DOI: 10.4149/BLL_2014_070

Bratisl Lek Listy 2014; 115 (6)
357 – 361

EXPERIMENTAL STUDY

The olive leaf extract attenuates bacterial translocation and liver damage in obstructive jaundice

Yildirim M[1], Amanvermez R[2], Polat C[1], Karadag A[3], Karayigit MO[4], Erzurumlu K[1]

Department of General Surgery, Faculty of Medicine, Ondokuz Mayis University, Samsun, Turkey.
aramazan@omu.edu.tr

Abstract: *Objectives*: The olive leaf extract (OLext) is known to possess many biological properties including a powerful antioxidant. This study aimed to investigate the protective effects of postoperative nutrition with OLext and glutamine on bacterial translocation (BT) and liver damage in obstructive jaundice.
Materials and methods: Totally, 50 rats were randomly divided into the five groups of 10 each. The common bile duct was ligated in all animals, excepting in the group 1. Postoperative nutrition was given to all groups for ten days. The rats in the Group 1 and 2 were fed a normal diet, Group 3 rats were fed an additional glutamine (1 g/kg/day), and Group 4 and 5 rats were fed an additional OLext (1 ml of 1/2 diluted and pure form/kg/day). Biochemical, microbiological and liver histopathological changes were evaluated.
Results: BT in the Groups 3, 4, and 5 was significantly lower than in the Group 2. The values of aspartate transaminase (AST), alanine transaminase (ALT), γ-glutamyl transferase (γ-GT) and alkalen phosphatase (ALP) in blood were increased in obstructive jaundice, but the levels of these tests were statistically lower in glutamine and OLext groups when compared to the Group 2. Histopathological changes were observed low in the liver in OLext and glutamine groups.
Conclusions: The present data has demonstrated that the supplementation of olive leaf extract and glutamine reduce the incidence of BT and liver damage in obstructive jaundiced rats *(Tab. 4, Fig. 1, Ref. 23)*. Text in PDF *www.elis.sk.*
Key words: olive leaf extract, obstructive jaundice, bacterial translocation, liver damage.

Obstructive jaundice subsequently leads to significant functional and pathological damage in the liver. After obstruction in the bile tree, a series of pathologic changes occur in the organism including diminishing of antibacterial and detergent function of bile salts, immune system suppression and intestinal barrier function failure, which all lead to BT (1, 2, 3). Not only BT occurred in obstructive jaundice, but also publications have reported BT in several clinical conditions, such as acute pancreatitis, cirrhosis, hemorrhagic shock, heart failure, cardiopulmonary bypass, abdominal surgery and bowel transplant (4). In relation to the issue, obstructive jaundice can be induced by common bile duct ligation, which causes parenchymal cell damage that may ultimately lead to hepatic injury and then fibrosis and cirrhosis (5).

As cited in literature, the amino acid glutamine is quantitatively the most important fuel for intestinal tissue. It is recognized as an important dietary component for the maintenance of gut integrity as well. Likewise, glutamine administration decreases bacterial translocation, being beneficial to critically ill and other patients (6, 7).

Several reports have indicated that olive leaf extract containing oleuropein possesses a wide range of pharmacologic and health promoting properties such as immune-stimulant, anti-inflammatory, antioxidant, antidiabetic, cardioprotective, and anti-thrombotic effects. Many of these properties have been described as resulting from the antioxidant character of oleuropein (8–10). Despite many reports on olive leaf or its major compound, oleuropein, the effects of OLext in terms of antimicrobial activity and protective nutritional agent are unknown well in obstructive jaundice. The goal of this study was to examine the protective effects of postoperative nutrition with OLext and glutamine on bacterial translocation and liver damage in experimental obstructive jaundice.

Material and methods

The study had the approval of the Ondokuz Mayis University Ethical Committee for animal welfare regulation. This study was carried out in the Experimental Research Center, Ondokuz Mayis University, Samsun / Turkey. Totally, 50 Sprague-Dawley male rats, weighing 250–300 g, were allowed to adapt to our laboratory environment for 1 week before the onset of the experiment. The rats were housed in a cage with wood chip bedding and fed on standard laboratory chow (SLC) and water ad libitum. They were maintained on a 12 h light: dark cycle with a constant room tem-

[1]Department of General Surgery, Faculty of Medicine, Ondokuz Mayis University, Samsun, Turkey, [2]Medical Biochemistry, Faculty of Medicine, Ondokuz Mayis University, Samsun, Turkey, [3]Medical Microbiology, Faculty of Medicine, Ondokuz Mayis University, Samsun, Turkey, and [4]Department of Pathology, Faculty of Veterinary, Ondokuz Mayis University, Samsun, Turkey

Address for correspondence: R. Amanvermez, Dr, Department of Medical Biochemistry, Faculty of Medicine, Ondokuz Mayis University, TR-55139 Samsun, Turkey.
Phone: +90.362.3121919, Fax: +90.362.4576041

The authors have no any personal or company support for supplying chemical substances and no conflicts of interest o declare

perature at 22 ± 1 °C. The rats were randomized into the five groups (n = 10): *Group 1*, sham-operated group: A midline laparotomy was performed; the common bile duct was dissected and isolated. The rats were fed with SLC for ten days after operation. *Group 2*, control group: The common bile duct was dissected and ligated with 4/0 silk suture. The rats were fed with SLC for ten days after operation. *Group 3*, glutamine (purchased from Sigma Co.) group: The common bile duct ligated rats were fed with SLC for three days, and then animals were fed on SLC along with L-glutamine (1 g/kg/day, dissolved in saline) solution given intragastrically by feeding tube to rats for the next seven days postoperatively. *Group 4*, OLext (1/2 diluted liquid form) group: After bile duct ligation, rats were fed with SLC for three days, and then animals were fed on SLC along with 1 ml/kg/day of OLext (supplied free of charge from Kale Naturel Limited Co., Edremit-Balikesir/Turkey) solution (1/2 diluted or suspended in saline) administered intragastrically by feeding tube to rats during the next seven days postoperatively. *Group 5*, OLext (pure liquid form) group: similar procedures to the Group 4, except that OLext (pure liquid form/kg/day) was given. It should be noted that all of the rats whose common bile duct was ligated, developed clinically obstructive jaundice within 2–3 days after operation. Blood and tissue samples were collected on the tenth postoperative day under anesthesia (ketamine, 50 mg/kg and xylazine, 5 mg/kg i.p.) and sterile conditions, and then all rats were sacrificed by exsanguinations.

Biochemical Measurements

An aliquot of 2 ml blood from intra-cardiac was obtained for the measurement of AST, ALT, ALP, γ-GT, total and direct bilirubin. The levels of these tests were measured by a clinical chemistry laboratory.

Bacterial Translocation

Using an aseptic technique the tissue samples obtained from the mesenteric lymph nodes (MLNs), liver, and spleen were harvested with sterile instruments, weighed and placed in special eppendorf tubes containing sterile saline, and then homogenized during 5 minutes at 50 Hz with Tissuelyzer (QIAGEN®, Germany). 0.1 ml of homogenized organ samples was inoculated on blood agar (Oxoid, UK) and Eosin Metilen Blue agar (GBL, Turkey). All plates were incubated at 37 ºC during 24–48 hours and observed for growth daily. The growth of bacteria in culture-plate was observed at 24–48 hours. Any growth in the plates was considered positive and expressed as colony-forming units per mg tissue (cfu/mg). 0.1 ml blood samples taken from the vena porta of rats were inoculated on the media of anaerobic and aerobic blood cultures. These blood cultures were observed by incubation in Bact/alert (Biomerieux inc. Durham USA) blood culture system at 37 ºC for 7 days. During incubation, 0.1 ml specimen taken from flasks given growth signalling was cultured on blood-agar and EMB-agar. Vitek 2 (bioMerieux, France) system was used to identify the type of all cultured bacteria.

Histopathology

The liver tissue samples were washed in an isotonic sodium chloride solution, dried with filter paper and fixed in 10 % neutral buffered formalin, embedded in paraffin, and sectioned at 4–6 μm. Tissue sections were stained histochemically with hematoxylin and eosin (HE) and evaluated under a light microscope (Nikon Eclipse E600) by a pathologist blind to the experimental procedures. The scoring of the histopathological changes of the liver was composed with respect to the scoring system developed by Yetim et al (12). Microscopic changes in the liver were noted and then pathological findings categorized as necrosis: 0 (none); I (focal (single focus in single lobule); II (Marked (single focus in all lobules) and III (widespread (panlobular or several foci per lobule in all lobules) and severity of micro-abscess was classified as 0 (none); I (mild); II (moderate), and III (severe) grades.

Statistical analysis

The statistical analyses were performed using the SPSS 15.0 for Windows. All data was checked for the normality of distribution by Shapiro–Wilk test. Those that had normal distribution were statistically evaluated by Tukey HSD test. p values < 0.05 were accepted to be statistically significant. Statistical differences were set at a 95 % confidence interval. Non-normally distributed histological score evaluations were analyzed by Kruskal–Wallis test, and then, group comparisons were performed by the Mann–Whitney U-test corrected with Bonferroni.

Results

All rats in the experimental groups survived the surgical procedures and there were no deaths during the experimental period. On the 10th day, all BDL animals became clinically overt icteric, at that time, total and direct bilirubin values increased significantly in all animals of the Groups 2, 3, 4, and 5 compared to the sham group ($p < 0.001$). There was no significant difference

Tab. 1. The levels of biochemical laboratory test in the experimental groups.

Groups (n=10)	AST (U/L)	ALT (U/L)	ALP (U/L)	γ-GT (U/L)	D.Bilirubin (mg/dl)	T.Bilirubin (mg/dl)
1. Sham	152 ± 76	91 ± 23	196 ± 27	32 ± 7	0.5 ± 0.1	0.6 ± 0.1
2. Control-(BDL)	553 ± 109	272 ± 56	578 ± 47	102 ± 19	7.6 ± 0.2	9.2 ± 0.2
3. Glutamin (1 g/kg)-BDL	437 ± 67	138 ± 33	447 ± 64	49 ± 24	6.9 ± 0.1	9.3 ± 0.1
4. OLext (½ diluted form/kg)-BDL	462 ± 80	146 ± 35	487 ± 63	58 ± 22	7.3 ± 0.1	9.0 ± 0.1
5. OLext (pure form/kg)-BDL	460 ± 79	146 ± 29	489 ± 67	58 ± 26	7.2 ± 0.2	9.4 ± 0.2

Data are expressed as mean ± standard deviation.

Tab. 2. Incidence of BT in blood and cultured tissue materials (cfu/mg) in the groups.

Groups (n=10)	Cultured Materials			
	Blood	Liver	Spleen	MLNs
1.Sham	*1/10 (10%)	BT not	BT not	BT not
2.Control-(BDL)	9/10 (90%)	1.25 (0.3-4.0)x10^4	0.35 (0.0-1.6)x10^4	2.0 (0.02-7)x10^4
3.Glutamin (1 g/kg)-BDL	4/10 (40%)	0.02 (0.0-0.8)x10^4	0.00 (0.0-0.2)x10^4	0.0 (0.0-0.8)x10^4
4. OLext (½ diluted form/kg)-BDL	4/10 (40%)	0.01 (0.0-0.2)x10^4	0.00 (0.0-0.2)x10^4	0.0 (0.0-0.5)x10^4
5. OLext (pure form/kg)-BDL	4/10 (40%)	0.0 (0.0-0.1)x10^4	0.00 (0.0-0.1)x10^4	0.0 (0.0-0.3)x10^4

*One plate was contaminated in sham. Data are expressed as median (min-max) for liver, spleen and MLNs.

Tab. 3. Pathologic grade rates according to necrosis in the liver.

Groups	Grade 0	Grade I	Grade II	Grade III
1.	10/10 (%100)	0/10 (0 %)	0/10 (0 %)	0/10 (0 %)
2.	3/10 (%30)	3/10 (%30)	2/10 (%20)	2/10 (%20)
3.	6/10 (%60)	4/10 (%40)	0/10 (0 %)	0/10 (0 %)
4.	5/10 (%50)	4/10 (%40)	1/10 (%10)	0/10 (0 %)
5.	6/10 (%60)	4/10 (%40)	0/10 (0 %)	0/10 (0 %)

Tab. 4. Pathologic grade rates according to micro-abscess in the liver.

Groups	Grade 0	Grade I	Grade II	Grade III
1.	10/10 (%100)	0/10 (0%)	0/10 (0%)	0/10 (0%)
2.	5/10 (%50)	3/10 (%30)	2/10 (%20)	0/10 (0%)
3.	7/10 (%70)	3/10 (%30)	0/10 (0%)	0/10 (0%)
4.	6/10 (%60)	4/10 (%40)	0/10 (0%)	0/10 (0%)

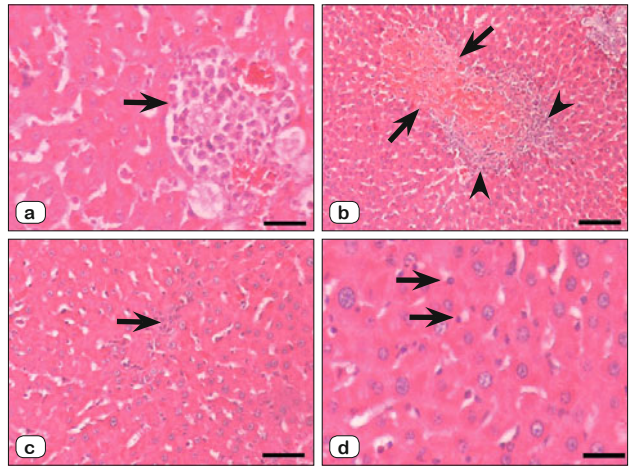

Fig. 1. Morphologic changes of obstructive jaundice in the liver on 10 days after operation. Representative photo of liver: a) Shows micro-abscess in the liver (arrow) (Group 2), Haematoxylin-Eosin stain, Bar: 15 µm. b) Shows an intense inflammatory area (arrowheads) around a large necrosis area (arrows) in the liver (Group 2), Haematoxylin-Eosin stain, Bar: 30 µm. c) Shows little necrosis in the liver (arrow), Haematoxylin-Eosin stain, Bar: 30 µm, and d) Rats that were treated with OLext (pure form) had a smaller degree of inflammation in the liver (arrows) (Group 5), Haematoxylin-Eosin stain, Bar: 15 µm.

between glutamine and OLext supplemented groups ($p > 0.05$). As shown in the Table 1, the levels of AST, ALT, ALP, and γ-GT in serum were statistically higher in the Group 2 when compared to the sham group ($p < 0.001$). However, the levels of these tests were decreased statistically in the Groups 3, 4, and 5 compared to the Group 2 ($p < 0.001$). No statistically significant difference was obtained for any of enzymatic parameters studied among the supplemented postoperative nutrition groups ($p > 0.05$).

The incidence of BT to liver, spleen, MLNs, and blood for all groups are summarized in the Table 2. The most common encountered pathogens in the MLNs, spleen, liver and blood were *E. coli, Enterobacter spp, K. pneumoniae,* and *P. myrabilis*. The instances of BT in the Group 2 were significantly higher than those of the Group 1 ($p < 0.007$). However, BT in liver and MLNs was lower in the Groups 3, 4, and 5 compared to the Group 2 ($p < 0.0001$). In addition to this, BT in the spleen was lower in the Groups 3, 4, and 5 when compared to the Group 2 ($p < 0.05$).

The findings of necrosis and micro-abscess in the liver are shown in Figure 1 and Tables 3–4. Histopathological examination of liver specimens revealed intense inflammatory areas and mild to moderate abscesses in the Group 2 as compared to the Group 1. These pathological features were lower in the Groups 3, 4, and 5 compared to the Group 2. Also, rats in the group 5 that were treated with OLext had a smaller degree of inflammation in the liver.

Discussion

Bacterial translocation from the gastrointestinal system to extra-intestinal organs has been shown to occur under a variety of conditions like intestinal obstruction, intestinal ischemia, burns, hemorrhagic shock, and obstructive jaundice (13–16). Raised intestinal permeability and bacterial translocation were reported following both experimental biliary obstruction and jaundiced patients (17, 18). Also, icteric patients undergoing invasive diagnostic and treatment procedures are at risk of serious preoperative or postoperative complications and death. Sepsis and systemic endotoxaemia that constitute the bulk of this morbidity and mortality, can occur in obstructive jaundice (19, 20).

Experimental studies have indicated that several substances such as glucan, glutamine, pentoxifylline, growth hormone, propolis, and bile salts reduce the incidence of bacterial translocation in disease conditions like intestinal obstruction, intestinal ischemia-reperfusion, and obstructive jaundice. In the present study, we hypothesized whether treatment with OLext or glutamine could be given in the early stages of obstructive jaundice and whether BT or the pathological features of the liver during obstructive jaundice might be diminished or prevented postoperatively. Previous reports have implied that glutamine is a metabolic fuel for small intestine mucosal cells, lymphocytes, macrophages and fibroblasts. It also stimulates gut mucosal cell proliferation, and maintains intestinal

mucosal integrity and immunostimulating activity (18, 20). In this study, positive cultures of blood, liver, spleen and mesenteric lymph nodes in the Group 3 (glutamine) were significantly lower than those of the Group 2. OLext, used for the first time in this study, decreased bacterial translocation in jaundiced rats of the groups 4 and 5 as shown in Table 2. These results may show that glutamine and OLext supplemented to rat diet possessed antimicrobial properties in obstructive jaundice.

Apart from bacterial translocation, endotoxemia and physical injury of intestinal mucosa, intestinal barrier failure in obstructive jaundice is associated with significant intestinal oxidative stress (2, 21). In addition, it has been reported that bile duct obstruction induces oxidative stress and decreases the production of different enzymes with antioxidant properties and reduced glutathione as well as vitamin A and E in the liver (5, 22). If OLext containing oleuropein supplemented to rat diet has a beneficial effect on intestinal function and liver damage in obstructive jaundice, therefore, these protective effects may be due to its potent antioxidant action as well as many of the pharmacologic features of OLext or oleuropein (8–10).

In cholestatic diseases or physical obstruction of the biliary tract (extrahepatic cholestatic or obstructive jaundice), where bile flow is obstructed due to obstruction to bile duct flow (extrahepatic cholestasis) or impairment of bile synthesis by the hepatocytes (intrahepatic cholestasis), increased direct bilirubin levels are thought to be associated with a higher elevation of ALP than of serum transaminases (AST and ALT). Transaminase elevations usually imply liver cell injury and death, which may or may not be associated with cholestasis (11). In the current study, it seems that mostly direct bilirubin levels along with total bilirubin lead to be obstructive icterus visually because of hyperbilirubinemia in rats. Also, we observed bacterial translocation to liver, spleen, mesenteric lymph nodes, and blood and the harmful effect of obstructive jaundice on the biochemical and histopathological parameters in the serum and liver tissue. OLext at two different doses in jaundiced rats exhibited a pronounced decrease in the serum AST, ALT, ALP, and γ-GT enzymes activities compared to those of the Group 2, ameliorated bacterial translocation in the Groups 4 and 5. Histological examinations showed that OLext treatment attenuated the harmful effects of obstructive jaundice in terms of necrosis and micro-abscess in liver tissue with respect to the Group 2 as shown in Figure1, Tables 3 and 4. Omagari et al have recently reported that olive leave extract prevents spontaneous occurrence of non-alcoholic steatohepatitis in rats as well (23). From these findings, it may be concluded that OLext has a protective effect in obstructive jaundice.

In view of literature along with the above statements, any abdominal and liver surgery or in bilio-pancreatic obstructive states included obstructive jaundice can lead to a diminished glutamine concentration and the formation of oxidative stress on the intestinal mucosa and liver (21–23). As a result, oxidative stress may be decreased by the administration of OLext in both tissues. It is known that large patients with obstructive jaundice have been treated and followed up in clinics. These patients might be at risk of bacterial translocation and liver damage as the diagnoses and preparation before surgery takes time. So, we may suggest that nutritional support containing OLext and glutamine might be administered to a patient in bilio-pancreatic obstructive diseases, because our results indicate that supplementation of oral glutamine and OLext in the presence of obstructive jaundice seems to ameliorate the bacterial translocation and the liver histo-pathological features.

Consequently, it may be concluded that glutamine, given orally, reduces bacterial translocation and liver damage in obstructive jaundice. The administration of OLext attenuated bacterial translocation considerably and liver injury because of its protective effects on intestinal mucosa integrity and liver tissue of jaundiced rats. Controlled randomized clinical studies are necessary for the routine use of OLext plus glutamine.

References

1. Sakrak O, Akpinar M, Bedirli A, Akyurak N, Aritas Y. Short and long-term effects of bacterial translocation due to obstructive jaundice on liver damage. Hepato-gastroenterology 2003; 50 (53): 1542.

2. Assimakopoulos SF, Vagianos CE, Patsoukis N, Georgiou C, Nikolopoulou V, Scopa CD. Evidence for intestinal oxidative stres in obstructive jaundice-induced gut barrier dysfunction in rats. Acta Physiol Scand 2004; 180: 177.

3. Abdeldayem H, Ghoneim E, Ahmad-El Refaei A, Abou-Gabal A. Obstructive jaunduce promotes intestinal barrier dysfunction and bacterial translocation: experimental study. Hepatol Int 2007; 1: 444.

4. Balzan S, Quadros CA, Cleva R, Zilberstein B, Cecconello I. Bacterial translocation: Overview of mechanisms and clinical impact. J Gasroenterol Hepatol 2007; 22: 464.

5. Liu TZ, Lee KT, Chern CL, Cheng JT, Stern A, Tsai LY. Free radical-triggered hepatic injury of experimental obstructive jaundice of rats involves overproduction of proinflammatory cytokines and enhanced activation of nuclear factor κB. Annals of Clin Lab Sci 2001; 31 (4): 383.

6. Erbil Y, Berber E, Ozarmagan S, Seven R, Eminoglu L, Calis A et al. The effects of sodium deoxycholate, lactulose and glutamine on bacterial translocation in common bile duct ligated rats. Hepatogastroenterelogy 1999; 46: 2791.

7. Neu J, DeMarco V, Li N. Glutamine: clinical applications and mechanisms of action. Current Opinion in Clinical Nutrition and Metabolic Care 2002; 5: 69.

8. Jemai H, El Feki A, Sayadi S. Antidiabetic and antioxidant effects of hydroxytyrosol and oleuropein from olive leaves in alloxan-diabetic rats. J Agric Food Chem 2009; 57: 8798.

9. Visioli F, Poli A, Galli C. Antioxidant and other biological activities of phenols from olives and olive oil. Med Res Rev 2002; 22: 65.

10. Somova LI, Shode FO, Ramnanan P, Nadar A. Antihypertensive, antiatherosclerotic and antioxidant activity of triterpenoids isolated from olea europaea, subspecies africana leaves. J Ethnopharmacol 2003; 84: 299.

11. Hayat JO, Loew CJ, Asrress KN, Mcintyre AS, Gorard DA. Contrasting liver function test patterns in obstructive jaundice due to biliary structures and stones. Q J Med 2005; 98: 35.

12. Yetim I, Erzurumlu K, Hokelek M, Baris S, Dervisoglu A, Polat C et al. Results of alcohol and albendazole injections in hepetic hydatidosis: Experimental Study. J Gastroenterel Hepatol 2005; 20: 1442.

13. Kocdor MA, Kocdor H, Gulay Z, Gokce O. The effects of pentoxifylline on bacterial translocation after intestinal obstruction. Shock 2002; 18: 148.

14. Medeiros AC, Chacon DA, Sales VSF, Egito EST, Brandao-Neto J, Pinheiro LAM et al. Glucan and glutamine reduce bacterial translocation in rats subjected to intestinal ischemia-reperfusion. J Invest Surg 2006; 19: 39.

15. Margaritis VG, Filos KS, Michalaki MA, Scopa CD, Spiliopoulou I, Nicoloulou VN et al. Effect of oral glutamine administration on bacterial translocation, endotoxemia, liver and ileal morphology, and apoptosis in rats with obstructive jaundice. World J Surg 2005; 29: 1329.

16. Demirbag AE, Turhan N, Ercis S, Hamaloglu E. Does granulocyte-macrophage colony-stimulating factor prevent bacterial translocation in rats surgical and obstructive jaundice? Turk J Gastroenterol 2009; 20: 31.

17. Slocum MM, Sittig KM, Specian RD, Deitch EA. Absence of intestinal bile promotes bacterial translocation. Am Surg 1992; 58: 305.

18. Aldemir M, Geyik MF, Kökoglu ÖK, Büyükbayram H, Hoşoglu S, Yagmur Y.Effects of ursodeoxycholic acid, glutamine and polyclonal immunoglobulins on bacterial translocation in common bile duct ligated rats. ANZ J Surg 2003; 73: 722.

19. Su CH, Peng FK, Lui WY. Factors affecting morbidity and mortality in biliary tract surgery. World J Surg 1992; 16: 536.

20. Miller AL. Therapeutic considerations of L-glutamine: a review of the literature. Altern Med Rev 1999; 4: 239.

21. Assimakopoulos SF, Thomopoulos KC, Patsoukis N, Georgiou CD, Scopa CD, Nikolopoulou VN et al. Evidence for intestinal oxidative stress in patients with obstructive jaundice. Eur J Clin Invest 2006; 36: 181.

22. Aydin S, Aytac E, Uzun H, Altug T, Mansur B, Saygili S et al. Effects of *Ganodermalucidum* on obstructive jaundice-induced oxidative stress. Asian J Surg 2010; 33: 173.

23. Omagari K, Kato S, Tsuneyama K, Hatta H, Sato M, Hamasaki M et al. Olive leafextract prevents spontaneous occurrence of non-alcoholic steatohepatitis in SHR/NDmcr-cp rats. Pathology 2010; 42: 66.

Received January 25, 2013.
Accepted March 8, 2014.