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Ameliorated Effects of *Camellia sinensis* Extract on Acute Toxicity of Ethanol on Haematological Profile of Albino Rats

Poonam Lodhi*[1], Neeraj Tandan[2], Neera Singh[1], Monu Kumar[2] and Divayansh 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. (P.G.) College, Bulandshahr, U.P., 203001, India.*
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Abstract

The present study was aimed to investigate the ability of some natural antioxidants present in CSE on haematological profile of male albino rats against ethanol toxicity. The rats were divided into 4 groups. Group 1 fed on a standard diet (controls), group 2 received a dose of 0.25ml/100g body weight (ethanol treated), group 3 and group 4 treated with ethanol and CSE (5mg/kg bw and 10mg/kg bw) for 15 days. The ethanol administration significantly ($P<0.05$) decreased the levels of red blood cell count (RBCs count) counts, haemoglobin percentage (Hb%), packed cell volume (PCV%), total leucocyte count (TLC) and differential leucocyte count (DLC count) comparing with the control group of rats. However, the oral feeding of CSE to rats after 30 minutes of ethanol ingestion caused significant ($P<0.001$) improvement in the levels of above parameters. Ethanol metabolism led to the formation of acetaldehyde, a highly cytotoxic compound responsible for the oxidation of proteins, erythrocyte abnormalities and hemolysis. It causes an oxidative stress resulting from increased free radical production and decreased antioxidant defence. The data of this study suggested that, CSE has the ability of natural antioxidants to prevent the changes in the above blood profile against the ethanol toxicity by enhancing the levels of total antioxidant status.

Keywords: Ethanol, *Camellia sinensis* extract (CSE), RBCs, Hb%, PCV, TLC

Contents

***Corresponding author**
Poonam Rani
Department of Zoology,
Meerut College, Meerut, India.
E-mail: poonamlodhi@gmail.com
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1. Introduction

The excessive intake of alcohol is widely consumed in alcoholic beverages all over the world. Ethanol is a principal ingredient of alcoholic drinks which produces many ethanol-related health complications, including those affecting the red blood cells, a variety of behavioral, biological, cognitive changes and the liver damage (MA Leo et al., 1982; HS Ballard, 1989). Ethanol diffuses into all body water through blood uniformly after its consumption (C Trandum et al., 1999). After stomach mucosa cells ethanol affects red blood cells and other cells in blood and un-metabolized ethanol causes hemolysis (G Lahajnar et al., 1995; E Padmini et al., 2008; KS kumar et al., 2013). The ethanol is primarily metabolized in the liver (JJ Maher, 1997) which caused release of acetaldehyde in the blood where it causes abnormality in erythrocyte (OV Tyulina et al., 2006). Thus, excess alcohol consumption may accelerate an oxidative mechanism directly or indirectly, which eventually produces cell death and tissue damage (PE Hartman et al., 1990; AY Sun et al. 2001). Therefore, alternative treatments for liver disorders are needed to replace the existing synthetic drugs.

Medicinal plant products are known to modify different aspects of human physiology and exert an alleviating influence on several patho-physiological states, and concepts of psychological disorder (G Ganu et al., 2013; K Prasad et al., 2013;T Gopal et al., 2013). Nowadays, tea is considered as a source of dietary constituents with biological and pharmacological activities with potential benefits to human health. *Camellia sinensis* (L.) O.Kuntze, (Family Theaceae) is the second most popular beverage worldwide and contains six primary catechins or polyphenol compounds (CS Yang et al. 2001). The tea extract and its main catechin polyphenols have medicinal value for prevention and therapeutics in several diseases (S Mandel et al., 2006; J Ostrowska and Skrzydlewska, 2006). The tea extract also displays antioxidants and free radicals scavenger properties (V Crespy and Williamson, 2004). Much of the effects of green tea are believed to be mediated by the polyphenolic constituents most notably epigallocatechin-3-gallate (EGCG) present in it. (SK Katiyar and Mukhtar, 1996). In the present study, an attempt has been made to find out the effects of *C. sinensis* on haematological profile against ethanol toxicity.

2. Materials and Method

Preparation of extract:

Green tea was procured from Tea State of Tata Group of Company, TALAT, Assam. Preparation of aqueous extract of *Camellia sinensis* was done according to the method described by (D Dahiru et al., 2007).

Experimental animals:

All Male Wistar strain albino rats (7-8 weeks old, 100-150gm bw) purchased from Animal house of IVRI, Izatnagar, were acclimatized for laboratory conditions at room temperature and kept on normal diet (tap water *ad libitum*) in the animal facility of the Zoology Department of Meerut College, Meerut. All animals were cared for according to guidelines of the Institutional Animal Ethics registered by IAEC (384/PO/a/01/CPCSEA 28-03-2001). Committee (IAEC) and experiments were also approved.

Experimental design:

After 2 weeks, all experimental animals were divided into following 4 groups of 6 animals each. The experiment duration was 2 weeks. The animals of all groups accept normal control received 0.25ml ethanol dose orally by oral gavage.

Group I: Normal rats.

Group II: Ethanol control group.

Group III: 0.25ml/100gm ethanol + CSE dose of 5.0 mg/kg body weight.

Group IV: 0.25ml/100gm ethanol + CSE dose of 10 mg/kg body weight.

Haematological studies- The animals of different groups were sacrificed under light anesthesia (diethyl ether) 1 hour after the end of the treatment. The blood sample from each rat was collected from the orbital-vein in fluoride tubes for analysis. Haematology was done according to standered methods (L Chen et al., 2002).

Statistical analysis

Results were presented as Mean ± SD. The Differences between groups were analyzed using student's t-test. Statistical significance was accepted at $p < 0.05$). All the data was calculated by using the software sigma plot (version 11).

3. Results and Discussion

Results

In the present study the body weight was non-significantly decreased with the ethanol treatment and restored in a dose dependant manner to near normal level with CSE treated compared with normal control. It appears that green tea exerts improvement in body weight (Figure 1).

The level of haematological parameters The packed cell volume (PCV), haemoglobin concentration Hb%, and RBC counts in the ethanol treated rats were significantly reduced compared with the control group. The CSE doses were

able to elevate significantly ($p < 0.05$) these values in all the treated groups treated compared with the ethanol treated group (Table 1). The percentage of MCV, MCH and MCHC was based on PCV, Hb% and RBC count. The level of these parameters showed changes according to these changes (Table 2). Ethanol administration caused a significant decrease in the level of TLC Count and came down near to normal levels after both doses of C. sinensis as compared to normal control group. DLC also showed changes according to the changes in TLC (Table 1). DLC also showed changes according to the change in TLC. Ethanol treatment in rats significantly decreased neutrophil and eosinophil counts. But in 5 mg C. sinensis group number of neutrophils and eosinophils was comparatively lower than the normal control group. Percentage of monocytes and basophils was comparatively unaffected in all treated groups. In 10 mg C. sinensis group of both sets, DLC was observed normal (Table 2).

Comparison of initial and final body weight after 15 days treatment

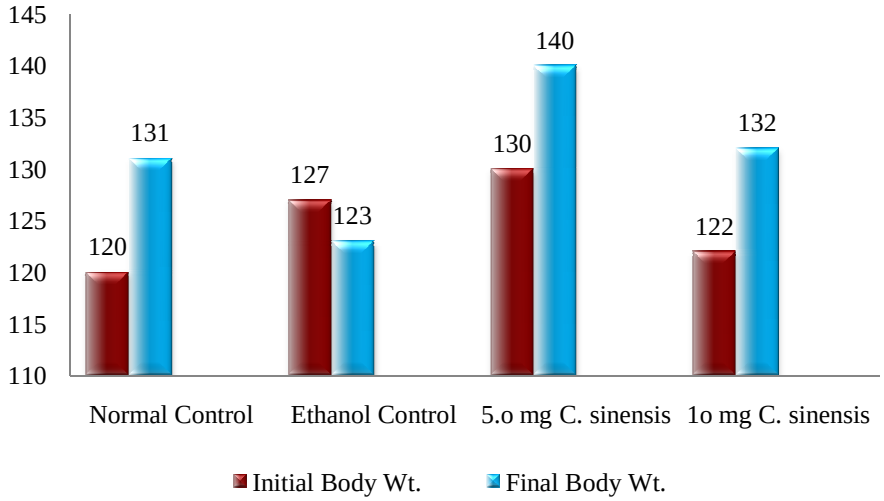

Figure 1. Comparison of body weight of control, ethanol treated (0.25 ml/100 g. of C_2H_5OH) and *C. sinensis* (5 mg and 10 mg) treated albino rats.

Table 1. Effect of CSE on concentrations of blood parameters in acute ethanol-induced acute toxicity in rats.

Analyzed parameters	Control group	EtOH group 0.25ml/100gm	EtOH + CSE group 0.25ml+5mg/kg bwt	EtOH + CSE group 0.25ml+10mg/kg bwt
Hb gm/dl	10.44±0.75	8.56±0.46[b]	12.11±0.49[c]	12.91 ± 0.41[c]
RBCs million/cumm	4.37 ± 0.87	3.08±0.78[b]	4.76 ± 0.29[c]	5.4 ± 0.7[c]
PCV%	44.49 ± 2.5	24.16 ± 2.22[b]	31.83 ± 2.7[c]	41.5 ± 4.88[c]
TLC cumm	8445 ± 287	3791 ± 3791[b]	5400 ± 718.3[c]	8246 ± 2018[c]

Table 2. Effect of CSE on concentrations of blood parameters in acute ethanol-induced acute toxicity in rats.

Analyzed parameters		Control group	EtOH group 0.25ml/100gm	EtOH + CSE group 0.25ml+5mg/kg bwt	EtOH + CSE group 0.25ml+10mg/kg bwt
DLC %	N	42.64 ± 4.16	32.11 ± 2.63[c]	35.11 ± 1.41	41.61 ± 3.61[c]
	L	53.46 ± 5.9	52.68±3.61	53.32±5.56	55.01±7.49
	E	2.33±0.51	1.82±0.74	1.65±0.81	1.79±0.72
	M	2.16±0.72	2.15±0.51	2.34±1.31	2.45±1.25
	B	0.66±0.55	0.58±0.54	0.52±0.17	0.51±0.55
MCV μm^3		89.16 ± 5.6	68 ± 4.19	80 ± 3.16[c]	83.5 ± 83.5[c]
MCH Pg		25.04 ± 7.3	16.5 ± 1.87	18.83 ± 1.83	22.5 ± 2.51
MCHC %		23.58 ±2.8	18 ± 4.14	19.5 ± 3.56	21 ± 3.89

Values are mean ± SD Rats for each reading = 6, Significance as per Student "t" test, a = P<0.01, b = P<0.05, c = P<0.001.

Discussion

In the present study the Comparison between the initial and final weights of rats in each group showed that the body weight of rats in the control and ethanolgroup had a mean increase of 8.61 %, 0.70% respectively whereas with 5 mg *C. sinensis* dose the body weight was 6.24% and with 10 mg dose it was 8.34% after 15 days treatment with 0.25 ml ethanol (50% v/v) (Figure 1). IO Macdonald et al. (2010) have also reported no significant changes in the body weight of experimental animals. The decrease in body weight can be attributed to that the excessive alcohol intake can impair the utilization of nutrients by altering their storage and excretion. Alcohol impairs nutrient absorption by damaging cells lining the stomach and intestine, and disabling transport of some nutrients into the blood (L Feinman, 1998). Alcohol also inhibits the breakdown of nutrients into usable substances, by decreasing the secretion of digestive enzymes from the pancreas (MA Korsten, 1989; CS Lieber, 2003).

During the present work, there was significant decrease ($p< 0.05$) observed in haemoglobin percentage erythrocytes counts and PCV% after the administration of both 0.25 ml dose of ethanol for 15 days treatment (OA Osonuga et al., 2010). So ethanol induces anemia due to destruction of Hb% and RBCs. The reduced number of RBCs and decreased haemoglobin percentage are most probably due to suppressive effect of ethanol on erythrogenic tissues or destruction of RBCs by altering membrane function of erythrocytes (VD Reddy et al., 2009). Ethanol metabolism which occurred, in major part, in liver led to the formation of acetaldehyde, a highly cytotoxic compound responsible for the oxidation of proteins, erythrocyte abnormalities and hemolysis (J Latvala et al., 2001; OV Tyulina et al., 2006). It causes an oxidative stress resulting from increased free radical production and/or decreased antioxidant defence, though erythrocytes are prone to oxidative damage due to presence of polyunsaturated fatty acids (PUFA), heme, iron and oxygen (B Halliwell and JM Gutteridge, 1986). Administration of malathion + green tea extract caused increase in Hb% and RBCs count when compared to malathion treated groups (AF El-kott et al., 2008). M Saoudi et al., (2011) reported that *Opuntia vulgaris* fruit extract (OE) treatment caused significant increase in the levels of RBC and Hb% against alcohol induced toxicity when compared with methanol-treated group. They suggested that the protective effects of OE may be due to the modulation of antioxidant enzymes activities. Tea polyphenols (ECG and EGCG) protects of red blood cells against oxidative damage and increase the level of RBC, Hb % and PCV% levels (LK Grinberg et al., 1997). Our findings are also in agreement with these researchers. The MCV, MCH and MCHC percentage are based on the Hb%, RBC count and PCV%. The level of these parameters was also changed according to these values (Table 2).

MCV and MCH showed significant ($p<0.05$) decrease with 0.25 ml dose of ethanol and increase with the both doses of CSE after 15 days whereas MCHC showed no significant decrease.The TLC count is regarded a non-specific predictor of various pathologic conditions including stress (SM Lewis et al., 2006). The normal levels of RBCs, Hb %, PCV% and leucocytes in 10 mg CSE co- treated with ethanol, indicate that no damage to RBCs or WBCs is caused by ethanol, when CSE is given along with ethanol (OA Osonuga et al., 2010). ER Eichner and Hillman (1971) observed that ethanol depresses the hemopoiesis in an organism by producing vacuolation in the granulocyte precursors in the bone marrow. Neutropenia and eosinopenia have been reported in patients consuming alcohol. It may be due to the direct effect of ethanol on adrenal gland secretion. Ethanol-induced corticosterone secretion might be the cause of decreased eosinophil count (TL Sipp et al., 1993; Nagaraja et al., 2006). In presence of polyphenols and catechins in *C. sinensis* aqueous extract probably ethanol is not able to exert its toxic effects and able to bring back this change to normal level.

4. Conclusion

In conclusion, in the present study, we have used whole extract of *C. sinensis*. The reason for using whole extract was that green tea is commonly consumed in this form only. We investigated the natural antioxidants present in C. sinensis and evaluated for the first time the effect of C. sinensis against ethanol-induced toxicity in rats. According to our findings, the antioxidant properties of CSE reversed the alterations in haematological parameters and thus ameliorate the toxic effects of ethanol.

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