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Short Communication

Bilirubin lowering potential of *Orthosiphon stamineus* in temporarily jaundiced adult rats

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Bilirubin (BR) lowering potential of *Orthosiphon stamineus* (OS) aqueous extract was evaluated in temporarily jaundiced adult rats. Treatment of these rats with OS aqueous extract for three days reduced the BR level significantly to the normal value. Whereas smaller dose (50 mg/kg body weight) resulted in the reduction in BR level from 2.53 ± 0.16 to 1.12 ± 0.17 mg/dL, higher doses of 500 and 1250 mg/kg body weight were found to be more effective in reducing the BR level from 2.44 ± 0.12 to 0.52 ± 0.12 mg/dL and from 2.67 ± 0.29 to 0.32 ± 0.21 mg/dL, respectively. Therefore, OS aqueous extract can be used to reduce BR concentration to a normal level in jaundiced subjects.

Key words: *Orthosiphon stamineus*, Misai Kuching, hyperbilirubinemia, jaundice, bilirubin.

INTRODUCTION

Bilirubin (BR), a catabolic product of hemoglobin in mammals is transported to the liver by albumin for further metabolism (Ostrow et al., 2004; Zunszain et al., 2008) . In a healthy adult, approximately 3 - 4 mg BR per kg of body weight is produced per day. However, in certain metabo-lic disorders of liver or in newborn infants with genetic deficiency or low levels of albumin, the amount of unconjugated BR in blood increases and when it exceeds 1 mg/ dL, hyperbilirubinemia develops (Jansen and Bittar, 2004). When the BR level reaches a certain concentra-tion (≥ 2.5 mg/dL), it diffuses into the tissues, causing jaundice in adults and kernicterus in infants. Treatment for neonatal hyperbilirubinemia includes either photothe-rapy for mild condition or exchange transfusion under se-vere conditions (Jansen and Bittar, 2004).

Medicinal plants and herbs contain substances known to the ancient civilizations for their healing properties. People without access to modern medicine rely on these medicinal plants and herbs for treating diseases, as they are similar in terms of active compound. *Orthosiphon stamineus* (OS), locally known as Misai Kuching is a popular traditional medicinal plant and has been extensively used for the treatment of several ailments such as urinary lithiasis, edema, eruptive fever, influenza, hepatitis, jaundice, diabetes, hypertension, rheumatism, tonsillitis, menstrual disorder, etc (WHO, 1990; PT Eisai Indonesia, 1995). Recently, studies on the antioxidant and hepatoprotective effects of OS have been carried out which indicated that the hepatoprotective effect of OS might be ascribed to its antioxidant and free radical scavenging property (Yam et al., 2007). The essential oils, methanol extract and deriv-ed fractions of methanol extract have displayed great po-tential of anti-fungal activity (Hossain et al., 2008). The acute toxicity LD_{50} of OS has been estimated to be >5000 mg/kg body weight (Abdullah et al., 2009). Although OS is known traditionally for curing jaundice, no scientific stu-dy has been carried out on OS for checking its BR lower-ing action. In this study, BR clearing potential of OS aqu-eous extract has been tested in temporarily jaundiced rats.

MATERIALS AND METHODS

Grinded dried leaves (500 g) of OS were treated with water (2 L) at 50-60°C for 8 h. The mixture was filtered in batches of 200 ml through a muslin cloth and subjected to rotary evaporation to get the aqueous extract. About 22 g of aqueous extract with a yield of 4.4% (w/w) was obtained. It was stored in a capped bottle at 25°C. For preparation of different doses, desired quantity of aqueous extract was dissolved in 0.9% sodium chloride solution.

Sprague Dawley adult rats weighing 150 - 200 g were treated with phenylhydrazine (PHZ) for five days to develop jaundiced con-

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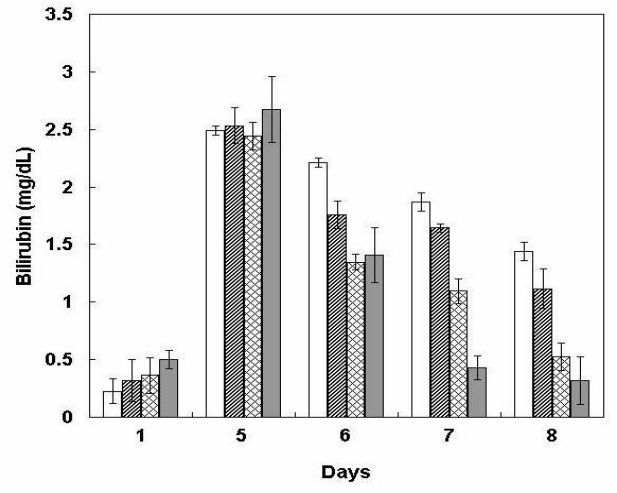

Figure 1. BR level over eight days in the sera of control group( )and other three groups of PHZ-treated rats re-ceiving OS aqueous extract in different doses of 50 ( ), 500( ) and 1250 ( ) mg/kg body weight. Each value of the bar represents the mean ± S.D. of six different measurements. Each group was comprised of six rats.

dition following standard procedure (Cekic et al., 2003) with slight modification. PHZ solution was prepared by mixing 100 mg of PHZ with 10 ml of 0.01 M sodium phosphate buffer, pH 7.4 containing 0.138 M NaCl. Each animal received 0.1 ml of PHZ solution (5 mg/kg body weight) as a single dose through intraperitoneal route for five consecutive days. The concentration of total serum BR was determined by Fog's method (Fog, 1958) both prior (on 1st day) and after PHZ treatment (on 5th, 6th, 7th and 8th days). Measurement of BR in the animal sera after 5 doses of PHZ confirmed the jaundiced condition. The treatment of jaundiced rats with OS aqueous extract (orally; once a day) was started 6 h after the last injection of PHZ. Animals were divided into four groups each consisting of six rats. Three groups of rats were treated with 50 mg (lower range), 500 mg (medium range) and 1250 mg (higher range) of OS aqueous extract respectively per kg body weight. The fourth group did not receive any treatment and served as control. Blood was collected from the tail of the rats on the first (normal) day, fifth day (after 6 h of PHZ treatment) and the following three (6th, 7th and 8th) days after aqueous extract administration to determine the BR concentration. Since BR level became normal on 8th day using two different doses of OS aqueous extract, the study was conducted up to 8 days.

The mean value and standard deviation were calculated for each group. The p-value was determined by Student's t-test and a p-value less than 0.05 was considered statistically significant.

RESULTS AND DISCUSSION

Figure 1 shows the concentration of BR (mg/dL) in four groups of rats receiving PHZ treatment followed by OS treatment except for the first group (control group) that received PHZ treatment only. Panel I represents the control group; while panels II, III and IV represent other three groups which received OS treatment with a dose of 50, 500 and 1250 mg/kg body weight respectively. On 1st day of the study before PHZ treatment, BR level of rats in these groups was found in the normal range (0.23 ± 0.10 to 0.50 ± 0.08 mg/dL) . Treatment of these rats with PHZ for five days resulted in the development of jaundice as BR level was found to be higher than 2 mg/dL in all four groups, falling in the range of 2.44 ± 0.12 to 2.67 ± 0.29 mg/dL. There was no significant difference in the BR level of these rats of four different groups ($p > 0.05$).

Panel I shows the concentration of BR (mg/dL) in control group rats receiving PHZ treatment but without any OS treatment. As can be seen from the figure, BR concentration increased from 0.23 ± 0.10 to 2.49 ± 0.04 mg/dL on the fifth day upon PHZ treatment. The serum BR level was reduced to 1.44 ± 0.08 mg/dL in the next three days if no further treatment was given to these rats. However, this concentration of BR was still higher than the normal value suggesting prevalence of the hyperbilirubinemic condition up to eight days.

The effect of three different doses of OS aqueous extract treatment on the serum BR level of jaundiced rats is shown in panels II, III and IV. OS aqueous extract treatment with a dose of 50 mg/kg body weight (panel II) for three consecutive days reduced the serum BR level of jaundiced rats from 2.53 ± 0.16 to 1.12 ± 0.17 mg/dL (panel II, day 5 and 8). Although this decrease (56%) in BR level was highly significant ($p = 0.0005$) compared to jaundiced condition, it was not sufficient to bring back the BR level back to the normal value as the BR concentration was still higher than 1 mg/dL.

Selection of a higher dose (500 mg/kg body weight) of OS aqueous extract was found to be successful in reducing the BR level of jaundiced rats significantly ($p = 0.00004$) from 2.44 ± 0.12 to 0.52 ± 0.12 mg/dL in three days. This has been clearly shown in panel III, day 5 and 8. The reduced value (0.52 ± 0.12 mg/dL) of BR concentration was similar to the BR level (0.36 ± 0.16 mg/dL), found before the PHZ treatment ($p = 0.23$). Although this dose was found to be effective in lowering down the BR level close to the normal value, it took three days to achieve this level with three similar doses of OS aqueous extract. About 45% (from 2.44 ± 0.12 to 1.35 ± 0.07 mg/dL) and 55% (from 2.44 ± 0.12 to 1.09 ± 0.10 mg/dL) reduction in BR concentration was achieved after giving the first and second dose, respectively (panel III, day 5, 6 and 7). The second dose of 500 mg/kg body weight (pa-nel III, day 7) produced a similar decrease in BR level ($p = 0.85$) as obtained by the third dose of 50 mg/kg body weight (panel II, day 8).

Panel IV shows the effect of OS aqueous extract treatment of jaundiced rats with a dose of 1250 mg/kg body weight on the BR level. As can be seen from the figure (panel IV, day 5 and 7), normal level of BR was achieved in two days only since the BR concentration was reduced from 2.67 ± 0.29 to 0.43 ± 0.10 mg/dL ($p = 0.0002$). This BR lowering effect was remarkable as compared to the effects shown by smaller doses. A comparison of results shown in panel IV, day 6, 7 and 8 suggests that there is a direct correlation between the dose amount of OS aqu-

eous extract and BR clearing potential, as two doses of 1250 mg/kg body weight were sufficient to reduce the BR level to the normal value against three dose requirement with a dose amount of 500 mg/kg body weight to achieve the same level.

All these results suggest that OS aqueous extract has the potential to reduce BR concentration to a normal level in jaundiced rats. The possible mechanisms of BR reducing action of OS aqueous extract might be the increased activity of glucuronyl transferases (Ostrow et al., 2003) to facilitate hepatic conjugation of BR or increased BR binding by albumin (Greige-Gerges et al., 2007) or some other mechanism. Therefore, OS aqueous extract can be used successfully to develop a future drug for the management of hyperbilirubinemia/jaundiced condition.

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REFERENCES

Abdullah NR, Ismail Z, Ismail Z (2009). Acute toxicity of *Orthosiphon stamineus* Benth standardized extract in Sprague Dawley rats. Phytomed. 16: 222–226.

Cekic D, Bellarosa C, Garcia-Mediavilla MV, Rigato I, Pascolo L, Ostrow JD, Tiribelli C (2003). Upregulation in the expression of multi-drug resistance protein Mrp1 mRNA and protein by increased biliru-bin production in rat. Biochem. Biophys. Res. Commun. 311: 891– 896.

Eisai Indonesia PT 1995). Medicinal Herb Index in Indonesia, second ed. Dian Rakyat, Jakarta.

Fog J (1958). Determination of bilirubin in serum as alkaline azobilirubin. Scand. J. Clin. Lab. Invest. 10: 241–245.

Greige-Gerges H, Khalil RA, Chahine R, Haddad C, Harb W, Ouaini N (2007). Effect of cucurbitacins on bilirubin-albumin binding in human plasma. Life Sci. 80: 579–585.

Hossain MA, Ismail Z, Rahman A, Sun CK (2008). Chemical composition of anti-fungal properties of the essential oils and crude extracts of *Orthosiphon stamineus* Benth. Ind. Crops Prod. 27: 328–334.

Jansen PLM, Bittar EE (2004). Bilirubin metabolism. In: The Liver in Biology and Disease. Principles of Medical Biology, Elsevier Press 15: 257–289.

Ostrow JD, Pascolo L, Shapiro SM, Tiribelli C (2003). New concepts in bilirubin encephalopathy. Eur. J. Clin. Invest. 33: 988–997.

Ostrow JD, Pascolo L, Brites D, Tiribelli C (2004). Molecular basis of bilirubin-induced neurotoxicity. Trends Mol. Med. 10: 65–70.

WHO Regional Publications (1990). Medicinal Plants in Viet Nam. Western Pacific Series No.3, Ed. Institute of Materia Medica, Hanoi pp. 271.

Yam MF, Basir R, Asmawi MZ, Ismail Z (2007). Antioxidant and hepatoprotective effects of *Orthosiphon stamineus* Benth. Standardized extract. Am. J. Chin. Med. 35: 115–126.

Zunszain PA, Ghuman J, McDonagh AF, Curry S (2008). Crystallographic analysis of human serum albumin complexed with 4Z,15E-bilirubin-IXα. J. Mol. Biol. 381: 394–406.