

Short Communication

Direct *ex vivo* effects of herbal extracts on serum bilirubin in neonatal blood samples

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Herbal drug therapy is a common practice adopted in traditional and alternative medicine and has been used in the treatment of neonatal jaundice or hyperbilirubinemia from ancient times. This is done with the help of different herbal extracts and using different treatment methods. The knowledge of new possibilities and the fact that herbal drugs have been used in some communities for a long time, clarifies the importance of studying herbal drugs. Herbal drug therapies are used commonly which amplifies the need to evaluate the efficacy of these drugs. This study was performed to evaluate the effect of commonly used herbal drugs in neonatal jaundice, through *in vitro* techniques. In this study 0.5 cc (50 µg/ml) of different herbal extracts such as *Chi corium intybus*, *Fumaria parviflora*, *Zizyphus jujuba* and *Alhagi pseudoalhagi*, *Purgative manna* which were obtained through hydrochloric instillation and were added to 1 cc of neonatal serum with hyperbilirubinemia. Then the level of bilirubin was checked by diazo blank method. Among all of the herbal extracts, only *Chi corium intybus* demonstrated a lowering effect on indirect serum bilirubin. Although *Chi corium intybus* showed lower levels than the control group, but statistically there was no significant difference in direct bilirubin levels between these groups. Herbal drugs can decrease the bilirubin level through different mechanisms, such as increasing the activity of liver enzymes and decreasing the enterohepatic circulation. But the extract of the herb *Chi corium intybus* imposes its affect directly on bilirubin. Therefore further investigation is needed in order to separate the active agent in *chi corium intybus*.

Key words: Hyperbilirubinemia, *Fumaria parviflora*, *Zizyphus jujuba*, *Chi corium intybus*, *Alhagi pseudoalhagi*, *Purgative manna*.

INTRODUCTION

Hyperbilirubinemia is a common problem among neonates, observed during the first week of life in approximately 60% of term infants and 80% of pre-term infants (Behrman, 2008). This means that two out of every three newborns develop clinical jaundice after birth.

The yellow color usually results from the accumulation of unconjugated, lipid soluble bilirubin pigment in skin which is formed from hemoglobin by the action of hemeoxygenase and biliverdin reductase in reticuloendothelial cells. Increase in the amount of unconjugated bilirubin in neonatal serum increases the risk of kernicterus, resulting from the deposition of unconjugated bilirubin in the brain cells (Behrman, 2008). Treatment of hyperbilirubinemia, for which conventional phototherapy is applied

causes many complications such as damage to retina and genitalia of the neonate, dehydration, diarrhea and bronze baby syndrome.

The knowledge of new possibilities and the fact that herbal drugs have been used in some communities for a long time, clarifies the importance of studying herbal drugs. On the other hand, due to some side effects of conventional phototherapy, there is a need to somehow decrease the amount of time exposed to phototherapy or rather use some alternative methods (Fayaz, 2000). In the western world, many studies have been done on the use of protoporphyrin as an alternative to conventional phototherapy. Protoporphyrin inhibits the conversion of biliverdin to bilirubin by hemeoxygenase, but the effect is no greater than that achieved with phototherapy (Kappas et al., 1988). Treatment with herbal drugs has been in use since ancient times, and herbs have been an effective source of treatment regimens for different diseases.

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Table 1. Comparison of bilirubin levels between the sera to which herbal extracts were added with the control group(mg/dl).

Extracts	Total	Bilirubin	Direct	Bilirubin	Indirect	Bilirubin
	Mean \pm SD	P. value	Mean \pm SD	P. value	Mean \pm SD	P. value
<i>Fumaria parviflora</i>	12.39 \pm 0.8	0.328	2.54 \pm 0.34	0.103	9.84 \pm 2.46	0.181
<i>Cichorium intybus</i>	11.11 \pm 0.9	0.021	2.13 \pm 0.25	0.222	8.93 \pm 1.38	0.029
<i>Alhagi pseudoalhagi</i>	12.38 \pm 0.7	0.267	2.36 \pm 0.36	0.213	10.01 \pm 2.32	0.166
<i>Purgative manna</i>	13.3 \pm 0.8	0.758	2.41 \pm 0.22	0.144	10.88 \pm 2.06	0.906
<i>Zizyphus jujuba</i>	12.32 \pm 1.1	0.176	2.43 \pm 0.31	0.118	9.89 \pm 2.23	0.102
Distilled water(control group)	13.0.8 \pm 1.2	-----	2.28 \pm 0.21	-----	10.79 \pm 2.11	-----

In modern medicine, medicinal herbs are an integral part of alternative therapy. Statistics show that in the US, the use of herbal therapy had a five fold increase between the years 1990 - 1997 (Deter and Green, 2002). The importance of studying herbal drug therapy in jaundice should be in the knowledge of new possibilities and to clarify its real value since it's been used by some communities.

Among the medical herbs used by Iranians to treat hyperbilirubinemia those which have been incorporated in our study include *Fumaria parviflora* (whole), *Zizyphus jujube* (Fruite), *Chi corium intybus* (whole), *Alhagi pseudoalhagi* (Fruite), and *Purgative manna* (Zargari, 1998; Tavakoli and Sedaght, 1999). These herbs have been widely used as drugs and food in Iran for generations. No side effects have been reported, so they seem to be safe to use. In Iran, limited studies on the effects of herbal medicines in the treatment of hyperbilirubinemia have been conducted. A good example is the study of the effects of *Purgative manna* done in Shiraz and Kurdistan, both of which showed positive results (Fayaz, 2000; Shekiba, 2000). All these studies were conducted in-vivo and therefore factors like prematurity, birth weight, IUGR, liver and kidney integrity, concurrent use of phototherapy with different intensities and duration, methods of patient care including change of patients position during phototherapy and breast feeding all have effectively modulated the results.

In this study, the direct effects of medical herbs in lowering the levels of bilirubin will be evaluated and no environmental or patient-related factor will interfere.

MATERIALS AND METHODS

This study was conducted at Imam Sajad Hospital in Yasuj, South-western Iran on the sera of normal 3 - 7 days old neonates > 2500 g with hyperbilirubinemia, without any other disease and without any maternal drug history. A 20 cc blood sample from the umbilical cord of neonates with hyperbilirubinemia was recovered just before the exchange transfusion therapy. These samples were protected from light. After centrifugation and serum harvesting in 1 cc of the serum, 0.5 cc of the extracts from the medical herbs of *Chi corium intybus*, *Fumaria parviflora*, *Zizyphus jujuba*, *Alhagi pseudoalhagi* and *Purgative manna* (obtained by hydrochloric instillation) were added separately. The aerial parts of each of *C. intybus*, *F. parviflora*, *Z. jujube*, *A. pseudoalhagi*, and *P. manna* were collected from

Kakan in Yasuj, Iran in the spring of 2004. A voucher specimen was deposited in the herbarium of Kohkyloeyeh and Boyerahmad Agricultural Research Center. The plant segments were dried far from direct light, and then powdered. The powder was kept in a closed container in 4C. The powdered material (500 g) was extracted three times with ethanol-water (1:1). The accumulated extract was concentrated under reduced pressure and dried. The dried extract was suspended in distilled water and the volume was adjusted to 500 ml.

After three hours, the level of bilirubin was checked by Diazo Blank method. Other more reliable methods are expensive and not widely available in Iran. It should be noted that 3 - 4 h is essential to allow cell attachment and interaction (Venkataraman et al., 2006). The results were compared to that of a control serum in which 0.5 cc of distilled water was added. Statistical analysis was done using a paired T-test method. P values less than 0.05 were considered significant.

After consulting with a statistical analyst, 21 sera samples were considered significant. The five extracts were tested in each of the serum samples collected from the 21 patients and also the distilled water, used as the control group. Then the level of bilirubin was checked. The mean (average) level of bilirubin checked for every extract was compared with the mean (average) of the control group.

RESULTS

Total bilirubin levels were significantly decreased only using *Chi corium intybus*. Although *Chi corium intybus* showed lower levels than the control group, but statistically there was no significant difference in direct bilirubin levels between these groups.

Considering indirect bilirubin levels, only in the case of *Chi corium intybus* is the difference significant. Table 1 shows the comparison of bilirubin levels after addition of herbal extracts with the control group.

DISCUSSION

Our study was performed to evaluate the effect of commonly used herbal drugs in neonatal jaundice, through *in vitro* techniques. Among all of the herbal extracts, only *Chi corium intybus* demonstrated a lowering effect on indirect serum bilirubin.

Several research studies on medical herbs have been conducted. In one that was performed on mice, it was noted that the extracts of *Fumaria parviflora* had a protective effect on liver against acetaminophen poisoning. It

was shown that the administration of 1 g/Kg of paracetamol killed 100% of the mice and in the study group in which *Fumaria parviflora* had been administered, the death rate was significantly decreased to 50.3% (Gilani et al., 1996). In another study conducted in Iran, the effects of *Fumaria Parviflora*, *Coriandrum sativum* on some biochemical parameters in the sera of diabetic mice were tested. The results showed that *Fumaria parviflora* extract had an effective role in lowering the levels of glucose, cholesterol, creatinin and liver enzyme activity and therefore, this herbal medicine was recommended for diabetic patients by the researchers (Jelodar and Habibabady, 2000).

Moreover, the effect of this herb in causing good prognosis of senile patients with chronic hepatitis has been reported (Hany and Rimm, 1999). In studies conducted on Purgative manna extracts in Iran (Shiraz and Sanandaj), this herbal medicine was administered orally to neonates with hyperbilirubinemia and reported to have positive effects in decreasing bilirubin levels (Fayaz, 2000; Shekiba, 2000).

Treatment of hyperbilirubinemia with herbal extracts has been used since ancient times. The mechanisms behind their action are thought to be the laxative effect of these extracts causing increased intestinal motility and also binding to bilirubin in the GI system which causes a decrease in the enterohepatic circulation, and finally excretion of bilirubin from the GI system. *Zizyphus jujuba*, *Purgative manna* and *Alhagi pseudoalhagi* are thought to carry out this action by their laxative nature (Zargari, 1998). Therefore, these herbal drugs were reported to have positive results in the studies carried out in Shiraz and Sanandaj (Fayaz, 2000; Shekiba, 2000). In our study which was conducted in-vitro, nothing was seen suggesting that their mechanism of action was through their laxative nature and binding to bilirubin in the intestine. Some studies showed that *Fumaria parviflora* had a protective effect on the liver and caused an increase in liver enzymes which is thought to be the mechanism behind their serum bilirubin lowering property (Gilani et al., 1996; Jelodar and Habibabady, 2000). In our study, *Fumaria parviflora* extracts were used only in-vitro, so their action on liver could not be evaluated. In our study, only *Chi corium intybus* was shown to decrease the total bilirubin, actually the indirect bilirubin, in a significant level.

Conclusion

Although all these herbal extracts could probably decrease the bilirubin level through their laxative nature, causing increased liver activity and binding to bilirubin in the

intestine, but the extracts of *chi corium intybus* in addition to the actions above, could have an important role in decreasing the bilirubin levels.

The pigments in these extracts probably impose an increasing effect on laboratory results therefore, in future research these colored pigments need to be separated from the herbal extracts. This procedure could reveal the efficacy of some extracts which seemed to have been ineffective before. In the case of *chi corium intybus*, its active agents must be separated through chromatography technique.

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