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Original Article

Medical-grade Spore-free Natural Honey is an Effective Choleretic in Neonatal Cholestasis: A Pilot Single-center Trial

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Abstract

Background and objectives: Liver damage in cholestasis is multifactorial, yet bile acid-mediated hepatotoxicity is pivotal. Honey consumption has many physiological effects; it influences detoxification processes (phase I, II and III), has antioxidant, anti-inflammatory, immune-stimulating, anti-ulcer, wound/burn healing effects and others. The bile acid ursodeoxycholic acid (UDCA) is currently used off-label to treat neonatal cholestasis. This study aimed to assess the effectiveness of spore-free natural honey in treating neonatal nonobstructive cholestasis.

Methods: Thirty infants with cholestasis received spore-free natural honey. Their progression was compared to a control group with cholestasis (28 infants) and a historical group (31 infants) on UDCA.

Results: The mean ± standard deviation (SD) follow-up duration was 29.68 ± 18.68 months. At presentation, the total bilirubin concentrations were 9.5 ± 5.9 mg/dL, 10.9 ± 7 mg/dL and 14 ± 9 mg/dL in the honey, control and UDCA groups, respectively (p = 0.064), and their direct bilirubin concentrations were 6 ± 4 mg/dL, 6.7 ± 4 mg/dL and 8.4 ± 6.9 mg/dL, respectively (p = 0.169). The final total bilirubin concentrations were 1.2 ± 1.7 mg/dL, 4.6 ± 8.2 mg/dL and 7.3 ± 8.9 mg/dL, (p = 0.006), and their final direct bilirubin concentrations were 0.8 ± 1 mg/dL, 2.77 ± 5.2 mg/dL and 5.1 ± 7.2 mg/dL (p = 0.008), with cure achievement in 25, 16 and 16 (p = 0.023), improvement in 3, 5 and 3 (p = 0.565), failure in 2, 3 and 10 (p = 0.016), and death in 0, 4 and 2, respectively (p = 0.095). None suffered from botulism or flaccid paralysis.

Conclusions: Spore-free honey is effective in clearing cholestasis in neonates and infants. UDCA use in cholestasis in the pediatric age group should be abandoned as it is less effective and is associated with a worse outcome.

Introduction

Cholestatic liver disorders include a spectrum of hepatobiliary diseases of diverse etiologies with impaired hepatocellular secretion of bile, resulting in the accumulation of bile acids, bilirubin and cholesterol. Causes of cholestasis include extrahepatic biliary obstruction (*e.g.*, stones, tumors, biliary atresia), intrahepatic biliary obstruction (*e.g.*, primary biliary cholangitis, primary sclerosing cholangitis, paucity of intrahepatic biliary radicals) and intrahepatic cholestasis (*e.g.*, drug-induced, genetic transporter defects, metabolic or infection-induced). The major abnormalities observed in patients with cholestasis include an elevation of circulating levels of primary bile acids and an increase in the formation of sulfated bile acids.[1] The mechanisms of liver damage associated with cholestasis are complex and multifactorial. Bile acid-mediated hepatotoxicity is central and crucial in the pathogenesis of liver damage.[2] In addition, oxidative stress and lipid peroxidation are also involved.[3] In obstructive jaundice models, reactive oxygen species generation, mitochondrial permeability transition, cytochrome c release, Kupffer cell activation and increased neutrophil chemotaxis are critical for induction of liver injury by bile acids.[4,5] Congenital aflatoxicosis-induced cholangiopathy also has been reported as a cause of biliary atresia; Kotb disease that has been observed in infants with the null glutathione S-transferase mu 1 genotype with disrupted p53 and glutathione S-transferase pi to mothers heterozygous for the glutathione S-transferase mu 1 polymorphism.[6–8]

There is no known treatment for cholestasis.[9] Bile acids are known to be hepatotoxic, yet ursodeoxycholic acid (UDCA) is

Keywords: Spore-free honey; Ursodeoxycholic acid; Neonate; Cholestasis; Botulism; Kotb disease; Biliary.

Abbreviations: ALT, alanine amino transferase; AST, aspartate aminotransferase; SD, standard deviation; UDCA, ursodeoxycholic acid.

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used off-label as a choleretic to treat neonatal cholestasis even though there is poor evidence for its safety or effectiveness.[9–11] UDCA is a physiologic hydrophilic dihydroxy bile acid[12] that is partially metabolized into lithocholic acid, which is toxic and may cause intrahepatic cholestasis and bile infarcts.[13,14]

Honey has bactericidal, bacteriostatic, antifungal, antiviral, scolicidal, anti-oxidant, antitumoral, and anti-inflammatory effects due to its content of monosaccharides, its ability to inhibit prostaglandins, its ability to increase nitric oxide production, and its contents of anti-oxidants and trace elements.[15] Moreover, it has a potential role in phase II detoxification attributed to augmentation of the process of glucuronidation through its content of glucuronic acid.[16] The biological role of honey is not limited to its carbohydrate components and is very much related to its components of simple phenolics, phenolic acids, flavonoids, amino acids, enzymes, vitamins and minerals such as potassium, calcium, copper, iron, magnesium, manganese, phosphorus, sodium, zinc and selenium.[17] These components have greatly contributed to its antioxidant and anti-inflammatory role, inhibition of the bronchial hyperplasia of goblet cells,[18] supportive role in allergic rhinitis[19] and recovery following gastroenteritis in children, *etc*.[20]

The aim of this work was to investigate the choleretic effect of spore-free honey in neonatal cholestasis and to compare its effect to the outcomes of infants with cholestasis who received other treatments.

Subjects and methods

Subjects

Thirty neonates and infants suffering from nonobstructive cholestasis were enrolled in this study. They were compared to a control group of 28 gender and age-matched neonates and infants suffering from nonobstructive cholestasis. The neonates and infants were enrolled at initial presentation with cholestasis prior to the diagnosis of the etiology and were assigned to either the honey or control group. Assignment was subject to parent approval. Those who refused to try honey were assigned to the control group and vice versa. All underwent examination and investigations to rule out biliary atresia. The inclusion criteria included nonobstructive cholestasis in a neonate or infant, not complicated by ascites or liver cell failure, sepsis and not associated with other congenital anomalies.

The results of both groups were compared to data of a historical group of neonates on UDCA since their initial presentation who were referred to our unit. UDCA is not approved for use in neonates or infants in Egypt, yet self-medication is a common practice.[21] All lawful caregivers consented to the trial. This study was in compliance with Helsinki declaration guidelines, and was approved by the Pediatric Department Committee for Postgraduate Studies and Research as well as the Faculty of Medicine Committee for Postgraduate Studies and Research, Cairo University, Cairo, Egypt. Parents of enrolled children consented to the study.

Study design

This study was a prospective case–control trial performed at the Hepatology Clinic, Children's Hospital, Cairo University, Egypt.

Diagnosis of etiology of cholestasis

All infants underwent clinical examination; initial lab investigations of total and direct serum bilirubin, aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase, gamma glutamyl transpeptidase and serum albumin concentrations; prothrombin time; international normalized ratio; and conventional ultrasonography imaging. Details observed on abdominal ultrasonography included the liver size, echogenicity, triangular cord sign of the portal and hepatic veins, gall bladder size, spleen size, sizes of other abdominal organs (especially the kidneys) and the presence of ascites. A percutaneous biopsy was performed as the gold standard to exclude the diagnosis of extrahepatic biliary atresia. Those with extrahepatic biliary atresia were excluded from the trial, and they continued with their surgical management plan.

Further investigations guided by the clinical situation were performed in neonates with hepatitis cholestasis, including fundus and slit-lamp examination as well as screening for toxoplasmosis, syphilis, varicella-zoster, parvovirus B19, rubella, cytomegalovirus and herpes infections and reducing substances in the urine, alpha-1-antitrypsin, uridyl-6-galactose transferase, alfa-fetoprotein, succinyl acetone, serum ferritin, *etc*. Diagnosis of vanishing bile duct syndrome, paucity of intrahepatic biliary radicals and progressive familial intrahepatic biliary radicals were based on the levels of gamma glutamyl transpeptidase and bile acids as well as the histopathologic examination of a percutaneous liver biopsy.

Intervention

All infants received treatment for treatable underlying causes whenever indicated, *e.g.*, infants with galactosemia were advised to replace breast feeding or artificial milk with lactose-free milk. All groups received fat-soluble and water-soluble vitamins throughout their period of cholestasis. Those assigned to the spore-free honey group received daily aliquots of 5–10 cc/kg/day of natural medical-grade spore-free honey, divided into 3–4 doses for at least 6 months. When the patient was diagnosed with a treatable condition, he/she received his/her treatment together with the honey. Their results were compared to those who were concurrently on UDCA.

The spore-free honey used in this trial was unprocessed floral honey that was free from *Clostridium botulinum* spores. The spore-free honey was cultured from a single sample of a batch on blood agar grown under anaerobic conditions and revealed no growth for any anaerobic organisms. Samples from each batch were analyzed for traces of pesticides and heavy metals. It was given as such or freshly diluted in the feeding formula.

The neonates and infants were followed up prospectively for a minimum of 6 months.

Outcome grading

The patients outcome was graded according to the clinical examination and investigations as successful, improved, failed outcome, or death due to the liver condition or its complication. The outcome was considered "successful" when the infant or child was anicteric and maintained alanine aminotransferase levels at less than double the highest normal level; "improved" when there was persistent jaundice, stable disease and stable alanine aminotransferase levels of less than four times the highest normal level, "failed" when there was stationary disease and/or progressive disease, chronic hepatitis, or liver cell failure.[10]

Statistical analysis

The statistical analyses in this study were conducted using the Statistical Package for Social Sciences, version 10 (Chicago, IL, USA). Simple frequency, cross-tabulation, descriptive analysis and tests of significance (the t-test for parametric data and the chi-squared test for nonparametric numbers) were used. Parametric

Table 1. Demographic, follow-up and clinical presentations of subjects

	Honey group (n = 30)	Control group (n = 28)	UDCA group (n = 31)	p-value
Age at onset (days)	19.5 ± 35.4	20.2 ± 49	16.5 ± 30.3	0.928
Age at presentation (days)	60.4 ± 47.1	58.2 ± 58.6	85.2 ± 75	0.173
Age at resolution (days)	148.7 ± 100.6	135 ± 87.4	153.8 ± 102.7	0.451
Cholestasis total duration (days)	234.1 ± 322.8	234.3 ± 235.1	438.8 ± 390.8	0.023*
Follow-up duration (months)	26.6 ± 7.2	20.1 ± 10.1	22.1 ± 8.2	0.016
		No.		p-value
Dark color of urine	15	28	29	0.000
Clay color of stools	8	7	3	0.192
Hepatomegaly	16	15	20	0.603
Splenomegaly	6	4	11	0.136
Initial presentation				
Total bilirubin (mg/dL)	9.5 ± 5.9	10.9 ± 7	14 ± 9.1	0.064
Direct bilirubin (mg/dL)	6 ± 4.01	6.7 ± 4.06	8.4 ± 6.9	0.169
AST	5.1 ± 5.7	3.4 ± 2.7	4.7 ± 4.7	0.385
ALT	2.9 ± 2.7	2.3 ± 1.6	3.6 ± 3.8	0.253
Final visit				
Total bilirubin (mg/dL)	1.2 ± 1.7	4.6 ± 8.2	7.3 ± 8.9	0.006*
Direct bilirubin (mg/dL)	0.8 ± 1	2.77 ± 5.2	5.1 ± 7.2	0.008*
AST	1.3 ± 1.1	3.4 ± 5.3	4.9 ± 8.6	0.073*
ALT	1 ± 0.7	2.7 ± 5.6	2.6 ± 3.8	0.181

Values are presented as the mean ± SD. * indicates a statistically significant difference. AST and ALT are expressed as the fold of the upper limit of normal. AST, aspartate aminotransferase; ALT, alanine aminotransferase; UDCA, ursodeoxycholic acid.

quantitative data were expressed as the mean ± standard deviation (SD) and compared using the Student's t-test. Logistic regression analysis was used to predict the influence of each intervention studied on the outcomes. Analysis of variance was used to analyze the differences among means.

Results

Demographic data and clinical presentations

The 89 neonates included in this study were grouped as follows: 30 received spore-free honey, 28 served as the control group and 31 received UDCA. Males comprised 75% (66 infants) of the study population, while females accounted for only 25% (23 infants). They were followed up for a mean duration ± SD of 29.68 ± 18.68 months. The mean ages ± SD at onset of cholestasis, at presentation were 18.53 ± 37.88 days and 67.57 ± 61.43 days, respectively. The studied groups were matched in terms of gender (p = 0.513), age at onset (p = 0.173), age at presentation (p = 0.928) and severity of cholestasis (p = 0.064) (Table 1). There was a statistically significant difference between the total bilirubin levels among those who received spore-free honey and those who received UDCA (p = 0.026) but not for the direct bilirubin levels or the biopsy findings (p = 0.101). Those who received honey had worse liver histopathology than those who received UDCA (p = 0.002).

Lab, imaging and histopathological findings

Table 1 presents the means ± SD of bilirubin and liver enzyme levels among the studied groups. Of them, 49 patients underwent a percutaneous liver biopsy to exclude extrahepatic biliary atresia: 16 in the spore-free honey group, 12 in the control group and 21 in the UDCA group. The three groups had similar findings for the presence of giant cells (p = 0.969), paucity of bile ducts (p = 0.264), inflammatory cells (p = 0.615) and cirrhosis (p = 0.632).

The initial diagnostic liver histopathology of those prior to receiving honey was significantly more severe than among the other 2 groups. Fibrosis was encountered in 20 biopsies: 9 in the spore-free honey group, 5 in the control group and 6 in the UDCA group (p = 0.017). Necrosis was only encountered in 3 of 49 biopsies, and all were in the spore-free honey group (p = 0.018). Proliferated bile ducts were encountered in 15 biopsies: 9 in the spore-free honey group, 2 in the control group and 4 in the UDCA group (p = 0.025). The histopathological findings and final diagnoses are presented in Figures 1 and 2.

Idiopathic neonatal hepatitis was the predominant diagnosis in the 3 groups, 8 patients in the spore-free honey group, 15 patients in the control group and 15 patients in the UDCA group, followed by inspissated bile syndrome and idiopathic neonatal hepatitis with underlying proliferative cholangiopathy in the liver biopsy. The diagnosis of underlying disease was not possible for three patients in the control group and six in the UDCA group (p = 0.003).

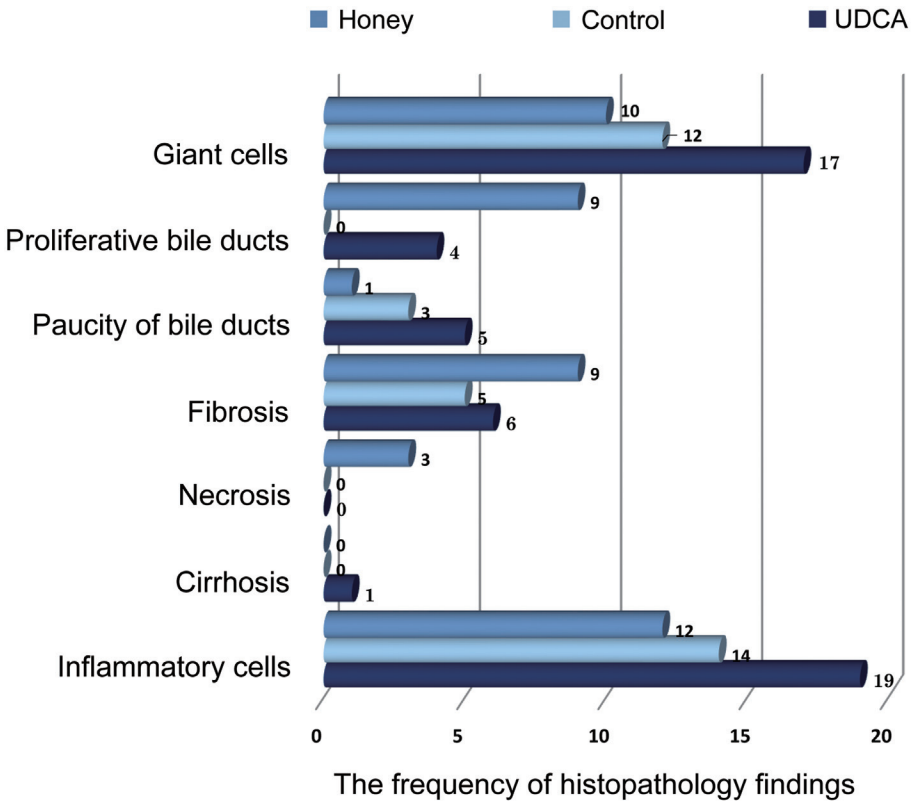

Fig. 1. Liver biopsy histopathology of the subjects. UDCA, ursodeoxycholic acid.

Outcome of cholestasis among the studied infants

Figure 3 and Table 2 outline the final diagnoses and outcomes of the studied infants. According to the outcome of cholestasis in the 3 groups, the numbers of cured patients in the spore-free honey, control and UDCA groups were 25, 16 and 16, respectively (p = 0.023), improved patients were 3, 5 and 3, respectively (p = 0.565), and failure in patients were 2, 3 and 10, respectively (p = 0.016). No patients died in the spore-free honey group, while four patients died in the control group and two patients died in the UDCA group (p = 0.095).

Complications were encountered in all infants. Bronchitis complicated the course in two infants in the spore-free honey group, two infants in the control group, and three infants in the UDCA group (p = 0.982). No cases of liver cell failure were reported in the spore-free honey group, while two infants developed liver cell failure in the control group and three infants developed liver cell failure in the UDCA group (p = 0.238). The spore-free honey and control groups had an equal number of cases (two patients) suffering from ascites, compared to three patients in the UDCA group (p = 0.891). The numbers of patients who encountered complications in the spore-free honey, control, and UDCA groups were as follows: diarrhea, 4, 0, and 2 (p = 0.129); urinary tract infection, 1, 0, and 0 (p = 0.370); achieved head support late, 5, 0 and 1 (p = 0.026); achieved walking later, 4, 0 and 1 (p = 0.054); bleeding from orifices, 5, 3 and 8 (p = 0.313); and pruritus, 1, 0 and 9 (p = 0.001), respectively.

Correlation of age at presentation

The age at presentation correlated positively with the age at resolution of cholestasis (p = 0.007) and the total duration of cholestasis (p = 0.000), while an older age at presentation correlated positively with a poor outcome (p = 0.021) and negatively with cure (p = 0.014).

Correlations of history, examination and lab parameters

Hepatomegaly (p = 0.009) and splenomegaly (p = 0.005) correlated positively with an older age at presentation. Meanwhile, splenomegaly correlated positively with hepatomegaly (p = 0.000). Furthermore, the total and direct bilirubin levels at presentation correlated positively with the AST (p = 0.008) and ALT fold of the upper limit of normal at presentation (p = 0.026).

Outcomes

An older age at presentation correlated positively with a poor outcome (p = 0.021) and negatively with a cure (p = 0.014). The total bilirubin level at presentation correlated positively with a poor outcome (p = 0.029). Cured patients had a total bilirubin level equal or less than 10.3 ± 6.6 mg%, while those who failed to have their cholestasis resolved had a higher mean total bilirubin level of 13.9 ± 9.7 mg% (p = 0.04). The AST fold of the upper limit of normal at presentation correlated negatively with chance of cure of cholestasis (p = 0.03); however, the ALT fold of the upper limit of normal did not (p = 0.054). Those with resolved cholestasis had 3.7 ± 4.3-fold of the upper limit of normal for AST, compared to a 6 ± 5.3-fold rise among those who failed to clear their cholestasis. Receiving spore-free honey correlated positively with a cure of cholestasis (p = 0.006) and negatively with a poor outcome (p = 0.007). In contrast, UDCA use was associated with a poor outcome (p = 0.014).

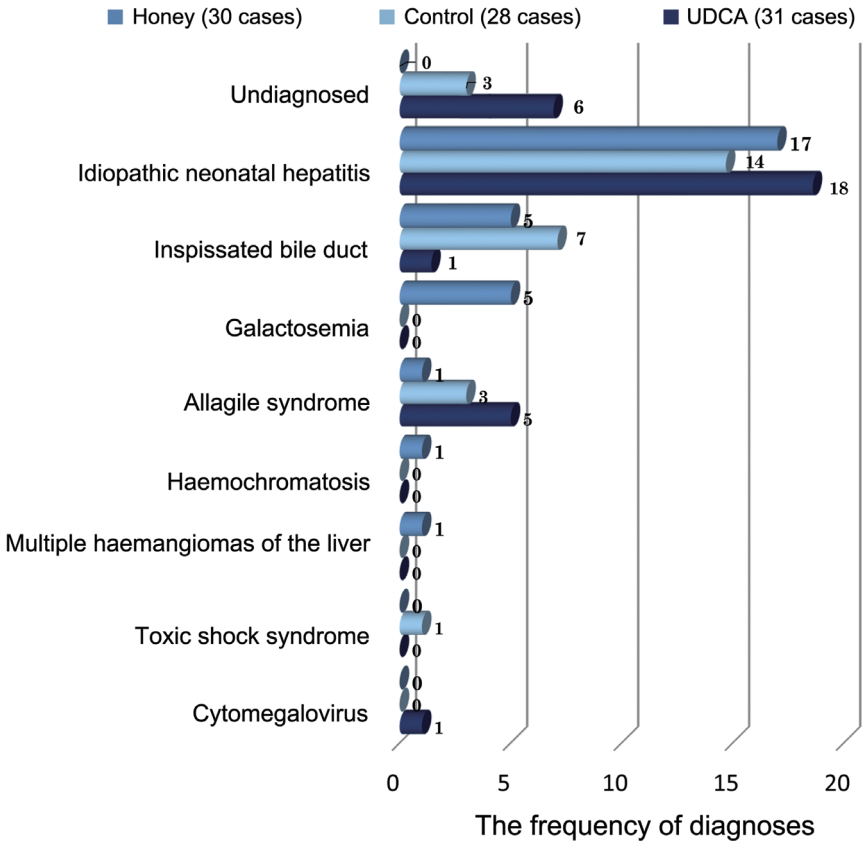

Fig. 2. Final diagnoses of the studied infants who received honey or ursodeoxycholic acid compared to the control group. UDCA, ursodeoxycholic acid.

According to logistic multi-regression analysis, the predictors of a successful outcome in infants with neonatal cholestasis were the intake of spore-free honey ($p = 0.008$), an earlier age at presentation ($p = 0.014$), and a lower fold increase of the upper limit of normal for AST at presentation ($p = 0.011$). Meanwhile, the predictors of a failed outcome for infants with neonatal cholestasis were the intake of UDCA ($p = 0.013$) and a longer duration of cholestasis ($p = 0.001$). ALT was not prognostic of a cure at any

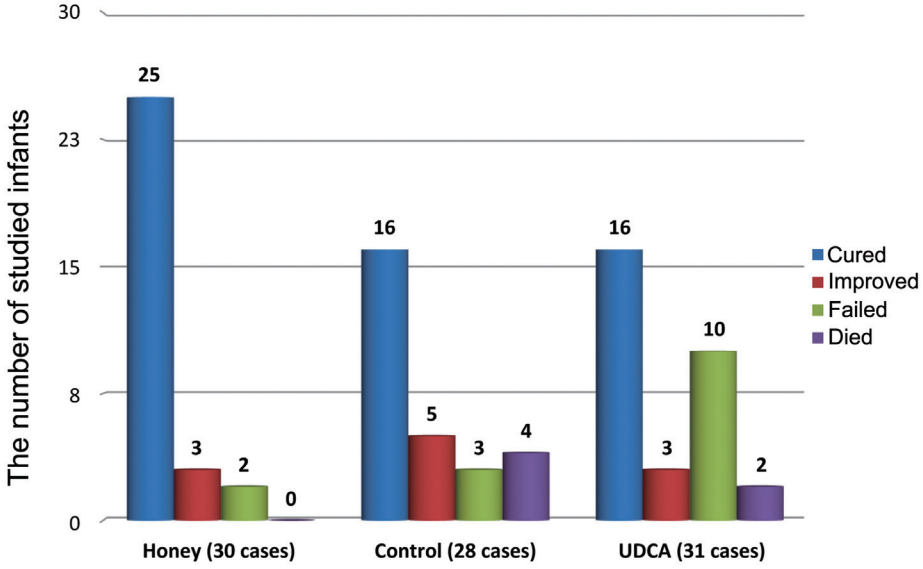

Fig. 3. Outcomes of the infants who received spore-free honey compared to the control group and the group that received ursodeoxycholic acid. UDCA, ursodeoxycholic acid.

Table 2. The diagnoses and outcomes of the studied infants

Outcome	No.	%	Diagnosis	No.	%
Honey group (*n* = 30)					
Cured	25	83.3	Idiopathic neonatal hepatitis with underlying proliferative cholangiopathy in the liver biopsy	7	23.3
			Neonatal hepatitis	6	20
			Inspissated bile syndrome	5	16.6
			Galactosemia	5	16.6
			Paucity of intrahepatic bile ducts	1	3.3
			Neonatal hemochromatosis	1	3.3
Improved	3	10	Idiopathic neonatal hepatitis with underlying proliferative cholangiopathy in the liver biopsy	2	6.6
			Neonatal hepatitis	1	3.3
Failed	2	6.6	Neonatal hepatitis	1	3.3
			Multiple hemangiomas of liver	1	3.3
Died	0	0			
Control group (*n* = 28)					
Cured	16	57.1	Neonatal hepatitis	8	28.6
			Inspissated bile syndrome	6	21.4
			Toxic shock syndrome	1	3.6
			Paucity of intrahepatic bile ducts	1	3.6
Improved	5	17.8	Neonatal hepatitis	4	14.3
			Inspissated bile syndrome	1	3.6
Failed	3	10.7	Neonatal hepatitis	1	3.6
			Paucity of intrahepatic bile ducts	1	3.6
			Undiagnosed	1	3.6
Died	4	14.2	Undiagnosed	2	7.1
			Neonatal hepatitis	1	3.6
			Paucity of intrahepatic bile ducts	1	3.6
UDCA group (*n* = 31)					
Cured	16	51.6	Neonatal hepatitis	8	25.8
			Undiagnosed	3	9.7
			Paucity of intrahepatic bile ducts	2	6.5
			Inspissated bile syndrome	1	3.2
			Idiopathic neonatal hepatitis with underlying proliferative cholangiopathy in the liver biopsy	1	3.2
			Cytomegalovirus infection	1	3.2
Improved	3	9.6	Idiopathic neonatal hepatitis with underlying proliferative cholangiopathy in the liver biopsy	1	3.2
			Neonatal hepatitis	1	3.2
			Paucity of intrahepatic bile ducts	1	3.2
Failed	10	32.2	Neonatal hepatitis	5	16.1
			Idiopathic neonatal hepatitis with underlying proliferative cholangiopathy in the liver biopsy	2	6.5
			Paucity of intrahepatic bile ducts	2	6.5
			Undiagnosed	1	3.2
Died	2		Undiagnosed	2	6.5

UDCA, ursodeoxycholic acid.

setting ($p = 0.253$). UDCA intake correlated positively with pruritus ($p = 0.000$).

Discussion

Medical grade spore-free honey is safe in neonates with cholestasis. Infant botulism is a rare disease that presents as flaccid paralysis or even death. Honey has been incriminated as a carrier of botulinum spores; accordingly, honey consumption has been discouraged in infants.[22] No cases of infant botulism have ever been reported in Africa, and the only case of infant botulism reported in Africa was confirmed to be not caused by honey.[23,24] No cases of infant botulism or flaccid paralysis have been reported (botulism is a reportable disease in Egypt),[25,26] while the average annual incidence of infant botulism in Argentina is 2.2 per 100,000 live births[27] and that in the United States is 2.1 per 100,000 live births.[28] We advise strict analysis for botulinum spores in honey prior to its use in infants with cholestasis. Anaerobic cultures and polymerase chain reaction detection of botulinum spores and toxins are currently available.[29] None of the analytical reports of honey in our study came back positive for any bacterial contaminant, and none of the infants developed any side effects during the study duration. The same findings have been reported when medical-grade honey was studied among preterm infants.[30]

Medical grade spore-free honey is effective in neonatal cholestasis. Spore-free honey cured cholestasis in 83.3% of studied infants and improved cholestasis in another 9.9% of studied infants, compared to cure rates of 57.1% in the control group and 51.6% in the UDCA group. Spore-free honey did not shorten the duration until resolution compared to the control group in our studied population. Spore-free honey was not a successful treatment in only 6.6% of studied infants. We did not try honey in infants with obstructive cholestasis. It was effective in curing cholestasis in 7/9 infants with neonatal hepatitis with underlying proliferative cholangiopathy according to the liver biopsy as well as in infants with galactosemia. The infants were assigned to each group at the initial visit before diagnosis, and this resulted in an unequal distribution of diseases across the three groups. There are no previous reports of honey used to treat neonatal cholestasis, yet honey has been reported to prevent hepatic damage induced by obstruction of the common bile duct in 30 rats and to have a protective role in rats with experimental aflatoxicosis.[31,32] The researchers attributed the prevention of liver damage to (1) the anti-oxidant activity of the flavonoid components of honey, such as luteolin, quercetin, apigenin, fisetin, kaempferol, isorhamnetin, acacetin, tamarixetin, chrysin and galangin; and (2) the antibacterial, anti-inflammatory, immune-stimulant, anti-ulcer and wound/burn healing (regenerative) effects of honey. Others have reported that honey ameliorates the influence of hemorrhage and food restriction on renal and hepatic functions as well as hematological and biochemical variables.[33–35] The *in-vitro* cytoprotective effect of honey against chromosomal breakage in patients with Fanconi anemia also has been reported; however, further confirmation *in vivo* is needed.[36]

Honey is unique in its ability to augment the process of detoxification. Honey influences the detoxification process (phases I, II and III)[37] and anti-oxidative stress genes; in addition, it can transcriptionally activate several enzymes involved in cellular protection,[38,39] such as glutathione peroxidase, superoxide dismutase, catalase or glutathione reductase.[40] Moreover, honey has been reported to enhance the levels of glutathione within liver cells[41] and to protect against carbon tetrachloride-induced hepatoxicity and lipid peroxidation.[42] The role of honey in phase II detoxification could be attributed to augmentation of the process of glucuronidation through its content of glucuronic acid.[43] In the infants studied, the levels of glutathione S transferase were not assessed.

It is interesting that the best outcome was in the spore-free honey group, despite this group having more fibrosis, necrosis and bile duct proliferation in the pretreatment biopsies than the other two groups. In particular, the infants in the honey group with signs of necrosis in their biopsies had a successful outcome (one infant was cured and two infants were improved), and one infant with fibrosis had a failed outcome and the remaining had successful outcomes (six infants were cured and two infants were improved). In the UDCA group, of those with fibrosis, three infants were cured and three infants had a failed outcome; while in the control group, three infants were cured and two infants had a failed outcome.

Among the 30 infants in the control group, seven (23.3%) infants did not achieve a cure or improvement, including three infants who failed to resolve their cholestasis and four infants who died, compared to two (6.5%) infants who failed to resolve their cholestasis in the honey group. Twenty-five (80.6%) infants in the honey group achieved a cure compared to 16 infants in the control group. The difference is statistically significant. Therefore, spore-free honey is effective in resolving cholestasis, yet achievement of head support was often delayed in the spore-free honey group. This can be attributed to an unequal distribution of diseases as no infants in the control group suffered from galactosemia or hemochromatosis, while the honey group included five infants with galactosemia, one infant with hemochromatosis, nine infants with neonatal hepatitis with underlying proliferative cholangiopathy in the liver biopsy, one infant with Crouzon syndrome and one infant with dysmorphism.

UDCA is ineffective in management of cholestasis. The better outcome was associated with spore-free honey consumption than with UDCA use, and this finding was irrespective of the disease type or underlying pathology. UDCA is only approved and indicated in primary biliary cholangitis. In adults with primary biliary cholangitis, a prolonged intake of UDCA has been reported to be associated with some histopathological and biochemical effects but not with the halt of cirrhosis;[44] therefore, debate continues as to whether UDCA enhances long-term survival, slows the progression of the disease versus progression of the disease, or causes liver cell failure or hepatocellular carcinoma.[44–46] In patients with sclerosing cholangitis, UDCA has been demonstrated to be seriously hepatotoxic at a dose of 28 mg/kg/day.[13] Some mechanisms of UDCA liver injury are well characterized.[12] In the current study, it is important to highlight that those on UDCA did not have more severe cholestasis than the other groups and were followed up for a longer duration to allow them to clear their cholestasis. Hence, we cannot attribute the poor outcome of cholestasis in the UDCA group to a worse initial presentation. UDCA has been reported to be ineffective and unsafe among neonates and children with cholestasis and liver disease and has been contraindicated among Egyptian children since 2018.[9–11,47] Handy off-label use of UDCA has precluded double-blind prospective clinical trials of this drug in children with different ethnicities, and there is a lack of evidence to support its use among children.

In cholestasis, there is incomplete absorption of UDCA and subsequent formation of hepatobiliary lithocholic acid, which is toxic. Lithocholic acid, when administered chronically to animals, leads to cholestatic liver injury and possibly death due to liver cell failure.[4,13,14] UDCA toxicity is related to its interference with drug detoxification, its long half-life, its transcriptional mutational abilities, its ability to downregulate cellular functions, its narrow

toxicity margin with a small difference between the recommended (13 mg/kg/day) and toxic dose (28 mg/kg/day), its typical transformation into lithocholic acid that may induce DNA strand breakage, its unique co-mutagenicity and its promotion of cell transformation.[12,48]

This study is limited by the relatively small sample size. Therefore, more research and insight are needed to verify the specific mechanism of action of honey as a treatment for cholestasis.

Conclusion

Medical-grade spore-free honey is safe and effective in resolving neonatal cholestasis, especially in those with neonatal hepatitis and underlying proliferative cholangiopathy according to the liver biopsy. Its mechanism of action awaits further research. UDCA is contraindicated, ineffective and not safe for the management of neonatal cholestasis, and its off-label use should be abandoned.

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Conflict of interest

The authors have no conflicts of interest to disclose.

Author contributions

Study concept and design, drafting of the initial manuscript, revision and approval of the final manuscript, and analysis and interpretation of data (MAK, EAES, AMB, IAA). All authors critically reviewed the final manuscript and approved the version to be published.

Ethical statement

This study was in compliance with Helsinki declaration guidelines, and was approved by the Pediatric Department Committee for Postgraduate Studies and Research as well as the Faculty of Medicine Committee for Postgraduate Studies and Research, Cairo University, Cairo, Egypt. Parents of enrolled children consented to the study.

Data sharing statement

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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