EFFECT OF RUTIN AGAINST HIGH-FAT DIET AND ALCOHOL-INDUCED ALTERATIONS IN HEMATOLOGICAL VARIABLES OF RATS

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ABSTRACT

Objectives: Alcohol abuse leads to several diseases and millions of death worldwide. High-fat diet (HFD) is major contributor of non-alcoholic liver diseases and obesity. Combined consumption of HFD and alcohol has deleterious effect on blood cells. This study was carried out to evaluate the protective effect of rutin against combined consumption of HFD and alcohol-induced hematological alterations.

Methods: HFD 30% and ethanol 10% were administered for 4 weeks for induction of toxicity. Rutin (10, 20, and 40 mg/kg) and 50 mg/kg dose of silymarin were administered along with HFD and alcohol for 4 weeks.

Results: Combined consumption of HFD and alcohol increased mean corpuscular volume, total leukocytes count, eosinophil and monocyte, and decreased hematoctrit and platelets. Administration of rutin improved hematological variables altered by HFD and alcohol consumption.

Conclusion: The present study concluded that administration of rutin may alleviate HFD and alcohol-induced hematological alterations by scavenging free radicals generation.

Keywords: High-fat diet, Alcohol, Rutin, Red blood cell, White blood cell.

INTRODUCTION

In the current century, modernization and busy lifestyle promote the consumption of fast food and alcohol which leads to several health, economic, and societal problems. Studies on non-alcoholic fatty liver disease (NAFLD) reported in which liver hypertrophy is initiated by accumulation of fat in liver leading to metabolic disorder. Studies on non-alcoholic fatty liver disease (NAFLD) reported in which liver hypertrophy is initiated by accumulation of fat resulting in oxidative stress [1]. Over 3 million deaths were reported in 2012 due to alcohol consumption, corresponding to 5.9% of the global or one in every 20 deaths worldwide [2]. Intake of alcohol induces expression and activity of cytochrome P4502E1 (CYP2E1) enzyme leads to conversion of ethanol to acetaldehyde. This activity of CYP2E1 enzyme needs oxygen activation, resulting in overproduction of reactive oxygen species (ROS), for example, hydrogen peroxide, hydroxyl radical, and superoxide anion radical, elevated ROS generation, along with the exhaustion or inhibition of endogenous non-enzymatic and enzymatic antioxidant by ethanol, can lead to oxidative stress in body and subsequent tissue damage [3].

Antioxidant supplements are one of the possible strategies for maintaining redox homeostasis through directly quenching excessive ROS or defending endogenous antioxidant enzyme activities against oxidative stress [4]. Polyphenolic compounds including flavonoids and phenolic acids functions as antioxidant, antihyperglycemic, and antihypertensive agent [5]. Rutin, also known as Vitamin P, is one of the major flavonoids and presents in onions, apples, tea, and red wine. It has antiviral, antihypertensive, antioxidant [6], antibacterial, antitumor, anti-inflammatory, anti-diarrheal, antiulcer, antimutagenic, vasodilator, immunomodulatory, and hepatoprotective activities [7,8].

Till now, there is no scientific report on protective effect of rutin against combined consumption of HFD and alcohol-induced hematological deviations. Therefore, the present investigation was an attempt to evaluate ameliorating effect of rutin against HFD and alcohol-induced hematological abnormalities in rats.

METHODS

Animals and chemical

Female albino Wistar rats (160±10 g) were obtained from Defense Research and Development Establishment, Gwalior, and acclimatized in departmental animal facility under standard husbandry conditions (25±2°C temperature, 60–70% relative humidity, and 12 h photoperiod). Rats were fed with standard diet (Pranav Agro Industries, India) and water ad libitum. Experiments were conducted in accordance with the guidelines set by the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), India, and experimental protocols were approved by the institutional animal ethics committee (994/ER/06/CPCSEA). Rutin, silymarin, and other chemicals were procured from Sigma-Aldrich, Ranbaxy, and Hi-Media Laboratories Ltd., India, ERBA Diagnostics, Germany. HFD was procured from VRK Nutritional Solutions, Pune (India).

Experimental design

4 weeks duration of exposure to HFD and alcohol was selected from our previous studies to induce hepatorenal disorder (Communicated). 42 animals were divided into seven groups of six animals each as follows: Group 1 and 2 received regular diet and water. Group 1 received vehicle according to body weight and served as control. Group 2 received rutin per se (40 mg/kg, p.o.) for 4 weeks (5 days in a week). Groups 3-7 received 30% HFD and 10% alcohol simultaneously and Group 3 served as experimental control. Group 4, 5, 6, and 7 were treated individually with rutin at different doses of 10 mg/kg, 20 mg/kg, and 40 mg/kg and silymarin (50 mg/kg, p.o.) considering 5 days in a week respectively. Animals of all the groups
**Hematological analysis**

Red blood cell (RBC), red cell distribution width (RDW), hematocrit (HCT), mean corpuscular volume (MCV), hemoglobin, mean corpuscular hemoglobin (MCH), MCH concentration (MCHC), platelets, platelet distribution width (PDW), mean platelet volume (MPV), total leucocytes count (TLC), monocyte, eosinophil, and lymphocyte were done by blood analyzers manual instruction guide.

**Statistical analysis**

Data were expressed as mean ± standard error of six animals used in each group. Statistical analysis was carried out using one-way analysis of variance considering significant at 5% level followed by Student’s t-test considering statistically significant at p≤0.05 and p≤0.01 [9].

**RESULTS**

4 weeks combined consumption of HFD and alcohol did not cause significant alteration in RBC count and RDW. HFD and alcohol consumption significantly decreased HCT and increased MCV (p≤0.05) as compared to control. Administration of 20 and 40 mg/kg doses of rutin and 50 mg/kg dose of silymarin improved HCT count at 5% level of significance. MPV was improved only at 40 mg/kg dose of rutin (p≤0.05) (Table 1).

Hemoglobin, MCH, and MCHC were not affected significantly after 4 weeks combined consumption of HFD and alcohol (Table 2).

| Groups | RBC (10^6/mm³) | RDW (%) | HCT (%) | MCV (µm³) |
|--------|----------------|---------|---------|------------|
| Group 1| 7.5±0.58       | 16.6±1.58| 52.1±3.70| 33.0±5.79  |
| Group 2| 7.54±0.62      | 16.7±1.58| 52.2±3.70| 33.0±5.71  |
| Group 3| 6.59±0.62      | 14.8±1.33| 37.3±2.82*| 43.0±5.20* |
| Group 4| 6.86±0.65      | 14.9±1.36| 44.3±2.44| 38.30±3.08 |
| Group 5| 6.91±0.62      | 15.1±1.04| 49.5±2.84*| 36.10±2.92 |
| Group 6| 7.29±0.70      | 15.2±1.17| 51.3±3.20*| 35.40±2.20*|
| Group 7| 7.24±0.67      | 15.0±1.03| 48.5±2.99*| 37.30±2.95 |
| ANOVA  | 0.37           | 0.45     | 3.59*    | 1.83       |

Data are expressed as mean±SE; n=6; Significant ANOVA at p≤0.05. For Student’s t-test, Significant P value HFD+alcohol versus control *p≤0.05, and therapy versus HFD+alcohol *p≤0.05. Group 1: Control; Group 2: Rutin per se (rutin 40 mg/kg); Group 3: HFD+alcohol; Group 4: HFD+alcohol+Rutin 10 mg/kg; Group 5: HFD+alcohol+Rutin 20 mg/kg; Group 6: HFD+alcohol+Rutin 40 mg/kg; Group 7: HFD+alcohol+Silymarin 50 mg/kg. RBC: Red blood cell, RDW: Red cell distribution width, HCT: Hematocrit, MCV: Mean corpuscular volume, HFD: High-fat diet, SE: Standard error.

**DISCUSSION**

Alcohol abuse is a rising epidemic in India, especially among men, and nowadays, it is becoming a major problem with young adults. The clinical manifestations of alcohol-induced hematological disorders are profoundly influenced by the patient’s social and economic status and other factors such as nutritional deficiency or alcoholic cirrhosis. Most of these changes may severely affect either directly or indirectly and may cause anemia; however, a patient with extensive liver disease may develop an abnormally functioning fibrinogen or other coagulation disorders, which may initiate or exacerbate bleeding [16]. In modern century, people prefer to a sedentary lifestyle and a lack of exercise which are associated with increased prevalence of diabetes mellitus, obesity, hypertension, and hypertriglyceridemia. These are considered to be important causes of NAFLD and its more destructive form, i.e., non-alcoholic steatohepatitis [11,12].

Blood is a connective tissue which consists of fluid portions or plasma that is suspended by some formed elements (erythrocytes, leucocytes, and thrombocytes). Blood provides the basic connections between the organs and cells of the body and maintains a constant cellular environment by circulating through every tissue providing nutrients to them and eliminating waste products [13,14]. The 4 weeks combined consumption of HFD and alcohol caused significant changes in hematological variables of animals. During the metabolism of alcohol and HFD, generation of ROS takes place leading to tissue and cells damage. Previous reports suggested that ethanol intake may lead to oxidative damage to erythrocytes and other tissues [15].

Chronic drinking of alcohol changes RBC in many aspects not only regarding changes in size of RBC (macrocytosis) but also even the presence of defective RBC in the blood and their production from the bone marrow [16]. As a result of these changes, anemia is a common found in alcoholics [16]. In the present finding, HFD and alcohol consumption did not alter the RBC, hemoglobin, MCH, and MCHC, due to low concentration of alcohol and also may be short time duration of consumption. Devi and Rajkumar reported that in hyperlipidemic condition RBC and hemoglobin were not altered significantly in rats [17].

In case of tissue damage or inflammation, the platelets stick to the lesions of the blood vessels and adhere to exposed endothelial tissues [18]. The HCT is one of the key factors influencing platelet adherence to the vessel wall, and the elevation of the HCT causes rise in platelet accumulation [19]. Reduction of platelets count is associated with thrombocytopenia. In the present study, HFD and alcohol consumption decreased platelets count and HCT. The finding is in agreement with previous reports [20-22]. Rutin administration increased platelet count and HCT, which indicate the ability of rutin to ameliorate bleeding tendencies. Previous finding showed that the rutin improved platelet count against doxorubicin [23]. The MCV estimates the average erythrocyte volume and increased MCV serves
The result of the present study concluded that rutin prevents deleterious changes in total WBC counts, HCT, platelet count, lymphocytes, eosinophil and monocyte in HFD, and alcohol-fed rats due to its effective free radical scavenging property. Rutin may be used as a therapeutic agent to combat blood toxicity.

### Table 3: Therapeutic effect of rutin against HFD and alcohol-induced alterations in blood parameters

| Groups          | TLC (%) | Lymphocyte (%) | Eosinophil (%) | Monocyte (%) |
|-----------------|---------|----------------|----------------|--------------|
| Group 1         | 1020±474| 78±4.66        | 1.5±0.11       | 1.0±0.09     |
| Group 2         | 1020±419| 81±5.00        | 1.6±0.08       | 1.1±0.11     |
| Group 3         | 1480±938*| 90±6.79        | 4.5±0.30**     | 2.0±0.18**   |
| Group 4         | 1305±910| 75±5.22        | 4.0±0.28       | 1.5±0.13*    |
| Group 5         | 1230±785*| 72±6.47        | 3.8±0.26       | 1.5±0.13*    |
| Group 6         | 1065±611*| 76±6.32        | 2.4±0.17**     | 1.5±0.13*    |
| Group 7         | 1120±747*| 78±4.91        | 3.0±0.30**     | 1.5±0.13*    |
| ANOVA           | 5.68**  | 6.65           | 3.27**         | 10.5**       |

Data are expressed as mean±SE; n=6; Significant ANOVA at *p≤0.05. For Student’s test significant P value HFD+alcohol versus control *p≤0.05, and therapy versus HFD+alcohol *p≤0.05 and **p≤0.01. Group 1: Control; Group 2: Rutin per se (rutan 40 mg/kg); Group 3: HFD; Group 4: HFD+alcohol; Group 5: HFD+alcohol+Rutin 20 mg/kg; Group 6: HFD+alcohol+Rutin 40 mg/kg; Group 7: HFD+alcohol+Silymarin 50 mg/kg. TLC=Total leukocytes count, HFD: High-fat diet, SE: Standard error

### Table 4: Therapeutic effect of rutin against HFD and alcohol-induced alterations in blood parameters

| Groups          | Platelet (10^12/mm^3) | PDW (%) | MPV (μm^3) |
|-----------------|-----------------------|---------|------------|
| Group 1         | 951±78.25             | 17.4±1.61| 5.30±0.33  |
| Group 2         | 955±75.12             | 17.3±1.63| 5.55±0.37  |
| Group 3         | 690±39.72*            | 16.5±1.47| 5.70±0.38  |
| Group 4         | 711±62.02             | 16.6±1.42| 5.60±0.38  |
| Group 5         | 732±44.77             | 16.7±1.43| 5.55±0.39  |
| Group 6         | 874±44.49*            | 16.8±1.49| 5.55±0.39  |
| Group 7         | 819±45.91*            | 17.3±1.62| 5.45±0.37  |
| ANOVA           | 4.50**                | 0.07     | 0.17       |

Data are expressed as mean±SE; n=6; Significant ANOVA at *p≤0.05. For Student’s test significant P value HFD+alcohol versus control *p≤0.05, and therapy versus HFD+alcohol *p≤0.05. Group 1: Control; Group 2: Rutin per se (rutan 40 mg/kg); Group 3: HFD; Group 4: HFD+alcohol; Group 5: HFD+alcohol+Rutin 10 mg/kg; Group 5: HFD+alcohol+Rutin 20 mg/kg; Group 6: HFD+alcohol+Rutin 40 mg/kg; Group 7: HFD+alcohol+Silymarin 50 mg/kg. PDW: Platelet distribution width, MPV: Mean platelet volume, HFD: High-fat diet, SE: Standard error

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The first, second, and third author carried out of the experimental part of the work and data compilation. The fourth and fifth authors guided and monitored the experimental design, as well as corrected the manuscript.

### CONFLICTS OF INTEREST

The authors declare that they have no conflicts of interest on any issues.

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