Clinicopathological Studies On The Effect Of Ribavirin, Silymarin and CCl_4 In Rabbits

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ABSTRACT

A total of one hundred apparently healthy one month age male New Zealand rabbits,500 gm body weight and were used in this investigation.The rabbits were divided into 8 groups. Clinical examination of these rabbits revealed that rabbits treated with ribavirin& CCl_4 in gps.(2,5 and 6)showed anorexia, depression, loss of weight,salivation and loss of fur.Hematological and biochemical examination revealed that ribavirin caused hemolytic anemia with compensatory reticulocytosis, thrombocytopenia, leukopenia, hyperbilirubinemia with increased total and indirect bilirubin levels.Ribavirin and silymarin combination minimize the previous changes. CCl_4 administration caused hemolytic anemia with compensatory reticulocytosis, thrombocytopenia, leukocytosis, neutrophilia, eosinophilia ,monocytosis. increased serum ALT, AST, AP activities, total, direct, indirect bilirubin, globulin,creatinine levels and decrease in serum albumin level.Using ribavirin in treatment of liver damage showed mild improvement in these parameters.Using ribavirin in combination with silymarin in treatment of liver damage showed marked improvement in the parameters.

INTRODUCTION

Ribavirin (1-β-D-ribofuranosyl-1H – 1,2,4 – triazole – 3- carboxamide) is an antiviral agent that has shown *in vitro* activity against a broad spectrum of DNA and RNA viruses *(1)*, the antiviral activity is due to the resemblance of the compound to nucleosides of the virus and inhibition of nucleic acid synthesis of the virus thus inhibition of virus replication.Also , Ribavirin at the therapeutic doses markedly suppressed the production of tumor necrosis factors-alpha(TNF-α), interleukin-10(IL-10) and interleukin-12 (IL-12) which may explain the reduction in hepatic inflammation observed during ribavirin monotherapy *(2)*. Oral or intravenous administration of ribavirin causes anemia and elevated bilirubin value *(3)*.

Silymarin is a favoured drug for different liver diseases because of its oral effectiveness and good safety profile.It had established efficacy in the restoration of liver function and regeneration of liver cells *(4)*.

CCl_4 is known as a potent hepatotoxic agent causing hepatic fibrosis. The liver injury induced by CCl_4, secondry hepatic injury occurs from inflammatory processes originated from products released by activated Kupffer cells,which play a central role in hepatic inflammation *(5)*.

The aim of the present work is to:

- Study the side effects of ribavirin on hematology and organs function.
- Study the addition of silymarin in declining the side effect of ribavirin.
- Evaluation of efficacy of ribavirin as anti-inflammatory and anti-fibrotic drug alone and after addition of silymarin. The evaluation was performed by the changes in the hematology, biochemical parameters and histopathology.

MATERIAL AND METHODS

1- Experimental animals

A total of 100 white male New Zealand apparently healthy rabbits of 500 gm average body weight was obtained from the animal house, Faculty of Veterinary Medicine, Zagazig University. The animals were housed in metal cages under hygienic conditions and fed balanced ration and water *ad – libidum*.

2-Preparation of the used drugs

The recommended therapeutic dose of ribavirin is 1200 mg daily *(6)* 56mg/kg B.wt daily *(7)*.While,silymarin therapeutic dose is 420 mg daily *(8)* 20mg/ kg B.wt daily *(7)*.

3- CCl_4 induced hepatic damage

The recommended dose of CCl_4 was 2.8 ml / kg for one week followed by 1.4 ml/kg for another week *(5)* 1.5ml/ kg B.wt. diluted in liquid paraffin(1:1) *(7)*.

4- Experimental design

Table 1. Summary of the experimental design

Design	Gps.	No. of rabbits	Treatments The treatment was completed after 3 months			Blood samples
			Ribavirin orally(56mg/kg B.wt) daily	Silymarin orally(20mg/kg B.wt) daily	CCl_4(1.5ml/ kg B.wt. for one week followed by 0.75ml/kg B.wt. for another week)	
Control group	1	10	-	-	-	At 1st, 2nd and 3rd months from starting treatment
Experimental groups	2	10	+	-	-	
	3	10	-	+	-	
	4	10	+	+	-	
	5	15	-	-	+	
	6	15	+	-	+	
	7	15	-	+	+	
	8	15	+	+	+	

A- Sample collection

Blood samples were collected after 1st, 2nd and 3rd months from starting the treatment from five rabbits in each group. Two blood samples were collected from the marginal ear vein. The 1st sample was five ml of blood without anticoagulant in a sterile test tube for serum. The 2nd sample was 1ml of blood was taken in clean Wasserman tubes containing disodium salts of EDTA for hematological examination *(9)*.

B- Hematological studies:

1-Erythrogram and Platelets count

Erythrocytic count was carried out using a hemocytometer *(9)*, hemoglobin (Hb) was determined *(10)*, packed cell volume (PCV) was estimated , MCV and MCHC were calculated. Blood films were stained with new methylene blue for the reticulocytic count. Platelets count was performed *(9)*.

2-Leukogram

Blood smears were prepared, fixed with absolute methyl alcohol (95%) and stained with Giemsa's stain for differential leukocytic count and the absolute differential leukocytic counts were calculated *(11)*.

C-Biochemical studies:

The serum alanine aminotransferase (ALT) , aspartate amino transferase (AST) and alkaline phosphatase (AP) activities were measured. Serum total protein, albumin, globulin, bilirubin (total, direct and indirect) and the serum creatinine were determined.

D- Statistical analysis

Was carried out using F- test according to *(12)*.

RESULTS AND DISCUSSION

Concerning the clinical signs. Rabbits in gp.(2)showed anorexia, depression, loss of weight & fur and salivation. Gp.(4) showed mild loss of weight & fur. The mortality rate was 50% in gp.(2) and 30% in gp.(4) Gps. (1 and 3) showed no mortalities during the experimental periods. .Rabbits in gp.(5) showed anorexia, depression and loss of weight. Rabbits in gp.(6) showed anorexia, depression , loss of weight and fur. Rabbits in gp.(7and 8) showed an improvement of appetite and body weight. The mortality rate

was53.33 % in gp.(5),60 % in gp.(6) and 40% in gps.(7 & 8).

Macrocytic hypochromic anemia with compansatory reticulocytosis, Tables (2 and 4) in rabbits treated with ribavirin gp.(2) may be due to accumulation of ribavirin in erythrocytes and metabolities to the triphosphate metabolite that cause oxidative injury to red blood cell membranes inducing hemolysis *(13)*. In addition to hemolysis caused by CCl_4 gp.(5) which increases susceptibility of erythrocyte cell membrane to oxidative stress *(14)*. Reticulocytosis may be due to regenerative response of bone marrow after hemolysis *(15)*.Gp.(4) administered ribavirin in combination with silymarin by half the therapeutic dose showed the same changes in erythrogram as gp.(2)but by a little degree. This may be due to ribavirin caused a dose-related decrease in circulating red blood cell mass *(16)*.Gp.(6) treated with ribavirin after liver damage showed macrocytic hypochromic anemia with compansatory reticulocytosis. This may be due to the effect of both CCl_4 and ribavirin.There was significant improvement in gps .(7 and 8) treated with silymarin or the combination when compared with gps.(5 and 6)but did not reach to the normal level .

Thrombocytopenia , Tables (2 and 4) was recorded in varying degrees in all groups except gp.(3) which may be due to progressive severe degrees of megakaryocytes hypoplasia and dysplasia, a synchronous megakaryocytes maturation caused by ribavirin *(17)*.Gps.(7 and 8) showed an improvement in platelets count after liver damage but still did not return to the control level.

Leukopenia with neutropenia, lymphopenia and eosinopenia, Tables (2 and 4) were found in gps.(2 and 6),may be due to destruction of circulating leukocytes.The same results previously obtained *(17)*. Gp.(4)administered ribavirin in. combination with silymarin by half the therapeutic dose showed the same changes in leukogram as gp.(2), but by a low degree. This may be due to the reduced dose of ribavirin that reduce the destruction of circulating leukocytes *(16)* and the immunomodulatory effect of silymarin *(18)*. Gp.(5) administered CCl_4 showed leukocytosis which may be due to neutrophilia occurred due to response to mediators related to products of tissue injury or necrosis *(11)*. Eosinophilia may be due to tissue fibrosis as eosinophils have the ability to secrete collagenase enzyme which has fibrinolytic power,where eosinophils could digest and lyse the fibrous tissue in the liver *(19)*. Monocytosis may be due to tissue inflammation and phagocytic activity of monocyte as it give rise to tissue macrophage . Gps.(7 and 8) showed an improvement in these parameters .

The present work, Tables (3 and 5) showed marked increase in liver enzymes activities which were pronounced in gp.(5). ALT is an intracellular cytoplasmic enzyme *(20)*. So, any inflammation of the hepatic parenchyma are likely to increase the permeability of the hepatic cell membrane and allow the cytoplasmic enzymes to leak out into the blood *(21)*. Gp.(5) showed significant increase in serum AP activity may be due to cholestasis and acute hepatocellular necrosis. But it may be released also from renal tubules in addition to biliary obstruction *(9)*.Using of ribavirin for treatment of hepatic damage in gp.(6) improves the increase in the serum ALT,AST and AP activities when compared with damaged non treated gp.(5) throughout the experimental periods which may be attributed to the anti-inflammatory and anti-fibrotic effect of ribavirin *(2)*. The benefical effects of ribavirin are mediated by inhibition of induction of macrophage pro-inflammatory cytokines . Gp.(7) showed significant improvement in the serum ALT, AST and AP activities all over the experimental periods when compared with damaged non treated gp.(5) due to the anti-inflammatory and anti-fibrotic effect of silymarin by its inhibitory effect on 5-lipoxygenase pathway resulting in inhibition of leukotriene synthesis *(22)*. At the same time, gp. (8) showed highly significant improvement in serum ALT, AST and AP activities throughout the experimental periods.

Hyperbilirubinemia with increased indirect bilirubin and normal direct bilirubin levels ,table(3 and 5) in gp.(2) along the experimental periods may be due to hemolytic anemia caused by ribavirin.Gp.(4) showed the same changes in the serum bilirubin level but by a little degree. This may be due to the reduced dose of ribavirin caused decrease in hemolysis *(16)* and due to the anti-oxidant properties of silymarin *(22)*. This study, showed highly significant increase in total, direct and indirect bilirubin levels along the experimental periods in gp.(5).These results may be attributed to the hepatocellular damage which led to an intrahepatic cholestasis and so the difficulty to uptake and conjugate the bilirubin *(9)*, also due to hemolysis caused by CCl_4. Gp.(6) showed mild improvement in serum total, direct and indirect bilirubin levels especially at the 3rd month of treatment but the values still high and did not reach the control level. Gp.(7) showed significant improvement in the serum total, direct and indirect bilirubin levels throughout the experimental periods when compared with damaged non treated rabbits (gp.5).Gp.(8) administered ribavirin in combination with silymarin by half the therapeutic dose showed an improvement in serum bilirubin level when compared with damaged non treated rabbits gp.(5).

Protein profile, Tables (3 and 5) in gp.(5) hypoalbuminemia and hyperglobulinemia were observed. Hypoalbuminemia may be attributed to liver damage as the liver considers the main organ responsible for synthesis of the most types of plasma proteins *(9)* . Hypoproteinemia could be attributed to hypoalbuminemia occurs in hepatic disease and albumin represent mostly the largest level of plasma proteins. Hyperglobulinemia occurred due to inflammation and liver disease. Using of ribavirin gp.(6) and silymarin gp.(7) for treatment of hepatic damage induced by CCl_4 improves the decrease in serum albumin level when compared with gp.(5).At the same time, gp.(8) returned serum albumin to the control level. This result may be due to the anti-inflammatory effect of the drugs that reduce hepatic inflammation and anti-fibrotic action which reduce hepatic cell necrosis and fibrosis that help liver cell repair and return its function to synthesis plasma proteins and albumin *(2,22)*.

The was significant increase in creatinine throughout the experimental periods, Tables (3 and 5) in gp. (5) may be due to renal damage induced by CCl_4 administration. The damaged then treated with ribavirin gp.(6) and silymarin gp.(7) showed significant improvement in creatinine when compared with gp.(5). At the same time, gp.(8) showed highly significant improvement in creatinine throughout the experimental periods. This result may be due to the anti-inflammatory effect of the drugs that reduce inflammation of the renal tubules and anti-fibrotic action which reduce renal tubules necrosis and fibrosis *(2,22)*.

Table 2. Erythrogram , platelets count and Leukogram (mean values ± S E) in rabbits in gps. (1-4) after 1st , 2nd and 3rd months of treatment.

	Parameters / Groups	RBCs 10^6/µl	Hb gm%	PCV %	MCV fl	MCHC %	Retic %	Platelets 10^3/µl	TLC X10^3/ µl	Neut cell/ µl	Lymph cell/ µl	Eosin cell/ µl
1st month	Control gp.(1)	5.35 a ±0.01	12.12a ±0.04	32.22a ±0.04	60.17c ±0.07	37.61 a ±0.09	0.96c ±0.06	444.0 a ±9.88	6.09a ±0.02	2.08 a ±0.012	3.48 a ±0.004	0.09 a ±0.004
	Ribavirin gp.(2)	4.09 c ±0.08	10.64c ±0.07	30.72 ±0.11	75.13a ±1.24	34.62 c ±0.16	2.70a ±0.33	364.0 c ±2 .88	5.06c ±0.02	1.98 c ±0.024	2.56 c ±0.014	0.07 c ±0.001
	Silymarin gp.(3)	5.35 a ±0.04	12.04a ±0.09	32.46a ±0.17	60.66c ±0.09	37.08 a ±0.12	0.84c ±0.16	442.2 a ±7.49	6.08a ±0.01	2.06 a ±0.016	3.49 a ±0.004	0.09 a ±0.004
	combination gp.(4)	4.88 b ±0.01	11.16b ±0.11	31.46b ±0.06	64.40b ±0.14	35.47 b ±0.36	2.36b ±0.34	413.2 b ±3 .54	5.87b ±0.01	2.01 b ±0.004	3.33 b ±0.009	0.08 b ±0.001
	F-test	*	*	*	*	*	**	*	*	*	*	*
	LSD	0.43	0.46	0.73	2.89	0.63	0.26	29.82	0.20	0.03	0.03	0.01
2nd month	Control gp.(1)	5.38 a ±0.14	12.12a ±0.17	32.30a ±0.25	60.10c ±1.34	37.51 a ±0.34	0.76c ±0.11	447.0 a ±14.55	6.14a ±0.06	2.12 a ±0.044	3.50 a ±0.011	0.08 a ±0.006
	Ribavirin gp.(2)	3.88 b ±0.04	10.08b ±0.16	29.36b ±0.54	75.53a ±0.61	34.18 b ±0.21	3.42a ±0.04	336.4 c ±2 .60	4.94c ±0.21	1.93 c ±0.024	2.51 c ±0.214	0.06 c ±0.003
	Silymarin gp.(3)	5.40 a ±0.10	12.14a ±0.09	32.54a ±0.14	60.29c ±1.17	37.30 a ±0.15	0.76c ±0.12	449.0 a ±17.84	6.12a ±0.03	2.08 a ±0.027	3.52 a ±0.007	0.08 a ±0.003
	combination gp.(4)	4.31 b ±0.28	10.24b ±0.47	29.84b ±0.92	69.90b ±2.61	34.24 b ±0.54	2.72b ±0.20	380.2 b ±9.93	5.64b ±0.01	1.95 b ±0.027	3.18 b ±0.037	0.07 b ±0.005
	F-test	**	**	*	**	*	**	**	*	*	*	*
	LSD	0.50	0.80	1.66	4.84	1.03	0.41	37.80	0.34	0.08	0.31	0.01
3rd month	Control gp.(1)	5.62 a ±0.07	12.94a ±0.11	33.06a ±0.44	58.78 ±0.39	39.14 a ±0.21	0.72c ±0.15	451.2 a ±8.24	6.12a ±0.12	2.21 a ±0.076	3.37 a ±0.082	0.09 a ±0.003
	Ribavirin gp.(2)	3.39 c ±0.07	8.92 c ± 0.10	27.04c ±0.27	79.85a ±1.00	32.98 b ±0.22	4.60a ±0.33	312.6 c ±5.22	4.08c ±0.30	1.71 c ±0.150	1.87 c ±0.177	0.06 c ±0.005
	Silymarin gp.(3)	5.63 a ±0.10	12.88a ±0.29	32.90a ±0.59	58.43c ±0.25	39.13 a ±0.15	0.68c ±0.11	446.6 a ±7.96	6.07a ±0.27	2.19 a ±0.085	3.36 a ±0.167	0.09 a ±0.009
	combination gp.(4)	4.21 b ±0.25	10.00b ±0.52	29.28b ±0.79	70.15b ±2.77	34.04 b ±0.92	2.82b ±0.31	347.6 b ±17.15	4.74b ±0.33	1.80 b ±0.061	2.43 b ±0.283	0.07 b ±0.003
	F-test	**	**	**	**	**	**	**	**	**	**	**
	LSD	0.44	0.92	1.68	4.48	1.59	0.74	31.90	0.51	0.08	0.47	0.01

L S D:Least significant difference.
**: Significant at 0.05 probability.*
***:Highly significant at 0.01 probability.*

Table 3. Some liver and kidney function tests (mean values ± S E) in rabbits in gps. (1-4) after 1st ,2nd and 3rd months of treatment.

Parameters / Groups		ALT µ/l	AST µ/l	ALP µ/l	Total bilirubin mg%	Direct bilirubin mg%	Indirect bilirubin mg%	Total protein g/dl	Albumin g/dl	Globulin g/dl	Creatinine mg/dl
1st month	Control gp.(1)	20.6 ±0.50	22.8 ±1.43	33.82 ±1.33	1.049 c ±0.05	0.354 ±0.04	0.695 c ±0.02	5.46 ±0.02	3.51 ±0.04	1.95 ±0.04	0.817 ±0.04
	Ribavirin gp.(2)	22.0 ±1.07	23.8 ±1.72	35.17 ±0.45	1.482 a ±0.03	0.356 ±0.01	1.126 a ±0.02	5.61 ±0.02	3.66 ±0.02	1.95 ±0.06	0.901 ±0.02
	Silymarin gp.(3)	20.2 ±1.20	22.5 ±1.69	32.90 ±0.99	1.068 c ±0.06	0.335 ±0.06	0.733 c ±0.04	5.34 ±0.16	3.27 ±0.15	2.07 ±0.02	0.804 ±0.04
	combination gp.(4)	21.2 ±0.24	24.0 ±0.48	34.05 ±0.70	1.274 b ±0.07	0.348 ±0.01	0.926 b ±0.07	5.25 ±0.06	3.25 ±0.04	2.00 ±0.02	0.878 ±0.07
	F-test	N.S	N.S	N.S	*	N.S	*	N.S	N.S	N.S	N.S
	LSD	-	-	-	0.05	-	0.04	-	-	-	-
2nd month	Control gp.(1)	21.9 ±1.80	24.0 ±2.16	32.01 ±0.55	1.042 c ±0.08	0.336 ±0.09	0.706 c ±0.02	5.49 ±0.01	3.46 ±0.06	2.03 ±0.07	0.864 ±0.05
	Ribavirin gp.(2)	23.1 ±1.36	25.4 ±1.65	33.57 ±1.51	1.998 a ±0.08	0.362 ±0.06	1.636 a ±0.02	5.70 ±0.03	3.55 ±0.07	2.15 ±0.04	0.944 ±0.10
	Silymarin gp.(3)	21.2 ±1.81	23.8 ±1.62	31.94 ±0.88	1.039 c ±0.01	0.324 ±0.01	0.715 c ±0.02	5.38 ±0.04	3.23 ±0.01	2.15 ±0.01	0.818 ±0.10
	combination gp.(4)	22.6 ±1.15	24.8 ±1.11	32.59 ±1.13	1.360 b ±0.05	0.337 ±0.09	1.023 b ±0.04	5.39 ±0.04	3.30 ±0.01	2.09 ±0.01	0.900 ±0.08
	F-test	N.S	N.S	N.S	**	N.S	**	N.S	N.S	N.S	N.S
	LSD	-	-	-	0.15	-	0.08	-	-	-	-
3 rd month	Control gp.(1)	23.2 ±1.82	24.2 ±1.44	32.50 ±1.05	1.041 c ±0.01	0.338 ±0.01	0.703 c ±0.02	5.51 ±0.01	3.29 ±0.01	2.22 ±0.08	0.873 ±0.08
	Ribavirin gp.(2)	24.5 ±1.95	25.6 ±0.77	33.20 ±0.56	2.400 a ±0.10	0.378 ±0.04	2.022 a ±0.09	5.71 ±0.04	3.59 ±0.02	2.12 ±0.02	0.920 ±0.07
	Silymarin gp.(3)	22.8 ±2.37	24.0 ±1.01	31.93 ±1.25	1.045 c ±0.06	0.341 ±0.07	0.704 c ±0.02	5.42 ±0.06	3.23 ±0.09	2.19 ±0.03	0.795 ±0.09
	combination gp.(4)	24.0 ±1.74	25.0 ±1.24	32.85 ±1.33	1.672 b ±0.17	0.354 ±0.03	1.318 b ±0.14	5.44 ±0.04	3.26 ±0.03	2.18 ±0.07	0.891 ±0.06
	F-test	N.S	N.S	N.S	**	N.S	**	N.S	N.S	N.S	N.S
	LSD	-	-	-	0.30	-	0.26	-	-	-	-

L S D:Least significant difference. *N.S: Non significant changes.*
: Significant at 0.05 probability.* *:Highly significant at 0.01 probability.*

Table 4. Erythrogram , platelets count and Leukogram (mean values ± SE) in rabbits in gps. (1&5-8) after 1st , 2nd and 3rd months of treatment.

Parameters / Groups		RBCs 10^6/µl	Hb gm%	PCV %	MCV fl	MCHC %	Retic %	Platelets 10^3/µl	TLC X10^3/ µl	Neut cell/ µl	Lymph. cell/ µl.	Eosin cell/ µl	Mono cell/ µl
1st month	Control gp.(1)	5.35 a ±0.01	12.12a ±0.04	32.22a ±0.04	60.17c ±0.07	37.61 a ±0.09	0.96c ±0.06	444.0 a ±9.88	6.09 c ±0.02	2.08 c ±0.112	3.48 a ±0.003	0.09 c ±0.004	0.28 b ±0.004
	CCl_4 gp.(5)	3.60 b ±0.19	8.84 b ±0.57	28.50b ±1.27	79.16b ±1.69	31.48 b ±0.85	4.20b ±0.95	339.6 c ±15.36	7.79a ±0.15	3.31 a ±0.146	3.27ab ±0.129	0.65 a ±0.029	0.40 a ±0.010
	Ribavirin gp.(6)	2.50 c ±0.08	6.50 c ±0.24	23.00c ±0.59	92.00a ±2.14	28.26 c ±0.60	6.12a ±0.36	286.4 d ±11.51	6.82b ±0.18	2.58 b ±0.130	3.26ab ±0.183	0.42 b ±0.022	0.38 a ±0.011
	Silymarin gp.(7)	3.78 b ±0.18	9.40 b ±0.44	29.88b ±1.32	79.13b ±2.06	31.48 b ±0.70	3.48b ±0.51	386.2 b ±9.15	7.17ab ±0.25	2.68 b ±0.123	3.55 a ±0.244	0.44 b ±0.003	0.33ab ±0.003
	combination gp.(8)	3.38 b ±0.32	8.36 b ±0.80	27.80b ±1.89	82.24b ±3.74	30.18 b ±0.94	4.34b ±0.38	341.8 c ±21.78	6.98 b ±0.09	3.05ab ±0.123	2.96 b ±0.182	0.43 b ±0.044	0.36 a ±0.011
	F-test	**	**	**	**	**	**	**	*	*	*	*	*
	LSD	0.56	1.46	2.31	6.69	1.89	1.25	42.24	0.68	0.40	0.45	0.08	0.08
2nd month	Control gp.(1)	5.38 a ±0.14	12.12a ±0.17	32.30a ±0.25	60.10d ±1.34	37.51 a ±0.34	0.76d ±0.11	447.0 a ±14.55	6.14bc ± 0.06	2.12 b ±0.144	3.50 ±0.011	0.08 d ±0.006	0.28 b ±0.005
	CCl_4 gp.(5)	3.60 d ±0.19	8.84 d ±0.57	28.50d ±1.27	79.16b ±1.69	31.48 d ±0.85	4.20 ±0.95	339.6 d ±15.36	7.79 a ±0.15	3.31 a ±0.146	3.27 ±0.129	0.65 a ±0.029	0.40 a ±0.010
	Ribavirin gp.(6)	2.76 e ±0.26	7.16 e ±0.59	23.60e ±1.68	86.37a ±3.22	30.28 e ±0.87	5.44a ±0.32	299.0 e ±12.23	5.96 c ±0.31	1.95 b ±0.154	4.29 ±0.344	0.22 c ±0.008	0.29 b ±0.010
	Silymarin gp.(7)	4.19 b ±0.09	10.28b ±0.17	31.90b ±0.55	76.14c ±0.64	32.22 b ±0.18	2.80c ±0.37	408.2 b ±20.13	6.55 b ±0.14	2.14 b ±0.141	4.56 ±0.147	0.38 b ±0.025	0.27 b ± .006
	combination gp.(8)	3.83 c ±0.15	9.40 c ±0.44	30.26c ±1.03	78.99b ±1.18	31.01 c ±0.55	3.42bc ±0.42	365.0 c ±28.83	6.38bc ±0.11	2.33 b ±0.176	4.17 ±0.112	0.11 d ±0.009	0.30 b ±0.007
	F-test	**	**	**	**	**	**	*	*	*	N.S	*	*
	LSD	0.20	0.50	0.09	2.42	0.04	1.17	20.51	0.53	0.46	-	0.05	0.07
3rd month	Control gp.(1)	5.62 a ±0.07	12.94a ±0.11	33.06a ±0.44	58.78d ±0.39	39.14 a ±0.21	0.72 d ±0.15	451.2 a ±8.24	6.12 b ± 0.12	2.21 b ±0.176	3.37 ±0.082	0.09 d ±0.003	0.28 b ± .004
	CCl_4 gp.(5)	3.60 d ±0.19	8.84 d ±0.57	28.50c ±1.27	79.16b ±1.69	31.48 d ±0.85	4.20 a ±0.95	339.6 d ±15.36	7.79 a ±0.15	3.31 a ±0.146	3.27 ±0.129	0.65 a ±0.029	0.40 a ±0.010
	Ribavirin gp.(6)	2.98 e ±0.03	8.10 e ±0.28	25.50d ±1.10	85.57a ±2.72	30.59 e ±0.20	5.10 a ±0.12	305.0 e ±1.58	5.60 c ±0.11	1.90 b ±0.129	3.10 ±0.095	0.20 b ±0.009	0.22 b ±0.007
	Silymarin gp.(7)	5.08 b ±0.17	11.52b ±0.15	32.92a ±0.19	65.00c ±1.93	34.98 b ±0.28	1.90 c ±0.20	424.0 b ±9.17	6.31 b ±0.03	2.14 b ±0.140	3.57 ±0.162	0.15 c ±0.005	0.28 b ±0.011
	combination gp.(8)	4.14 c ±0.21	10.50c ±0.28	30.85b ±0.58	75.77b ±2.46	33.28 c ±0.52	3.00 b ±0.25	381.5 c ±5.85	6.19 b ±0.11	2.16 b ±0.125	3.49 ±0.120	0.09 d ±0.003	0.28 a ±0.007
	F-test	*	*	*	*	*	**	*	*	*	N.S	*	*
	LSD	0.45	0.70	1.44	4.93	0.23	0.93	27.20	0.34	0.42	-	0.04	0.08

L S D:Least significant difference.
N.S: Non significant changes.
**: Significant at 0.05 probability.*
***:Highly significant at 0.01 probability.*

Table 5. Some liver and kidney function tests (mean values ± S E) in rabbits in gps. (1&5-8) after 1st ,2nd and 3rd months of treatment.

	Parameters / Groups	ALT µ/l	AST µ/l	AP µ/l	Total bilirubin mg%	Direct bilirubin mg%	Indirect bilirubin mg%	Total protein g/dl	Albumin g/dl	Globulin g/dl	Creatinine mg/dl
1st month	Control gp.(1)	20.6 c ± 0.50	25.6 c ±1.43	33.82d ±1.33	1.049 d ± 0.05	0.354 c ± 0.04	0.695 c ± 0.02	5.45 ± 0.02	3.22 a ±0.04	2.23 b ±0.04	0.817 b ± 0.04
	CCl_4 gp.(5)	76.0 a ±5.66	50.8 a ±6.36	50.49a ±4.05	3.700 ab ±0.20	1.540 a ±0.11	2.160 b ±0.10	4.97 ±0.19	2.11 c ±0.09	2.86 a ±0.12	3.780 a ±0.28
	Ribavirin gp.(6)	66.2ab ±1.68	47.6 a ±4.84	44.60b ±5.90	4.200 a ± 0.27	1.600 a ± 0.15	2.600 a ± 0.23	5.00 ±0.36	2.10 c ±0.21	2.90 a ±0.25	3.240 a ± 0.34
	Silymarin gp.(7)	60.4 b ±3.66	43.4 ab ±5.24	39.47c ±3.40	3.000 c ± 0.13	1.200 b ± 0.04	1.800 b ± 0.12	5.29 ±0.24	2.36 bc ±0.15	2.93 a ±0.11	3.020 a ± 0.33
	combination gp.(8)	57.0 b ±3.06	31.6 bc ± 2.44	38.46c ±3.89	3.300 bc ± 0.19	1.160 b ± 0.12	2.140 b ± 0.17	5.60 ±0.21	2.60 b ± 0.15	3.00 a ±0.07	2.870 a ± 0.46
	F-test	**	**	**	**	**	**	N.S	*	*	**
	LSD	10.05	13.16	1.94	0.54	0.30	0.43	-	0.42	0.20	0.95
2nd month	Control gp.(1)	20.4 d ± 1.80	26.0 c ±2.16	34.56b ±0.55	1.042 d ± 0.08	0.336 c ± 0.09	0.706 d ± 0.02	5.49 a ± 0.01	3.49 a ±0.06	2.00 b ±0.07	0.944 d ± 0.05
	CCl_4 gp.(5)	76.0 a ±5.66	50.8 a ±6.36	50.49a ±4.05	3.700 a ±0.20	1.540 a ±0.11	2.160 a ±0.10	4.97 ab ±0.19	2.11 c ±0.09	2.86 a ±0.12	3.780 a ±0.28
	Ribavirin gp.(6)	50.2 b ±5.20	40.2 ab ±4.48	40.15b ±3.63	3.000 b ± 0.15	1.100 b ± 0.10	1.900 ab ± 0.07	4.26 c ±0.15	2.30 c ±0.11	1.96 b ±0.06	2.900 b ± 0.11
	Silymarin gp.(7)	39.4 c ±4.20	37.6abc ±4.91	37.58b ±3.83	2.500 c ± 0.17	1.000 b ± 0.07	1.500 c ± 0.10	4.80 b ±0.20	2.80 b ±0.20	2.00 b ±0.09	2.100 c ± 0.21
	combination gp.(8)	35.6 c ±3.07	30.2 bc ± 1.77	35.44b ±0.91	2.820 bc ± 0.15	1.040 b ± 0.14	1.780 bc ± 0.21	4.99 ab ±0.08	2.90 b ± 0.14	2.09 b ±0.05	1.900 c ± 0.20
	F-test	**	**	*	**	**	**	*	*	*	**
	LSD	10.49	12.70	8.9	0.45	0.29	0.35	0.53	0.39	0.15	0.56
3rd month	Control gp.(1)	26.2 c ± 1.82	31.0 b ±1.44	34.10b ±1.05	1.041 d ± 0.01	0.338 c ± 0.01	0.703 d ± 0.02	5.47 ± 0.01	3.29 a ±0.01	2.18 b ±0.08	0.873 c ± 0.08
	CCl_4 gp.(5)	76.0 a ±5.66	50.8 a ±6.36	50.49a ±4.05	3.700 a ±0.20	1.540 a ±0.11	2.160 a ±0.10	4.97 ±0.19	2.11 c ±0.09	2.86 a ±0.12	3.780 a ± 0.28
	Ribavirin gp.(6)	40.2 b ±0.80	37.0 b ±0.94	37.40b ±0.18	2.700 b ± 0.09	0.820 b ± 0.11	1.880 b ± 0.02	4.90 ±0.09	2.70 b ±0.09	2.20 b ±0.02	2.000 b ± 0.06
	Silymarin gp.(7)	35.4bc ±3.66	34.2 b ±3.29	35.06b ±0.55	1.800 c ± 0.08	0.560 c ± 0.06	1.240 c ± 0.01	5.00 ±0.14	2.80 b ±0.23	2.20 b ±0.11	1.010 c ± 0.02
	combination gp.(8)	30.0 c ±1.58	33.2 b ± 1.11	34.98b ±0.73	2.020 c ± 0.15	0.910 b ± 0.01	1.110 c ± 0.13	5.30 ±0.02	3.18 a ± 0.03	2.12 b ±0.06	0.900 c ± 0.03
	F-test	*	*	*	*	*	*	N.S	*	*	*
	LSD	9.51	9.83	5.66	0.38	0.23	0.22	-	0.36	0.22	0.40

N.S: Non significant changes.
**: Significant at 0.05 probability.*
***:Highly significant at 0.01 probability.*
L S D:Least significant difference.

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الملخص العربي

دراسات باثولوجية إكلينيكية علي تأثيرالريبافيرين و السيليمارين ورابع كلوريد الكربون في الأرانب

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أجريت هذه الدراسة لمعرفة تأثير الريبافيرين والسيليمارين والخليط منهم في علاج العطب الكبدي الناتج من استخدام رابع كلوريد الكربون بالإضافة إلى دراسة الأثار الجانبية لكلا منهما.

لقد أظهرت المجاميع (الثانيه والخامسه والسادسه) فقدان للشهيه وخمول ونقص شديد فى الاوزان بالاضافه الى نزول اللعاب وتساقط الفرو فى المجموعتين الثانيه والسادسه المعالجه بالريبافيرين وقد اظهرت تحاليل الدم والمصل أن الريبافيرين فى الارانب السليمه يسبب انيميا التحلل الدموى مع زياده فى الخلايا الشبكيه ونقص فى الصفائح الدمويه وكرات الدم البيضاء وزياده البليروبين الكلى وغير المباشر.استخدام الخليط من الريبافيرين والسيليمارين يقلل ويحسن هذه التغيرات.اعطاء رابع كلوريد الكربون يحدث انيميا التحلل الدموى مع زياده الخلايا الشبكيه ونقص فى الصفائح الدمويه وزياده فى عدد كرات الدم البيضاء والخلايا متعدده الصبغه والخلايا حامضيه الصبغه والخلايا أحاديه النواه مع زياده فى انزيمات الألانين أمينوترانسفيريز والاسبرتيت أمينوترانسفيريز والفوسفاتيز القلوى والبليروبين الكلى والمباشر وغير المباشر والجلوبيولين والكرياتينين ونقص فى الالبيومين.استخدام الريبافيرين للعلاج يحدث تحسن بسيط فى هذه التغيرات.استخدام الخليط من الريبافيرين والسيليمارين يحدث تحسن ملحوظ فى هذه التغيرات. ولذلك نوصى باستخدام الخليط من الريبافيرين والسيليمارين للوقاية من الآثار الجانبية السيئة الناتجة من استخدام الريبافيرين وللزيادة من تأثيره لعلاج العطب الكبدي.