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EXPRESSION OF AQUAPORIN 1, 4, 8 AND 9 IN THE LIVER OF RATS FED WITH THE STANDARD DIET AND SUPPLEMENTED WITH DRIED SEA-BUCKTHORN LEAVES (*HIPPOPHAE RHAMNOIDES* L.). A PILOT STUDY

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Abstract

The purpose of this study was to investigate the effect of a diet supplemented with *Hippophae rhamnoides* L. on the expression of AQP1, AQP4, AQP8 and AQP9 in the liver. The study was carried out on 8 male Wistar rats. Both control (n = 3) and experimental (n = 5) groups were fed ab libitum with a standard diet. The diet of the experimental animals was supplemented with dried sea buckthorn leaves. The rats were sacrificed after 14 days, which included a 7-day introductory period for acclimatization and habituation to diets, and 7 days of the actual experimental period. Western blot technique was used to analyze aquaporin expression in liver homogenates. It was found that the expression of AQP8 and AQP9 was higher than that of AQP1 and AQP4 in all animals tested. The present study found no statistically significant differences in the expression of individual AQPs between the control and experimental groups.

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Introduction

The discovery of water channels – aquaporins (AQPs), which was awarded the Nobel Prize in 2003, initiated a period of intensive research on the role of these proteins in the processes of membrane water transport and other small particles. Presently, it is known that these 'unusual' small proteins, with a molecular weight of about 30 kDa, located in practically all types of cells building the entire body, are involved in many key processes. AQPs play an important role among others in renal water reabsorption and urine concentration, in the regulation of water flow in brain, spinal cord and interstitial fluid surrounding the neurons, in airway hydration, salivary and gastric acid secretion, skin hydration and sweat secretion, cell differentiation and proliferation, milk synthesis during lactation and production of male semen (DAY et al. 2014, AGBANI and ALASTAIR 2021, AZAD et al. 2021, MICHAŁEK et al. 2021). To date, 13 of aquaporins (AQP0-AQP12) have been identified in mammals, 8 of which are located in the liver (AQP0, AQP1, AQP3, AQP4, AQP5, AQP8, AQP9 and AQP11) (MASYUK and LARUSSO 2006, GREGOIRE et al. 2015, CALAMITA et al. 2018, CHENG et al. 2021) In the light of recently published new data on aquaporins, these proteins are of particular interest in the context of bile production and secretion. It is well known that water makes up more than 98% of bile (GREGORIE et al. 2015). Although the transport and synthesis of many of its components is widely described in the literature, the molecular mechanism of water flow within hepatocytes and cholangiocytes is still not fully understood. Among all aquaporins localized in the liver, the best described in the literature are AQP1, AQP4, AQP8 and AQP9. AQP8 and AQP9 are located in hepatocytes, which make up about 80% of all cells that build the liver (TANI et al. 2001, HUEBERT et al. 2002). While AQP1 and AQP4 are present in the cholangiocytes, the epithelial cells of the bile duct (MARINELLI et al. 1997, MARINELLI et al. 2000, TIETZ et al. 2003). The available data show that these aquaporins play a key role in bile secretion, and changes in their expression may affect its canalicular and ductal formation (MARINELLI et al. 1997, HUEBERT et al. 2002, MARINELLI et al. 2004).

The development of civilization and the accompanying increased consumption of processed food, containing numerous preservatives, an increase in the consumption of drugs, as well as environmental pollution negatively affect the liver function. Therefore, people are looking for new, natural and safe factors, the use of which will neutralize the negative impact and will have a protective effect on this organ. The sea buckthorn (*Hippophae rhamnoides* L.), which has long been known in folk medicine as a medicinal plant, is of particular interest in this regard. The multidi-

rectional and wide health-promoting effect of oil, fruit, juices and dried *Hippophae rhamnoides* L. includes anti-inflammatory, anti-cancer, antioxidant and immunomodulating properties (HOU et al. 2017, SHI et al. 2018, GÂLTAN and GUTT 2021). The studies published to date show that the use of sea buckthorn in the diet also affects the liver and protects it against the histopathological changes (GAO et al. 2003, GEETHA et al. 2003, MAHESHWARI et al. 2011, SOLCAN et al. 2013, CZAPLICKI et al. 2017, ZHANG et al. 2018, RAN et al. 2021). It has been shown, inter alia, that liver histology after dosed of sea buckthorn oil showed a reduction in necrosis and fat formation. In addition, taking of oil from sea buckthorn berries decrease the toxic effects of aflatoxin B1, which leads to a reduction in total serum proteins and specifically reduced albumin (SOLCAN et al. 2013). It was also found that the dietary additive of compounds from sea buckthorn berries significant effects in inhibiting the activation of liver stellate cells and reduced the level of inflammatory factors (ZHANG et al. 2018). Particularly noteworthy are results of the study by GAO et al. (2003) on the effect of sea buckthorn in patients with cirrhosis of the liver. It has been shown that after the treatment with sea buckthorn, the levels of laminin, hyaluronic acid, type III and IV collagen, total bile acid in serum decreased significantly compared to before and after treatment in the control group. Sea buckthorn significantly shortened also the time of normalization of transaminases. These findings allowed the authors to suggest that sea buckthorn may be a promising drug for the prevention and treatment of liver cirrhosis. Despite many interesting data, the available literature lacks information regarding the effect of dietary application of *Hippophae rhamnoides* L. on aquaporins located in the liver. Among the many health-promoting properties of this plant, can a positive effect on the expression of AQP1, AQP4, AQP8 and AQP9, and thus on the production and secretion of bile be also observed? Preliminary studies were conducted in response to this question and wide possibilities associated with the use of sea buckthorn in order to identify and analyze the aforementioned aquaporins in the liver of rats fed a standard diet enriched with dried sea buckthorn leaves (*Hippophae rhamnoides* L.).

Materials and methods

Animals and experimental design

All experiments were performed in accordance with the principles and procedures of local Commission of Ethics for Care and use of Laboratory Animals (No. 43/2014). The study was carried out on 8 male Wistar rats.

During the experiment the animals, were remained unified and controlled environmental condition. The rats were kept individually in metabolic cages with 12 hours of light and 12 hours of darkness per day. The ambient temperature was 25°C ± 2°C and relative humidity was ~70%. From the 65 – 70th day of life (animals had a body weight 150 ± 5g) rats were divided into two nutrition groups. Rats from the control group (n = 3) were fed ab libitum with the standard diet (Table 1).

Table 1

Ingredient composition [g/kg] and analyzed nutrient content [%] of test diets fed in experiment (as fed basis)

Item	Control	Experimental
Corn starch	671.40	620.73
Sea buckthorn leaves	–	85.08
Soybean meal	303.17	271.07
Mineral mixture[1]	15.43	13.12
Vitamin mixture[2]	10	10
Analyzed nutrient content [%]		
Dry matter	90.11	89.99
Crude protein	15.86	15.40
Ash	3.73	3.58
Crude fat	0.54	0.82
Crude fibre	0.64	0.24
Total carbohydrates	79.23	79.96

[1] Provided per kg of diet: $CaHPO_4$, 735 g; K_2HPO_4, 81.8 g; K_2SO_4, 68 g; NaCl, 30.6 g; $CaCO_3$, 21.0 g; Na_2HPO_4, 21.4 g; MgO, 25 g; ferric citrate, 5.6 g, $ZnCO_3$, 8.1 g, $MnCO_3$, 4.2 g; $CuCO_3$, 0.33 g, KJ, 7.2 mg, citrid acid, 7.06 g.

[2] Provided per kg of diet: vitamin A, 20 000 IU; vitamin D, 2000 IU; vitamin E, 100 IU; vitamin K, 5 mg; choline, 2 g, para-aminobenzoic acid, 100 mg, inositol, 100 mg; niacin, 40 mg; calcium pantothenate, 40 mg; vitamin B_2, 8 mg; thiamine, 5 mg; vitamin B_6, 5 mg; folic acid, 2 mg; biotin, 0.4 mg; vitamin B_{12}, 0.03 mg

Experimental group (n = 5) were fed ab libitum with the standard diet supplemented with sea buckthorn leaves. After 14 days, which included a 7-day introductory period for the acclimatization of the animals and habituation to diets, and 7 days of the actual period, the rats were sacrificed.

SDS PAGE and Western blot

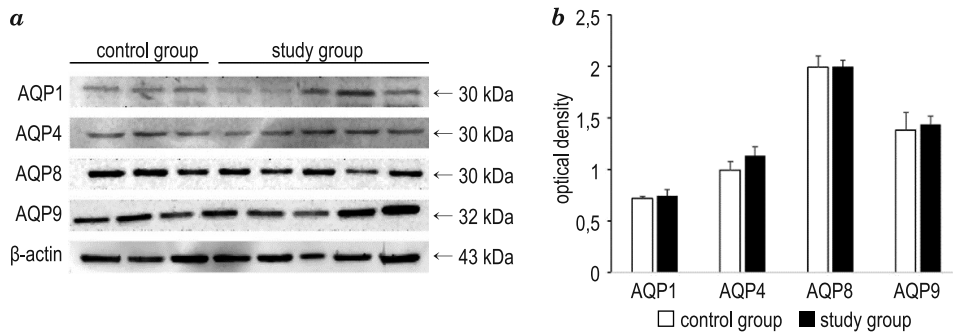
Immediately after slaughter, the livers were rapidly removed, washed with the 0.9% NaCl and cut into representative, small uniform pieces using dissecting tools. The liver samples were placed in lysis buffer (1% SDS, 150 mM NaCl, 50 mM Tris-HCl, pH 7.8, 2 mM PMSF, 1 mM EDTA) containing protease inhibitor cocktail at 1 : 200 (ab201111, Abcam).

Afterwards, the tissue samples were frozen in liquid nitrogen and were homogenized using a TissueLyser (QIAGEN). The homogenates were centrifuged at 20.800×g for 45 min at 4°C. The total protein in the obtained supernatants was determined by the modified Bradford method (Protein Assay Dye Reagent Concentrate, Bio-Rad). Subsequently liver homogenates were mixed with the Laemmli buffer (60 mM Tris HCl pH 6.8, 2% w/v SDS, 10% v/v glycerol, 4% betamercaptoethanol, 0.0005% bromophenol blue) in such proportions, so that after applying 10 µl of the sample to the wells, each of them contained 10 µg of total protein. The samples were warmed at 37°C for 15 min and loaded on 12% polyacrylamide gels and run for 120 min at 100 V. Subsequently, the proteins were then electrotransfered (12 V, 14 min) from the gels to PVDF membranes. The membranes were blocked with 5% non-fat-milk in PBS-T (80 mM Na_2HPO4, 20 mM NaH_2PO_2, 100 mM NaCl, and 0.1% Tween 20, pH 7.5) for 1 h and incubated overnight at 4°C with anti-AQPs primary antibodies diluted 1 : 500 – 1 : 2000 (depending on prior optimization experiments). In the current experiment, the following antibodies and dilutions were used: mouse monoclonal anti-AQP1 (Santa Cruz Biotechnology, sc-25287) 1 : 500, mouse monoclonal anti-AQP4 (Abcam, ab9512) 1 : 2000, rabbit polyclonal anti-AQP8 (Abcam, ab203682) 1 : 500, and anti-AQP9 (Santa Cruz Biotechnology, sc-74409) 1 : 500. The membranes were then incubated with a secondary rabbit anti-mouse or goats anti-rabbit horseradish peroxidase conjugated antibody (Santa Cruz Biotechnology, sc-516102; Dako, P 0448) diluted 1 : 100 or 1 : 200 respectively. The labeling was visualized by an enhanced chemiluminescence system (ECL Plus, Thermo Fisher Scientific) and exposure to a CCD camera (Versadoc 4000 MP, Bio-Rad). The obtained images were recorded in a digital form and modified (auto-scale was used, speckles were removed, and a representative band was cut out) using the Quantity One and PDQuest program (Bio-Rad). The expression of AQPs were normalized against β-actin (Santa Cruz Biotechnology, sc-47778) which was used as an internal control.

The results of the optical density were analyzed using STATISTICA 13 software (StatSoft, Kraków, Poland). The arithmetical means and standard deviations (X ± SD) were calculated. Due to the unequal number of variables between the groups and small number of variables in each group, nonparametric tests were used for further analysis. To assess the differences between the control and experimental groups, the nonparametric Mann-Whitney U-test were used. To evaluate the differences between the experimental groups, the nonparametric Kruskal–Wallis test with Dunn's multiple comparison test for post hoc analysis were used. The cut-off level for statistical significance was $p \leq 0.05$.

Results

The presence of four isoforms of aquaporins in rat livers was confirmed using Western blot technique. Figure 1 shows selective and representative results for each of them. In the present experiment all AQPs were detected as a single unglycolysated band with the molecular weight 30–32 kDa.



* Significance of differences ($p < 0.05$)

Fig. 1. Representative results of Western blot analysis of AQP1, AQP4, AQP8 and AQP9 in the rat's liver of control and experimental group

Weak expression of AQP1 and AQP4 were observed in all study animals. There were no significant changes in expression of these proteins between the control and experimental groups. Expression of AQP8 and AQP9 in rat's livers were higher compared to the remaining identified aquaporins. In comparison to AQP4 expression of AQP8 was statistically significant higher ($p < 0.05$). There were no statistically significant changes observed in the expression of AQP8 and AQP9 between the control and the experimental group. It is noteworthy that despite the lack of statistically significant differences observed between the studied groups, a detailed analysis of all band optical density seemed to indicate that the expression of AQP4 and AQP9 was slightly higher in rats fed a diet enriched with dried sea buckthorn leaves.

Discussion

There is no information in the literature regarding the effect of a diet supplemented with dried *Hippophae rhamnoides* L. leaves on the expression of AQPs in the liver. The results presented in this study are the first data in this field. It should be emphasized, however, that these were preliminary studies carried out on a small number of animals and the exper-

imental period was relatively short. The lack of recent data on liver AQPs in the context of bile production further complicates the interpretation of the results. It is widely known that bile formation is initiated by hepatocytes and is modified by secretory and absorptive processes in cholangiocytes within of bile duct. Final modification of bile composition occurs in gallbladder (BOYER 2013)). Synthesized in hepatocytes osmotically active substance such as bile salts and glutathione, are secreted into to canaliculus via the bile salt transporters Bsep and via the glutathione organic anion transporters Mrp2 (LEE et al. 2000, LEHMANN et al. 2008). Hydrocarbonates are also transposed to the light of the canalicus via the Cl^-/ HCO_3^- exchanger AE2, which together with the bile salts and glutathione constitute the major driving force for water movement from the sinusoidal blood to the bile canaliculus across hepatocytes (LEHMANN et al. 2008). This targeted water flow is mainly determined by the AQPs located in hepatocytes, among which AQP8 and AQP9 seem to play a particularly important role (Figure 2) (Marinelli et al 2004). In rat hepatocytes under basal conditions AQP8 is located mainly intracellularly to a lesser extent to the canalicular membrane, while AQP9 at the basolateral membrane (GREGOIRE et al. 2015, ELKJAER et al. 2001, NICCHIA et al. 2001). The study demonstrated that in response to stimulation by choleretic agonist such as dibutyryl cyclic adenosine monophosphate (cAMP) or glucagon, AQP8 is redistributed to the canalicular plasma membrane, thus increasing the permeability apical surface cell for water (GARCIA et al. 2001, HUEBERT et al. 2002, GRADILONE et al. 2003, MARINELLI et al. 2003). This mechanism seems to be similar to that seen in the kidney collecting duct (CD). In the absence of vasopressin (AVP) stimulation, present in the principal cells of CD AQP2 is mainly located in intracellular vesicles. Under AVP stimulation, the intracellular level of cAMP increases, as a result, the transport and fusion of this protein with apical plasma membrane and increased inflow of water to the renal cells of CD take place (MICHAŁEK et al. 2014). While AQP8 modulates the canalicular water flow, AQP9 localized in basolateral of hepatocytes enables its uptake (ELKJAER et al. 2000, NICCHIA et al. 2001, HUEBERT et al. 2002, LEHMANN et al. 2008). It is believed, that during the bile formation AQP9 facilitates the water transport from the sinusoidal blood to the liver hepatocytes (LEHMANN et al. 2008, MARINELLI et al. 2003). In the present experiment it was observed relative stable expression of total AQP8 expression in both control and experimental group, while in animals fed with dried *Hippophae rhammoides* L. leaves expression of AQP9 was slightly higher. This slight increase in a total amount of AQP9 may suggest that dietary use of dried sea buckthorn leaves will increase water transport via AQP9 from the sinusoidal blood to

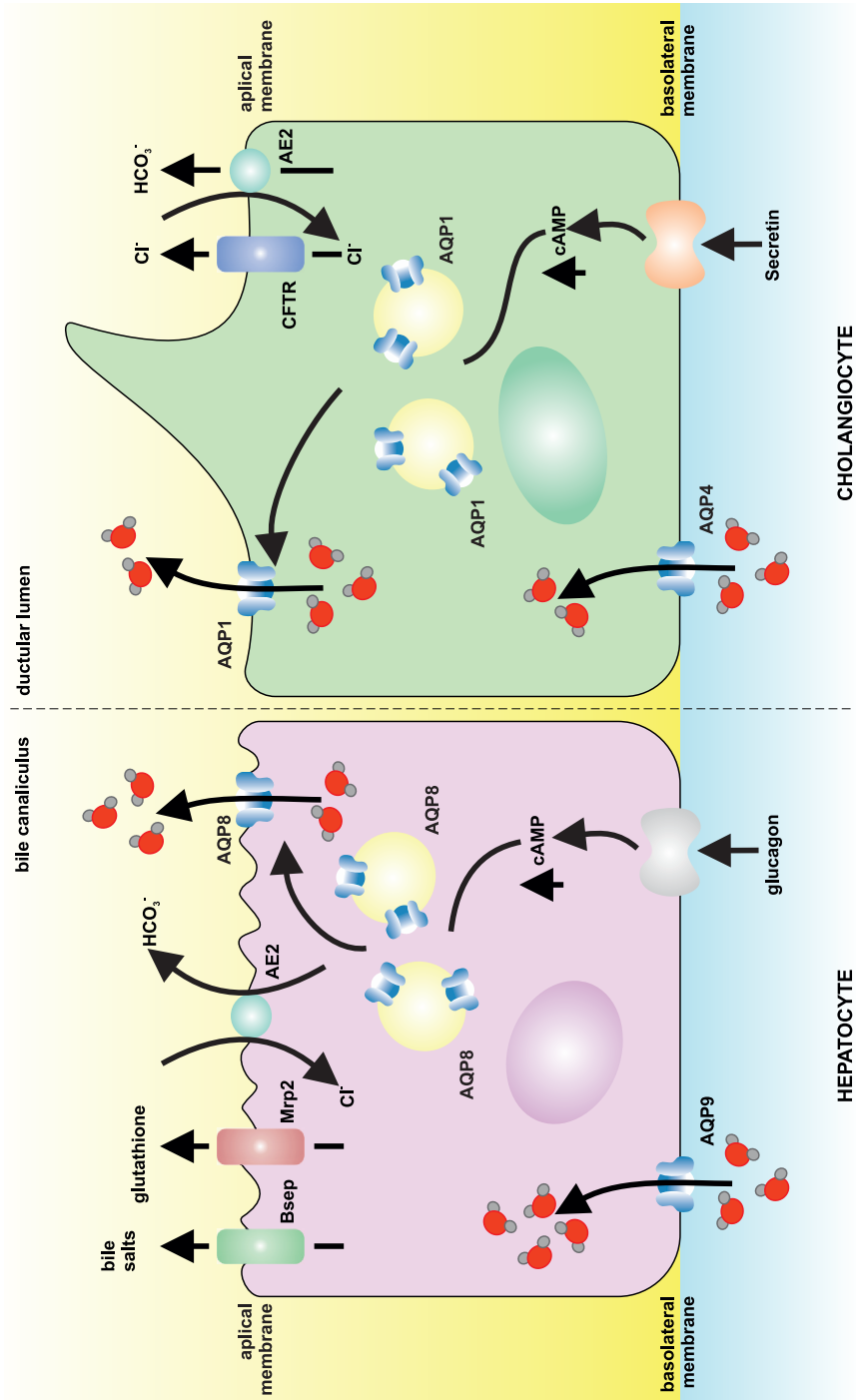


Fig. 2. Diagram showing the potential mechanism of water transport in hepatocytes and cholangiocytes

Source: own study based on MARINELLI et al. (2004)

the hepatocytes in rats from the experimental group. A consequence of the increase in water flow across the basolateral membrane should be its increased apical transport to the bile canaliculus. In the present study, no increase in the expression of AQP8 was found in animals fed a diet supplemented with dried sea buckthorn leaves, whatever the localization of this protein may have changed from intracellular compartments to the canalicular membrane.

AQP1 and AQP4 are mainly located in the cholangiocytes, that account only 3–5% the liver cell population (MARINELLI et al. 2004). Although cholangiocytes make up a small percentage of all cells that make up the hepatobiliary system, they play a significant role in bile formation, producing as much as 40% of total bile volume in some species (MASYUK and LARUSSO 2006). According to MARINENELLI and coworkers (2004) intensive processes taking place within bile duct related to bile formation, suggest that the amount of transcellular water movement across an individual cholangiocyte is potentially up to 10 times higher than across individual hepatocytes. Driving force for the water transport across cholangiocytes to the lumen of bile duct is osmotic gradients, created by secreted Cl^- via CFTR channels and HCO_3^- via AE2 transporter (MASYUK and LARUSSO 2006). Like AQP8 in hepatocytes, under basal conditions in cholangiocytes AQP1 is mainly located in intracellular vesicles (MARINELLI et al. 1997, MARINELLI et al. 1999). Under secretin stimulation and an increase intracellular cAMP synthesis, this protein undergoes translocation to the apical plasma membrane and thus promotes the water transport across the biliary epithelial cells (MARINELLI et al. 1997, MARINELLI et al. 1999). Unlike AQP1, whose subcellular distribution depends on physiological conditions, AQP4 is exclusively expressed in basolateral membrane of cholangiocytes (MARINELLI et al. 2000). During the bile formation, water enters the cholangiocytes via AQP4 located in the basolateral membrane and exits via AQP1 present in apical cell surface (Figure 2.) (MARINELLI et al. 2000). In the present experiment, the total expression of both AQP1 and AQP4 in liver homogenates were significantly lower than AQP8 and AQP9. The observed low expression of AQP1 and AQP4 is most likely associated with a significantly lower number of cholangiocytes compared to hepatocytes in liver. In the present preliminary study, only a slightly increase in the expression of total amount of AQP4 in the liver homogenates of animals fed with the diet supplemented with the dried sea buckthorn leaves was found, which may indicate a slight increased inflow of water to the cholangiocytes across the basolateral membrane. As in hepatocytes, increased transport of water inside the cell should be accompanied by an increase of water movement across the apical plasma membrane. It is possible that in

the tested animals, the slight increase of the total amount of AQP4 was accompanied by a change in AQP1 localization and an increase in its expression in apical plasma membrane of cholangiocytes. Despite the lack of significant changes in total amount of the studied AQPs, a slight increase in the expression of AQP4 and AQP9 suggests that perhaps long-term use of dried sea buckthorn leaves in the diet will have a positive effect on bile formation and secretion. Among the few data that can be used in the analysis of the presented results is information from research carried out by XING and coworkers (2012). According to these authors, one of the effects of using sea buckthorn pulp oils (SPBO) in the diet is the stimulation of the secretion of enterohormones, such as secretin. As mentioned earlier, this hormone accumulates translocation of the AQP1 from intracellular compartment to the apical plasma membrane and thus promotes the water transport across the biliary epithelial cells.

Conclusion

The results of the preliminary analysis of the dietary effect of the addition of dried sea buckthorn leaves on the expression of selected aquaporins in the livers of rats, obtained in the present study, seem to be quite promising and prompt to undertake further research in this direction. The observed slight increase in the total amount of AQP9 and AQP4 suggest that perhaps long-term dietary use of *Hippophae rhamnoides* L. may have a positive effect on liver function in terms of bile production and secretion. However, this issue requires further research, which will take into account a larger group of animals, the duration of the experiment and the use of the diet will be longer, and the WB analysis will be supplemented with studies enabling the detailed location of these proteins.

References

AGBANI E.O., POOLE A.W. 2021. *Aquaporins in platelet function*. Platelets, 32(7): 895–901.

AZAD A.K., RAIHAN T., AHMED J., HAKIM A., EMON T.H., CHOWDHURY P.A. 2021. *Human aquaporins. Functional diversity and potential roles in infectious and non-infectious diseases*. Front Genet., 16(12): 654865.

BOYER J.L. 2013. *Bile formation and secretion*. Compr. Physiol., 3(3): 1035–1078.

CALAMITA G., PERRET J., DELPORTE C. 2018. *Aquaglyceroporins. Drug targets for metabolic diseases?* Front Physiol., 9: 851.

CHENG Q., DING H., FANG J., FANG X., LIU H., WANG J., CHEN C., ZHANG W. 2021. *Aquaporin 9 represents a novel target of chronic liver injury that may antagonize its progression by reducing lipotoxicity*. Oxid. Med. Cell Longev., 6: 5653700.

CZAPLICKI S., OGRODOWSKA D., ZADERNOWSKI R., KONOPKA I. 2017. *Effect of Sea-Buckthorn (Hippophaë rhamnoides* L.*) pulp oil consumption on fatty acids and vitamin A and E accumulation in adipose tissue and liver of rats.* Plant Foods Hum. Nutr., 72(2): 198–204.

DAY R.E., KITCHEN P., OWEN D.S., BLAND C., MARSHALL L., CONNER A.C., BILL R.M., CONNER M.T. 2014. *Human aquaporins: regulators of transcellular water flow*. Biochim. Biophys. Acta, 1840(5): 1492–1506.

ELKJAER M., VAJDA Z., NEJSUM L.N., KWON T., JENSEN U.B., AMIRY-MOGHADDAM M., FROKIAER J., NIELSEN S. 2000. *Immunolocalization of AQP9 in liver, epididymis, testis, spleen, and brain*. Biochem. Biophys. Res. Commun., 276: 1118–1128.

GÂTLAN A.M., GUTT G. 2021. *Sea buckthorn in plant based diets. An analytical approach of sea buckthorn fruits composition. Nutritional value, applications, and health benefits.* Int. J. Environ. Res. Public. Health, 18(17): 8986.

GAO Z., GU X., CHENG F., JIANG F. 2003. *Effect of sea buckthorn on liver fibrosis. A clinical study*. World J Gastroenterol., 9(7): 1615–1617.

GARCÍA F., KIERBEL A., LAROCCA M.C., GRADILONE S.A., SPLINTER P. LA RUSSO N.F., MARINELLI R.A. 2001. *The water channel aquaporin-8 is mainly intracellular in rat hepatocytes and its plasma membrane insertion is stimulated by cyclic AMP.* J. Biol. Chem., 276(15): 12147–12152.

GEETHA S., SAI R.M., SINGH V. 2003. *Evaluation of antioxidant activity of leaf extract of sea buckthorn (Hippophaë rhamnoides L.) on chromium (VI) induced oxidative stress in male albino rats*. J. Ethnopharmacol., 87(2–3): 247–51.

GRADILONE S.A., GARCÍA F., HUEBERT R.C., TIETZ P.S., LAROCCA M.C., KIERBEL A., CARRERAS F.I., LA RUSSO N.F., MARINELLI R.A. 2003. *Glucagon induces the plasma membrane insertion of functional aquaporin-8 water channels in isolated rat hepatocytes.* Hepatology, 37(6): 1435–1441.

GREGOIRE F., LUCIDI V., ZERRAD-SAADI A., VIRREIRA M., BOLAKY N., DELFORGE V., LEMMERS A., DONCKIER V., DEVIERE J., DEMETTER P., PERRET J., DELPORTE C. 2015. *Analysis of aquaporin expression in liver with a focus on hepatocytes*. Histochem. Cell. Biol., 144(4): 347–363.

HOU D., WANG D., MA X., CHEN W., GUO S., GUAN H. 2017. *Effects of total flavonoids of sea buckthorn (Hippophae rhamnoides* L.*) on cytotoxicity of NK92- MI cells*. Int. J. Immunopathol. Pharmacol., 30(4): 353–361.

HUEBERT R.C., SPLINTER P.L., GARCÍA F., MARINELLI R.A., LA RUSSO N.F. 2002. *Expression and localization of aquaporin water channels in rat hepatocytes. Evidence for a role in canalicular bile secretion*. J. Biol. Chem., 277(25): 22710–22717.

LEE J.M., TRAUNER M., SOROKA C.J., STIEGER B., MEIER P.J., BOYER J.L. 2000. *Expression of the bile salt export pump is maintained after chronic cholestasis in the rat*. Gastroenterology, 118(1): 163–172.

LEHMANN G.L., LAROCCA M.C., SORIA L.R., MARINELLI R.A. 2008. *Aquaporins. Their role in cholestatic liver disease*. World J. Gastroenterol., 14(46): 7059–7067.

MAHESHWARI D.T., YOGENDRA K. M.S., VERMA S.K. 2011. *Antioxidant and hepatoprotective activities of phenolic rich fraction of sea buckthorn (Hippophaë rhamnoides* L.*) leaves*. Food Chem. Toxicol., 49(9): 2422–2428.

MARINELLI R.A., GRADILONE S.A., CARRERAS F.I., CALAMITA G., LEHMANN G.L. 2004. *Liver aquaporins: Significance in canalicular and ductal bile formation.* Ann. Hepatol., 3(4): 130–136.

MARINELLI R.A., PHAM L., AGRE P., LARUSSO N.F. 1997. *Secretin promotes osmotic water transport in rat cholangiocytes by increasing aquaporin-1 water channels in plasma membrane. Evidence for a secretin-induced vesicular translocation of aquaporin-1.* J. Biol. Chem., 272(20): 12984–12988.

MARINELLI R.A., PHAM L.D., TIETZ P.S., LARUSSO N.F. 2000. *Expression of aquaporin-4 water channels in rat cholangiocytes*. Hepatology, 31(16): 1313–1317.

MARINELLI R.A., TIETZ P.S., CARIDE A.J., HUANG B.Q., LARUSSO N.F. 2003. *Water transporting properties of hepatocyte basolateral and canalicular plasma membrane domains*. J. Biol. Chem., 278(44): 43157–43162.

Marinelli R.A., Tietz P.S., Pham L.D., Rueckert L., Agre P., LaRusso N.F. 1999. *Secretin induces the apical insertion of aquaporin-1 water channels in rat cholangiocytes*. Am. J. Physiol., 276(1): G280–G286.

Masyuk A., LaRusso N. 2006. *Aquaporins in the hepatobiliary system*. Hepatology, 43(1): 75–81.

Michałek K., Dratwa-Chałupnik A., Ciechanowicz A.K., Malinowski E. 2014. *Aquaporin 2. Identification and analysis of expression in calves' urine during their first month of life.* Can. J. Anim. Sci., 94(4): 653–659.

Michalek K., Oberska P., Malkowska P., Bartkiene E. 2021. *In seaarch of new potential markers for male fertility and semen quality control. Aquaporins in reproductive system and metabolomic profiling of semen.* J. Physiol. Pharmacol., 72(3): 309–319.

Nicchia G.P., Frigeri A., Nico B., Ribatti D., Svelto M. 2001. *Tissue distribution and membrane localization of aquaporin-9 water channel: evidence for sex-linked differences in liver*. J. Histochem. Cytochem., 49(12): 1547–1556.

Ran B., Guo C., Li W., Li W., Wang Q., Qian J., Li H. 2021. *Sea buckthorn (Hippophae rhamnoides* L.*) fermentation liquid protects against alcoholic liver disease linked to regulation of liver metabolome and the abundance of gut microbiota*. J. Sci. Food Agric., 101(7): 2846–2854.

Shi H., He J., Li X., Han J., Wu R., Wang D., Yang F., Sun E. 2018. *Isorhamnetin, the active constituent of a hinese herb Hippophae rhamnoides* L.*, is a potent suppressor of dendritic-cell maturation and trafficking*. Int. Immunopharmacol., 55: 216–222.

Solcan C., Gogu M., Floristean V., Oprisan B., Solcan G. 2013. *The hepatoprotective effect of sea buckthorn (Hippophae rhamnoides) berries on induced aflatoxin B1 poisoning in chickens*. Polut. Sci., 92(4): 966–974.

Tani T., Koyama Y., Nihei K., Hatakeyama S., Ohshiro K., Yoshida Y., Yaoita E., Sakai Y., Hatakeyama K., Yamamoto T. 2001. *Immunolocalization of Aquaporin-8 in Rat Digestive Organs and Testis*. Arch. Histol. Cytol., 64(2): 159–168.

Tietz P., Marinelli R., Chen X., Huang B., Cohn J., Kole J., McNiven M., Alper S., LaRusso N. 2003. *Agonist-induced coordinated trafficking of functionally related transport proteins for water and ions in cholangiocytes*. J. Biol. Chem., 278(22): 20413–20419.

Xing J., Jinyao S., Hu S., Wang B., Dong Y., Yang B., Kallio H. 2012. *Effects of sea buckthorn (Hippophaë rhamnoides* L.*) pulp oils on the gastric secretion, gastric emptying and its analgesic activity*. J. Med. Plants Res., 6(16): 3240–3245.