A REVIEW OF THE HEPAPROTECTIVE EFFECTS OF THE NTX PRODUCT

By:  **Date: _08/19/2015_____**

Dr. Gavin E. Arteel

Qualifications of the reviewer

Gavin Arteel is Professor of Pharmacology & Toxicology, Distinguished University Scholar, and Associate Chair for Research in the Department of Pharmacology & Toxicology at the University of Louisville. He received his BS degree from the University of Wisconsin and his PhD from the University of North Carolina, Chapel Hill. He also was a post-doctoral fellow at the Institut für Physiologische Chemie I, Heinrich Heine Universität in Düsseldorf, Germany and at the Bowles Center for Alcohol Studies at the University of North Carolina, Chapel Hill. His research has resulted in publication of over 100 journal articles/book chapters, and over 100 abstracts. His has also made over 100 invited research presentations throughout the world. The main goal of his research for over 20 years is to study the mechanisms by which liver disease initiates and progresses, with a particular emphasis on alcoholic liver disease.

Alcohol consumption and defining the risk to the liver

The liver plays multiple roles in the intact organism. It is critical for maintaining metabolic homeostasis and synthesis of lipids and carbohydrates, it is the major site of synthesis of several key proteins (e.g., albumin and clotting factors), and it synthesizes and excretes bile acids, which is critical for normal uptake of vitamins, lipids and excretion of many xenobiotics. Additionally, the strategic location of the liver between the intestinal tract and the rest of the body makes it a critical organ for clearance of xenobiotics and toxins that enter the portal blood. As the main detoxifying organ in the body, the liver has a high likelihood of toxic injury. To compensate for this injury, the liver also has a tremendous regenerative capacity, which distinguishes it from other vital organs (e.g., the brain, heart and lungs) that are far less able to replace functional tissue once it has been

destroyed. However, if this regenerative process is impaired and/or overwhelmed, damage can accumulate and cause liver disease.

Alcohol is highly prevalent in most societies and more than 50% of Americans consume alcohol at least once a month.[1] Most people are aware that heavy alcohol consumption associated with alcohol dependence (i.e., "alcoholism") is potentially detrimental to your health. Alcoholic liver disease (ALD) affects more than 10 million Americans each at a cost of more than $166 billion annually to treat the medical consequences of this disease.[2] Progression of the disease is well characterized and is actually a spectrum of liver diseases, which ranges initially from simple steatosis, to inflammation and necrosis (steatohepatitis), to fibrosis and cirrhosis. Therapeutic options remain limited to palliative care in the late stages of these devastating diseases. Luckily, ALD is observed in only a minority of even heavy drinkers and is not a major concern for most moderate/social drinkers.

Whereas most Americans are aware of the risks of prolonged heavy drinking, the negative impact of moderate or "social" alcohol consumption on health is less well understood. Indeed, most attention has focused on the potential beneficial effects of moderate alcohol consumption (e.g., preventing cardiovascular disease). The National Institute of Alcohol Abuse and Alcoholism defines moderate alcohol consumption for women or men as no more than 3 or 4 drinks/day, respectively.[3] These limits can be easily surpassed with social drinking patterns; for example, 1 pint of a local microbrew ale or a standard martini is actually the equivalent of ~2 "drinks" (a standard drink is defined as 0.6 ounces of pure alcohol). Furthermore, the speed with which this alcohol is consumed is also important; if these 3-4 daily drinks are consumed too quickly (i.e., < 2 hrs), it is defined as a "binge" episode.[4] Binge alcohol consumption is considered risky behavior legally, as it usually translates to a Blood Alcohol Concentration (BAC) above the legal driving limit. Furthermore, this level of alcohol appears to be a threshold for negative health effects of alcohol consumption.[5] More than 20% of all adults and 40% of college age adults report

participating in such risky drinking patterns at least once a month in the US.[1] Therefore, a large portion of the populace are at risk from such consumption behavior.

The risks of drinking on the liver described above only take into account alcohol consumption alone. However, damage to the liver caused by alcohol has long been known to be enhanced by other diseases (e.g., hepatitis virus infection)[6] and drugs (e.g., Tylenol)[7] that may also damage the liver. Recently, being overweight or obese, which is also a major cause of liver disease in the US population,[8] has been shown to increase the risk of liver damage from even moderate alcohol consumption.[9-11] These data suggest that ethanol exposure, even at "safe" levels can serve as the second 'hit' on the background of underlying liver disease.[6] It is therefore not uncommon for casual or social drinkers to engage in periodic risky behavior, even if they are not an "alcoholic." By extension, protecting against acute alcohol-induced liver injury may also translate to an effective therapy to protect against the progression of chronic alcohol-induced liver disease.[12]

What is NTX product?

The NTX compound is marketed to be an additive to alcoholic beverages to protect the liver from potential damage caused by moderate/social alcohol consumption. The predominant active ingredients in this product are glycyrrhizin and mannitol. Glycyrrhizin (or glycyrrhizic acid or glycyrrhizinic acid) is a plant metabolite found in *G. glabra* (licorice root) and is the main sweet component of licorice. Structurally, the compound is a triterpenoid saponin glycoside. It has been used for several millennia as a traditional herbal medicine, and the compound has several established or potential therapeutic uses in modern medicine.[13] The compound is Generally Recognized as Safe (GRAS) for use in foods by the US FDA.[13] Notable current or historical uses for glycyrrhizin include using it as an anti-ulcer agent, antiviral agent, and to enhance glucocorticoid (cortisone) and mineralocorticoid (aldosterone) levels. Several studies have indicated that glycyrrhizin may have hepatoprotective properties under several conditions.[14]

Indeed, the compound has been used in Japan to treat hepatitis C for several decades; the compound appears to synergistically work with antiviral therapies (e.g., interferon), as well as potentially directly blunting hepatic inflammation and necrosis.[15] Experimentally, the compound has shown to be protective in several models of toxic liver injury[16-18] and metals-induced liver injury.[19] Glycyrrhizin has also been shown to blunt injury in a model of T-cell mediated inflammation and fibrosis.[20, 21] Mannitol is a sugar alcohol, derived from the reduction of the sugar, mannose. It is used clinically to treat increases in intracranial pressure (e.g., after stroke[22]), as it does not cross the blood: brain barrier. Mannitol is a well-known diuretic and antioxidant.[23, 24]

NTX product protects against liver damage caused by social drinking

In a recent placebo-controlled cross-over study, the effect of NTX product on liver damage caused by social drinking in human subjects was determined. Healthy adults with exclusions for potential confounding factors (*i.e*., underlying liver disease) were investigated in this study. The experimental design was to compare placebo and NTX product in healthy patients that were exposed to repeated alcohol administration over the course of 12 visits. Each test subject consumed ethanol until they reached a blood alcohol concentration (BAC) of 0.12%; this BAC level is what would be expected in an individual consuming 3-4 drink equivalents within a 2 hour period. Chemical detection of aspartate aminotransferase (AST) and alanine aminostransferase (ALT) in plasma were used to measure liver damage; AST and ALT are released from damaged or dying liver cells into the blood. Both AST and ALT values increased ~2-fold over the 12 exposure periods for the placebo arm, and both were above the Upper Limits of Normal (ULM) for the US populace.[25] The NTX product significantly blunted these increases.

Potential mechanisms by which NTX product protects the liver from alcohol.

Alcohol exposure can cause a 'perfect storm' that favors oxidative stress and inflammatory liver damage. The inflammatory response stimulated, and more cytotoxic mediators (e.g., free radicals and cytokines). Furthermore, fatty liver in liver cells sensitizes them to cytotoxic killing

by the mediators released by macrophages. Glycyrrhizin and mannitol individually have several mechanisms of action that may explain how NTX product protects the liver from damage effects alcohol. These potential effects will be highlighted in the context of the known or hypothesized mechanisms by which alcohol causes liver damage.

Fatty liver. The first and most common hepatic change caused by alcohol consumption is fatty liver (i.e., steatosis), which occurs rapidly even after one dose of alcohol.[26-28] Steatosis is considered a benign pathology in the liver, which readily reverses with abstinence.[29] However, fatty livers are more sensitive to injury and appear to be a key step in liver damage caused by ethanol.[30] The major mechanism by which alcohol causes fatty liver is via the metabolic stress on the organ during metabolism. The net effect is to favor fat accumulation in liver cells. Furthermore, alcohol consumption can induce fat accumulation in the liver by increasing the deposition of fatty acids, and decreasing the breakdown and secretion of lipids into the circulation. Ultimately, these changes stimulate fat accumulation in the liver by increasing fatty acid supply, and concomitantly impairs the liver's capacity for fatty acid metabolism and secretion. Glycyrrhizin has been shown to prevent fatty liver in other experimental and clinical studies. For example, in both clinical and experimental models of hepatitis C infection, glycyrrhizin has shown to blunt steatosis.[31, 32] A test compound containing glycyrrhizin also protected against steatosis in a model of liver damage caused by the combination of carbon tetrachloride (CCl_4) and ethanol.[33] Glycyrrhizin also protects against insulin resistance,[34, 35] which may contribute to fatty liver caused by alcohol.[11]

Inflammation. Inflammation is key factor in liver damage caused by alcohol consumption. Alcohol activates macrophages (important inflammatory cells in the liver) to respond more aggressively when stimulated and increases the damage caused by inflammation to the liver cells.[36-38] Glycyrrhizin exerts many effects that may blunt/prevent the inflammatory response caused by ethanol. The compound directly inhibits activation of macrophages and production of

proinflammatory mediators.[39-41] Furthermore, other studies have shown that glycyrrhizin prevents liver cell death.[42] Lastly, glycyrrhizin inhibits increases the level of the potent anti-inflammatory hormone, cortisol, by blocking it's breakdown; this hormone is often used to treat acute alcoholic hepatitis.[29]

Oxidative stress. Oxidative stress is also proposed to be critically involved in ALD. Alcohol consumption causes an overall decreased antioxidant status and increases the production of reactive species (e.g., free radicals) in the liver.[43, 44] Several studies have identified protection against oxidative stress by glycyrrhizin and mannitol.[23, 45] Furthermore, glycyrrhizin has been shown to inhibit the prooxidant enzyme, CYP2E1,[46] which is a key source of free radicals after alcohol exposure.[44] The protection against oxidative stress is clearly a potentially important mechanism by which NTX product protects the liver from damage.

Summary and Conclusions

The risk that alcohol consumption can damage your liver extends well beyond chronic heavy consumption (i.e., alcoholic liver disease), as binge alcohol abuse is on the rise Worldwide.[47] Furthermore, there is a large portion of the US populace that may already have subclinical liver disease that can be exacerbated by even moderate alcohol consumption. There is an increasing need to identify new therapies to treat pathophysiology associated with this drinking behavior. The main components of the NTX product have established safety and efficacy in humans that is relevant for the likely mechanisms by which alcohol damages the liver, even in social drinkers. The results of the NTX study and review of basic and clinical literature suggests that the NTX product may well be hepatoprotective against alcohol-induced liver injury in moderate/social drinkers.

Reference List

1. U.S.Department of Health and Human Services. Results from the 2010 National Survey on Drug Use and Health. 2010.

2. Nelson S, Kolls JK. Alcohol, host defence and society. *Nature Rev.Immunol* 2002; **2**:205-209

3. National Institute of Alcohol Abuse and Alcoholism. Beyond hangovers: understanding alcohol's impact on your health. 13-7604 ed. Washington, DC: National Institutes of Health, 2010.

4. National Institute of Alcohol Abuse and Alcoholism. NIAAA council approves definition of binge drinking. 3 ed. 2004:3.

5. Shukla SD, Pruett SB, Szabo G, Arteel GE. Binge ethanol and liver: new molecular developments. *Alcohol Clin.Exp.Res.* 2013; **37**:550-557

6. Reuben A. Alcohol and the liver. *Curr.Opin.Gastroenterol.* 2007; **23**:283-291

7. Draganov P, Durrence H, Cox C, Reuben A. Alcohol-acetaminophen syndrome. Even moderate social drinkers are at risk. *Postgrad.Med.* 2000; **107**:189-195

8. Lazo M, Hernaez R, Eberhardt MS, Bonekamp S, Kamel I, Guallar E, Koteish A, Brancati FL, Clark JM. Prevalence of nonalcoholic fatty liver disease in the United States: the Third National Health and Nutrition Examination Survey, 1988-1994. *Am.J.Epidemiol.* 2013; **178**:38-45. PMCID:PMC3698993.

9. Alatalo PI, Koivisto HM, Hietala JP, Puukka KS, Bloigu R, Niemela OJ. Effect of moderate alcohol consumption on liver enzymes increases with increasing body mass index. *Am.J.Clin.Nutr.* 2008; **88**:1097-1103

10. Ruhl CE, Everhart JE. Joint effects of body weight and alcohol on elevated serum alanine aminotransferase in the United States population. *Clin.Gastroenterol.Hepatol.* 2005; **3**:1260-1268

11. Diehl AM. Obesity and alcoholic liver disease. *Alcohol* 2004; **34**:81-87

12. Massey VL, Arteel GE. Acute alcohol-induced liver injury. *Frontiers in Physiology* 2012; **3**:1-8. PMCID:PMC3372892.

13. Isbrucker RA, Burdock GA. Risk and safety assessment on the consumption of Licorice root (Glycyrrhiza sp.), its extract and powder as a food ingredient, with emphasis on the pharmacology and toxicology of glycyrrhizin. *Regul.Toxicol.Pharmacol.* 2006; **46**:167-192

14. Zhang A, Sun H, Wang X. Recent advances in natural products from plants for treatment of liver diseases. *Eur.J.Med.Chem.* 2013; **63**:570-577

15. van Rossum TG, Vulto AG, de Man RA, Brouwer JT, Schalm SW. Review article: glycyrrhizin as a potential treatment for chronic hepatitis C. *Aliment.Pharmacol.Ther.* 1998; **12**:199-205

16. Jeong HG, You HJ, Park SJ, Moon AR, Chung YC, Kang SK, Chun HK. Hepatoprotective effects of 18beta-glycyrrhetinic acid on carbon tetrachloride-induced liver injury: inhibition of cytochrome P450 2E1 expression. *Pharmacol.Res.* 2002; **46**:221-227

17. Lee CH, Park SW, Kim YS, Kang SS, Kim JA, Lee SH, Lee SM. Protective mechanism of glycyrrhizin on acute liver injury induced by carbon tetrachloride in mice. *Biol.Pharm.Bull.* 2007; **30**:1898-1904

18. Lin G, Nnane IP, Cheng TY. The effects of pretreatment with glycyrrhizin and glycyrrhetinic acid on the retrorsine-induced hepatotoxicity in rats. *Toxicon* 1999; **37**:1259-1270

19. Lee JR, Park SJ, Lee HS, Jee SY, Seo J, Kwon YK, Kwon TK, Kim SC. Hepatoprotective Activity of Licorice Water Extract against Cadmium-induced Toxicity in Rats. *Evid.Based.Complement Alternat.Med.* 2009; **6**:195-201. PMCID:PMC2686628.

20. Yoshida T, Abe K, Ikeda T, Matsushita T, Wake K, Sato T, Sato T, Inoue H. Inhibitory effect of glycyrrhizin on lipopolysaccharide and d-galactosamine-induced mouse liver injury. *Eur.J.Pharmacol.* 2007; **576**:136-142

21. Tu CT, Li J, Wang FP, Li L, Wang JY, Jiang W. Glycyrrhizin regulates CD4+T cell response during liver fibrogenesis via JNK, ERK and PI3K/AKT pathway. *Int.Immunopharmacol.* 2012; **14**:410-421

22. Jeon SB, Koh Y, Choi HA, Lee K. Critical care for patients with massive ischemic stroke. *J.Stroke* 2014; **16**:146-160. PMCID:PMC4200590.

23. Gilmour PS, Brown DM, Lindsay TG, Beswick PH, MacNee W, Donaldson K. Adverse health effects of PM10 particles: involvement of iron in generation of hydroxyl radical. *Occup.Environ.Med.* 1996; **53**:817-822. PMCID:PMC1128615.

24. Lang F. Osmotic diuresis. *Ren Physiol* 1987; **10**:160-173

25. Ruhl CE, Everhart JE. Upper limits of normal for alanine aminotransferase activity in the United States population. *Hepatology* 2012; **55**:447-454. PMCID:PMC3268908.

26. Donohue TM, Jr., Osna N, Trambly C, Whitaker N, Thomes P, Todero S, Davis JS. Early growth response-1 contributes to steatosis development after acute ethanol administration. *Alcohol Clin Exp Res* 2011. PMCID:PMC3297709.

27. Kaiser JP, Beier JI, Zhang J, David HJ, von Montfort C, Guo L, Zheng Y, Monia BP, Bhatnagar A, Arteel GE. PKCepsilon plays a causal role in acute ethanol-induced steatosis. *Arch.Biochem.Biophys.* 2009; **482**:104-111. PMCID:PMC2672568.

28. Korkusuz H, Keese D, Raschidi BA, Hubner F, Namgaladze D, Hintereder G, Hammerstingl R, Korkusuz Y, Monch C, Vogl TJ. Detection of a fatty liver after binge drinking: correlation of MR-spectroscopy, DECT, biochemistry and histology in a rat model. *Acad.Radiol.* 2011; **18**:1349-1357

29. Bergheim I, McClain CJ, Arteel GE. Treatment of alcoholic liver disease. *Dig.Dis.* 2005; **23**:275-284

30. Day CP, James OF. Steatohepatitis: a tale of two "hits"? *Gastroenterology.* 1998; **114**:842-845

31. Korenaga M, Hidaka I, Nishina S, Sakai A, Shinozaki A, Gondo T, Furutani T, Kawano H, Sakaida I, Hino K. A glycyrrhizin-containing preparation reduces hepatic steatosis induced by hepatitis C virus protein and iron in mice. *Liver Int.* 2011; **31**:552-560

32. Melhem A, Stern M, Shibolet O, Israeli E, Ackerman Z, Pappo O, Hemed N, Rowe M, Ohana H, Zabrecky G, Cohen R, Ilan Y. Treatment of chronic hepatitis C virus infection via antioxidants: results of a phase I clinical trial. *J.Clin.Gastroenterol.* 2005; **39**:737-742

33. Wang JY, Guo JS, Li H, Liu SL, Zern MA. Inhibitory effect of glycyrrhizin on NF-kappaB binding activity in CCl4- plus ethanol-induced liver cirrhosis in rats. *Liver* 1998; **18**:180-185

34. Lim WY, Chia YY, Liong SY, Ton SH, Kadir KA, Husain SN. Lipoprotein lipase expression, serum lipid and tissue lipid deposition in orally-administered glycyrrhizic acid-treated rats. *Lipids Health Dis.* 2009; **8**:31. PMCID:PMC2729303.

35. Sil R, Ray D, Chakraborti AS. Glycyrrhizin ameliorates insulin resistance, hyperglycemia, dyslipidemia and oxidative stress in fructose-induced metabolic syndrome-X in rat model. *Indian J.Exp.Biol.* 2013; **51**:129-138

36. McClain CJ, Cohen DA. Increased tumor necrosis factor production by monocytes in alcoholic hepatitis. *Hepatology* 1989; **9**:349-351

37. Enomoto N, Ikejima K, Bradford BU, Rivera CA, Kono H, Brenner DA, Thurman RG. Alcohol causes both tolerance and sensitization of rat Kupffer cells via mechanisms dependent on endotoxin. *Gastroenterology* 1998; **115**:443-451

38. Yang SQ, Lin HZ, Lane MD, Clemens M, Diehl AM. Obesity increases sensitivity to endotoxin liver injury: implications for the pathogenesis of steatohepatitis. *Proc.Natl.Acad.Sci.U.S.A.* 1997; **94**:2557-2562

39. Ohuchi K, Kamada Y, Levine L, Tsurufuji S. Glycyrrhizin inhibits prostaglandin E2 production by activated peritoneal macrophages from rats. *Prostaglandins Med.* 1981; **7**:457-463

40. Schrofelbauer B, Raffetseder J, Hauner M, Wolkerstorfer A, Ernst W, Szolar OH. Glycyrrhizin, the main active compound in liquorice, attenuates pro-inflammatory responses by interfering with membrane-dependent receptor signalling. *Biochem.J.* 2009; **421**:473-482

41. Ogiku M, Kono H, Hara M, Tsuchiya M, Fujii H. Glycyrrhizin prevents liver injury by inhibition of high-mobility group box 1 production by Kupffer cells after ischemia-reperfusion in rats. *J.Pharmacol.Exp.Ther.* 2011; **339**:93-98

42. Gwak GY, Moon TG, Lee DH, Yoo BC. Glycyrrhizin attenuates HMGB1-induced hepatocyte apoptosis by inhibiting the p38-dependent mitochondrial pathway. *World J.Gastroenterol.* 2012; **18**:679-684. PMCID:PMC3281226.

43. Lieber CS. Alcohol: its metabolism and interaction with nutrients. *Annu.Rev.Nutr.* 2000; **20**:395-430

44. Arteel GE. Oxidants and antioxidants in alcohol-induced liver disease. *Gastroenterology* 2003; **124**:778-790

45. Ming LJ, Yin AC. Therapeutic effects of glycyrrhizic acid. *Nat.Prod.Commun.* 2013; **8**:415-418

46. Jeong HG, You HJ, Park SJ, Moon AR, Chung YC, Kang SK, Chun HK. Hepatoprotective effects of 18beta-glycyrrhetinic acid on carbon tetrachloride-induced liver injury: inhibition of cytochrome P450 2E1 expression. *Pharmacol.Res.* 2002; **46**:221-227

47. Mathurin P, Deltenre P. Effect of binge drinking on the liver: an alarming public health issue? *Gut* 2009; **58**:613-617