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Original article

Erythroleukemia treated effects of rat plasma profile and erythrocyte membranes

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ABSTRACT

Erythroleukemia disease is caused by over production of malignant blood and immature large number of blood cells enters into peripheral compartment. Biophysical and biochemical changes in plasma and erythrocyte membrane in erythroleukemia treated rats were identified. Our study, leukemia is experimentally exposed in rats were injecting erythroleukemia cells (FLC) (H-2d) intravenously in adult rats and normal control rats were maintained. Significant increase in the activity of blood glucose, proteins levels, aspartate transaminase (AST) and alanine transaminase (ALT) values and significant decrease in haemoglobin (Hb), albumin levels in erythroleukemia treated rats were observed when compared with control rats. Cholesterol and low density liproprotein (LDL) levels increased significantly in erythroleukemia treated rats but triglycerides, high density lipoprotein (HDL) and very low density lipoprotein (VLDL) levels decreased significantly. Levels of red cell membrane cholesterol decreased in erythroleukemia treated rats in comparison with control while levels of phospholipids and proteins increased in erythrocytes of erythroleukemia treated rats. Red blood cell (RBC) and white blood cell (WBC) counts increased significantly and platelet count decreased. C/P (cholesterol/phospholipid) ratio decreased significantly in erythroleukemia treated rats. This study has been undertaken for the first time to investigate the effect of (FLC) (H-2d) erythroleukemia cells (treated) in intravenously in adult rats and normal control rats. Results indicate biophysical and biochemical alterations at molecular level in plasma and erythrocyte membrane.

1. Introduction

WHO reported myeloid neoplasms (Vardiman et al., 2002) and described the erythroleukemia diagnostic criteria for particular account of acute erythroid and myeloid leukemia interest. The scientific report available in (world health organigation) WHO (Jaffe et al., 2001), myeloid leukemia and acute erythroid is clear as have at least 50% erythroid precursors in the total bone marrow and population of nucleated cells and myeloblasts relation (Dale et al., 2003) for at least 20% of the (NEC) non erythroid cells populations (Doumas et al., 1971). It's uncommon in acute myeloid leukemia and the diagnosis condition were set up purposes contain change considerably in excess of point time in acute erythroid leukemia. The report of acute erythroid leukemia (AEL) shows to have been published first in year of 1912 by Eminent scientist (Copelli, 1912) a hematologic disorder described viz., erythromatosis. It was understanding the enlarge of this sequence of commentary disease by Giovanni, (Dameshek and Baldini 1958, Di Guglielmo 1917) at the beginning in year. He described 2 forms of acute erythroid leukemia of the extra ordinary alternative form, to pass on myelosis of erythremia, and consequently while erythroid, myeloid leukemia, erythroleukemia and by others, are comprised of immature elements of myeloid and erythroids. The gradually enhance myeloblasts, develop the disease in keen on acute myeloid leukemia (Dameshek 1969). He described and differentiates by a clean proliferation of normoblastic, delicate erythremia and afterward passes on to others as accurate erythroleukemia, simply distinguish erythroleukemia, and clean erythroid leukemia second variant. Afterwards the syndrome of this disease is honor of involvement to the sympathetic of this disease called Dameshek

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and further supporter the terms Di Guglielmo (Fouillard et al., 2002). The immature cells of blood are overproductions, entering the blood flow is leukemia and malignant disease blood-forming tissues causing (Azher and Shiggaon 2013). This is the most common cancer in twelfth one woman, and ninth one in men (Quinn et al., 2001). To the evolution of the disease basically it is off the record as myeloid or lymphoid, pact of the cell roots, when chronic or acute (Love 1936; Javed et al., 2012). Etiological factors include factors of environmental, smoke condition, consumption alcohol, genetic a1teration, various chemicals, viruses' infection, radiation impact, deficiency of immune system (Kinane 2000; Eden 2010). These types of diseases are regularly delighted by joint with rays and chemotherapy, hematopoietic stem cell remove therapy (Seibel 2008). Erythroleukemic treated rat blood pathophysiological situation contribute to morphological of erythrocytes, structural and functional alterations that enhance platelet reactivity, RBC procoagulant activity, endothelium adhesiveness and reduce deformability of erythrocytes (Straat et al., 2012). These alterations of red blood cell structural results are in the impaired, chronic anaemia, blood flow, endothelial dysfunction, hypertension, risk for cardiovascular diseases, ischemia, and haemolysis of red blood cells at physiological conditions (Tyulina et al., 2000; Maio et al., 2011; Mozos 2015). Hence our study was designed to investigate the erythroleukemic treated rats cause plasma biochemical and biophysical alterations particularly lipid profile, glucose mechanism and lipid peroxidation in plasma, AST, ALT and haemoglobin effects. We have also evaluated RBC, WBC and platelet count, erythrocyte membrane cholesterol, phospholipid modifications/ changes and membrane protein levels, C/P ratio.

2. Materials and methods

2.1. Experimental design and procuring animals

60 days old twenty four albino *Wistar* strain of male rats, body weight ranging from l20-140, were taken from NCLAS, Hyderabad, Telangana, India and quarters in cage separately AC room (25 + 1 °C) during 7.00 a.m to 7.00 p.m. Experimental animals divided into two groups in each one 12 rats. **Group I** consisted of normal/control rats (C) and **Group II** erythroleukemia treated rats were injected intravenously (erythroleukemia cells (FLC) (H-2d). All the rats were recorded daily weight and intake of food and water was followed on days of alternate. The finishes of the investigational period, in each group of rats were fasted overnight and then by using of cervical dislocation rats were sacrificed. Collected tissues and immediately for further analysis for processed. The animal study, we are followed all procedures of including the whole surgical, feeding, and raising process were based on approved methods by the Committee of Animal Ethical clearance for the treatment of animals and approved for the study protocol was obtained subsequent the guiding principle. This study was also accepted by institutional ethical committee (20181236) of The First People's Hospital of Yunnan Province, China.

2.2. Blood collection and sample study

Blood drawn from rats via cardiac puncture, were collected among 7–10 AM into heparinised tubes and immediately further analysis for plasma, other experimentation process. Plasma regular parameters glucose were projected process (Trinder 1969), using enzymatic kits triglycerides, cholesterol were measured (Allian et al., 1974), To determined HDL- cholesterol were subsequent to rainfall of LDL and VLDL in the midst of magnesium chloride. Concentrations of VLDL, LDL-cholesterol were calculated from the equation: plasma albumins and protein (Wootton 1974) were also determined spectrophotometrically using commercial kits. Hb estimation by (Eross et al., 1984), AST (Babson et al., 1962), ALT (Matsuzawa and Katunuma 1966), proteins of erythrocyte membrane were estimated (Lowry et al., 1951), by Zlatkis method estimation of cholesterol (Zlatkis et al., 1953), phospholipids by (Connerty et al., 1961). Isolation of erythrocyte and preparation of erythrocyte membrane were carried out by different techniques.

2.3. Estimation of plasma lipid peroxidation

Amount of plasma lipid peroxidation was intended MDA (Niehaus and Samuelsson 1968. one ml plasma added two ml of (15°/c w/v TCA, 0.375°/c w/v TBA and 0.25 N HCl) reserved 15 min in boiling water bath, then cool. For 10 min duration at 1000 g samples were centrifuged. Transferred supernatant to another tube sample read in spectrophotometer at 535 nm, followed by blank coefficient to be 1.56×10^5.

2.4. Erythrocytes isolation

Isolation of erythrocytes was using the method of (Beutler 1975). Anti-coagulated blood samples were passing through the cellulose column and the filtrate was collected to eliminate platelets, lymphocytes, etc. Erythrocytes were collected after filtrate and diluted with saline by centrifugation for 10 min at 1000 rpm. Step was repeated until the erythrocytes washing and collected erythrocytes and for study was obtained.

2.5. Preparation of erythrocyte membrane

Preparations of erythrocyte membranes were using (Dodge et al., 1963) method. The suspension of erythrocyte were cleaned with the help of saline buffer, after clean wash phosphate buffer of 5 mM, cells were lysed, centrifuge for 30 min at 15,000*g*. Removed the supernatant watchfully with the help of buffer used previously, finishing step were repeated to collect free ghosts for more analysis.

2.6. WBC count and growth performance

Every week weighed rats during the total experiment period. After the last week raise in weights was observed, later by using laparotomy and anesthesia the rats were sacrificed. Through needle-syringe by puncturing of heart the blood samples were collected for further study. By using standard protocol total WBCs were counted a hemocytometer, by staining technique after dilution factor. WBC count was carrying out Leishman's stain method, use with an oil lens, and standard protocol by staining and examining accordingly.

3. Results

Data presented in Fig. 1a–g, suggest significant increase in the activity of blood glucose, proteins levels, ALT and AST values and significant decrease in haemoglobin (Hb), albumin levels in erythroleukemia treated rats when compared with control rats. Plasma lipid peroxidation increased significantly in experimental subjects. Cholesterol and LDL levels increased significantly in erythroleukemia treated rats but triglycerides, HDL and VLDL decreased significantly when compared to control rats (Fig. 2a–e). Levels of red cell membrane cholesterol levels decreased in the experimental rats (eryhroleukemia treated rats) when compared with control rats while phospholipids and protein level increased in erythrocytes of experimental subjects viz., erythroleukemia treated rats group II compared with controls group

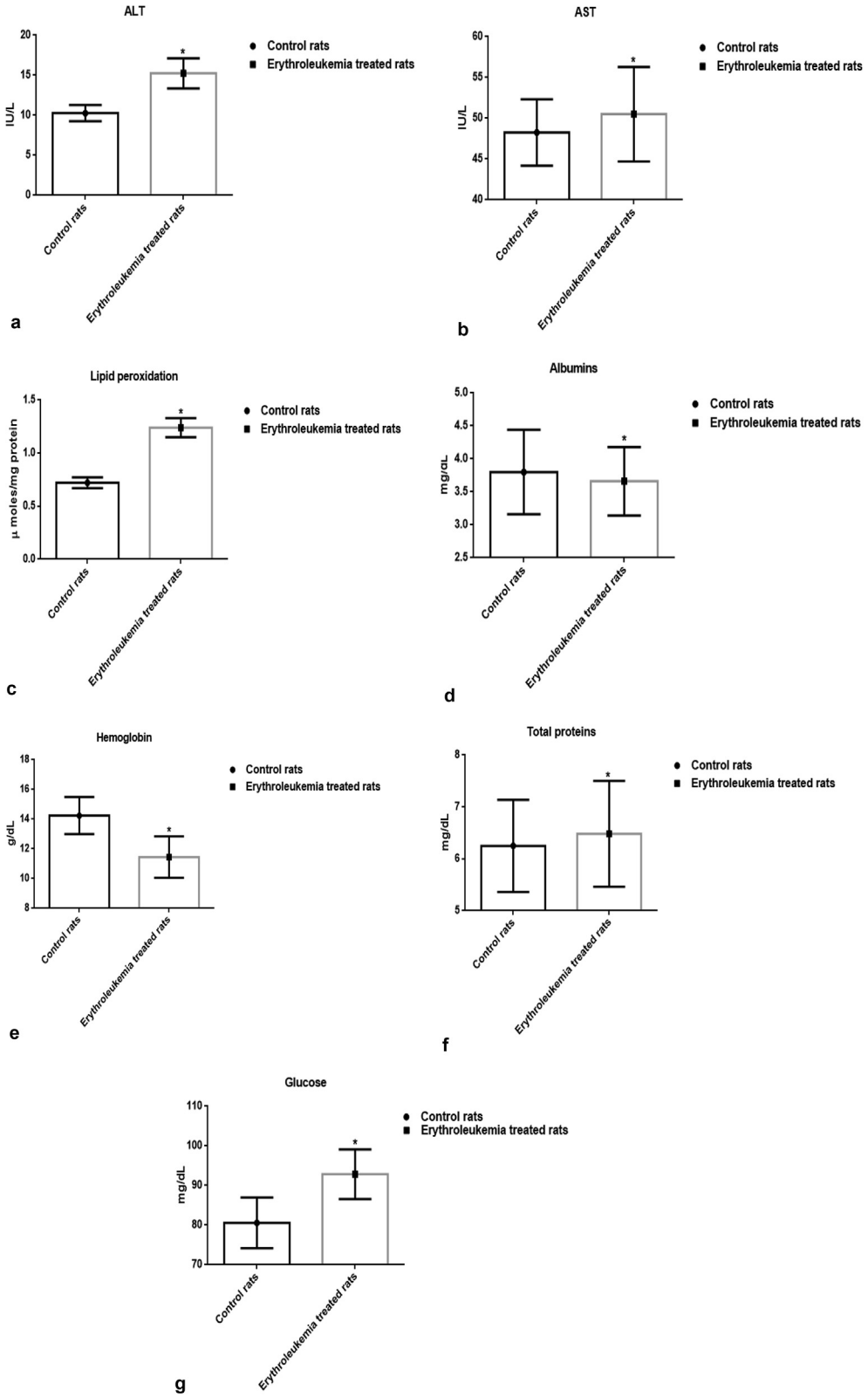

Fig. 1. a–g. Effects of rat plasma profile in erythroleukemia treatment. Values are expressed as Mean ± SEM, in significantly different ($P \leq 0.05$) from each other according to Duncan's Multiple Range (DMR) test, n = 12.

1 (Fig. 3a–g). RBC and WBC counts were significantly increased and platelet count decreased, C/P ratio decreased significantly in erythroleukemia treated rats compared with normal control (Fig. 3a–g).

4. Discussion

Erythroleukemia in mice differentiate to, enlargement of splen, novel retroviral disease, death was described in the year 1957 by

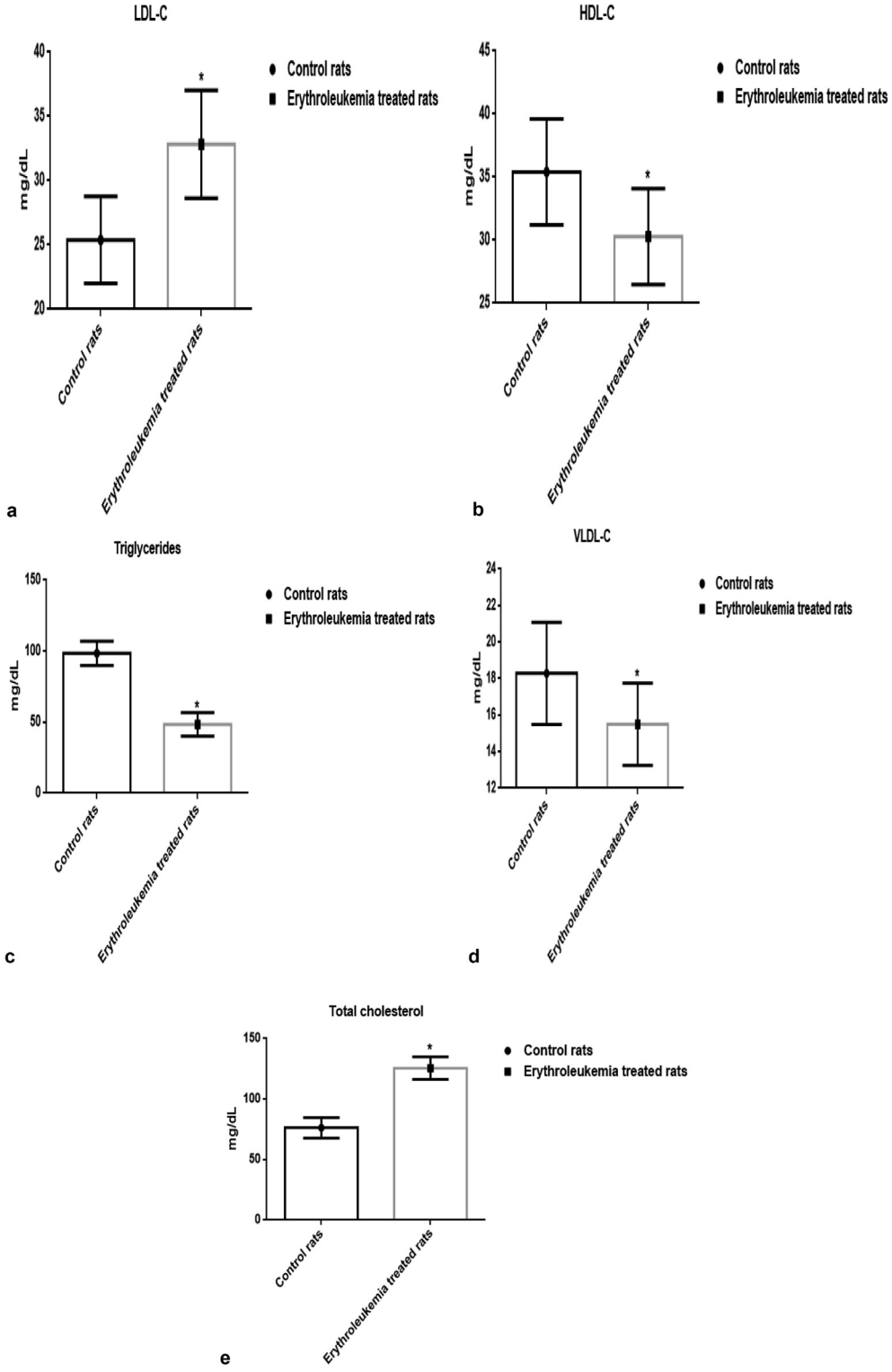

Fig. 2. a–e. Effects of rat lipid composition in erythroleukemia treatment. Values are expressed as Mean ± SEM, in significantly different ($P \leq 0.05$) from each other according to Duncan's Multiple Range (DMR) test, n = 12.

Charlotte (Friend 1956). The disease progressed rapidly, now it has called new disease (Friends), and multi stage of caicinogenesis study of afford a dominant tools. Isolated altered cells of MEL, in virus contaminated by mice, were supply a multipurpose model scheme *in vitro* study, signal transduction and erythroblast differentiation was observed in erythropoietin induced cells. Above the existence, disease has supply main on the mode, leukemia disease involved in molecular mechanisms and resistance, Epo receptor (EpoR) regular mechanisms and activation, molecular evolution in most recently observed in leukemia. Molecular insights provide and expand from the study of this disease. Friend erythroleukemia cells (FLC) (H-2d) were injected intraperitoneally in rats. The data presented reveal that FLC (H-2d) induced leukemia in rats successfully. Make this disease mixed from spread erythroblastic stem

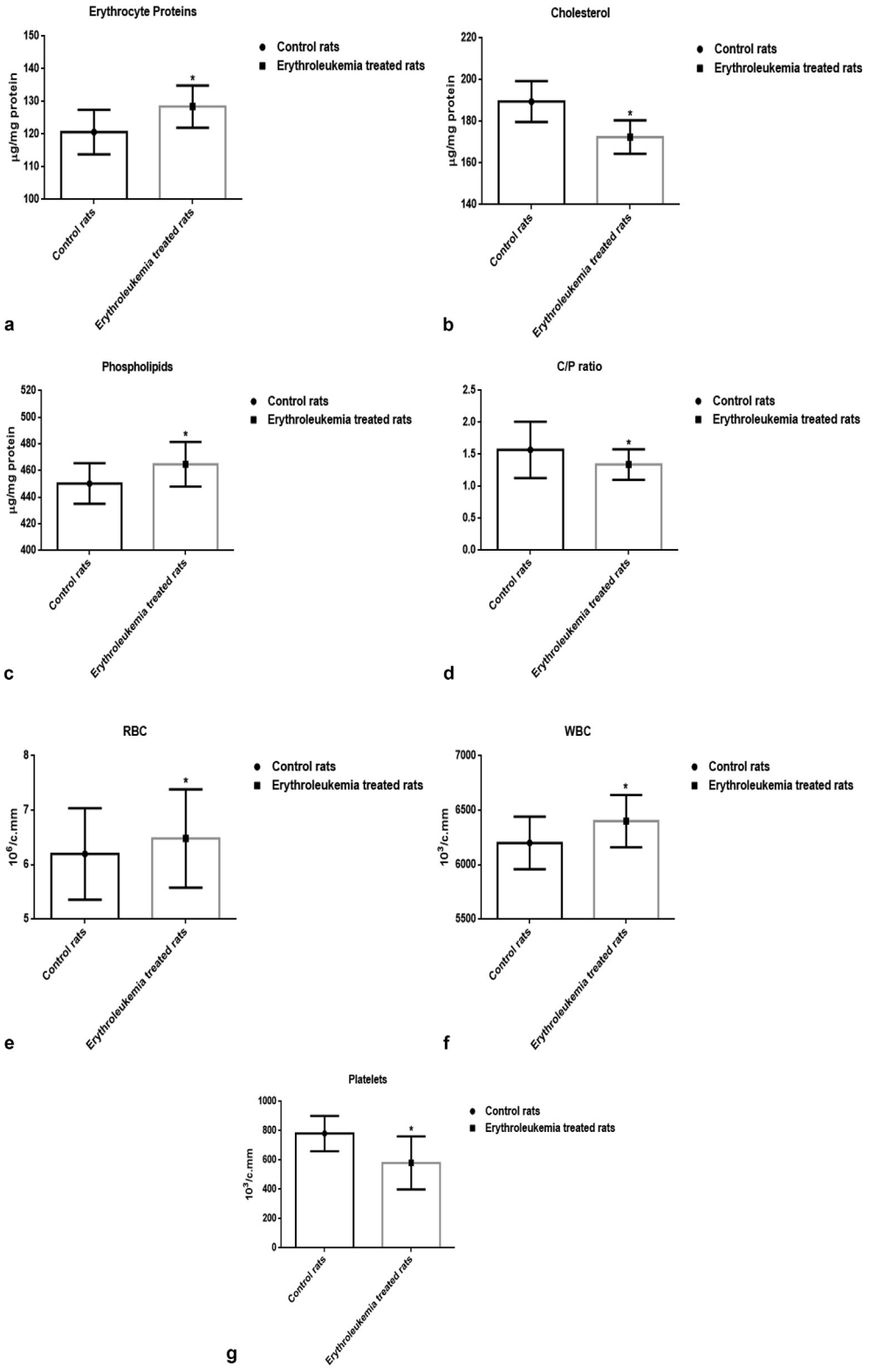

Fig. 3. a–g. Alterations of blood components and erythrocyte membrane in experimental rats (Erythroleukemia treated rats and controls). Values are expressed as Mean ± SEM, in significantly different ($P \leq 0.05$) from each other according to Duncan's Multiple Range (DMR) test, n = 12.

cells of hepatic, lymphoblastic, thymic and myelogenous leukemia (Huggins and Sugiyama 1966). Most of the proteins and lipids of the membranes be exaggerated through the placing of this complex origin alter in functional organization and structural changes of biomembranes. Current study, phospholipid levels were increased and contents of cholesterol were decreased in membrane of erythrocyte in experimental subjects match up to controls and increased modify in protein level modification in organization of membrane affecting lipid-protein, connections inside the membranes of erythrocyte (Manno et al., 2002). Glucose levels within

plasma increased observe group II match up to controls in glucose metabolism. In generally, glucose in blood a multipart route delicately keeping pace homeostatic machinery in different hormones, enzymes, tissues and issue catch division and maintenance of stable levels (Ex-ton et al., 1970; Kahn 1985; Anderson and Geil 1994). In addition, the glucose homeostasis mechanism adjustments involved metabolic accents in intracellular of compassion in insulin action receptors are accountable. Our report strongly supports earlier studies. Obviously, decrease in values VLDL-C go after decreased levels in triglyceride through observed significantly alter in increased cholesterol and decrease in HDL-C and LDL-C 1evels significantly propose some risk cardiovascular in group II compared with control group I. Though, increases values of LDL-C and plasma protein were improved albumins point are decreased, and decrease in hemoglobin (Hb), suggest some protective measure (Doumas et al., 1971; Yousef et al., 2003) in lesser LDL-C values in plasma plus rising LPO. Alter observed lipoprotein patterns (HDL-C and VLDL-C), other lipids (triglycerides), cholesterol, plasma lipid peroxidation and AST and ALT in plasma (Figs. 1a–g and 2a–e) might reveal damage of liver, investigative of cardiovascular risk and coronary disease. Expand levels and movements observed ALT and AST, erythroleukemia treated rats indicated liver damage were experiential in plasma. Levels of red cell membrane cholesterol levels decreased (erythroleukemia treated rats) when compared with controls and phospholipids, protein level increased in erythrocytes of experimental subjects to erythroleukemia treated rats group II match up to controls. RBC and WBC counts significantly increased and platelet count decreased. C/P ratio decreased significantly in erythroleukemia treated rats of experimental compared with normal control.

Statistical investigation

The consequences are states that mean ± SEM. (DMR) impact was set at ($P \leq 0.05$).

Declarations

Availability of data and materials

Not applicable.

Authors' contributions

LY and NM participated in interpreting the obtained results and organizing the manuscript. All authors read and approved the final manuscript.

Informed consent

Informed consent was obtained from all individual participants included in the study.

Declaration of Competing Interest

All authors declare that they have no conflict of interest.

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Ethical approval

All applicable international, national, and/or institutional guidelines for the care and use of animals were followed.

All procedures performed in studies involving animals were in accordance with the ethical standards of the institution or practice at which the studies were conducted. This study was approved by institutional ethical committee (20181236) The First People's Hospital of Yunnan Province, China.

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