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# Herbal extracts as hepatoprotectants against acetaminophen hepatotoxicity

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## Abstract

Many plant-derived natural products have the potential to be hepatoprotective and therefore can be used to treat acute and chronic liver diseases. The challenge is to identify the most promising compounds and evaluate their protective mechanism. In a recently published article, Wang *et al* evaluated extracts of the plant *Gentiana manshurica* Kitagawa (GM) in a model of acetaminophen hepatotoxicity. The authors concluded that GM is hepatoprotective against acetaminophen-induced liver injury due to its antioxidant properties and anti-apoptotic capacity. We would like to discuss the limitations of this experimental approach and question the conclusion based on the data presented in this manuscript and the published literature.



**Key words:** Acetaminophen; Drug hepatotoxicity; Herbal extracts; N-acetylcysteine

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## TO THE EDITOR

We read with interest a recent paper published in *World Journal of Gastroenterology* by Wang *et al*[1], who showed that 2 h pretreatment with an extract of the plant *Gentiana manshurica* Kitagawa (GM) could protect mice against acetaminophen (APAP) hepatotoxicity (300 mg/kg). The authors concluded that GM is hepatoprotective against acetaminophen-induced liver injury due to its antioxidant properties and anti-apoptotic capacity. A comparison of GM to the well-established clinically used antidote *N*-acetyl-*L*-cysteine (NAC) resulted in similar beneficial effects. According to the authors, their data suggest that GM is a potent hepatoprotective agent with a comparable mechanism to NAC. However, some of these mechanistic conclusions are clearly not justified by the data and whether or not GM is actually hepatoprotective under clinically relevant conditions has not been established.

The first concern is the authors' conclusion that GM has an anti-apoptotic effect. Apoptotic cell death is characterized by cell shrinkage and nuclear condensation[2,3]. However, none of these morphological features was evident from the liver sections shown by Wang *et al*[1]. In contrast, extensive centrilobular necrosis was shown, which correlates with the extensive release of liver enzymes into the plasma. The cells that are stained positive for DNA strand breaks with the transferase-mediated

dUTP nick end-labeling (TUNEL) assay are not apoptotic. These cells show the characteristic nuclear and cytosolic staining of oncotic cells, which has been extensively described for APAP hepatotoxicity[2-4]. The nuclear fragmentation is caused by the mitochondrial release of endonuclease G and apoptosis-inducing factor[4,5], rather than by caspase-activated DNase, which is responsible for DNA fragmentation during apoptosis[6]. Although the authors claimed that caspase-3 was activated based on the appearance of a 17 kD cleavage product, no other bands (pro-caspase-3) have been shown and no positive controls as reference points were included. Most importantly, a dramatic increase in procaspase-3 cleavage should go along with a dramatic increase in caspase-3 enzyme activity, which has never been shown in APAP-induced liver injury[2,7-9]. Consistent with these observations, caspase inhibitors do not protect against APAP hepatotoxicity arguing against a relevant role of caspase activation in the pathophysiology[8,9]. Furthermore, in both primary cultured mouse[10] and human hepatocytes[11], APAP induces necrotic cell death. Taken together, oncotic necrosis is the predominant mode of cell death (> 95%) during APAP hepatotoxicity in animal models *in vivo* and in humans[2]. The only exception is APAP-induced cell death in metabolically incompetent hepatoma cell lines. However, these mechanisms have no relevance to the *in vivo* hepatotoxicity of this drug[7].

A second major concern is related to the extensive mechanistic conclusion including the hypothesis that GM acts as an antioxidant. There is direct evidence that reactive oxygen species and peroxynitrite are formed in mitochondria during APAP hepatotoxicity and play a critical role in cell death[4,12,13]. However, the fact that at 12 h after APAP administration, there was less lipid peroxidation, together with other evidence for reduced tissue injury, does not prove that GM acts as an antioxidant. The same results would be obtained if GM protected and improved cell viability through other mechanisms with the consequence of less oxidant stress. In fact, one of the most likely mechanisms of protection, i.e. that one or several compounds in this plant extract may have inhibited cytochrome P450 activities or may have competed with APAP for metabolism, was not investigated. Toxicity of APAP is entirely dependent on its metabolic activation[14], which means that any interference with its reactive metabolite formation will substantially reduce or even eliminate toxicity. In the absence of clear evidence that this extract does not affect reactive metabolite formation, any conclusion regarding more distal mechanisms is not justified.

The third concern is the conclusion that GM acts as a hepatoprotectant similar to NAC. However, in clinically relevant situations of drug overdose, the antidote has to be effective when administered after the insult not as a pretreatment. The effectiveness of GM against APAP hepatotoxicity when treated after drug overdose has not been investigated. Furthermore, the comparison to NAC is not justified. NAC given as pretreatment to fasted animals will support glutathione (GSH) synthesis in the liver resulting in much higher GSH levels than the respective controls 2 h later[15]. These elevated GSH levels will more effectively scavenge the reactive metabolite of APAP and therefore prevent initiation of liver injury[16]. This protection mechanism of NAC is independent of the antioxidant effect of GSH mainly because no oxidant stress is generated at this point. However, if NAC is administered several hours after APAP, i.e. at a time when hepatic GSH is depleted and mitochondria have already generated an oxidant stress, GSH synthesized at this time is used to scavenge reactive oxygen species and peroxynitrite[13,17]. In addition, some of the excess NAC will also be used to support the impaired mitochondrial energy metabolism[17]. Both mechanisms contribute to the late protection against APAP hepatotoxicity[13,17]. Thus, NAC can have 3 different mechanisms of action depending on the time of administration relative to APAP. The pretreatment with NAC as used by Wang *et al*[1] will mainly scavenge the reactive metabolite of APAP, an effect that is unlikely to be relevant to GM.

Taken together, the protective effect of GM against APAP hepatotoxicity is an interesting observation. However, GM as a hepatoprotectant against drug toxicity under clinically relevant conditions has not been demonstrated. In addition, the actual protection mechanism of GM remains unclear. More mechanistic studies considering clinically relevant conditions are needed to evaluate the potential of this plant extract as an antidote against drug hepatotoxicity.

## REFERENCES


1 **Wang AY**, Lian LH, Jiang YZ, Wu YL, Nan JX. Gentiana manshurica Kitagawa prevents acetaminophen-induced acute hepatic injury in mice via inhibiting JNK/ERK MAPK pathway. *World J Gastroenterol* 2010; **16**: 384-391

2 **Gujral JS**, Knight TR, Farhood A, Bajt ML, Jaeschke H. Mode of cell death after acetaminophen overdose in mice: apoptosis or oncotic necrosis? *Toxicol Sci* 2002; **67**: 322-328

3 **Jaeschke H**, Lemasters JJ. Apoptosis versus oncotic necrosis in hepatic ischemia/reperfusion injury. *Gastroenterology* 2003; **125**: 1246-1257

4 **Cover C**, Mansouri A, Knight TR, Bajt ML, Lemasters JJ, Pessayre D, Jaeschke H. Peroxynitrite-induced mitochondrial and endonuclease-mediated nuclear DNA damage in acetaminophen hepatotoxicity. *J Pharmacol Exp Ther* 2005; **315**: 879-887

5 **Bajt ML**, Cover C, Lemasters JJ, Jaeschke H. Nuclear translocation of endonuclease G and apoptosis-inducing factor during acetaminophen-induced liver cell injury. *Toxicol Sci* 2006; **94**: 217-225

6 **Nagata S**, Nagase H, Kawane K, Mukae N, Fukuyama H. Degradation of chromosomal DNA during apoptosis. *Cell Death Differ* 2003; **10**: 108-116

7 **Jaeschke H**, Gujral JS, Bajt ML. Apoptosis and necrosis in liver disease. *Liver Int* 2004; **24**: 85-89

8 **Lawson JA**, Fisher MA, Simmons CA, Farhood A, Jaeschke H. Inhibition of Fas receptor (CD95)-induced hepatic caspase activation and apoptosis by acetaminophen in mice. *Toxicol Appl Pharmacol* 1999; **156**: 179-186

9 **Jaeschke H**, Cover C, Bajt ML. Role of caspases in acetaminophen-induced liver injury. *Life Sci* 2006; **78**: 1670-1676

10 **Bajt ML**, Knight TR, Lemasters JJ, Jaeschke H. Acetaminophen-induced oxidant stress and cell injury in cultured

mouse hepatocytes: protection by N-acetyl cysteine. *Toxicol Sci* 2004; **80**: 343-349
11 **McGill MR**, Yan HM, Jaeschke H. Acetaminophen-induced injury in HepaRG cells: a novel human cell line for studies of drug hepatotoxicity (abstract). *FASEB J* 2010; **24**: 759
12 **Jaeschke H**, Knight TR, Bajt ML. The role of oxidant stress and reactive nitrogen species in acetaminophen hepatotoxicity. *Toxicol Lett* 2003; **144**: 279-288
13 **Knight TR**, Ho YS, Farhood A, Jaeschke H. Peroxynitrite is a critical mediator of acetaminophen hepatotoxicity in murine livers: protection by glutathione. *J Pharmacol Exp Ther* 2002; **303**: 468-475
14 **Zaher H**, Buters JT, Ward JM, Bruno MK, Lucas AM, Stern ST, Cohen SD, Gonzalez FJ. Protection against acetaminophen toxicity in CYP1A2 and CYP2E1 double-null mice. *Toxicol Appl Pharmacol* 1998; **152**: 193-199
15 **Wendel A**, Jaeschke H. Drug-induced lipid peroxidation in mice--III. Glutathione content of liver, kidney and spleen after intravenous administration of free and liposomally entrapped glutathione. *Biochem Pharmacol* 1982; **31**: 3607-3611
16 **Corcoran GB**, Racz WJ, Smith CV, Mitchell JR. Effects of N-acetylcysteine on acetaminophen covalent binding and hepatic necrosis in mice. *J Pharmacol Exp Ther* 1985; **232**: 864-872
17 **Saito C**, Zwingmann C, Jaeschke H. Novel mechanisms of protection against acetaminophen hepatotoxicity in mice by glutathione and N-acetylcysteine. *Hepatology* 2010; **51**: 246-254

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