Cholestasis impaired spatial and non-spatial novelty detection in mice

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Abstract

  Bile duct ligation (BDL) is shown to induce cholestasis-related liver function impairments as well as consequent cognitive dysfunctions (i.e. impaired learning and memory formation). This study investigates the effects of cholestasis (14, 21 and 28 days post bile duct ligation) on spatial and non-spatial novelty detection, using a non-associative task. Male mice weighing 30-35 g were used. Cholestasis was induced by ligation of the main bile duct using two ligatures and transecting the duct at the midpoint between them. Open field paradigm was employed to assess the spatial and non-spatial memories retention. Our data showed that cholestasis (28 days after bile duct ligation) decrease and increased duration time of displace and non-displace objects respectively, indicating spatial memory deficit. Moreover, this intervention (28 days after bile duct ligation) decreased and did not alter duration time of substitute and non-substitute objects respectively, suggesting non-spatial memory deficit. Moreover, the data postulated that 14 and 21 days post bile duct ligation both spatial and non-spatial memories did not alter. Our results suggested that cholestasis (28 but not 14 and 21 days post bile duct ligation) impaired spatial and non-spatial memory in the mice.

Keywords: Cholestasis; Spatial memory; Non-spatial memory; Mice

INTRODUCTION

   Cholestasis is described by various degrees of symptoms mainly jaundice, pruritus, elevated serum levels of alkaline phosphatase, GGT (γ-glutamyl transpeptidase), 5'-nucleotidase, bile acids, and cholesterol. Bile acid retention reduces new bile acid synthesis, which in turn results in decreased bile salt pool and dysregulation in the enterohepatic recirculation. There are several experimental models trying to elicit hepatic encephalopathy in lab animals [1]. The two of the most frequently used models are the administration of carbon tetrachloride, and the common bile duct ligation (BDL) [1]. Patients with liver diseases, also animal models of chronic liver failure [2] may show hypothermia as well as notable impairment in cognitive functions [3].
Acute or chronic liver failure may induce hepatic encephalopathy (HE), which may present a wide range of different grades from minimal HE to coma and death [2]. The disorder has been extensively studied using the model of common bile duct ligation in the rodents. In (BDL) model, the hepatocellular excretion of bile constituents is markedly impaired eliciting its retention within hepatocytes. Thus, accumulation of bile salts in the body and by deficiency of bile salts in the intestinal lumen causes cholestasis [4]. Several investigations indicated that HE induced several cognitive and non-cognitive symptoms such as the impairment of learning and memory [2, 3, 5, 6] anxiolytic-like behaviors [7], alteration in sleep pattern [8] and tremor [9]. It has been reported that after HE, all classical neurotransmitter systems such as opioidergic, glutamatergic, GABAergic, cholinergic, serotoninergic can be altered [2, 5, 10-12]. A marked elevation of endogenous opioid levels in plasma of patients with cholestatic liver diseases and also with animal models of cholestasis have been shown [13]. Thus, there is suggestion that endogenous opioids are implicated in the pathophysiology of cholestasis [14, 15]. According to the above studies the aim of present study is investigation the effect of different days post BDL on spatial and non-spatial memories.

MATERIALS AND METHODS
Animals

   Subjects were male NMRI mice weighing 30-35 g, bred at the Institute for Cognitive

Science Studies (ICSS), Tehran, Iran. Mice were kept in the animal house with a 12/12-h light-dark cycle and controlled temperature (22±2ºC). They were housed in groups of 10, in Plexiglas cages and had free access to food and water except during the limited periods of experiments. Eight animals were in each group and each animal was used once only. Behavioral experiments were done during the light phase of the light/dark cycle. All animal experimentations reported in this study, were conducted in accordance with the guidelines laid down by the NIH (NIH Guide for the Care and Use of Laboratory Animals) in the USA. The Research and Ethics Committee of the Faculty of Science of the University of Tehran approved the experimental protocol.

Bile Duct Ligation surgery and induced cholestasis

There were three experimental groups: sham-operated and bile duct ligated (BDL) mice. Mice receiving a BDL or sham-ligation surgery were anesthetized by an intraperitoneal injection of ketamine hydrochloride (50 mg/kg) plus xylazine (5 mg/kg). In mice receiving BDL, the common bile duct was located and ligated using 4-0 silk at two points anterior to the pancreas and posterior to the hilum of the liver. One ligation was made just above the duodenum; the second ligation was made approximately 2 mm above the first ligation and then transected at the midpoint between the two ligatures [3, 16]. Sham-ligation surgery was performed by locating and manipulating the common bile duct. Sterile 0.9% NaCl solution (1mL/mice) was injected intraperitoneally immediately after the surgery. All surgeries were performed using aseptic technique. Immediately after the operation, each animal was placed in a cage by itself to prevent wound dehiscence and was moved to its original cage 4 h after the surgery [3, 7, 17]. Operative mortality was less than 10%.

Apparatus

We used a circular open field apparatus (Borj Sanat Co, Tehran, Iran) as a spatial and non-spatial learning and memory task. For his apparatus, there were minor modifications made in objects described in Roullet's study [18]. Briefly, the task consisted of a metal circular box, 60 cm in diameter and 20 cm high. The arena's ground area was divided into equal sectors. The wall of the arena was white, with a black striped pattern (1.5 cm thick line), 20 cm wide, 10 cm high. The six objects used in the open field were: A cube (a metal-plated parallelepiped, measuring 7×4×4 cm with irregular holes distributed on all sides), a cone (a plastic cone on a transparent cylinder base, 8 cm in diameter and 6 cm high), a ladder (a small plastic white ladder, 5 cm wide and 16 cm high, having 10 steps connected to the two parallel arms, 3 cm thick), a cylinder (a black cylinder,10 cm high and 4 cm in diameter, having a 2 cm in diameter hole on the top), a steel glass (12 cm high and 5 cm in diameter having a black handle) and a corner [this object consisted of two gray iron pieces (10 cm sides) with regularly pierced squares, forming a 90° angle, fixed on a quadrate 5 cm thick in sides. The corner object was used to examine the reactivity to non-spatial changes.

Testing procedure and data collection

Animals were placed into the empty open field for a 6 min period (session 1, S1). 5 min after S1 session, five successful sessions with 6 min period and 3 min interval were in the open field containing the objects. The animals were returned to their home cages immediately after each session. The objects were placed in a square configuration with a central object (cone) in sessions 2 to 4 (S2–S4). This configuration was changed by means of transposing the cone with the cylinder in session 5 (spatial change session, S5), in such a way that the initial square arrangement was changed (objects displacement). To measure the non-spatial memory, the glass object (as a familiar non-displaced) was replaced by the corner as a new object, at the same location (object substitution, S6 session) [18-20].

The apparatus was illuminated using two red lights (80 W) fixed on the ceiling. The animal behaviors were recorded by a video camera while a monitor and a computer-recording system were installed in an adjacent room. The observed behaviors were manually recorded as raw data. The locomotor activity defined as mean sector crossings in all sessions (S1 to S6). Moreover, the time spent by the mouse in contact with an object (when the animal touched an object by its snout and made rearing against the object) was carefully recorded during S2-S6 [18-20]. To measure the spatial novelty detection, the mean time that mouse contacted with objects in S5 [both with displaced objects (DO) and non-displaced objects (NDO)] was subtracted from the mean time spent in contact

with the same object in S4. In addition, to measure the non-spatial novelty detection, the mean time that the animal contacted with objects in S6 [both substituted object (SO) and non-substituted object (NSO)] was deducted from the mean time spent in contact with the same object in S5. Experiments were carried out by someone blinded to doses of drugs and statistical measure.

Drug treatment

Four groups of mice were used in this experiment. Following S4, the spatial (S5-S4) and non-spatial (S6-S5) novelty detection were assessed in 24 h time, on the test day. The animals' locomotor activities were recorded during S1 to S6. The data from cholestatic animals was compared with those from sham operated animals.

Statistical analysis

Given the normality of distribution and the homogeneity of the data variance, the results were statistically evaluated by one-way ANOVA repeated measures for the analysis of spatial and novelty. Further analyses for individual 'between-group' comparisons were carried out through post hoc Tukey's test. In all comparisons, $P<0.05$ represented a statistical significance.

RESULTS

One-way ANOVA and post hoc Tukey's analysis revealed that cholestasis decrease both spatial [displaced object-DO; $F_{(3, 28)}= 5.2$, $P<0.01$, Fig.1B] and non-spatial novelty detection [substitute object-SO; $F_{(3, 28)}= 4.2$, $P<0.01$, Fig.1C], while decreased non-displaced object-NDO [$F_{(3, 28)}= 9.1$, $P<0.001$, Fig.1B]. The data showed that laparotomy did not alter locomotor activity [$F_{(3, 28)}= 3.12$, $P>0.05$, Fig.1A] and non-substituted object- NSO [$F_{(3, 28)}= 1.02$, $P>0.05$, Fig.1C]. In conclusion, the data showed that cholestasis (28 days post BDL) impaired spatial and non-spatial memories.

DISCUSSION

The present data indicated that cholestasis impaired both spatial and non-spatial memories formation only 28 but not 14 and 21 days after BDL, meanwhile laparotomy did not alter locomotor activity in all memories testing days, suggesting the uniformity of data on memory formation. However the locomotor activity tends to decrease which was not significant.
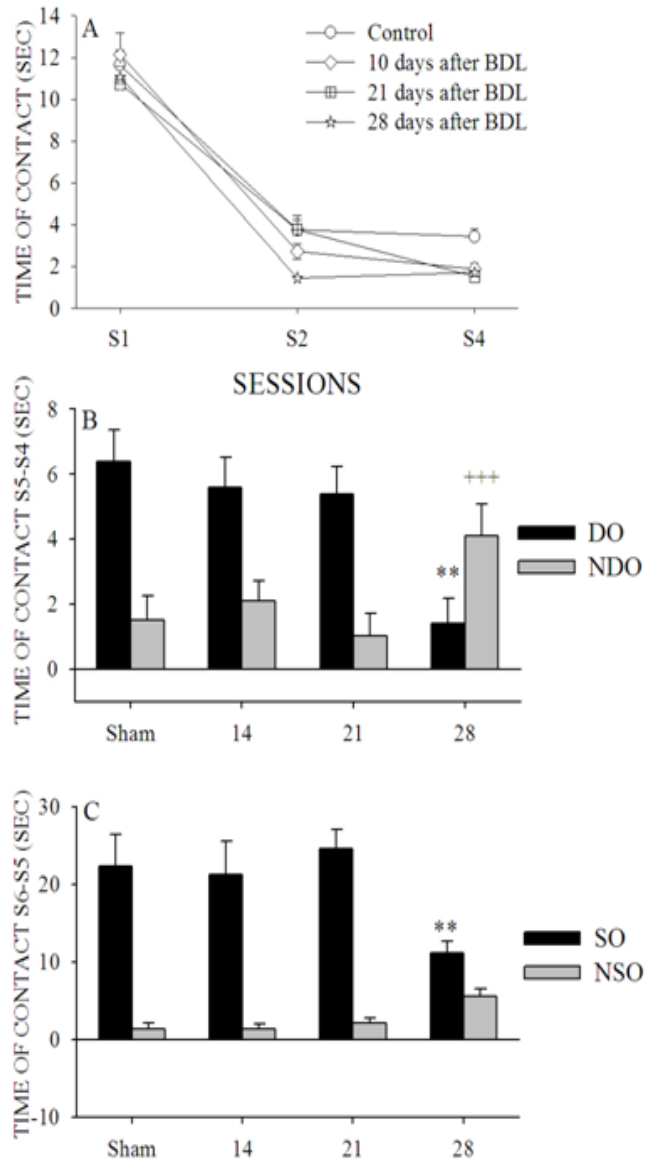

Figure.1. Effects of cholestasis on the time spent in contact with objects. Panel A: habituation profile in sessions 2–4. Panel B: reaction to object displacement is measured as time spent in contact with either displaced (DO) or non-displaced (NDO) objects in session 5 minus the same in session 4. Panel C: reaction to object substitution is measured as time spent in contact with either substituted (SO) or non-substituted (NSO) objects in session 6 minus the same in session 5. Each bar represents mean±S.E.M. **$P <0.01$ and +++$P <0.001$, as compared to sham-treated group.

There are also reports showing that cognitive function and locomotor activity decreased 21 days after BDL in the mice [5, 21]. If there is any controversy may be due to the method used, which may influence on the BDL response on locomotor activity.

Cholestasis may result from various liver diseases. The impaired bile flow is secondary to structural or biochemical abnormalities of the

liver and/or the biliary tree. These alterations may be involved in: 1- rapid changes in transporter function, e.g. due to drug-induced direct inhibition of relevant transporters or changes in their localization; and 2- altered transporter expression, due to the inhibition of carrier synthesis or exacerbated degradation. As a consequence of the hepatocellular cholestasis, or blockade of intra- or extra-hepatic bile transit, the liver develops a number of secondary adaptive changes to minimize the detrimental effects of toxic biliary compounds retained as a consequence of the secretory failure. Although endogenous opioid peptide alterations under impaired bile secretion have been proven [22], the role of the opioid system in the regulation of hepato-biliary functions is yet not clear.

As it has been indicated previously, BDL leads to biliary cirrhosis within 3 to 4 weeks [23], which occurs in conjunction with fibrosis, portal hypertension, portal-systemic shunting and immune system dysfunction [23-25]. Cholestasis-induced HE would correspond to type C [26] which associated with liver cirrhosis [27]. The liver cirrhosis in patients without clinical symptoms of HE may show mild cognitive impairment [28]. However, patients with manifestations of HE may present impairment of attention, memory and cognitive function, and also some alterations in motor function, including psychomotor slowing, bradykinesia or hypokinesia and asterixis. These behaviors in patients with cholestatic liver disease are central and not peripheral origin [29].

Some studies have shown an impaired ability to discriminate between the novel and the previously encountered sample objects [26] as well as impaired spatial memory acquisition in the Morris water maze task two to three weeks

after BDL in cholestatic rodents [30]. There are other reports suggesting deficits in visuo-spatial abilities [31, 32] and working memory in patients with hepatic encephalopathy (HE) [33] which are suggested to be possibly due to the changes in the hippocampal formation [34].

Based on the reports which indicate an increase in central opioidergic neurotransmission/neuromodulation tonus upon cholestasis, there is compelling evidence supporting the role of opioid system, accordingly. These include: 1- an opiate withdrawal-like reaction is seen upon opiate antagonist administration in cholestatic patients [35, 36] or experimental animals with cholestasis, 2- bile duct resection exhibit a state of antinociception which is reversed by naloxone [16]. HE is characterized by deficits in several neurotransmitter systems in the brain [37, 38] including opioidergic, dopaminergic, cholinergic, adrenergic, glutamatergic, GABAergic and serotoninergic [39]. Meanwhile, some other investigators have suggested that deficits in the release of corticotrophin-releasing hormone [29, 40, 41] and an imbalance in manganese homeostasis in the brain [42] are involved in cholestasis-induced behaviors

Given the three phases of memory formation process including acquisition, consolidation and retrieval, it is not yet fully understood whether this phenomenon is related to deficits in memory acquisition, consolidation and/or retrieval. In conclusion, our data indicated that cholestasis (28 days post BDL) impaired spatial and non-spatial memory in the open field task but other investigations are need to clearing involvement of different brain sites, neurotransmitter and memory stages on this phenomenon.

REFERENCES

1. Rodriguez-Garay, EA. Cholestasis: human disease and experimental animal models. Ann Hepatol. 2003; 2(4): 150-8.

2. Cauli, O; Rodrigo, R; Llansola, M; Montoliu, C; Monfort, P; Piedrafita, B; El Mlili, Net al. Glutamatergic and gabaergic neurotransmission and neuronal circuits in hepatic encephalopathy. Metab Brain Dis. 2009; 24(1): 69-80.

3. Zarrindast, MR; Hoseindoost, S; Nasehi, M. Possible interaction between opioidergic and cholinergic systems of CA1 in cholestasis-induced amnesia in mice. Behav Brain Res. 2012; 228(1): 116-124.

4. Gartung, C; Ananthanarayanan, M; Rahman, MA; Schuele, S; Nundy, S; Soroka, CJ; Stolz, Aet al. Down-regulation of expression and function of the rat liver Na+/bile acid cotransporter in extrahepatic

cholestasis. Gastroenterology. 1996; 110(1): 199-209.

5. Magen, I; Avraham, Y; Ackerman, Z; Vorobiev, L; Mechoulam, R; Berry, EM. Cannabidiol ameliorates cognitive and motor impairments in bile-duct ligated mice via 5-HT1A receptor activation. Br J Pharmacol. 2010; 159(4): 950-7.

6. Huang, LT; Tiao, MM; Tain, YL; Chen, CC; Hsieh, CS. Melatonin ameliorates bile duct ligation-induced systemic oxidative stress and spatial memory deficits in developing rats. Pediatr Res. 2009; 65(2): 176-80.

7. Eslimi, D; Oryan, S; Nasehi, M; Zarrindast, MR. Effects of opioidergic systems upon anxiolytic-like behaviors induced in cholestatic rats. Eur J Pharmacol. 2011; 670(1): 180-5.

8. Newton, JL. Fatigue in primary biliary cirrhosis. Clin Liver Dis. 2008; 12(2): 367-83; ix.

9. Chung, CH; Wang, CH; Tzen, CY; Liu, CP. Intrahepatic cholestasis as a paraneoplastic syndrome associated with pheochromocytoma. J Endocrinol Invest. 2005; 28(2): 175-9.

10. Marrachelli, VG; Miranda, FJ; Alabadi, JA; Llorens, S; Alborch, E. Contribution of endothelin receptors and cyclooxygenase-derivatives to the altered response of the rabbit renal artery to endothelin-1 in diabetes. Eur J Pharmacol. 2006; 534(1-3): 178-86.

11. Marquez-Aguirre, AL; Canales-Aguirre, AA; Gomez-Pinedo, U; Galvez-Gastelum, FJ. [Molecular aspects of hepatic encephalopathy]. Neurologia. 2010; 25(4): 239-47.

12. Palomero-Gallagher, N; Bidmon, HJ; Cremer, M; Schleicher, A; Kircheis, G; Reifenberger, G; Kostopoulos, Get al. Neurotransmitter receptor imbalances in motor cortex and basal ganglia in hepatic encephalopathy. Cell Physiol Biochem. 2009; 24(3-4): 291-306.

13. Mombeini, T; Roushanzamir, F; Jorjani, M; Pourpak, Z; Gaskari, SA; Mehr, SE; Dehpour, AR. Alteration in specific opioid-receptor labeling on peripheral blood leukocytes of bile duct-ligated rat. Pathophysiology. 2006; 13(2): 111-7.

14. Talaenko, AN; Krivobok, GK; Pankrat'ev, DV; Goncharenko, NV. [Neurochemical mechanisms of dorsal pallidum in antiadverse effects of anxiolytics of different models of anxiety]. Ross Fiziol Zh Im I M Sechenova. 2005; 91(7): 776-84.

15. Ebrahimkhani, MR; Kiani, S; Oakley, F; Kendall, T; Shariftabrizi, A; Tavangar, SM; Moezi, Let al. Naltrexone, an opioid receptor antagonist, attenuates liver fibrosis in bile duct ligated rats. Gut. 2006; 55(11): 1606-16.

16. Bergasa, NV; Alling, DW; Vergalla, J; Jones, EA. Cholestasis in the male rat is associated with naloxone-reversible antinociception. J Hepatol. 1994; 20(1): 85-90.

17. Rastegar, H; Homayoun, H; Afifi, M; Rezayat, M; Dehpour, AR. Modulation of cholestasis-induced antinociception by CCK receptor agonists and antagonists. Eur Neuropsychopharmacol. 2002; 12(2): 111-8.

18. Roullet, P; Mele, A; Ammassari-Teule, M. Involvement of glutamatergic and dopaminergic systems in the reactivity of mice to spatial and non-spatial change. Psychopharmacology (Berl). 1996; 126(1): 55-61.

19. Adriani, W; Sargolini, F; Coccurello, R; Oliverio, A; Mele, A. Role of dopaminergic system in reactivity to spatial and non-spatial changes in mice. Psychopharmacology (Berl). 2000; 150(1): 67-76.

20. Roullet, P; Sargolini, F; Oliverio, A; Mele, A. NMDA and AMPA antagonist infusions into the ventral striatum impair different steps of spatial information processing in a nonassociative task in mice. J Neurosci. 2001; 21(6): 2143-9.

21. Magen, I; Avraham, Y; Ackerman, Z; Vorobiev, L; Mechoulam, R; Berry, EM. Cannabidiol ameliorates cognitive and

motor impairments in mice with bile duct ligation. J Hepatol. 2009; 51(3): 528-34.

22. Bergasa, NV; Jones, EA. The pruritus of cholestasis: potential pathogenic and therapeutic implications of opioids. Gastroenterology. 1995; 108(5): 1582-8.

23. Kountouras, J; Billing, BH; Scheuer, PJ. Prolonged bile duct obstruction: a new experimental model for cirrhosis in the rat. Br J Exp Pathol. 1984; 65(3): 305-11.

24. Aronson, DC; Chamuleau, RA; Frederiks, WM; Gooszen, HG; Heijmans, HS; James, J. Reversibility of cholestatic changes following experimental common bile duct obstruction: fact or fantasy? J Hepatol. 1993; 18(1): 85-95.

25. Garcia-Ayllon, MS; Silveyra, MX; Candela, A; Compan, A; Claria, J; Jover, R; Perez-Mateo, Met al. Changes in liver and plasma acetylcholinesterase in rats with cirrhosis induced by bile duct ligation. Hepatology. 2006; 43(3): 444-53.

26. Garcia-Moreno, LM; Conejo, NM; Gonzalez-Pardo, H; Aller, MA; Nava, MP; Arias, J; Arias, JL. Evaluation of two experimental models of hepatic encephalopathy in rats. Braz J Med Biol Res. 2005; 38(1): 127-32.

27. Ferenci, P; Lockwood, A; Mullen, K; Tarter, R; Weissenborn, K; Blei, AT. Hepatic encephalopathy--definition, nomenclature, diagnosis, and quantification: final report of the working party at the 11th World Congresses of Gastroenterology, Vienna, 1998. Hepatology. 2002; 35(3): 716-21.

28. Romero-Gomez, M; Cordoba, J; Jover, R; del Olmo, JA; Ramirez, M; Rey, R; de Madaria, Eet al. Value of the critical flicker frequency in patients with minimal hepatic encephalopathy. Hepatology. 2007; 45(4): 879-85.

29. Burak, KW; Le, T; Swain, MG. Increased sensitivity to the locomotor-activating effects of corticotropin-releasing hormone in cholestatic rats. Gastroenterology. 2002; 122(3): 681-8.

30. Roma, MG; Crocenzi, FA; Sanchez Pozzi, EA. Hepatocellular transport in acquired cholestasis: new insights into functional, regulatory and therapeutic aspects. Clin Sci (Lond). 2008; 114(9): 567-88.

31. Tarter, RE; Arria, AM; Carra, J; Van Thiel, DH. Memory impairments concomitant with nonalcoholic cirrhosis. Int J Neurosci. 1987; 32(3-4): 853-9.

32. Weissenborn, K; Heidenreich, S; Giewekemeyer, K; Ruckert, N; Hecker, H. Memory function in early hepatic encephalopathy. J Hepatol. 2003; 39(3): 320-5.

33. Thomas, A; Baillie, GL; Phillips, AM; Razdan, RK; Ross, RA; Pertwee, RG. Cannabidiol displays unexpectedly high potency as an antagonist of CB1 and CB2 receptor agonists in vitro. Br J Pharmacol. 2007; 150(5): 613-23.

34. Mechoulam, R; Peters, M; Murillo-Rodriguez, E; Hanus, LO. Cannabidiol--recent advances. Chem Biodivers. 2007; 4(8): 1678-92.

35. Thornton, JR; Losowsky, MS. Opioid peptides and primary biliary cirrhosis. BMJ. 1988; 297(6662): 1501-4.

36. Bergasa, NV; Alling, DW; Talbot, TL; Swain, MG; Yurdaydin, C; Turner, ML; Schmitt, JMet al. Effects of naloxone infusions in patients with the pruritus of cholestasis. A double-blind, randomized, controlled trial. Ann Intern Med. 1995; 123(3): 161-7.

37. Lozeva, V; Montgomery, JA; Tuomisto, L; Rocheleau, B; Pannunzio, M; Huet, PM; Butterworth, RF. Increased brain serotonin turnover correlates with the degree of shunting and hyperammonemia in rats following variable portal vein stenosis. J Hepatol. 2004; 40(5): 742-8.

38. Butterworth, RF. The neurobiology of hepatic encephalopathy. Semin Liver Dis. 1996; 16(3): 235-44.

39. Celik, T; Goren, MZ; Cinar, K; Gurdal, H; Onder, FO; Tan, A; Terzioglu, Bet al. Fatigue of cholestasis and the serotoninergic neurotransmitter system in the rat. Hepatology. 2005; 41(4): 731-7.

40. Bearn, J; Allain, T; Coskeran, P; Munro, N; Butler, J; McGregor, A; Wessely, S. Neuroendocrine responses to d-fenfluramine and insulin-induced hypoglycemia in chronic fatigue syndrome. Biol Psychiatry. 1995; 37(4): 245-52.

41. Swain, MG; Patchev, V; Vergalla, J; Chrousos, G; Jones, EA. Suppression of hypothalamic-pituitary-adrenal axis responsiveness to stress in a rat model of acute cholestasis. J Clin Invest. 1993; 91(5): 1903-8.

42. Forton, DM; Patel, N; Prince, M; Oatridge, A; Hamilton, G; Goldblatt, J; Allsop, JMet al. Fatigue and primary biliary cirrhosis: association of globus pallidus magnetisation transfer ratio measurements with fatigue severity and blood manganese levels. Gut. 2004; 53(4): 587-92.