**Available online at http://jbcp.shahed.ac.ir/**

Journal of Basic and Clinical
PATHOPHYSIOLOGY

**Volume 6, Number 1, Winter- Spring 2018**

# The effect of acute and chronic administration of naloxone on spatial memory in male cholestatic rats

**Masoud Afshari, Mohsen Parviz*, Mansoor Keshavarz, Majid Ghaseminejad**

*Department of Medical Physiology, Faculty of Medicine, Tehran University of Medical Sciences, Tehran, Iran.*

Article info
Received: 14 Dec 2017
Revised: 11 Feb 2018
Accepted: 19 Feb 2018

**p-ISSN:2322-1895**
**e-ISSN: 2345-4334**

**Key Words:**
Naloxone
Cholestatic rat
Spatial memory
Morris water maze

## ABSTRACT

**Background and Objective:** A great body of evidences suggested a marked elevation of endogenous opioid levels in plasma of animals with acute cholestasis. Endogenous opioids are implicated in the pathophysiology of cholestasis. Also, many studies have shown that endogenous opioids modulate memory processes. To clarify possible role of endogenous opioid receptors in information processing in acute cholestatic rats, we administered acute (5 mg/kg, i.p.) and chronic (by implanted osmotic mini-pump, s.c.) naloxone as an opioid receptor antagonist to male cholestatic rats.

**Materials and Methods:** For this purpose, male rats were divided into eight groups. All the rats were assessed for spatial learning and memory (a major cognitive function in rats) by the Morris water maze task about 8 days after the first operation. Rats were subjected to 6 days of training in the Morris water maze (MWM): 4 days with the invisible platform to test spatial learning and on the 5th day, one day after the last trial, retention performance was examined in a single probe trial. On the 6th day, motivation and sensory-motor coordination was tested with the visible platform.

**Results:** During the four consecutive acquisition trial days of this behavioral test, acute and chronic naloxone-treated bile duct-ligated rats had a significantly longer latency to escape than the bile duct-ligated groups ($p<0.05$).

**Conclusion:** The results of this study suggest that blockade of opioid receptors, both acute and chronic, results in spatial memory deficits in cholestatic rats.

## 1. Introduction

Many investigators have documented a marked elevation of endogenous opioid levels in plasma of human subjects with biliary cirrhosis as well as in animal models of acute cholestasis (1-3). Also, it has been suggested that endogenous opioids are implicated in the pathophysiology of cholestasis (4). Observations compatible with this hypothesis include precipitation of an opiate withdrawal-like syndrome in patients with chronic cholestatic liver disease by administration of an opiate antagonist (5) and a global down-regulation of mu-opioid and kappa-opioid receptors in the brain of rats with cholestasis due to bile duct resection (6,7).

***Corresponding Author:** *Mohsen Parviz*
*Department of Medical Physiology, Faculty of Medicine, Tehran University of Medical Sciences, Tehran, Iran.*
*Email: parvizmo@tums.ac.ir*

According to the previous investigations, it seems that endogenous opioids exert dual effects in memory processing in the brain. While it has been shown that activation of mu-opioid receptors by endogenous opioids in the CA3 region facilitates mossy fiber long-term potentiation (LTP) and naloxone, an opioid receptor antagonist, blocks mossy fiber LTP (8,9), some other studies have indicated that endogenous opioids may impair memory processes (10). Systemic injection of naloxone was found to improve memory function in rats and β-endorphin or [Leu]-enkephalin given systemically is shown to impair memory formation (8, 11]. Children with end-stage liver disease have been found to have cognitive deficits (12). Early biliary decompression of obstructive jaundice improves spatial memory deficits in rats (13).

The present study was designed to investigate the role of endogenous opioids on spatial performance in cholestatic rats.

## 2. Materials and Methods

### 2.1. Animals

A total of 56 male Wistar rats (Pasteur Institute of Iran), 9 to l2-weeks-old and weighing 200-250 g at the time of surgery were used in this study. The animals were allowed to habituate to the animal house for at least five days before the start of the experiment. Before surgery, the animals were housed in groups of four-five per standard cages in a temperature controlled room (23±2°C), with food and water available *ad libitum* and a 12 h light/dark cycle (lights on at 6.00 a.m.).

The rats were randomly allocated to 8 experimental groups. each group consisted of 7 rats: Intact control (Control), Sham-operated (Sham-op), Bile duct ligated (BBL or Cholestatic), Chronic Saline Sham (CS-Sham), Acute naloxone treated sham-operated (AN-Sham), Chronic naloxone treated Sham-op (CN-Sham), Acute naloxone treated BDL (AN-BBL) and Chronic naloxone treated BDL rats.

### 2.2. Drugs

The following drugs were used: naloxone HCL was obtained from Sigma Chemical Co (St. Louis, MO) and dissolved in saline and ketamine HCl was obtained from Rotexmedica (Trittau, Germany).

### 2.3. Surgery

Laparotomy was performed under general anesthesia induced by intraperitoneal injection of ketamine (50 mg/kg). In the Sham-operated controls, the bile duct was left in situ after its manipulation with forceps. In the BDL (Cholestatic) animals, the bile duct was doubly ligated and then abdominal wound was closed in two layers. Operation mortality rate was <l0%. Experiments were conducted 8 days after the surgery in rats. Animal housing and all experimental procedures followed the relevant provisions and general recommendations of Tehran University of Medical Sciences animal protection legislation. The experiments were approved by a local Animal Ethics Committee.

In acute groups, 5mg/kg of naloxone was injected i.p. 20 min before the Morris water task. In chronic groups, Alzet osmotic minipumps (Alza. Palo Alto. Calif., USA) were filled either with naloxone or saline solution. For the naloxone group, 3.0 mg/kg per hour was delivered by the Model 2 MLl pump (rate l0 µl/h). The concentration of naloxone varied, depending on the body weight of rats. Pumps, preconditioned for 4-6 h in sterile saline immediately before use were implanted subcutaneously through an incision in the midscapular region immediately after first surgery that explained above. The incisions were closed with sutures. One week later, the pumps were removed through this incision using the same procedure. The volumes of the pumps were checked to ascertain that all pumps had delivered their contents. From the $8^{th}$ to $13^{th}$ day after the first operation, all rats were tested in the Morris water maze.

### 2.4. Morris water maze

A circular water tank, 140 cm in diameter and 60 cm in depth, was used in the Morris Water task procedure. The water tank was filled with tap-water, kept at 22±1, and the escape platform was submerged 1 cm below the water surface. Most previous investigators have made the water opaque by adding India ink. However, in our study the hidden escape platform (Ø 15 cm), had the same black color as the water tank and pilot

experiments showed that it was invisible for the rats. The water tank was located in a room which contained several visual cues, such as asymmetric lamp positions, a dark curtain and some designed paper on the wall. All cues were kept constant throughout the experiment. The test parameters were measured using a digital camera system connected to a computer.

All animals were allowed to habituate in the experimental room for 60 min prior to testing. During training the submerged escape platform was located in the centre of the southeast quadrant and therefore invisible for testing spatial learning. The platform position remained stable over 4 days and acquisition of this task was assessed. All rats of the 4 test groups were given four trials per day for four consecutive days. In each trial, the rat was placed in the water facing the pool wall at one of four starting points (north, south, east or west pole).

Once the rat located the platform, it was allowed to stay on it for 30 s. If the rat did not find the platform within 60 s, it was gently directed to the platform by hand and it was allowed to remain there for 30 s (total inter-trial time 90 s). After each trial the rat was placed at a different starting point that randomly chosen by computer, thus eliminating the use of a Simple response strategy. After completion of the 4$^{th}$ trial, rats were gently dried with a towel, kept warm for an hour and returned to their home cage.

On day 5, one day after the last trial, retention performance was examined in a single probe trial. In this test, the platform was removed from the pool and the animals were allowed to swim freely for a 60 s period. The rat was released into the water tank from a position opposite to the quadrant where the platform had been during the training sessions (14).

### 2.5. Statistics

All values were expressed as the mean ±SEM. Comparisons of the escape latencies during the 16 trials of place learning of the Morris water maze were analyzed by one-way analysis of variance (ANOVA) followed by the Newman-Keuls test. The swimming speed and the probe trial were analyzed with the non-parametric Kruskall-Wallis H test. Statistical significance was defined as $p<0.05$ for all tests.

## 3. Results

### 3.1. Effect of bile duct ligation on spatial learning

Figure 1 shows the results comparing unoperated rats (control or intact control) versus sham-op and BDL rats. The data show that there is no significant difference between control, sham and bile duct ligation groups in the same day ($p>0.05$). No differences in swim speed or retention performance were found between the groups (data not shown).

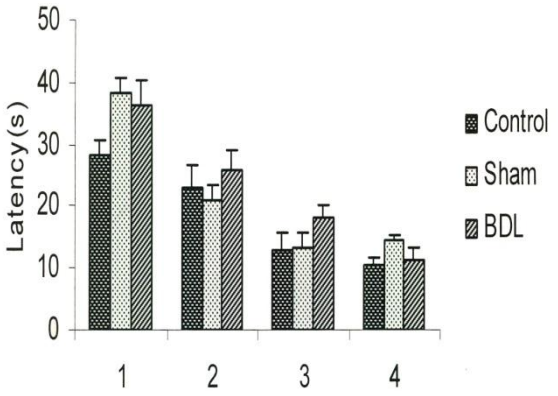



**Fig.1.** Effect of surgery and ligation of bile duct on spatial learning in the Morris Water Task.

The data are expressed as mean latencies+S.E.M.( Values averaged over four trials per session). No significant differences between the three groups in the same day were observed.

### 3.2. Effect of acute and chronic administration of naloxone in sham-operated rats

Figure 2 shows that there is no significant difference between both acute and chronic administration of naloxone and sham groups in the same day ($p>0.05$). No differences in swim speed or retention performance were found between the groups (data not shown).

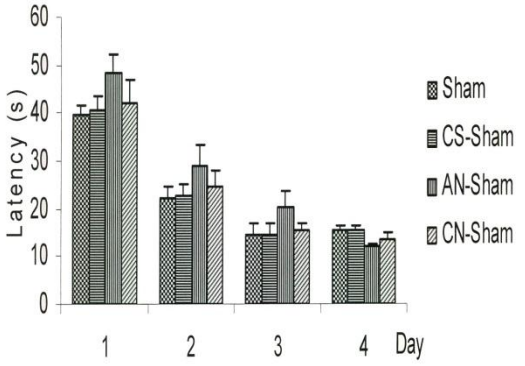
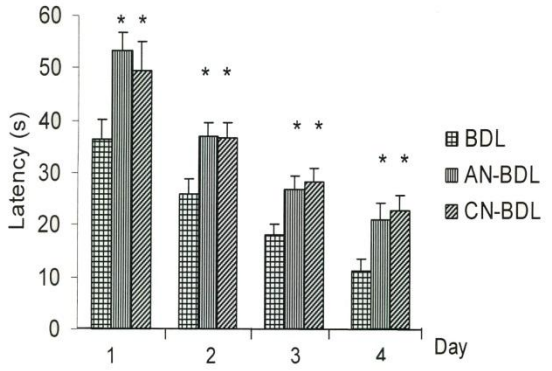



**Fig.2.** Effect of implantation of osmotic minipumps and injection of naloxone both acute (i.p.) and chronic (by osmotic minipumps) on spatial learning in the Morris Water Maze.

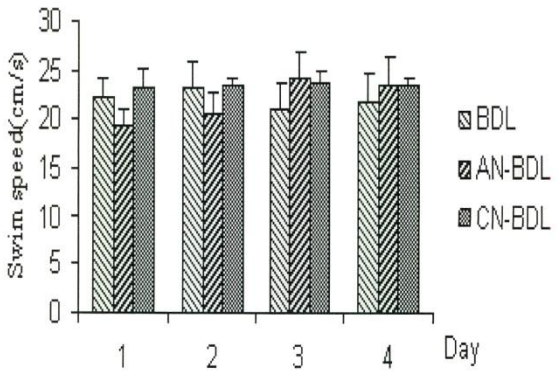
The data are expressed as mean latencies+S.E.M. (Values averaged over four trials per session). No significant differences between the four groups in the same day were observed.

### 3.3. Effect of acute and chronic naloxone administration on spatial learning in bile duct-ligated rats

Figure 3A shows the results comparing BDL rats versus AN-BDL and CN-BDL rats. A significant difference was found in escape latencies ($p<0.05$). BDL rats treated with acute (5 mg/kg) naloxone or chronic (3 mg/kg/h) naloxone showed a significantly ($p<0.05$) retarded learning compared to the BDL group. This finding demonstrated that administration of naloxone both acute and chronically impaired spatial learning of BDL rats. Figure 3B shows there was no significant difference in swimming speed among these groups ($p>0.1$).




A




B

**Fig.3.** (A) Mean escapes latencies in seconds per group and per session of four trials over 4 days in the Morris Water Maze. (B) Effect of injection of acute and chronic Naloxone on swim speed.

Three groups were tested: BDL, AN-BDL (5mg/kg, i.p.) and CN-BDL (3mg/kg/h by osmotic minipumps). The data are expressed as mean+S.E.M. A significant change of latency compared to corresponding BDL group is marked ($p<0.05$).

### 3.4. Retention

Retention performance was examined in all rats one day after the last trial of training days in a probe trial. No significant difference between groups with regard to time spent in target quadrant was found. Also, no significant differences were found in swim speed between the groups during the probe trial in all experiments (data not shown).

### 3.5. Motor activity and motivation test

Figure 4 shows mean escape latencies on day 6 with visible platform in all groups. There was no significant difference among the groups.

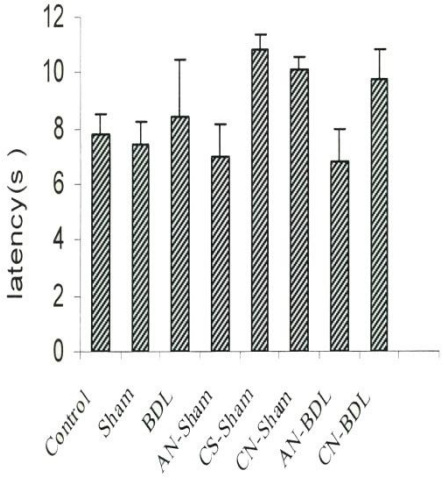



**Fig.4.** Mean escape latencies in seconds per group and session for trials on day 6 (visible platform) in the Morris Water Maze. All groups were tested.

## 4. Discussion

The results presented in this study showed that naloxone, as an opioid receptor antagonist in both acute (5 mg/kg, i.p.) 20 minute before test and chronic (3 mg/kg/h, s.c, continually 7 days) administration produces an impairment of spatial learning in the cholestatic rats without affecting motor performance as measured by swim speed. However, the retention test was not significantly affected, suggesting that naloxone mainly affects acquisition processes with marginal effects on storage and long-term memory processes. It is indicating that endogenous opioids play a marginal role in spatial learning in cholestatic rats. It is also notable that the spatial learning ability was not completely blocked, since the latency in the treated group decreased over the four consecutive days. The results also indicate different search patterns in swim path for different groups. The BDL (cholestatic) animals found the platform in a distinctive way without extensive searching. However, the animals treated with naloxone had to search the platform for a significantly longer time period than the control and cholestatic animals which indicates a more random search pattern compared to control and cholestatic rats.

Previous investigators have shown that opioid peptides, such as met-enkephalin, are increased in acute liver disease (15) and biliary cirrhosis (16) has also been reported and opioid receptor antagonists such as naloxone and nalmifen reduce the pruritus of cholestasis (17) as well as expression of some opioid withdrawal reactions on starting the drug (18). Swain et al reported that total opioid activity in plasma was threefold greater in bile duct resected rats than in sham operated and unoperated controls (19). It was also reported that adrenal secretion of opioid peptides is increased (20) and central mu-opioid receptors are down- regulated in a rat model of cholestasis (21).

Several studies have reported that opioid peptides and their receptors are abundant in the hippocampus and are likely to have a role in the physiological regulation of hippocampal function (10). Within the hippocampus, opioid peptides are particularly concentrated in the mossy fibers (MF) axons of the granule cells, and the release of both proenkephalin and prodynorphin-derived opioid peptides from mossy fibers in the CA3 region has been demonstrated (22). The release of endogenous opioids in the brain has been shown to play a dual role in the memory performance. It has been shown that activation of mu-opioid receptors by endogenous opioids in the CA3 region facilitates mossy fiber long-term potentiation (LTP) (23) and activation of kappa opioid receptors by endogenous opioids blocks LTP induction (24). Derrick and Martinez initially showed that in anesthetized rats, (D-Ala2. NMePhe4, Glyol5) enkephalin (DAMGO), a mu-opioid receptor agonist, facilitated mossy fiber LTP induction, and naloxone, a mu-opioid receptor antagonist, blocked mossy fiber LTP (25). This study is in agreement with previous results showing that naloxone block mossy fiber LTP. Consistent with that report, other researcher also showed that naloxone inhibited mossy-fiber LTP in rat hippocampus (23).

It has also been showed that using a highly selective endogenous mu-opioid receptor agonist can lead to impairment in acquisition (26). Also, some studies have shown that activation of μ-and δ-receptor can lead to memory impairments in various cognitive tests (27). Ukai et al reported that endomorphins l and 2 impair long-term memory through the mediation of μ-opioid receptors in the brain (28).

On the other hand, many studies have shown that circulating neurotoxins play a role in cognitive deficits in liver failure patients (29).

Long-term (more than 3 weeks) cholestasis results in spatial memory deficits in rats that correlate with anemia and hyperbilirubinemia encephalopathy and early biliary decompression of obstructive jaundice improves spatial memory deficits, possibly related to the recovery of the serum ammonia and hemoglobin levels (30).

We showed that there was no impairment of spatial learning in the 7-day BDL rats compare to controls. Therefore, we could conclude that it might be a balance between endogenous opioids LTP induction, besides down-regulation of opioid receptors and neurotoxic-induced cognitive deficits in 7-day BDL rats and so we could not detect any impairment in spatial memory. Acute naloxone treatment could block opioid receptors, therefore the protective effect of opioid peptide would be removed and neurotoxins impair spatial memory in cholestatic rats.

Another finding of this study was that chronic administration of naloxone, as an opioid receptor antagonist, also produces an impairment of spatial learning in the cholestatic rats without affecting motor performance as measured by swim speed (31). It has been reported that chronic treatment with opiate antagonists such as naloxone increases the number of opioid receptors in rat brain indicating that binding sites are up-regulated in these conditions (32). So, we could suggest that when we remove naloxone pump after 7 days, increased level of endogenous opioids besides up-regulation of opioid receptors and neurotoxins causes spatial memory impairment in BDL rats.

In conclusion, greater content of endogenous opioids in BDL rats treated with acute or chronic naloxone could play a dual role in spatial memory which might be due to possible changes in the number of opioid receptors.

## References


1. Zarrindast MR, Hoseindoost S, Nasehi M. Possible interaction between opioidergic and cholinergic systems of CA1 in cholestasis-induced amnesia in mice. Behavioural Brain Research 2012; 28(1):116-24.
2. Kremer AE, Namer B, Bolier R, Fischer MJ, Oude Elferink RP, Beuers U. Pathogenesis and Management of Pruritus in PBC and PSC. Digestive Diseases 2015;33 Suppl 2:164-75.
3. Hasanein P, Parviz M. Role of GABAA receptor in modulation of acute thermal pain using a rat model of cholestasis. Pharmacology Biochemistry & Behavior 2014 Sep;124:226-30.
4. Haj-Mirzaian A, Hamzeh N, Javadi-Paydar M, Abdollahzadeh Estakhri MR, Dehpour AR. Resistance to depression through interference of opioid and nitrergic systems in bile-duct ligated mice. European Journal of Pharmacology 2013;708(1-3):38-43.
5. Bunchorntavakul C, Reddy KR. Pruritus in chronic cholestatic liver disease. Clinical Liver Disease 2012; 16(2):331-46.
6. Swain MG. Alterations in hypothalamic mu-opiate receptor-mediated responses but not methionine enkephalin or proenkephalin messenger RNA levels in rats with acute cholestasis. Hepatology 1994; 20(3):700-5.
7. Inan S, Cowan A. Reduced kappa-opioid activity in a rat model of cholestasis. European Journal of Pharmacology 2005; 518(2-3):182-6.
8. Bodnar RJ. Endogenous opiates and behavior: 2015. Peptides. 2017 Feb;88:126-188.
9. Hawes SL, Salinas AG, Lovinger DM, Blackwell KT. Long-term plasticity of corticostriatal synapses is modulated by pathway-specific co-release of opioids through κ-opioid receptors. Journal of Physiology 2017;595(16):5637-5652.
10. Wang SY, Duan YL, Zhao B, Wang XR, Zhao Z, Zhang GM. Effect of delta opioid receptor activation on spatial cognition and neurogenesis in cerebral ischemic rats. Neuroscience Letters 2016;620:20-6.

11. Rizi AA, Reisi P, Naghsh N. Effect of forced treadmill exercise and blocking of opioid receptors with naloxone on memory in male rats. Advanced Biomedical Research 2016;5:20.

12. Gold A, Rogers A, Cruchley E, Rankin S, Parmar A, Kamath BM, Avitzur Y, Ng VL. Assessment of School Readiness in Chronic Cholestatic Liver Disease: A Pilot Study Examining Children with and without Liver Transplantation. Canadian Journal of Gastroenterology and Hepatology 2017;9873945.

13. Huang LT, Hsieh CS, Chou MH, Chuang JH, Liou CW, Tiao MM, Lai MC. Obstructive jaundice in rats: cause of spatial memory deficits with recovery after biliary decompression. World Journal of Surgery 2004;28(3):283-7.

14. Mokhtari Z, Baluchnejadmojarad T, Nikbakht F, Mansouri M, Roghani M. Riluzole ameliorates learning and memory deficits in Aβ25-35-induced rat model of Alzheimer's disease and is independent of cholinoceptor activation. Biomedicine & Pharmacotherapy 2017;87:135-144.

15. Bergasa NV, Sabol SL, Young WS 3rd, Kleiner DE, Jones EA. Cholestasis is associated with preproenkephalin mRNA expression in the adult rat liver. American Journal of Physiology1995;268(2 Pt 1):G346-54.

16. Owczarek D, Garlicka M, Pierzchała-Koziec K, Skulina D, Szulewski P. Met-enkephalin plasma concentration and content in liver tissue in patients with primary biliary cirrhosis. Przegl Lek 2003;60(7):461-6

17. Ebrahimkhani MR, Moezi L, Kiani S, Merat S, Dehpour AR. Opioid receptor blockade improves mesenteric responsiveness in biliary cirrhosis. Digestive Diseases and Sciences 2008;53(11):3007-11.

18. Jones EA, Bergasa NV. The pruritus of cholestasis: from bile acids to opiate agonists. Hepatology 1990;11(5):884-7.

19. Swain MG, Rothman RB, Xu H, Vergalla J, Bergasa NV, Jones EA. Endogenous opioids accumulate in plasma in a rat model of acute cholestasis. Gastroenterology 1992;103(2):630-5.

20. Swain MG, MacArthur L, Vergalla J, Jones EA. Adrenal secretion of BAM-22P, a potent opioid peptide, is enhanced in rats with acute cholestasis. American Journal of Physiology 1994;266(2 Pt 1):G201-5.

21. Ahmadi S, Karami Z, Mohammadian A, Khosrobakhsh F, Rostamzadeh J. Cholestasis induced antinociception and decreased gene expression of MOR1 in rat brain. Neuroscience 2015;284:78-86.

22. Zamir N, Quirion R, Segal M. Ontogeny and regional distribution of proenkephalin- and prodynorphin-derived peptides and opioid receptors in rat hippocampus. Neuroscience 1985;15(4):1025-34.

23. Harte-Hargrove LC, Varga-Wesson A, Duffy AM, Milner TA, Scharfman HE. Opioid receptor-dependent sex differences in synaptic plasticity in the hippocampal mossy fiber pathway of the adult rat. Journal of Neuroscience 2015;35(4):1723-38.

24. Bodnar RJ. Endogenous opiates and behavior: 2012. Peptides 2013;50:55-95.

25. Derrick BE, Martinez JL Jr. Opioid receptor activation is one factor underlying the frequency dependence of mossy fiber LTP induction. Journal of Neuroscience 1994 ;14(7):4359-67.

26. Laredo SA, Steinman MQ, Robles CF, Ferrer E, Ragen BJ, Trainor BC. Effects of defeat stress on behavioral flexibility in males and females: modulation by the mu-opioid receptor. European Journal of Neuroscience 2015 Feb;41(4):434-41.

27. Cao LQ, Wen J, Liu ZQ. Opioid μ receptors mediate the stress-induced spatial reference memory impairment. Sheng Li Xue Bao 2015;67(2):173-80.

28. Ukai M, Watanabe Y, Kameyama T. Effects of endomorphins-1 and -2, endogenous mu-opioid receptor agonists, on spontaneous alternation performance in mice. European Journal of Pharmacology 2000;395(3):211-5.

29. Freitas Castro V, Nascimento Alves P, Franco AC, Martins IP, Conceição I. Cognitive impairment in liver transplanted patients with transthyretin-related hereditary amyloid polyneuropathy. Amyloid 2017;24(2):110-114.

30. Huang LT, Hsieh CS, Chou MH, Chuang JH, Liou CW, Tiao MM, Lai MC. Obstructive jaundice in rats: cause of spatial memory deficits with recovery after biliary decompression. World Journal of Surgery 2004;28(3):283-7.

31. Ghiassy B, Rahimi N, Javadi-Paydar M, Gharedaghi MH, Norouzi-Javidan A, Dehpour AR. Nitric oxide mediates effects of acute, not chronic, naltrexone on LPS-induced hepatic encephalopathy in cirrhotic rats. Canadian Journal of Physiology and Pharmacology 2017;95(1):16-22.

32. Chen JJ, Dymshitz J, Vasko MR. Regulation of opioid receptors in rat sensory neurons in culture. Molecular Pharmacology 1997; 51(4):666-73.