JPRHC

Research Article

EFFECT OF BENZOIN RESIN ON THE SERUM BILIRUBIN LEVELS IN TEMPORARY JAUNDICE INDUCED BY PHENYLHYDRAZINE: A PRELIMINARY STUDY

RAJU.S.*[1], UMA MAHESHWARA RAO.V [2], SREERAMULU REDDY.K[3], RAMYA.G[3], VASANTH KUMAR. G[4]

For Author affiliations see end of the text

This paper is available online at www.jprhc.in

ABSTRACT

Bilirubin is the degradation product of heme, the bulk of which is derived from hemoglobin of senescent erythrocytes and hepatic hemoproteins. Bilirubin is potentially toxic, but is normally rendered harmless by binding to plasma albumin, and efficient hepatic clearance. Jaundice, (also known as icterus) is a yellowish pigmentation of the skin, the conjunctival membranes over the sclerae (whites of the eyes), and other mucous membranes caused by hyperbilirubinemia (increased levels of bilirubin in the blood). Complications of jaundice include sepsis especially cholangitis, biliary cirrhosis, pancreatitis, coagulopathy, renal and liver failure. Treatment of rats with Phenylhydrazine 5 mg/ kg body weight for five days resulted in the development of jaundice as BR level was found to be higher than 2 mg/dL. Bilirubin lowering potential of Benzoin ethyl alcohol extract was evaluated in temporarily jaundiced adult wistar rats. Treatment of these rats with Benzoin extract for seven days reduced the BR level significantly to the normal value. Whereas smaller dose (10mg/kg body weight) resulted in the reduction in BR level from 2.51 ± 0.02 to 0.90 ± 0.01 mg/dL, higher doses of 20 and 40 mg/kg body weight were found to be more effective in reducing the bilirubin level from 2.54 ± 0.01 to 0.82 ± 0.01 mg/dL and from 2.49±0.02 to 0.66±0.01 mg/dL, respectively. Therefore, Benzoin ethyl alcohol extract can be used to reduce bilirubin concentration to a normal level in jaundiced subjects.

KEY WORDS: Benzoin ethyl alcohol extract, hyperbilirubinemia, jaundice, Bilirubin.

INTRODUCTION

Bilirubin (from the Latin ``bilis," meaning bile, and ``rubor," meaning red) is a bile pigment formed during the catabolism of heme-containing compounds, primarily hemoglobin. Excessive accumulation of bilirubin, as a result of enhanced production or impaired elimination, results in yellow discoloration of the skin, sclera, and mucus membranes, which is termed jaundice[1].The management of severe unconjugated hyperbilirubinemias, including pathologic neonatal jaundice or that accompanying Crigler-Najjar (CN) disease, remains a clinical problem[2]. Several therapeutic approaches are available for the treatment of severe unconjugated hyperbilirubinemias, including phototherapy or exchange transfusions. However, these therapeutic options are costly, time-consuming and potentially risky[3, 4]. New efficient methods to decrease elevated serum bilirubin levels would be greatly appreciated. Prevention of bilirubin production results from any form of haemolysis, facilitation of glucuronyl transferase; the enzyme that converts bilirubin into a polar, water-soluble glucuronide, Prevention of enterohepatic circulation of bilirubin via enhancement of bilirubin sequestration or degradation in the intestinal lumen in newborn infants, patients with hereditary hyperbilirubinemias or those with bile salt malabsorption predisposing to pigment cholelithiasis is an alternative approach to these standard methods. The use of natural products with therapeutic properties is as ancient as human civilization and, for a long time, mineral, plant and animal products were the main sources of drugs[5].In recent years, there has been growing interest in alternative therapies and the therapeutic use of natural products, especially those derived from plants[6]. Benzoin a resin from Styrax *tonkinensis* (Benzoin Siam) and *Styrax benzoin* (Benzoin Sumatra) species locally known as Sambrani is a popular traditional medicine. Siam benzoin gum has previously been described as containing coniferyl benzoate (65-75%), *p*-coumaryl benzoate (10-15%), cinnamyl cinnamate (0.5-6%), benzoic acid (12%), vanillin (0.3%), and siaresinolic acid (6%) [7, 8, 9, 10, 11]. Externally; Benzoin possesses antiseptic & stimulant properties. Internally; it has carminative, antiseptic, expectorant & diuretic properties[12, 13]. People without access to modern medicine rely on these for the treatment of hyperbilirubinemia in rural areas of Andra Pradesh.

Although Benzoin is known traditionally for curing jaundice, no scientific study has been carried out on Benzoin for checking its BR lowering action. In this study, BR clearing potential of Siam Benzoin ethyl alcohol extract has been tested in temporarily jaundiced rats.

MATERIALS AND METHODS

To prepare the Siam Benzoin ethyl alcohol extract, 400gms of Benzoin lumps were broken into small pieces and ground to powder form in a ball mill. Then the powder was treated with ethyl alcohol (2 L) at 50-60°C for 8 h[14]. The mixture was filtered in batches of 200 ml through a muslin cloth and subjected to evaporation to get the brownish black mass as extract. About 42 g of ethanol extract with a yield of 10.2% (w/w) was obtained. It was stored in a capped bottle at 25°C. For preparation of different doses, desired quantity of ethanolic extract was dissolved in 0.9% sodium chloride solution[15].

Animals

Wistar adult rats weighing 180-200 g of either sex were used in the study. The animals were maintained in colony cages at 25±2 °C, relative humidity 50-55% maintained under 12:12 h light and dark cycle. The animals were fed with Standard animal feed (Hindustan Lever Ltd. Bangalore, India) and water; animals were acclimatized to the laboratory conditions for 5 days prior to experimentation. All experiments were carried out according to the guidelines for care and use of experimental animals, and are approved by Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA). Study proposal was approved by the Institutional Animal Ethical Committee.

Bilirubin letting down activity

Wistar adult rats weighing 180-200 g were treated with Phenylhydrazine 5 mg/ kg body weight. I.p. (PHZ) for five days to develop jaundiced condition following standard procedure[16] 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 PHZ solution at a dose of 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 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 Benzoin ethanolic 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. The first group (I) did not receive any treatment and served as control. The other three groups (II, III and IV) of rats were treated with 10 mg (lower range), 20 mg (medium range) and 40 mg (higher range) of Benzoin ethanolic extract respectively per kg body weight. No gross change in behavior of the animals was observed at these doses. 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 four (6th , 7th, and 8th) days after Benzoin ethanolic extract administration to determine the BR concentration. Since BR level became approximately normal on 8th day using three different doses of Benzoin ethanolic extract, the study was conducted up to 8days.

Statistical analysis

The results were reported as mean ± SEM. Differences in means were estimated by means of ANOVA. The mean values of the results from the control group were compared to the mean values of groups treated with extract using the (Dunnett post hoc test). The results were considered significant at $P < 0.05$.

RESULTS AND DISCUSSION

Group I represents the control group; while group II, III and IV represent other three groups which received Benzoin treatment with a dose of 10, 20 and 40 mg/kg body weight respectively **(Table-1).**

On 1st day of the study before PHZ treatment, BR level of rats in these groups was found in the normal range (0.39 ± 0.03 to 0.40 ± 0.02 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 2.49 ± 0.02 to 2.54 ± 0.01 mg/dL. There was no significant difference in the BR level of these rats of four different groups ($p > 0.05$).

Table 1: Effect of Benzoin extract on total serum bilirubin level

		Total serum Bilirubin level (mg/dL)				
Group	**Treatment**	**Day 1**	**Day 5**	**Day 6**	**Day 7**	**Day 8**
Group I	Control	0.39± 0.03ns	2.52±0.02 ns	2.41±0.03	2.22± 0.02	1.82±0.02
Group II	Benzoin extract 10 mg/kg	0.39± 0.02 ns	2.51± 0.02 ns	2.05± 0.12**	1.79± 0.01**	0.90±0.01**
Group III	Benzoin extract 20 mg/kg	0.40±0.02 ns	2.54±0.01 ns	1.96±0.01**	1.50±0.01**	0.82±0.01**
Group IV	Benzoin extract 40 mg/kg	0.40±0.02 ns	2.49±0.02 ns	1.84±0.01**	1.15±0.01**	0.66±0.01**

The results given are mean ± S.E.M; number of animals used (n=6) **P < 0.01 and *P < 0.05 was considered as significant in comparison to control

As can be seen from **(Table -1)** the concentration of BR (mg/dL) in control group rats receiving PHZ treatment but without any extract treatment the BR concentration increased from 0.39 ± 0.03 to 2.52 ± 0.02 mg/dL on the fifth day upon PHZ treatment. The serum BR level was reduced to 1.82 ± 0.02 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 nine days. The effect of three different doses of Benzoin extract treatment on the serum BR level of jaundiced rats is shown in **(Table-1)**. Benzoin extract treatment with a dose of 10mg/kg body weight for three consecutive days reduced the serum BR level of jaundiced rats from 2.51 ± 0.02 on day 5 to 0.90 ± 0.01 mg/dL on day 8. This decrease in BR level was highly significant ($p < 0.01$) compared to jaundiced condition. A selection of a higher dose (20 mg/kg body weight) of Benzoin ethanolic extract was found to be more successful in reducing the BR level of jaundiced rats significantly ($p < 0.01$) from 2.54 ± 0.01 on day 5 to 0.82 ± 0.01 mg/dL on day 8. A Selection of a still higher dose (40 mg/kg body weight) reduced the BR level from 2.49±0.02 on day 5 to 0.66±0.01 on day 8 which was highly significant ($p < 0.01$).Exposure to Phenylhydrazine cause damage to red blood cells, potentially resulting in anemia and consequentially hyperbilirubinemia[17]. PHZ increases reactive oxygen species (ROS). ROS production was associated with extensive binding of oxidized and denatured haemoglobin to the membrane cytoskeleton. Thus, PHZ-induce haemolytic injury seems to be derived from oxidative alterations to red blood cell proteins. [18]

All these results suggest that Benzoin ethanolic extract has the potential to reduce BR concentration to a normal level in jaundiced rats. The possible mechanisms of BR reducing action of Benzoin ethanolic extract might be the increased activity of glucuronyl transferases[19] to facilitate hepatic conjugation of BR or increased BR binding by albumin[20] or Prevention of enterohepatic circulation of bilirubin via enhancement of bilirubin sequestration or degradation in the intestinal lumen[21] or probably due to a shift in the distribution equilibrium of the pigment between serum, skin and other tissues or some other mechanism which may be attributed to its benzoic acid content[22,23]. Therefore, Benzoin ethanolic extract can be used successfully to develop a future drug for the management of hyperbilirubinemia / jaundiced condition.

REFERENCES

1. Weizheng W. Wang, Stephen D. Zucker Encyclopedia of Gastroenterology, 2005, Pages 219-222.
2. Vitek L, Jirsa Jr M, Brodanova.M, et al. Gilbert syndrome and ischemic heart disease: a protective effect of elevated bilirubin levels. Atherosclerosis 2002; 16:449–56.
3. Gies HP, Roy CR. Bilirubin phototherapy and potential UVR hazards. Health Phys 1990; 58:313–20.

4. Radermacher EH, Noirfalise A, Hornchen H, Maier RD, Bigalke KH. The bronze-baby syndrome: a complication of phototherapy. Klin Padiatr 1977; 189:379–84.
5. Rates S.M.K., Plants as source of drugs. Toxicon 2001; 39: 603–613.
6. Goldfrank, L., et al., The Pernicious Panacea: Herbal Medicine. Hospital Physician 1982; 10:64–86.
7. Coppen, J. J. W. Benzoin: production, uses and international trade. Perfume. Flavor. 1999, 24:11-22.
8. Moyler, D. A. The flavor of gum resins, their chemistry & uses. Riv. Ital. EPPOS 1998, Special Number, 351-360.
9. Pastorova, I.; de Koster, C. G.; Boon, J. J. Analytical study of free and ester bound benzoic and cinnamic acids of gum benzoin resins by GC-MS and HPLC-frit FAB-MS. Phytochem. Anal.1997; 8:63-73.
10. Reinitzer, F. Siamese benzoin. Arch. Pharm. 1914; 252:341- 349.
11. Schroeder, H. A. *p*-Hydroxycinnamyl compounds of Siam benzoin gum. Phytochemistry 1968; 7:57-61.
12. Jain S.K.and Sumita Srivastava.Traditional uses of some Indian plants among islanders of the Indian Ocean. Indian Journal of Traditional Knowledge2005; 4 (4):345-357.
13. Evans, W. C. Trease and Evans Pharmacognosy. 15th Edition, Elsevier, India, 2002.pp 27, 46, 183-184, 289-291, 411-413, 434, 485-486.
14. Harborne, J.B. Phytochemical methods. In: A guide to Modern Techniques of Analysis. London: Chapman and Hall Publishers, 1973:4-7.
15. Perez R.M., Perez J.A, Garcia L.M, Sossa H. Neuropharmacological activity of Solanum nigrum fruit. J. Ethnopharmacol1998; 62:43-48.
16. Cekic D, Bellarosa C, Garcia-Mediavilla MV, Rigato I, Pascolo L, Ostrow JD, Tiribelli C.Upregulation in the expression of multidrug resistance protein Mrp1 mRNA and protein by increased bilirubin production in rat. Biochem. Biophys. Res. Commun. 2003; 311: 891–896.
17. Stern A: Drug-induced oxidative denaturation in red blood cells. Semin. Hematol. 26:301–306, 1989.
18. McMillan et al. 2005. McMillan DC, Powell CL, BowmanZS, Morrow JD, Jollow DJ: Lipids versus proteins as major targets of prooxidant, direct-acting hemolytic agents. Toxicol. Sci. 2005; 88:274–283.
19. Ostrow JD, Pascolo L, Shapiro SM, Tiribelli C .New concepts in bilirubin encephalopathy. Eur. J. Clin. Invest. 2003; 33: 988-997.
20. Greige-Gerges H, Khalil RA, Chahine R, Haddad C, Harb W, Ouaini N. Effect of cucurbitacins on bilirubin-albumin binding in human plasma. Life Sci. 2007; 80: 579-585.
21. Libor Vitek, Lucie Muchova, Jaroslav Zelenka, Marie Zadinova, and Jir Malina. The Effect of Zinc Salts on Serum Bilirubin Levels in Hyperbilirubinemic Rats. Journal of Pediatric Gastroenterology and Nutrition 2005;40:135–140.
22. Bessard G, Chouraqui JP, Remy C, Rambaud P. Effect of sodium benzoate on the cutaneous bilirubin content of the adult Gunn rat. Biol Neonate. 1983; 44(5):315-20.
23. Nathenson G, Cohen MI, McNamara H. The effect of Na benzoate on serum bilirubin of the Gunn rat. J Pediatr. 1975; 86(5):799-803.

AUTHORS AFFILIATION AND ADDRESS FOR CORRESPONDENCE:

Assistant Professor, Vijaya College of Pharmacy, Munaganoor, Ranga Reddy Dist, Andhra Pradesh, India-505511.
Tel: +919966164766,
Email- ***rajenderreddysama@gmail.com***

[2]Nalla Narsimha Reddy College of Pharmacy, Korremula, Ranga Reddy Dist, Andhra Pradesh, India.

[3]Assistant Manager – Clinical R&D, Shantha Biotechnics Limited, Basheer Bagh, Hyderabad, Andhra Pradesh. India-500004.

[4]Health Management and Research Institute, Hyderabad, Andhra Pradesh. India.