Investigating the Efficacy and Safety of Silymarin in Management of Hyperbilirubinemia in Neonatal Jaundice

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Abstract

Unconjugated hyperbilirubinemia (UCB) is one of the most common conditions in neonates. Conventional treatment consists of phototherapy and exchange transfusion. Phototherapy is safe and effective but it has several disadvantages. Exchange transfusion is associated with a significant morbidity and even mortality. That indicates the need to develop an alternative pharmacological treatment strategies. It should be less invasive and at least as effective and safe as phototherapy. Herbal therapy has recently received special attention. Silymarin herbal drug has laxative, antioxidant, anti-inflammatory, hepatic protective, regenerative and enhancing of glucoronidation activities.

The study presented here aimed to investigate effect of Silymarin on duration of phototherapy. A prospective cohort trial performed on 170 full term healthy neonates with UCB, 85 received oral 3.75mg/kg of Silymarin twice daily plus phototherapy and 85 neonates received only phototherapy. Total serum bilirubin (TSB) was measured every 24h, Aspartate aminotransferase (AST), Alanine aminotransferase (ALT) and albumin were measured before and after therapy for both groups. The mean duration of phototherapy was found to be significantly reduced from 127.47±19.61 days in the control group to 100.66±18.30 days in Silymarin-treated group (p=0.001). ALT and AST were improved to normal levels significantly (p=0.001) also albumin (p=0.005).

Silymarin dose of 3.75mg/kg twice daily along with phototherapy was more effective than phototherapy alone in treating full term healthy neonates with UCB.

Key words: Silymarin, neonatal jaundice, hyperbilirubinemia, phototherapy, bilirubin.

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Introduction

Neonatal jaundice or hyperbilirubinemia reflects accumulation of the yellow-orange pigment bilirubin in the skin, sclera, and other mucous tissues. It is usually a normal physiologic condition occurring during the transitional period after birth that resulting from imbalance between production and elimination of bilirubin [1].

Newborn appear jaundiced when the serum bilirubin level is >7 mg/dl [2]. Severe elevation of serum bilirubin levels can result in brain damage, known as kernicterus which is a lifelong neurologic sequelae and may lead to death [2]. Serum bilirubin level increases to an estimated average of 8 to 10 mg/kg of body weight per day in newborn due to accelerated breakdown of heme-containing proteins or erythrocytes [3]. Also, serum bilirubin level increases due to the direct evidence that the liver is unable to function adequately to excrete all the extra-load of produced bilirubin [4]. Tazawa study [5], had shown that 31% of breast-fed jaundiced infants had at least one item of abnormal liver function that may suggest mild hepatic dysfunction. Treating indirect hyperbilirubinemia at the appropriate time is of high importance in neonates. The intensity and invasiveness of therapy is determined by many factors such as gestational age, relative health of neonate, the current level of total bilirubin and the etiology of jaundice. Phototherapy and exchange transfusion are two main interventions that are used to decrease total serum bilirubin (TSB). The basic mechanism of action of phototherapy is the photochemical transformation of bilirubin in the areas exposed to light into hydrosoluble products that can be eliminated by the kidney and liver without being metabolically transformed [6], Phototherapy, however, needs hospitalization and mostly mother separation. It may cause insensible water loss and it causes redistribution by changing velocity of blood flow through heart, kidney, lung and brain, which return to baseline after discontinuation of phototherapy [2]. It also may cause watery diarrhea, low calcium, retinal damage, bronze baby syndrome, mutation, sister chromatid exchange and DNA strand breaks [2]. Exchange transfusion is associated with a significant morbidity, and even mortality [7, 8].

Pharmacologic agents used in the management of hyperbilirubinemia can accelerate bilirubin clearance via the normal metabolic pathways, inhibit the enterohepatic circulation of bilirubin or interfere with bilirubin formation by either blocking the degradation of heme or inhibiting

hemolysis [9, 10]. Metalloporphyrin [11], d-penicillamine [12], phenobarbital and clofibrate [12] are pharmacological agents that can be used in management of hyperbilirubinemia.

Herbal therapy including silymarin has recently received special attention as a mode of complementary therapy. Silymarin is a flavonoid complex, which is extracted from seeds of Milk thistle Family: Asteraceae/Compositae) [13]. That was approved by FDA as herbal medicine. It is indicated as a dietary supplement that is widely used in traditional remedies for almost 2000 years as liver tonic in European medicine [14]. The main component of the silymarin complex is silybin, [15]. The extracts are still widely used to protect the liver against toxins and to control chronic liver diseases, hepatic viruses, fibroses and jaundice. Recent experimental and clinical studies suggest that milk thistle extracts also have anticancer, antidiabetic, cardioprotective effects, antihyperchlosteremic and induction of breast milk flow [13, 16]. Milk thistle extracts are known to be safe and well tolerated. Toxic or adverse effects observed in the reviewed clinical trials seem to be minimal [13]. Also oral administration of silibinin at daily doses up to 1.44 g over a week is safe [17].

Attempts to decrease the risk of hyperbilirubinemia should be directed at the early establishment of effective lactation and adequate caloric intake [18].

No clinical trials have been completed in neonate examining the effect of silymarin in treatment of neonatal jaundice. But it is used safely in treatment of neonatal lupus erythematosus with cholestatic hepatitis [19].

The present study was to investigate the efficacy and toxicity of silymarin as an adjunct therapy that decrease duration of phototherapy for treatment of neonatal jaundice.

Patients and methods

A prospective cohort study was performed in newborn services at Neonatal Intensive Care Clinical Center of Doctor Abdu Al-Naser Badawy in Sohag, Egypt. Local ethical approval was obtained for the study protocol and all patients were subjected to through history and clinical examination before enrollment in the study. 170 (73 Females) healthy, full term neonates were enrolled in this study. All infants were consecutive studied by one single investigator, after informed parental consents were obtained. The allocation notes were kept

in opaque sequentially numbered sealed envelope. Study group received phototherapy and silymarin [n=85 (40 Females)], and control group received phototherapy and placebo [n= 85 (33 Females)].

Inclusion Criteria were

1. Include patients who met the criteria of the 2004 AAP (American Academy of Pediatric) treatment guidelines of hyperbilirubinemia using phototherapy [20];
2. Only healthy neonates with unconjugated hyperbilirubinemia, non-hemolytic jaundice, and with no need for urgent exchange transfusion;
3. Healthy near-term and full-term newborns infants gestational age (38-42 weeks), who have jaundice in the age of 1-10 days;
4. Laboratory tests with negative direct combs test.

Exclusion criteria were:

1. Birth weight less than 2500gm;
2. Prior or current use of phenobarbitone by the mother or child [21];
3. Initial indication of double or triple phototherapy;
4. Newborn submitted to blood transfusion;
5. Newborn with congenital defect; hereditary disease of erythrocytes or autoimmune disease with intense haemolysis;
6. Newborn with conjugated hyperbilirubinemia or any other disease rather than jaundice such as sever sepsis, pneumonia, respiratory distress, anemia, ….etc. were excluded;
7. Newborn with ABO or Rh incompatability.
8. Newborn with decreased glucose-6-phosphate dehydrogenase (G6PD) determination.

Included patients could have been excluded from the research due to the following exclusion criteria:

1. Registering spectral irradiance below 4.0μW/cm²/nm in any of the measurements for phototherapy calibrations [22]
2. Changing modality of phototherapy to double or triple;
3. Technical or clinical impossibility to determine TSB;
4. Death during the period of phototherapy.

Laboratory tests:

TSB was measured on admission and at least every 24 hours until discharge. Aspartate aminotransferase (AST) and Alanine aminotransferase (ALT) were measured in all neonates both before and after the study.

Throughout the study it was suspected that silymarin has some effects on serum albumin. Hence serum albumin was also measured in 30 infants, 15 of silymarin-treated group (5 females) and 15 of control group (7 females) as a subsequent study to detect the significance of that effect.

A dose of 3.75 mg/kg of silymarin was administered twice daily orally to infants in the silymarin-treated group within 12 hours of admission. Laboratory tests including complete blood count, total and direct serum bilirubin, reticulocyte count, direct Coombs' agglutination test, maternal and neonatal blood group, G6PD determination and peripheral blood smear were performed and recorded routinely before the beginning of therapy for all jaundiced infants in both groups. Total and direct serum bilirubin levels were measured daily until phototherapy was discontinued.

Phototherapy was started immediately on admission for all studied patients case and control until TSB decreased to a safe level depending on the infant' s gestation and postnatal age. A nurse who was not involved in drug administration recorded duration of phototherapy. Each phototherapy unit contained 8 special white fluorescent tubes labeled TL 52/20w (Philips, Eindhoven, Netherlands) adjusted at a 20cm distance above the infant. During study all neonates had a careful physical observation of any symptoms appeared on neonate as vomiting, loose stools, skin rashes and hyperthermia. Laboratory tests were followed 48 hours and 1week after discharge included complete blood count, TSB, for detection of rebound hyperbilirubinemia. Lamps of phototherapy units were changed regularly after 1500 hours of usage to keep irradiance in the photo effective range. TSB measurement was performed on the basis of spectrophotometric principles by using Bilimeter3 (Pfaff Medical GmbH, Germany). Direct bilirubin measurement was performed by using Autoanalyser Random Access (Selectra E, Vital Scientific, Netherlands). The equipments were standardized periodically.

All data were analyzed using statistical package of social sciences SPSS V15.0 (SPSS Inc., Chicago, IL). Statistical analysis of data was performed by Student t- test to compare between the two groups and Paired t- test to compare within each group and p values less than 0.05 were considered significant for all checked results.

Results:

170 neonates studied, (73 Females) completed the study. Table 1 show the characteristics and clinical data of control and Silymarin-treated groups collected prior to therapy. Table 2 show the mean±S.D. data and statistical significance of both control and Silymarin-treated groups, from which there were no significant differences in age , gestational age, mean total serum bilirubin, ALT,AST and serum albumin values at the time of admition of neonates between both groups.

Table 1. Characteristics and clinical data of the two groups

Characteristics and clinical data	**Silymarin-treated group**	**Control group**
Gender (sex):		
- male	45	52
-female	40	33
Mode of delivery (labor):		
- normal	55	56
- cesarean section	30	29
Consanguinity:		
- YES:	62	60
- NO	23	25
Feeding:		
-Formula:	37	33
-Mixed:	48	52
Hyperbilirubinemia in previous siblings:		
-no previous sibling:	10	7
-present:	47	56
-Absent:	28	29

Table2: Mean±S.D. data and statistical significance of both control and Silymarin-treated groups

variables	Minimum	Maximum	Mean±S.D.	p-value
Age/days				
control	1	11	3.69±2.488	0.695
Silymarin-treated	1	13	3.54±2.58	
Gestational age/weeks				
control	38	42	38.94±4.201	0.904
Silymarin-treated	38	42	39.01±2.842	
TSB				
control	8.79	24.38	15.38±3.61	0.837
Silymarin-treated	9.43	24.35	15.26±3.80	
Duration of phototherapy				
Control	84	168	127.47±19.61	<0.001***
Silymarin-treated	64	132	100.66±18.30	
AST before				
Control	23	91	50.67±14.55	0.342
Silymarin-treated	17	89	48.35±17.04	
AST after				
Control	34	89	58.88±14.68	<0.001***
Silymarin-treated	63	123	94.84±14.26	
ALT before				
Control	10	31	19.51±4.84	0.721
Silymarin-treated	10	35	19.80±5.84	
ALT after				
Control	15	35	24.80±4.53	<0.001***
Silymarin-treated	12	43	35.15±5.23	
Albumin before				
Control	2.25	3.15	2.70±0.45	0.875
Silymarin-treated	2.2	3.14	2.67±0.47	
Albumin after				
Control	2.35	3.15	2.75±0.40	0.015*
Silymarin-treated	2.79	3.39	3.09±0.30	

* Significant at 0.05 level, ** Significant at 0.01 level, *** Significant at 0.001 level

The mean duration of phototherapy was significantly higher in control group in comparison with silymarin-treated group ($p<0.01$). Both ALT and AST was significantly increased ($p<0.01$) within normal ALT and AST serum level in silymarin-treated group at the end of therapy where it was increased insignificantly at the end of therapy in control group. Albumin was significantly ($p<0.05$) increased within normal albumin serum level in silymarin-treated

group at the end of therapy where it was increased insignificantly after the end of therapy in control group.

As shown in Table 3 and Figure 1 the reduction rate, amount removed per unit time, of total and indirect plasma bilirubin levels were significantly higher in the silymarin-treated group compared to the control group. The difference became significant ($p<0.05$) between mean TSB of the two groups in day two of therapy.

Table 3. Mean±S.D. total serum bilirubin (mg/dl) measured every 24hours along the duration of therapy in the two groups.

Time	**Silymarin-treated Mean±SD**	**Control Mean±SD**	**p-value**
0 (time of admission)	15.26±3.8	15.38±3.6	0.860
After 24h	12.32±2.73	12.86±3.09	0.180
After 48h	9.51±2.99	10.12±3.65	0.246
After 72h	6.2±1.34	9.06±2.87	0.001**
After 96hr	3.6±1.54	6±2.84	0.001**

* Significant at 0.05 level, ** Significant at 0.01 level, *** Significant at 0.001 level

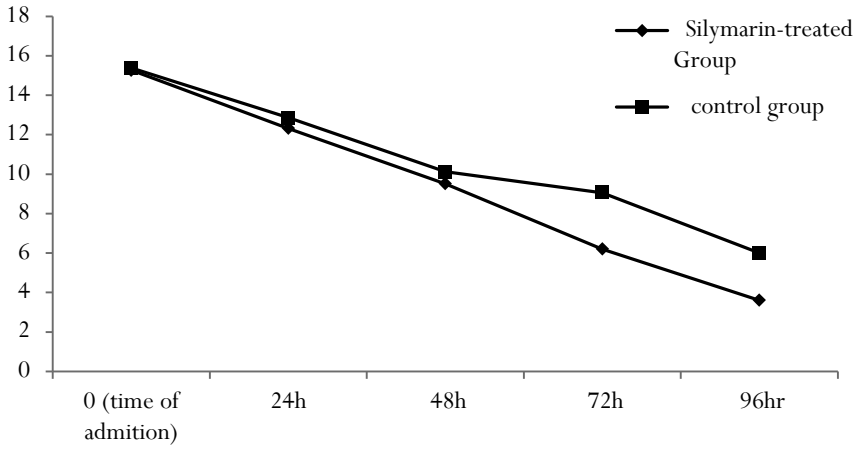

Figure 1. Mean total serum bilirubin (mg/dl) measured every 24hours along the duration of therapy in the two groups.

Table 4 demonstrates a comparison between Number and percent of symptoms those were recorded during duration of therapy in both groups.

During the duration of study, two cases of rebound hyperbilirubinemia were recorded from the control group.

Table 4. Number and percent of symptoms appeared during duration of therapy in each of control and silymarin-treated group.

Other symptoms	**Silymarin-treated group Number (%)**	**Control group Number (%)**	**p-value**
Number of neonates showed no other symptoms during study	24.0 (28.2)	16.0 (18.8)	0.205
Skin rash from (mild to severe)	7.0 (8.2)	36.0 (42.4)	0.001 **
Frequent loose stool	46.0 (54.1)	21.0 (24.7)	0.001 **
Hyperthermia	8.0 (9.4)	12.0 (14.1)	0.475

* Significant at 0.05 level, ** Significant at 0.01 level, *** Significant at 0.001 level

Discussion

Jaundice is the most common condition that requires medical attention in newborns. Conventional treatment of jaundice includes phototherapy and exchange transfusion in severe cases. They are having various and serious adverse effects. Development of intensified Phototherapy units and the use of drugs have contributed significantly to decrease the need for exchange transfusion due to its high risk of morbidity and mortality. Efficacy of phototherapy needs a lot of precautions to justify the required minimal effective dose. Hence numerous newborns still submitted to subtherapeutic doses of phototherapy, which may lead to neurological sequelae that may not be detected in childhood [22, 23]. Several pharmacological drugs are used to treat neonatal jaundice [12]. The belief that the natural medicines are much more safe than synthetic drugs has gained popularity in recent years and led to tremendous growth of phytopharmaceutical usage [24]. Physiological jaundice in neonates may be contributed to the fact that liver unable to function adequately so we need to support liver function [4]. Silymarin is a natural herbal supplement that supports liver activity with an evidence of wide margin of safety. It has several mechanism of action that may contribute to reduction of serum bilirubin. There was no previous study had been published using Silymarin in treatment of neonatal jaundice. Silymarin was used in treatment of a neonatal lupus erythmatosus with cholestatic hepatitis [19]. It has several mechanism of action one or more of them can reduce total serum bilirubin level. It can enhance Glucuronidation [25-27]. It inhibits reabsorption of bilirubin by enterohepatic circulation through Its mild laxative effect [16, 28, 29]. It stimulates ribosomal RNA polymerase and subsequent protein synthesis, and hence enhances hepatocyte regeneration which may promote the liver to function adequately to metabolize bilirubin. It has an antioxidant effect that may resemble the adaptive role of physiological neonatal jaundice in scavenging reactive oxygen species. It has the ability to regulate membrane permeability [25]sand so increasing membrane stability and decreasing excess heam metabolism by stabilizing RBCs.

Oral syrup dosage form with enhanced bioavailability preparation was used in the study so we avoid contamination of herbal medicines by any heavy metal, microbial toxins or any other contaminants. Silymarin increased the incidence of loose stools with phototherapy, which may have a beneficial effect in lowering hyperbilirubinemia.

In the present study, there was an increased numbers of appearances of jaundice and duration of therapy in breastfed infants and hyperbilirubinemia in previous sibling. Hence breastfeeding and hyperbilirubinemia in previous sibling might be considered as risk factors in neonatal jaundice. There was no correlation between sex, blood group of neonate and appearance of jaundice or duration of therapy. The duration of phototherapy and hospitalization was significantly shorter in infants that were treated with silymarin in addition to phototherapy in comparison with those treated with only phototherapy. As shown in Table 2, total serum bilirubin was significantly decreased in day three of silymarin therapy. No important side effect was determined during the short-term follow-up of infants. Data statistics demonstrated that duration of phototherapy was significantly reduced from 127.47±19.61 days in the control group to 100.66±18.30 days in silymarin-treated group ($p=0.001$). ALT and AST serum levels were followed as indicators of liver functions before and after therapy. In silymarin-treated group, first values of ALT and AST before therapy was either lower than the normal range or at the lower limit. At the end of therapy mean ALT and AST values were found to be increased significantly to higher values within the normal range. In control group there was no significant increase in mean ALT and AST values. This may indicate better activity of the liver which mean that silymarin can normalize ALT and AST [30]. After the statistical analysis and due to the significant increase of ALT and AST values in Silymarin-treated group, we suspected that silymarin may affect the serum albumin level. Albumin contributes to bilirubin conjugation and so it lowers serum blood level of the neurotoxic UCB [2]. Hence another 30 infants were subjected to the two regiments, 15 were enrolled to each of control and silymarin-treated group, to follow serum albumin level before and after therapy. It was found that silymarin increased the serum albumin level significantly ($p<0.05$).

Also there was a week correlation between the duration of therapy and ALT ($r=0.23$), AST ($r=0.43$) and albumin ($r=0.27$) in silymarin-treated group as shown in Figures 2-4. This correlation was not found in control group. This could mean that silymarin therapy enhanced ALT and AST serum levels hence enhanced liver function. There was no serious side effect observed during duration of therapy with silymarin. Similar to phenobarbital, silymarin has the same action of also enhance bilirubin conjugation and excretion [25-27] and is a better herbal drug with a wide margin of safety. Phenobarbital has a long half life [31] and many factors can affect the clearance of phenobarbital during the neonatal period [32]. In Heiman

& Gladlk study, phenobarbital half-life was significantly longer in neonates (118.6±16.1h), in babies (62.9±5.2h) or infants (68.5±3.2h) [33]. That mean its half life may reach more than two days, while clearance half-life of silymarin is six to eight hours [25, 34]. Phenobarbital also causes drowsiness in neonates and may slow down the oxidation of bilirubin in the brain leading to worse bilirubin toxicity [12]. From Table 4 it could be noted that silymarin significantly reduced the incidence of skin rash as a side effect of phototherapy. Silymarin significantly increased the incidence of loose stools with phototherapy, which may have a beneficial effect in lowering hyperbilirubinemia by reducing reabsorption of bilirubin through the enterohepatic circulation.

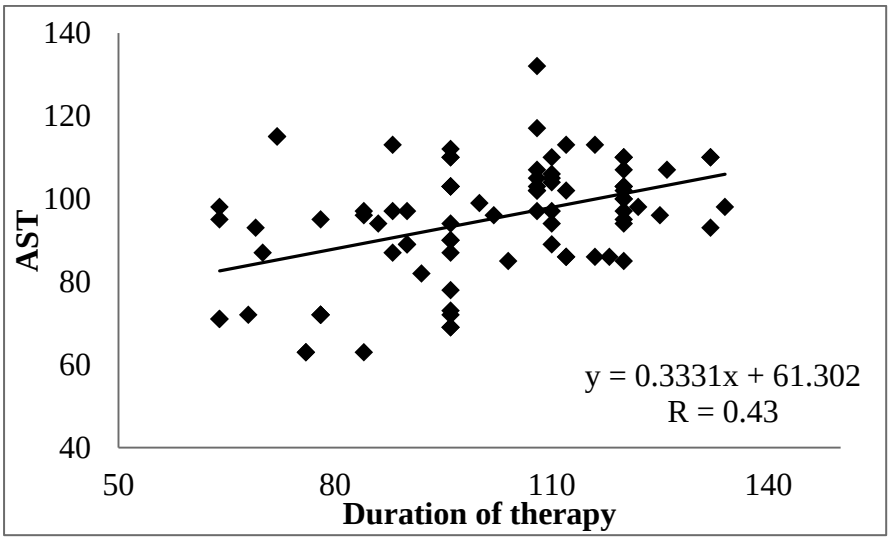

Figure 2. Correlation between AST and duration of therapy in silymarin treated group.

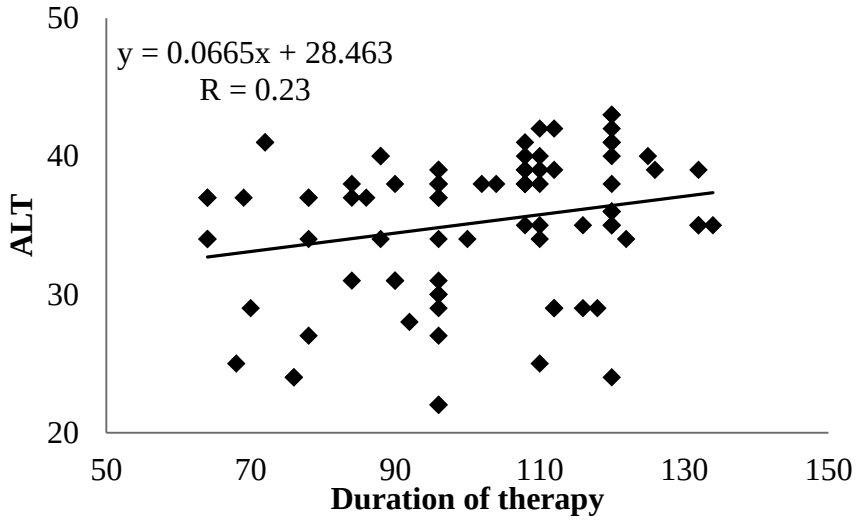

Figure 3. Correlation between ALT and duration of therapy in silymarin treated group.

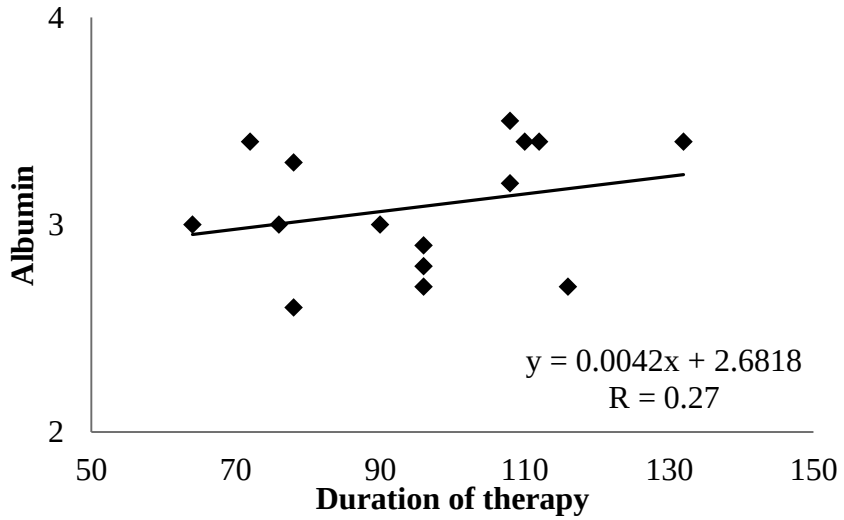

Figure 4. Correlation between serum albumin and duration of therapy in silymarin treated group.

Conclusion

Silymarin dose of 3.75mg/kg twice daily along with phototherapy is more effective than phototherapy alone in treating full term healthy neonates with UCB.

Further studies are required to fully understand Silymarin's role in treatment of neonatal jaundice and possibility to be used as a prophylactic therapy or to be used in managing pathological neonatal jaundice; also to determine the most effective dose.

Conflict of interest: None of the authors has any conflict of interest in this study, and there is no any commercial company involved in the current study.

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