Bilirubin Clearance in Temporarily Hyperbilirubinemic Rats Treated With Aqueous Extract of *Sida rhombifolia*

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Abstract: Bilirubin (BR)-clearing activity of *Sida rhombifolia* (SR) aqueous extract was assessed in temporarily hyperbilirubinemic rats. BR level of these rats was reduced to normal upon oral administration of SR aqueous extract for three consecutive days. Although both doses (50 and 500 mg/kg body weight) produced significant reduction in the BR level from 2.37 ± 0.30 and 2.15 ± 0.04 mg/dL to 0.89 ± 0.08 and 0.50 ± 0.20 mg/dL respectively, higher dose required only two days to reduce the BR level to normal. These results showed the potential of SR aqueous extract towards developing new drugs for hyperbilirubinemic subjects.
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1. Introduction

Bilirubin (BR), an endogenous toxic degradation product of heme catabolism in mammals, is carried to the liver by albumin for further metabolism and detoxification (Karp, 1979; Vanstapel et al., 1993). About 3-4 mg of BR per kg of body weight is produced every day in a healthy adult. Hyperbilirubinemia develops if the BR concentration in blood exceeds 1mg/dL while a BR concentration ≥ 2.5 mg /dL causes jaundice / kernicterus in adults / infants (Hauser et al., 1986; Gourley, 1997; Jansen and Bittar, 2004). Phototherapy and exchange transfusion are possible treatment strategies under mild and acute conditions, respectively. Epidemiological studies revealed that the administration of phototherapy in the newborn is not optimal; nor is it used when it should be, and sometimes used inappropriately (Atkinson et al., 2003).

Medicinal plants offer an alternative approach to treat various infections and diseases. *Sida rhombifolia* (SR), locally known as arrowleaf sida or ibu sida is a traditional plant used to cure or treat several ailments including pulmonary tuberculosis and rheumatism. Various parts of the plant are used as demulcent, emollient, diuretic, febrifuge and to treat skin diseases, rheumatism, leucorrhoea and swelling (Rao and Mishra, 1997; Poonam and Singh, 2009). The whole plant, its roots and aerial parts and their extracts have shown significant hepatoprotective, oedema suppressant and anti-arthritic activities (Rao and Mishra, 1997; Gupta et al., 2009). Antioxidant potential of SR has been proven with high free radical scavenging activity, reducing power and superoxide scavenging activity and the plant has been suggested as a potential source of natural antioxidants (Dhalwal et al., 2007). The antibacterial activity of SR against both Gram-positive and Gram-negative test organisms has also been demonstrated (Islam et al., 2003). Although SR is known to be effective in treating jaundice, but lacks any experimental proof. Therefore, we studied BR clearing activity of SR aqueous extract in temporarily hyperbilirubinemic rats.

2. Materials and Methods

Materials

Phenylhydrazine (PHZ), bilirubin (BR), sulfanilic acid, sodium nitrite and caffeine were purchased from Sigma-Aldrich Inc., USA. Sodium benzoate was the product of B.D.H. England. Fresh leaves of *Sida rhombifolia* (SR) were supplied by the local supplier and authenticated by a plant taxonomist. Sprague Dawley adult rats weighing 150–200 g were purchased from the Animal Research Centre and maintained by the staff of the Animal Centre Laboratory, Faculty of Medicine, University of Malaya.

Preparation of SR aqueous extract

Fresh SR leaves were dried under shade for 3 days and then grinded into powder form. About 500 g grinded dried leaves were treated with 2 L water at 70–90ºC for 8 h and filtered using a muslin cloth. Aqueous extract was obtained from the filtrate through rotary evaporation. A fixed amount of aqueous extract was dissolved in 0.15M sodium chloride to prepare different doses of SR aqueous extract.

Development of hyperbilirubinemia in rats

Hyperbilirubinemic condition was

developed in rats using PHZ treatment following the method described by Cekic *et al.* (2003) after slight modification. For the preparation of stock PHZ solution, 100 mg of PHZ was dissolved in 10 mL of 0.01 M sodium phosphate buffer, pH 7.4 containing 0.138 M NaCl. A single dose of 100 µL of stock PHZ solution (5 mg/kg body weight) was given intraperitoneally to each rat for five consecutive days. Total serum BR concentration was determined by the method of Fog (1958) both before (1st day) and after PHZ treatment (5th–8th days). Hyperbilirubinemic condition was confirmed by the measurement of BR in rat sera after 5 doses of PHZ treatment.

SR aqueous extract treatment of hyperbilirubinemic rats

Hyperbilirubinemic rats received SR aqueous extract in a single dose through oral route everyday starting from the 5th day (6 h after the last injection of PHZ) till 8th day. Two groups of rats (n = 6) received 50 mg (lower dose) and 500 mg (higher dose) of SR aqueous extract respectively per kg body weight. The third group served as control without receiving any dose of SR aqueous extract. Blood was collected from the tail of the rats on the 1st (normal) day, 5th day (6 h after PHZ treatment) and the following days after aqueous extract administration.

Statistical analysis

Different values in each group of rats were subjected to the calculation of mean value and standard deviation. Student's t-test was used to calculate the p-value and a p-value <0.05 was taken as statistically significant.

3. Results and Discussion

The concentration of BR (mg/dL) in control group rats is shown in Figure 1. Treatment of these rats with PHZ for five days resulted in the development of hyperbilirubinemia as BR concentration increased from 0.23 ± 0.10 to 2.49 ± 0.04 mg/dL on the 5th day upon PHZ treatment. Hyperbilirubinemic condition persisted for the next three days though the serum BR level was reduced to 1.44 ± 0.08 mg/dL.

BR level in hyperbilirubinemic rats, receiving a dose of SR aqueous extract (50 mg/kg body weight) is shown in Figure 2A. The BR concentration increased from 0.180 ± 0.07 (normal) to 2.37 ± 0.30 mg/dL after five days of PHZ treatment. Treatment of these rats with SR aqueous extract reduced the BR concentration to 0.89 ± 0.08mg/dL on the 3rd day of treatment. The decrease (62%) of BR level was highly significant (p = 0.001) compared to hyperbilirubinemic condition and was sufficient to bring down the BR level back to the normal range (< 1 mg/dL).

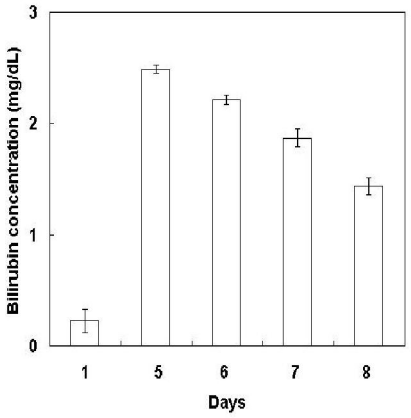

Figure 1. BR level in the sera of control group of PHZ-treated rats over a period of eight days both before and after PHZ treatment. Each value of the bar represents the mean ± S.D. of six different measurements

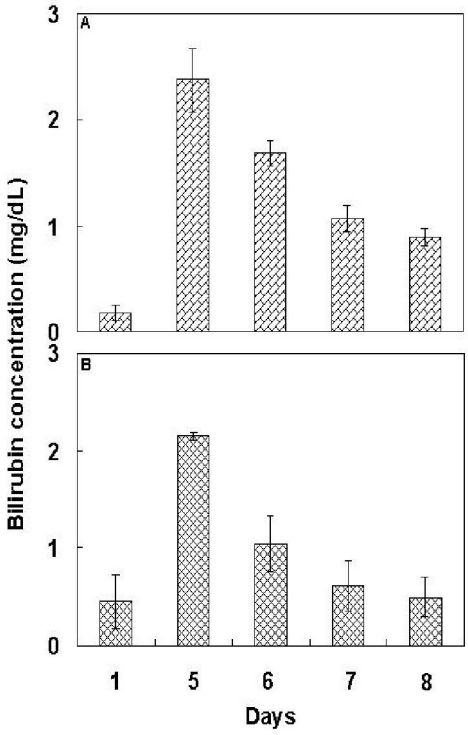

Figure 2. BR level in the sera of two groups of PHZ-treated rats over a period of eight days both before and after receiving different doses of SR aqueous extract such as 50 mg/kg body weight (A) and 500 mg/kg body weight (B). Each value of the bar represents the mean ± S.D. of six different measurements

The effect of a higher dose of SR aqueous extract (500 mg/kg body weight) treatment on the serum BR concentration of hyperbilirubinemic rats is shown in Figure 2B. The three days treatment significantly reduced the serum BR level of hyperbilirubinemic rats from 2.15 ± 0.04 to 0.50 ± 0.20 mg/dL ($p = 0.0001$). The higher dose (500 mg/kg body weight) was more effective in reducing the BR concentration compared to the smaller dose (50 mg/kg body weight) as it took only two days to lower down the BR level back to the normal value, from 2.15 ± 0.04 mg/dL to 1.05 ± 0.29 mg/dL and 0.61 ± 0.26 mg/dL with the first and second dose, respectively.

These results suggested the BR clearing activity of SR aqueous extract in hyperbilirubinemic rats and opened the way to develop future drug using SR aqueous extract for the treatment of hyperbilirubinemic/ jaundiced conditions.

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