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Protective Activity of *Tinospora cordifolia* and *Camellia sinensis* extract on Alcohol-Induced Toxicity in Mice

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ABSTRACT

Heavy alcohol intake depletes the plasma vitamins due to hepatotoxicity and decreased intestinal absorption. Medicinal plants may provide an effective remedy for the enormous health burden posed by alcohol abuse. This study was designed to observe the effects of alcohol on various hematological parameters in adult mice. A total of 30 mice were divided into six groups; control (n=6) and test groups II, III, IV, V, VI (n=6 comprise in each group). After treatments blood was collected on 0th, 30th and 60th days. Hematological parameters were assessed by using bioanalyzer. Statistical analysis was performed for comparison between the groups by using Dunnett's test. The white blood cells (WBC) was found significantly higher in a alcohol treated group compared to control group, while the significant decrease was observed in hemoglobin, hemocrait and platelets in the alcohol treated group. In contrast the differential count viz. neutrophil, eosinophil, monocyte, lymphocyte, basophil were found significant ($p \leq 0.05$) compared to control group and test groups II, III, IV, V and V respectively. However, administration of extract for 60 days was capable to slightly lower levels of all these parameter in alcohol groups. It is concluded that alcohol administration in mice resulted in toxic effects on various hematological parameters. *T. cordifolia* and leaf part of *C. Sinensis* helps more to wakeup immune system and boost the body of person who regularly consume alcohol.

Keywords: Alcohol, Hematological parameters, Dunnett's test

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INTRODUCTION

Alcohol is commonly consumed as a beverage, and is considered a socially acceptable toxic substance[1]. According to WHO, alcohol is a psychoactive substance with dependence-producing properties and the harmful use of alcohol ranks among the top five risk factors for disease, disability and death worldwide[2]. Much of alcohol-related mortality and morbidity is due directly to alcohol-induced disease[1]. Liver is the central organ to metabolize all foreign compounds and hence it's susceptible to many different disorders[3]. Among the compounds provoking such disorders, alcohol is one of the main causes of end-stage liver damage in a form of cellular necrosis and it is the second most common reason for liver transplantation in the United States[4]. The plants having antioxidants prevent the cell death and tissue damage resulting from chronic alcohol consumption[5].*Camellia sinensis* (Green tea, Theaceae) is the second most popular beverage worldwide[6]. It contains six primary catechins or polyphenol compounds, alkaloid, flavonoids, steroids and terpenoids which serve as valuable starting material for the medicine development. These constituents have potent antioxidant action and their putative disease preventive effects[7].These polyphenols prevent oxygen-free radicals-induced hepatocyte lethality, reduce the risk of liver disease and protect against liver injury that is fibrosis and liver cirrhosis in rats[8-10]. *Tinospora cordifolia* (Willd) Hook and Thoms (Guduchi) is a large, glabrous, deciduous, climbing shrub belonging to the family Menispermaceae[11-13]. It is distributed throughout the tropical Indian subcontinent and China, ascending to an altitude of 300 m. the plant is commonly known as Giloe, Amrita[14] is used as a medicine for centuries in the Ayurvedic and Unani systems of the medicine. *T. cordifolia* extract contains many constituents such as alkaloids, steroids, glycosides, diterpenoid, lactones, sesquiterpenoid, phenolics, aliphatic compounds and polysaccharides[15]. It has been shown to possess antidiabetic, antioxidant, antihepatotoxic and immunomodulatory properties[16, 17]. The active ingredient, G1-4A, of a dry stem of *T. cordifolia* protected mice against lipopolysaccharide- (LPS-) induced endotoxic shock by modulating the responses of macrophages[18]. It has been shown to control the drug-resistance Mycobacterium tuberculosis infection by inducing Th1immune responses[19]. *T. cordifolia* extract showed an antitumor potential against the skin carcinogenesis in a mouse model[20].

MATERIALS AND METHODS

Collection of plant materials

The tea leaves of *Camellia sinensis* were purchased from local market and whole plant material *Tinospora cordifolia* were purchased from Sanjivani Herbal Bhopal.

Chemicals

All chemicals used were of analytical grade and were supplied by the department.

Instruments

The instruments facility of Institute was utilized.

Extraction

The whole plant materials of *T. cordifolia* and leaf part of *C. sinensis* (30 g) were extracted three times for 30 min with distilled hot water in separating funnel. The temperature was maintained at 37°C. Ratio of plant material and solvent was 1:10. The extracts were filtered through Whatman, no.1 filter paper and evaporated to dryness under reduced pressure by the rotary evaporator. The obtained crude extracts were stored in dark glass bottles for further processing.

Preliminary phytochemical screening

The dried extract of *T. cordifolia and C. sinensis* was subjected to the preliminary phytochemical analysis for the presence of different phytoconstituents[21].

Experimental model

Experimental design and treatment protocol

The animals were divided into six groups of six animals each and as:

Group I: Normal control group mice received saline

Group II: Alcohol treated group received alcohol at a dose of 7mg /kg of body weight

Group III: received *C. sinensis* extracts at a dose of 200 mg/kg/p.o

Groups IV: received *T. cordifolia* extracts at a dose of 200 mg/kg/p.o

Groups V: received alcohol (1mg/kg/p.o.) and *C. sinensis* extracts at a dose of 200 mg/kg/p.o

Groups VI: received alcohol (1 mg/kg/p.o.) and *T. cordifolia* extracts at a dose of 200 mg/kg/p.o

Sample collection

Blood samples were collected in EDTA tubes by performing retro orbital plexus.

Hematological parameters

The blood samples collected into heparinized tubes were immediately used for determination of haematological parameters. Total red blood cell and white blood cell counts were estimated according to the visual method of Dacie and Lewis (1975)[22]. The percentage packed cell volume was determined according to the hematocrit method of Alexander and Griffiths (1993)[23], while the blood haemoglobin concentrations in all samples were estimated according to the cyanomethaemoglobin method of Alexander and Griffiths (1993) [23].

Mean cell volume (MCV)

MCV is a measure of the mean size of RBCs. Individual RBC volumes are measured directly by electrical impedance. The values are plotted on a histogram that is used to calculate mean cell volume. MCV is useful in classifying anemia a normocytic, microcytic or macrocytic.

Mean corpuscular hemoglobin (MCH)

MCH is the average hemoglobin content of a single RBC. It is the calculated by dividing hemoglobin by the RBC count.

Mean corpuscular hemoglobin concentration (MCHC)

MCHC is the average red cell hemoglobin concentration expressed as a percent. It is calculated by multiplying hemoglobin by 100 and dividing the product by the hematocrit. Elevated MCHC can be caused by spherocytosis, hyponatremia, cold agglutinin, lipemia or strongly discolored plasma.

Platelet count

Platelet counts in mice are measured mostly with automated cell counters but manual counting and flow cytometry are also used. As mouse platelets are smaller than human platelets, it is necessary to adjust the discriminators on the automated cell counter to ensure that all platelets are included in the analysis. Reports on platelet counts vary from a low of ~0.4 $x10^6$ μL^{-1} to a high of ~1.6 $x10^6$ μL^{-1}; however, hematological analysis of more than 30 commonly used inbred strains revealed an overall mean platelet count of ~1.1 $x10^6$ μL^{-1}.

Differential counts

These were estimated using the method of Osim et al (2004)[24]. An automated CBC with differential white blood cell count can determine 32 different hematological parameters. Automated cell counters incorporate a method for flagging white blood cell (WBC), red blood cell (RBC) and platelet abnormalities. The flagged abnormalities prompt a medical scientist to perform a manual peripheral smear review and differential to verify the abnormality flagged as well as allow identification of additional morphological abnormalities.

Statistical Analysis

All analysis was performed using graph pad prism for Windows. All statistical analysis is expressed as mean ± standard error of the mean (SEM). Data were analyzed by one way ANOVA, where applicable $p<0.05$ was considered statistically significant, compared with vehicle followed by Dunnett's test.

RESULTS

Phytochemical screening

Phytochemical screening revealed the presence of saponins, flavonoids, terpenoids, glycoside, phenol, carbohydrate, tannins in green tea extract as well as saponins, flavonoids, phenol, carbohydrate, tannins compounds in guduchi extract. In the present study mice were treated with *T. cordifolia and C. sinensis* induced by alcohol. Our finding shows significant results of hematological parameters in alcohol induced mice as expressed in different tables and figures. Hematological parameters on every 0^{th}, 30^{th} and 60^{th} day were performed. All the animals, which were used in the experiment, were very healthy and physically active. The values of various hematological parameters including WBC, RBC, Hgb concentration, Hct, MCV, MCH, MCHC and PLT were assessed in control and test group-III, IV, V and VI

respectively. In test group Dunnett's test showed significant decrease (p≤0.05) in Hgb concentration, hemocrait, platelets counts, monocyte, lymphocyte in as compared to the control group as shown in Table 1-5.

Table 1 Haemoglobin concentration of control, alcohol induced, green tea, guduchi, alcohol + green tea and alcohol + guduchi treated mice

Haemoglobin (gm/dL)	Control	Alcohol-Induced	Green Tea	Guduchi	Alcohol+ Green Tea	Alcohol+ Guduchi
0th Day	12.30 ± 3.26	9.90 ± 2.19	13.50 ± 3.80	14.10 ± 4.07	9.30 ± 1.92	9.30 ± 1.92
30th Day	11.70 ± 3.00	8.70 ± 1.65	14.70 ± 4.34	14.70 ± 4.34	10.50 ± 2.46	11.10 ± 2.72
60th Day	11.10 ± 2.73	6.90 ± 0.85	14.70 ± 4.34	15.30 ± 4.60	11.70 ± 3.00	12.30 ± 3.26

Values are expressed as the mean ± SEM. (One-way ANOVA followed by Dunnett's post hoc test).

Table 2 Hemocrait concentration of control, alcohol induced green tea, guduchi, alcohol + green tea and alcohol + guduchi treated mice

Hemocrait %	Control	Alcohol-Induced	Green Tea	Guduchi	Alcohol+ Green Tea	Alcohol+ Guduchi
0th Day	44.80 ± 17.80	38.40 ± 14.94	44.00 ± 17.44	44.80 ± 17.80	39.20 ± 15.30	36.80 ± 14.22
30th Day	43.20 ± 17.08	34.40 ± 13.15	45.60 ± 18.16	45.60 ± 18.16	40.00 ± 15.65	39.20 ± 15.29
60th Day	41.60 ± 16.37	32.80 ± 12.43	45.60 ± 18.16	46.40 ± 18.51	40.80 ± 16.01	41.60 ± 16.37

Values are expressed as the mean ± SEM. (One-way ANOVA followed by Dunnett's post hoc test).

Table 3Platelets counts of control, alcohol induced green tea, guduchi, alcohol + green tea and alcohol + guduchi treated mice

Platelets (x100000)	Control	Alcohol-Induced	Green Tea	Guduchi	Alcohol+ Green Tea	Alcohol+ Guduchi
0th Day	3.15 ± 1.39	2.15 ± 0.94	3.35 ± 1.48	3.15 ± 1.39	1.85 ± 0.80	1.55 ± 0.67
30th Day	2.85 ± 1.25	1.85 ± 0.16	3.65 ± 1.61	3.55 ± 1.57	2.15 ± 0.94	1.95 ± 0.85
60th Day	2.65 ± 1.16	1.45 ± 0.63	3.85 ± 1.70	3.95 ± 1.74	2.35 ± 1.03	2.55 ± 1.12

Values are expressed as the mean ± SEM. (One-way ANOVA followed by Dunnett's post hoc test).

Table4 Different platelet counts viz. Monocyte of control, alcohol induced green tea, guduchi, alcohol + green tea and alcohol + guduchi treated mice

Monocyte (%)	Control	Alcohol-Induced	Green Tea	Guduchi	Alcohol+ Green Tea	Alcohol+ Guduchi
0th Day	1.50 ± 1.57	1.70 ± 1.48	1.50 ± 1.57	1.70 ± 1.48	1.50 ± 1.57	1.30 ± 1.65
30th Day	1.70 ± 1.48	1.30 ± 1.65	1.70 ± 1.48	1.90 ± 1.39	2.10 ± 1.30	1.90 ± 1.39
60th Day	1.70 ± 1.48	0.90 ± 1.83	1.70 ± 1.48	1.90 ± 1.39	2.70 ± 1.03	2.50 ± 1.12

Values are expressed as the mean ± SEM. (One-way ANOVA followed by Dunnett's post hoc test).

Table 5Different platelet counts viz. Lymphocyte of control, alcohol induced green tea, guduchi, alcohol + green tea and alcohol + guduchi treated mice

Lymphocyte (%)	Control	Alcohol-Induced	Green Tea	Guduchi	Alcohol+ Green Tea	Alcohol+ Guduchi
0th Day	67.00 ± 27.73	57.00 ± 23.26	65.00 ± 26.83	61.00 ± 25.04	53.00 ± 21.47	51.00 ± 20.57
30th Day	69.00 ± 28.62	53.00 ± 21.47	63.00 ± 25.94	65.00 ± 26.83	67.00 ± 27.73	69.00 ± 28.62
60th Day	73.00 ± 30.41	47.00 ± 18.78	63.00 ± 25.94	63.00 ± 25.94	71.00 ± 29.52	73.00 ± 30.41

Values are expressed as the mean ± SEM. (One-way ANOVA followed by Dunnett's post hoc test).

In test group, Dunnett's test showed significant increase (p<0.05) , MCV/RBC counts, WBC counts, neutrophil, eosinophil and basophill concentration as compared to the control group as shown in Fig. 1-5.

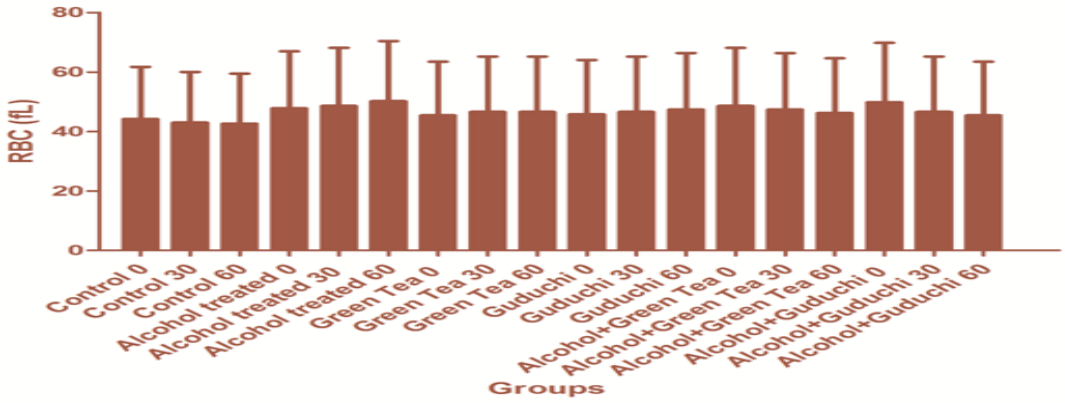

Fig.1MCV/RBC counts of control, alcohol induced green tea, guduchi, alcohol + green tea and alcohol + guduchi treated mice

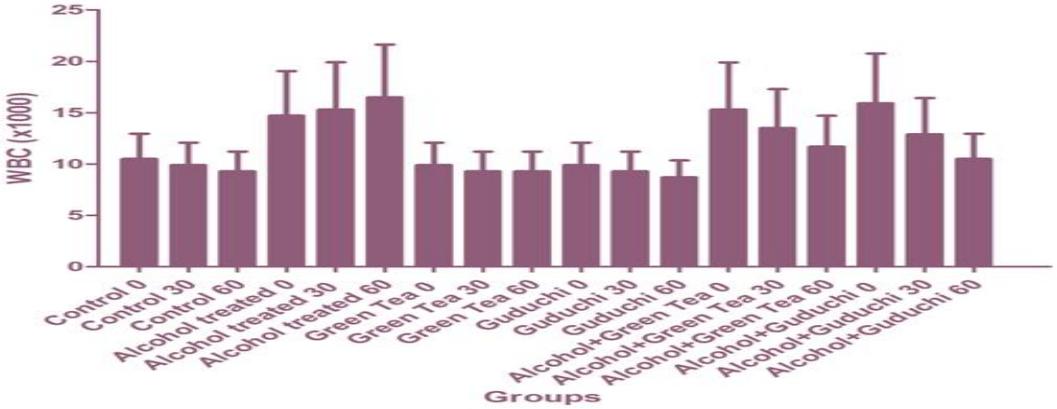

Fig. 2WBC counts of control, alcohol induced green tea, guduchi, alcohol + green tea and alcohol + guduchi treated mice

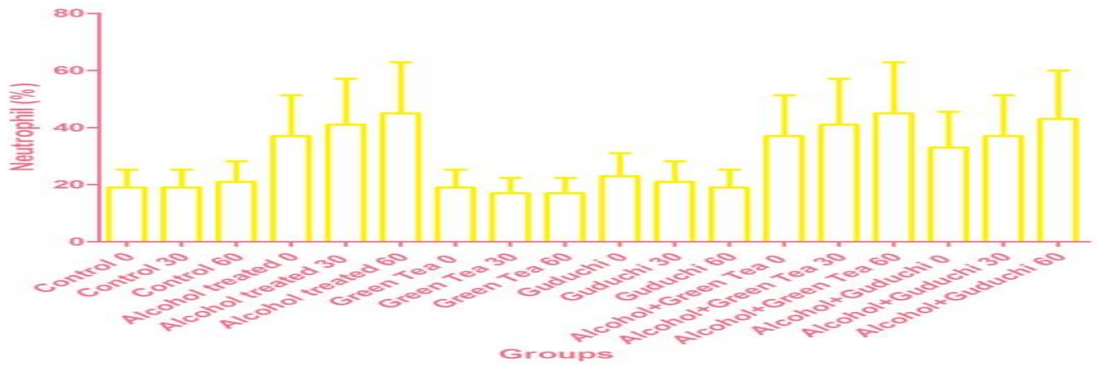

Fig. 3 Different platelet counts viz. neutrophil of control, alcohol induced green tea, guduchi, alcohol + green tea and alcohol + guduchi treated mice

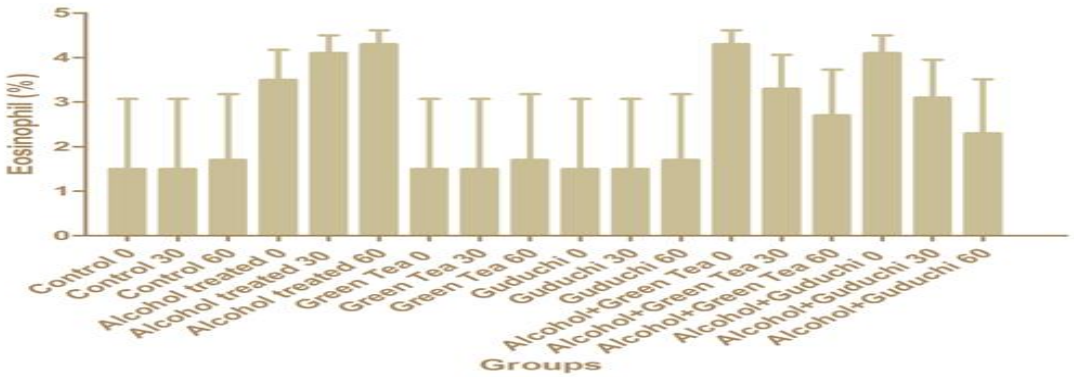

Fig. 4 Different platelet counts viz. Eosinophil of control, alcohol induced green tea, guduchi, alcohol + green tea and alcohol + guduchi treated mice

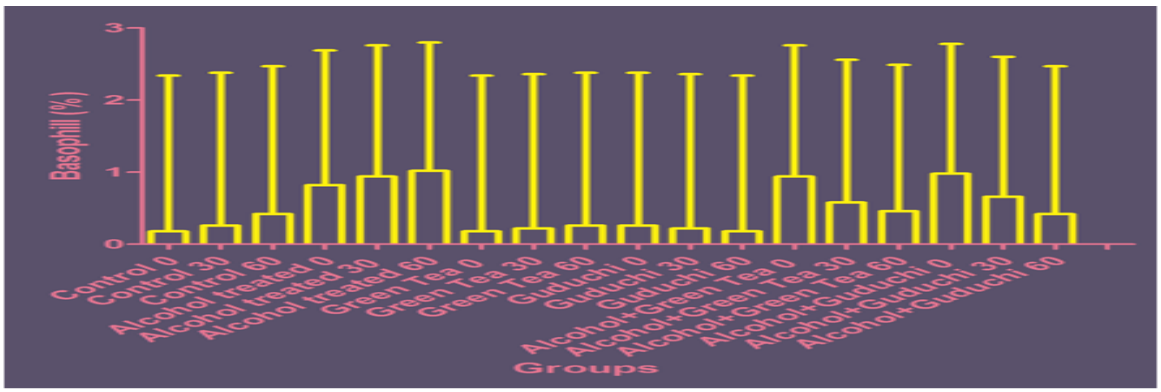

Fig. 5 Different platelet count viz Basophill of control, alcohol induced green tea, guduchi, alcohol + green tea and alcohol + guduchi treated mice

DISCUSSIONS

This study confirmed that administration of alcohol to adult mice significantly altered various hematological parameters including WBC, RBC, Hgb, HCT and PLT. A significant increase in WBCs count and decrease in RBCs count was observed. Green tea and guduchi extract prevent these changes and our results are also consistent with Maruthi et al. and Wan-Guo Yu et al[11, 12]. One of the major effects of alcohol on the physiology of body is that it greatly suppresses the function of immune system and sometimes paradoxical influences on immune function[13]. Ballard 1989, reported alcohol has numerous adverse effects on the various types of blood cells and their functions. Heavy alcohol consumption can cause generalized suppression of blood cell production and the production of structurally abnormal blood cell precursors that cannot mature into functional cells. Alcoholics frequently have defective RBC that is destroyed prematurely, possibly resulting in anemia. Alcohol also interferes with the production and function of WBC, especially those that defend the body against invading bacteria. Consequently, alcoholics frequently suffer from bacterial infections. Finally, alcohol adversely affects the platelets and other components of the blood-clotting system. Heavy alcohol consumption thus may increase the drinker's risk of suffering a stroke[14]. The study revealed that immunological boost up potency hypothesis of green tea and guduchi extract in alcohol induced toxicity.

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