



PROTECTIVE EFFECTS OF LUTEIN AGAINST RIBAVIRIN TOXICITY IN ALBINO WISTAR RATS

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ABSTRACT:

INTRODUCTION: Ribavirin is a broad-spectrum antiviral drug that is used to cure hepatitis C and other viral infections. **OBJECTIVE:** To demonstrate the protective effects of lutein against red blood cell membrane damage caused by ribavirin toxicity. **METHODS:** A quasi-experimental study was conducted at Isra University Hyderabad from October 2021-February 2022. Study animals were recruited from the animal husbandry of Sindh Agriculture University, Tando-Jam. Healthy male Albino Wistar rats of bodyweight between 200 and 250 grams were included in the study while female and unhealthy rats were excluded from the study. Animals were handled according to guidelines for handling laboratory animals provided by the national institute of health. Total of 30 rats (selected randomly) were allowed to acclimatize after which they were divided into three equal (n=10) groups. Group I, II and III. Group I (Control group): The animals received normal chow along with 0.9% normal saline as placebo drug. Group II (Ribavirin or experimental group): These animals received Ribavirin alone (4mg/Kg/day) along with normal chow. Group III (Ribavirin plus Lutein group): These animals received Ribavirin (4mg/Kg/day) with Lutein (150mg/ Kg/day) along with normal chow diet. **RESULTS:** There is a significant decline in the body weight of study animals in group II compared with the animals in groups I and III. There is no significant difference ($p > 0.05$) in body weight between the animals in all study groups. There was a significant decrease in mean RBC count, MCV, MCHC, MCH and hematocrit count in the ribavirin-treated experimental group (group II) compared with the counterparts in groups I and III. There was a statistically significant difference ($p < 0.05$) in mean RBC count, MCHC and hematocrit count. **CONCLUSION:** Ribavirin leads to marked red blood cell hemolysis as evident through peripheral blood smear and RBC indices. Lutein has a protective role against ribavirin-induced hemolysis in rats.

KEY-WORDS: Hematology, Ribavirin, RBC, Lutein

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INTRODUCTION:

Ribavirin is a broad-spectrum antiviral drug that is used to cure hepatitis C and other viral infections.^{1, 2} This drug is basically a

synthetic purine nucleotide analogue proposed by Sidwell in 1972. To cure the infection, it is typically used with the

combination of interferon- α .³ Ribavirin has low competence for drug-drug interaction and it cannot be metabolized via cytochrome P450 mediated mechanism.^(4, 5) Ribavirin accumulates in red blood cell and causes hemolytic anemia during ribavirin therapy, the hemolysis which is caused by ribavirin doesn't cause inflammation and is totally passive.³ The inability of erythropoiesis in bone marrow can also be the reason of hemolysis which leads to reduce hemoglobin level.⁵ The common side effect of ribavirin therapy is reversible anemia seen in several patient with unknown mechanism. Pharmacokinetic study of this drugs shows that it penetrates the erythrocyte through nucleoside transporters and reduces the erythrocyte level, it also decrease the ATP level because of transforming it into ribavirin triphosphate (RTP).^{6,7} Further this reduction leads to disruption of antioxidant defense mechanism because G6PD formation is interrupted which affect glycolysis and pentose phosphate pathway.⁸ Ribavirin has also its contribution to inhibiting inosine monophosphate dehydrogenase (IMPDH) , this enzyme is involved in the oxidation of inosine monophosphate to xanthosine monophosphate transforms NAD to thioNADP , hence when the enzyme is inhibited so these activities are affected in guanine nucleotide biosynthesis so there's no viral replication.⁹ Ribavirin has caused teratogenic effects in animals hence its contraindicated in pregnancy⁵ There have been adverse side effects reported after and during the treatment of hepatitis C which include retardation psychiatric symptoms , emotional liability, anorexia, sexual dysfunction etc.¹⁰

Phytochemical lutein is categorized as carotenoids. They are not essential for life but have contributed as being protective external danger such as UV lights, foreign bodies etc.⁶ Lutein also has antioxidant property because of that they fight with reactive oxygen species to destroy free radicals.¹¹ They are 500 times higher concentration in primate retinas macula where they act as antioxidants and blue light filters.^{7,12} Lutein is present in human body as well in eye , cervix , breast , brain and skin.^{13,}

¹⁴ It is present in central core of plasma

membrane bounded with protein, it is modestly lipophilic in nature.^{15, 16} It is noted that ribavirin causes impairment of membrane which led to lysis of rbc's that is similar that of hemolytic anemia.⁸ After viewing above points, it was proved that lutein is in anti-oxidant which give effectiveness to the therapy, so the main purpose was to see the combined effect on ribavirin causing membrane effects.

METHODS AND MATERIAL

This quasi-experimental study was conducted at Isra University Hyderabad from October 2021-February 2022. Study animals were recruited from the animal husbandry of Sindh Agriculture University, Tando-Jam. Healthy male Albino Wistar rats of body weight between 200 and 250 grams were included in the study while female and unhealthy rats were excluded from the study. All the animals were handled according to guidelines for handling the laboratory animals provided by the national institute of health. Rats were housed in stainless steel cages with sawdust bedding. The room temperature where cages were placed was maintained at $25 \pm 2^{\circ}\text{C}$ under 12/12hr light/dark cycle. For the period of one week, total 30 rats (selected randomly) were allowed to acclimatize after which they were divided into three equal (n=10) groups. Group I, II and III. Group I (Control group): The animals received normal chow along with 0.9% normal saline as placebo drug. Group II (Ribavirin or experimental group): These animals received Ribavirin alone (4mg/Kg/day) along with normal chow. Group III (Ribavirin plus Lutein group): These animals received Ribavirin (4mg/Kg/day) with Lutein (150mg/ Kg/day) along with normal chow diet. After the duration of four weeks, blood samples were collected from tail of rats from each group. Samples were collected in anti-coagulant treated CP bottles and shifted to the laboratory from performing different tests. Peripheral blood smear of all group rats were prepared. The prepared smear were placed in the staining rack and once they were dried, Leishman's stain was poured on them and left them for 2-3 minutes. Later the buffered water was added on slides for 10 minutes and dried. After preparation, the morphology of

stained blood smears were observed under the light microscope (Olympus, Japan) under 40 X lens power.

Blood CP was performed using SYSMAX XN 550 analyzer. The erythrocyte osmotic fragility was tested with NaCl solution of different osmotic concentrations such as. 0.1N NaCl solution, 0.2N NaCl solution, and etc. RBC and Platelets were measured by hydro-dynamically focused impedance measurement. Whereas, chemical and physiological aspects of cell are analyzed through flow cytometry.

All the gathered data was analyzed in SPSS version 23.0. Mean value of body weight and

hematological parameter were compared for difference between all three groups using ANOVA. Level of significance was set at p-value <0.05.

RESULTS:

Pre and post mean body weight and mean difference of study animals is mentioned in table I. Based on findings, there is a significant decline in body weight of study animals in group II compared with the animals in group I and III. There is no significant difference ($p > 0.05$) in body weight between the animals in all study groups. (Table I)

Table I: Pre and Post mean body weight of study animals in all three groups

Study groups	Mean weight		Mean difference	P-value
	Pre	Post		
Group I	202.3±2.3	203.5±3.6	+1.2	0.685
Group II	220.2±5.6	183.4±5.7	-36.8	
Group III	210.4±5.4	206.0±5.5	-4.4	

Hematological parameters including; mean of RBC count, Mean MCV, Mean MCH and hematocrit count is demonstrated in Table II. There was significant decrease in mean RBC count, MCV, MCHC, MCH and hematocrit count in ribavirin treated experimental group

(group II) compared with the counterparts in group I and III. There was statistically significant difference ($p < 0.05$) mean RBC count, MCHC and hematocrit count. (Table II)

Table II: Hematological parameters of animals in all three groups (n=30)

Hematological parameters	Mean	±SD	p-value
RBC count			
Group I	4.65	±1.01	0.036*
Group II	3.29	±0.22	
Group III	5.13	±0.91	
MCV			
Group I	53.4	±3.60	0.081
Group II	37.3	±1.52	
Group III	55.6	±2.08	
MCHC			
Group I	53.4	±3.60	0.0001*
Group II	37.3	±1.52	
Group III	55.6	±2.08	
MCH			
Group I	19.4	±0.6	0.092
Group II	18.7	±0.1	
Group III	19.1	±0.3	
Mean HCT			
Group I	34.2	±2.1	0.004*
Group II	25.7	±1.4	
Group III	34.2	±2.6	

Figure 1 is demonstrating the morphological findings of blood smear of all study groups of rats. Slide A is showing the normal findings in the control group (I) while in slide B there is a significant abnormal changes in the morphology of the

blood smear following the induction of Ribavirin alone. Slide C showing the conserving normal morphology of RBCs as result of induction of Lutein along with the Ribavirin. (Figure 1)

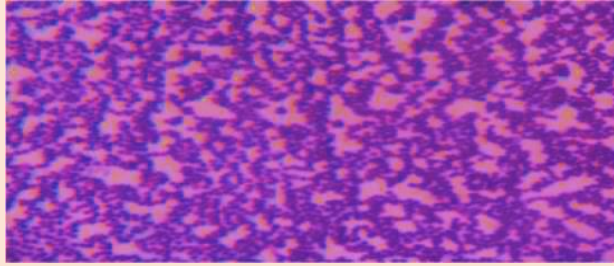
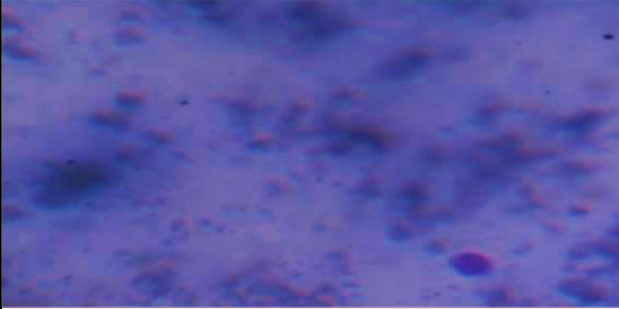
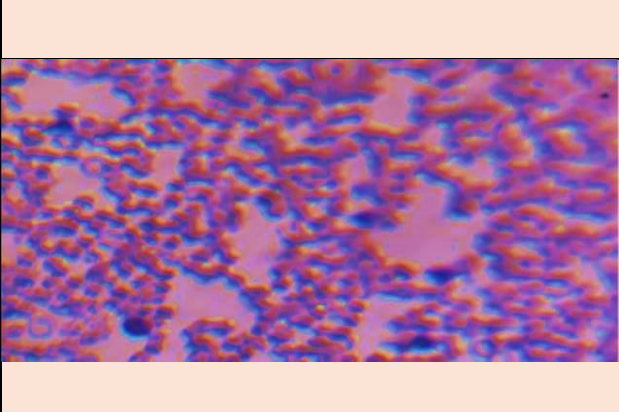
	<p style="text-align: center;">Slide A</p> <p style="text-align: center;">Group I: Normal RBC Morphology Normocytic, Normochromic (100x)</p>
	<p style="text-align: center;">Slide B</p> <p style="text-align: center;">Group II: Abnormal RBC Morphology Anisocytosis, Polychromasia , Fragmented Red Blood Cells (100x)</p>
	<p style="text-align: center;">Slide C:</p> <p style="text-align: center;">Group III: Normal RBC Morphology with minute RBC breakdown Normocytic, Normochromic (100x)</p>

Figure 1: Morphological findings of blood smear of study rats in all three groups

DISCUSSION:

Ribavirin is an antiviral drug which has shown its tremendous effects for the treatment of hepatitis C. though ribavirin has shown positive effects in viral treatment but it causes hemolytic anemia. The side effects shown in the study were reduces by collateral use of lutein with ribavirin. In the present study, hemolysis was evident in Group B which were treated with just ribavirin, while in Group C hemolysis was

less evident because ribavirin was accompanied by lutein. Uydu et al studies the consequences of ribavirin therapy in patient with dyslipidemia on the basis of rheological characteristics of erythrocyte membrane , oxidative status and serum lipid profile.¹⁷ likewise soumaya et al proposed a study in which rat's blood were observe to see the consequences of several anti-oxidant on osmotic fragility, he added that L_cartinine , vitamin C and curcumin have positive consequences on osmotic

fragility on preserved blood, in recent studies we hands on alpha tocopherol and lutein which are forms of antioxidant to analyze osmotic fragility and the results are same.¹⁸ Vidya et al in an experiment took human blood and treat it with lutein and result shows minimum hemolysis which proved that it defensive product against hemolysis thou the finding were same but decrease in hemolysis was observed in lutein group.¹⁹ Assem et al proposed a theory in which he took hepatitis C patient with ongoing ribavirin therapy, furthermore they added vitamin E to their routine which as compared to ribavirin betterment was observed in hemolytic anemia because of the improvement of MCV, HCT, MCH, RBC numbers, the study result was same as it was with alpha tocopherol.²⁰ Gabre et al also came up with his study and told that ribavirin induce hemolysis which become better with lutein and alpha tocopherol, these two are produced by same pathway of HMGCO A pathway of cholesterol.²¹ The weight of group B animals was reduced as compared to group A and C. the revelation were same as of shaimuna et al 2012, weight loss was also observed in the both studies taking ribavirin therapy, so wistar rats also reduces their weight.²² Pravastatin is a HMG CO A reductase inhibitor and identical to ribavirin so harisaet alhas did an in vitro study on erythrocyte conducted on electron microscope while talking human erythrocyte as potential carrier of pravastatin.²³ There are potential reason for the different results though ribavirin and pravastatin are HMG COA reductase inhibitors but they're overall very contrasting to each other such as their study strategies, molecular component, laboratory facilities, appliance and methodology.

CONCLUSION:

Ribavirin leads to marked red blood cells hemolysis as evident through peripheral blood smear and RBC indices. Lutein have a protective role against ribavirin induced hemolysis in rats.

ETHICS APPROVAL: The ERC gave ethical review approval

CONSENT TO PARTICIPATE: written and verbal consent was taken from subjects and next of kin

FUNDING: The work was not financially supported by any organization. The entire expense was taken by the authors

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AUTHORS' CONTRIBUTIONS: All persons who meet authorship criteria are listed as authors, and all authors certify that they have participated in the work to take public responsibility of this manuscript. All authors read and approved the final manuscript.

CONFLICT OF INTEREST: No competing interest declared.

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