumulation in the liver. Ethanol and acetaldehyde are known to induce cell injury and necrosis by enhancing the production of reactive oxygen species by the process of lipid peroxidation[11].

The level of acid phosphatase (ACP) increased with ethanol treatment[21]. In tissues, acid phosphatase is associated with break down and catalytic activities. High level of acid phosphatase is observed in tissues with cell destruction and lytic activities. ACP is a lysosomal enzyme which hydrolyses the ester linkage in phosphate esters and help in the autolysis of the cell after death. Experimental evidences show that it is not only restricted to lysosomal fraction out has also been found in golgi cisternae and specialized region of endoplasmic reticulum. Acid phosphatase reaction thus reflects some impressions about the structure and function of these organelles or components. The damaged organs might produce an augmented quantity of the enzymes[19]. Green tea extract restore the ACP level to normal[8]. Normal level of acid phosphatase in blood clearly indicates that no tissue damage is taking place.

The level of blood sugar showed significant increase with ethanol dose compared to control rats[2]. This increased level was almost in normal range with green tea extract. Our results are consistent with Mongi Saoudi *et al*[14]. who reported *Opuntia vulgaris* fruit extract (OE) treatment could significantly decrease the level of glucose because of a potential source of natural antioxidants. The protective effects of OE may be due to the modulation of antioxidant enzymes activities and inhibition of lipid per oxidation. Same mode of action can be suggested for green tea.

The level of total and direct bilirubin showed significant increase with ethanol when compared with control group[9,6]. Green tea extract prevent these changes and our results are also consistent with Maruthi *et al*. and Wan-Guo Yu *et al*.[13,25]. 2′,4′-Dihydroxy-6′-methoxy-3′,5′-dimethylchalcone has potential hepatoprotective effects against $CCl_4$ and decreased activity of total bilirubin and the aqueous extract of *Ziziphus mauritiana* leaves with alcohol resulted in reduction of bilirubin level[6,25]. Which cause attenuation of oxidative stress and inhibition of lipid peroxidation.

## CONCLUSION

The liver can be injured by many chemicals and drugs. In the present study ethanol was selected as a hepatotoxicant to induce liver damage, since it is clinically relevant. These findings indicate that antioxidant or flavanols and polyphenols (catechins) present in *C. sinensis* probably inhibit ethanol uptake by tissues and exert their protective effects. The green tea exerts improvement in liver function by preventing the production of reactive oxygen species and enhancing the antioxidant defence system capacity. In conclusion, the results of this study indicate that treatment of rat with green tea extract had a marked protective effect against ethanol toxicity.

## ACKNOWLEDGEMENT

The authors are grateful to the Director, Scientific and Applied Research Center, Meerut for providing research facilities and encouragement to P.R.

## REFERENCES

1. Arulmozhi V, Mani K, Kandhan K, Ganesan D, Sankaran M. Antioxidant and antihyperlipidemic effect of Solanum nigrum fruit extract on the experimental model against chronic ethanol toxicity. Pharmacogn Mag. 2010; 6(21): 42–50.
2. Arun R, Praveen KV, Sheeba V, Mukkadan JK, Joseph PK. Biochemical effects of feeding soft drink and ethanol. *Indian J Exp Bio*. 2009; 47: 333-337.
3. Ashakumary L, Vijayammal PL. Additive effect of alcohol and nicotine on lipid metabolism in rats. *Indian J Exp Biol*. 1993; 31: 270-4.
4. Bashir M, Javed MT. Effects of ethanol on brain and pancreas weights, serum sodium and potassium and haematological parameters in quail (*Coturnix japonica*). Avian Pathol. 2005; 34: 96-100.
5. Blancho CD, Gentil S. Alternations of glucose metabolism in chronic liver disease. Diab Res Clin Prac.1990; 8: 29-36.
6. Dahiru D, Obidoa O. Pretreatment of albino rats with aqueous leaf extract of *Ziziphus mauritiana* protects against alcohol induced liver damage. *Tropical J Pharma Res*. 2007; 6(2): 705.
7. Devaki T, Venmadhi S, Govindaraju P. Alterations in protein metabolism in ethanol-ingested rats treated with garlic oil. Med Sci Res. 1992; 20: 725-727.
8. Geidam MA, Daudam E, Hamza HG. Effects of aqueous stem- bark extract of *Momordica balsamina* Linn on some serum enzymes in normal and ethanol fed rats. J of Biological Sciences. 2007; 7(2): 397-400.
9. Gujrati V, Patel N, Rao VN, Nandkumar K, Gouda TS, Shalam Md, Shanta KSM. Hepatoprotective activity of alcoholic and aqueous extracts of leaves of *Tylophora indica* (linn.) in rats. *Indian J Pharmacol*. 2006; 39(1): 43-47.
10. Klassen RW, Persaud T V. Influence of alcohol on the reproductive system of the male rat. *Intern. J. Fertil*. 1978; 23: 176 - 184.
11. Kurose I, Higuchi H, Kato S, Miura S, and Ishii H. Ethanlo induced oxidative stress in the liver. Alcohol Clin Exp Res. 1996; 20: 77-85.
12. Mandel S, Weinreb O, Reznichenk L, Kafon L, Amit T. Green tea catechins as brain-permeable, non toxic iron chelators to 'iron out iron' from the brain. *J Neural Transm*. 2006; 71: 249–57.
13. Maruthi TE, Ramesh CK, Mahmood R, Thippeswamy BS, Verapur V. Hepatoprotective and antioxidant effect of *Azima tetracantha* Lam. leaves extracts against $CCl_4$- induced liver injury in rats. Indian Journal of Natural Products and Resources. 2010; 1(4): 493-499.
14. Mongi S, Samira J, Kamel J, Ghada BS, Choumous K, Abdelfattah EF. Haematological and biochemical toxicity induced by methanol in rats: Ameliorative effects of *Opuntia vulgaris* fruit extract. Hum Exp Toxicol. 2011; 30(12): 1963-1971.
15. Ogbera AO, Dada O, Adeyeye F, Jewo PI. Complementary and alternative medicine use in diabetes mellitus. *West Afr. J. Med*. 2010; 29(3): 158-162.
16. Ostrowska J, Skrzydlewska E. The comparison of effect of catechins and green tea extract on oxidative modification of LDL in vitro. Adv Med Sci. 2006; 5:1 298–303.
17. Ozaras R, Tahan V, Aydin S, Uzun H, Kaya S, Senturk H. N-acelylcysteine attenuates alcohol-induced oxidative stress in rats. World J Gastroenterol. 2003; 9:791-794.
18. Ray G, Hussain SH. Oxidants, antioxidants and carcinogenesis. *Ind J. Exp. Biol*. 2002; 40: 1213-1232.
19. Rees, KR, Sinha KP. Blood enzymes in liver injury. J Pathol Bacteriol. 1960; 80: 297-307.
20. Sadrzadeh SM, Nanji AA, Meydani M. Effect of chronic ethanol feeding on plasma in normal and vitamin E-deficient rats. Relationship to lipid peroxidation. Biochem Pharmacol. 1994; 47: 2005- 2010.

21. Soliman KM, Hamed MA, Ali SA. Hepatoprotective effect of carnosine on liver biochemical parameters in chronic ethanol intoxicated rat. Medical J Islamic World Aca. Sci. 2006); 16(2): 77-86.
22. Sriram N, Kalayarasan S, Sudhandiran G. Enhancement of antioxidant defense system by epigallocatechin-3-gallate during bleomycin induced experimental pulmonary fibrosis. Biol Pharm Bull. 2008; 31(7): 1306.
23. Suzuki Y, Cherian MG. Effect of ethanol on brain metallothionein in transgenic mice. Alcohol Clin. Exp. Res. 2000; 24: 315-321.
24. Vilstrup H, Tygstrup N. Glucoregulation in acute liver failure. Eur J Clin Invest.1985; 16: 193-7.
25. Wan-Guo Yu, Jie Q,and Yan-Hua Lu (2011). Hepatoprotective effects of 2',4'-Dihydroxy-6'-methoxy-3',5'-dimethylchalcone on CCl4-induced acute liver injury in mice. *J Agri. Food Chem.* 2011;59(24):12821-12829.

**Table 1.** Effect of GTE on liver weight of ethanol-induced liver injury in rats

| Parameters Liver wt.gm | Controls | EtOH group 0.25ml / kg B wt. | EtOH + GTE group 0.25ml + 5mg/ kg B wt. | EtOH + GTE group 0.25ml + 10mg/ kg B wt. |
|---|---|---|---|---|
| 30 Days | 1.65± 0.14 | 1.81± 0.40$^{d}$ | 1.72± 0.09$^{d}$ | 1.62± 0.12$^{d}$ |
| 30 Days+ reversibility | 1.52±0.20 | 1.72±0.10$^{d}$ | 1.67± 0.15$^{d}$ | 1.65±0.17$^{d}$ |

Values represent mean ± S.D.; n = 6; a = P<0.01, b = P<0.005, c = P<0.001, d = non significant, e= no change

**Table 2.** Effect of GTE on biochemical parameters in serum in ethanol-induced liver injury in rats

| Parameters | Controls | EtOH group 0.25ml / kg B wt. | EtOH + GTE group 0.25ml + 5mg/ kg B wt. | EtOH + GTE group 0.25ml + 10mg/ kg B wt. |
|---|---|---|---|---|
| ACP (KA unit) | 1.17± 0.39 | 1.55± 0.15$^{c}$ | 1.15± 0.18$^{d}$ | 0.93± 0.121$^{c}$ |
| Sugar (gm/dl) | 94±14.79 | 128±9.89$^{c}$ | 125± 9.58$^{d}$ | 98±19.08$^{c}$ |
| Total Bilirubin (gm/dl) | 0.65±0.18 | 1.43±0.18$^{b}$ | 1.15±0.25$^{d}$ | 0.66±0.21$^{c}$ |
| Direct Bilirubin (gm/dl) | 0.74 ± 0.16 | 1.1 ± 0.22$^{b}$ | 1.1± 0.29$^{d}$ | 0.91±0.24$^{c}$ |
| Urea (mg/dl) | 24.33± 6.12 | 24.16± 5.15$^{e}$ | 23.5± 4.03 | 23.33± 3.44 |

Values represent mean ± S.D.; n = 6; a = P<0.01, b = P<0.005, c = P<0.001, d = non significant, e= no change

 ISSN 2321 – 2748

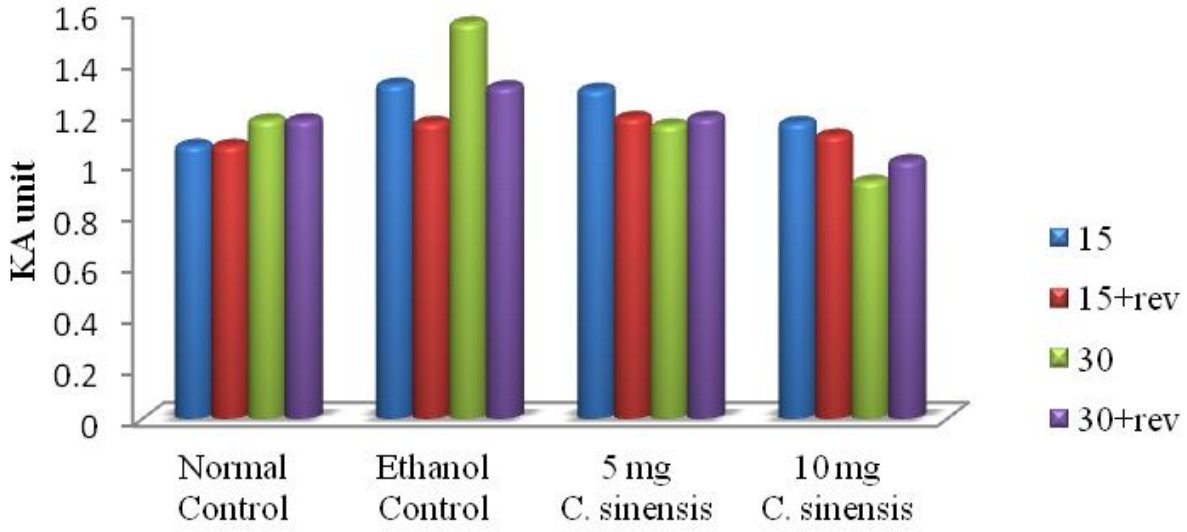


**Figure 1.** Histogram showing comparison of acid phosphataselevel in blood of 0.25 ml ethanol treated and *C. sinensis* aqueous extract treated group.