

# Protective effects of natsumikan (*Citrus natsudaïdai*) extract on acetaminophen-induced lethal hepatotoxicity in mice

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## ABSTRACT

**Background:** Like other citrus fruits, natsumikan (*Citrus natsudaïdai*) contains several antioxidative nutrients which occur in higher concentrations in the peel than in the pulp. A high dose of acetaminophen (APAP) generates highly reactive intermediates and causes fatal liver injury. In this study, we examined whether an extract from immature natsumikan peel prevents lethal hepatotoxicity induced by a lethal dose of APAP in mice. **Materials and Methods:** Male ICR mice were treated orally with natsumikan extract (300 and 1,000 mg/kg) 2, 26, and 50 h before single oral APAP (300 mg/kg) administration. Mice were fasted for 18 h before APAP treatment, but given tap water *ad libitum*. Survival was assessed for 24 h after APAP treatment. **Results:** Following administration of 300 mg/kg APAP, all mice died within 6 h. However, pretreatment with natsumikan extract (300 and 1,000 mg/kg) or silymarin (300 and 1,000 mg/kg) increased the survival rate to 16.7%, 33.3%, 16.7%, and 50%, respectively, at 24 h. **Conclusion:** The results suggest that natsumikan has a protective effect on APAP-induced lethal hepatotoxicity.

**Key words:** Natsumikan, acetaminophen (APAP), lethal hepatotoxicity, hepatoprotective effect

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## INTRODUCTION

Acetaminophen (APAP) is one of the most commonly used drugs for reducing fever in adults and children and is generally considered a safe drug. However, a large single dose can cause potentially fatal acute liver failure in both humans and experimental animals.<sup>[1-3]</sup> APAP poisoning currently represents the most common cause of acute liver failure in North America and Europe.<sup>[4]</sup> Between 400 and 500 deaths occur annually in the United States due to APAP-related liver failure.<sup>[5]</sup> Unintentional liver injury from self-medication for pain or fever that leads to daily doses exceeding the 4 g/day package recommendation is also well recognized. However, unintentional overdosing is usually only recognized after symptoms have developed.<sup>[6]</sup>

*Citrus natsudaïdai* (natsumikan) is a well-known citrus fruit recognized in many countries for its medicinal properties.

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Because natsumikan contains a large amount of antioxidative nutrients such as vitamin C and flavonoids, regular consumption of citrus fruit has been considered good for maintaining health. Natsumikan is rich in many bioactive components, such as hesperidin, neohesperidin, naringin, nobiletin, tangeretin, and aurapten, which have all been well studied for their pharmacological anti-inflammatory,<sup>[7,8]</sup> anticarcinogenic,<sup>[9]</sup> and antioxidant properties.<sup>[10]</sup>

It has been reported that some medicinal plants, such as *Phyllanthus urinaria*,<sup>[11,12]</sup> *Dioscorea alata* L,<sup>[13]</sup> and *Acanthopanax koreanum*<sup>[14]</sup> have protective effects with regard to APAP-induced acute liver failure. As these medicinal plants are known to have antioxidative properties, we hypothesized that natsumikan may also have a protective effect on APAP-induced hepatotoxicity.

Therefore, we designed the present study to investigate the potential usefulness of natsumikan focusing on its preventive effects against lethal hepatotoxicity induced by an overdose of APAP.

## MATERIALS AND METHODS

### Plant material

Immature fresh natsumikan were collected in Minamiboso city, Chiba, Japan in August 2009 and stored at 4°C prior to extraction of the active ingredients from the peel. The natsumikan peel contained the following active components per 100 g: hesperidin 11 mg, neohesperidin 370 mg, naringin 780 mg, nobiletin 1.6 mg, tangeretin 3.2 mg, and auraptin 26 mg.

### Chemicals

Silymarin was obtained from Sigma Chemical (St. Louis, MO) and APAP was obtained from Wako Pure Chemical Institute (Osaka, Japan).

### Extraction procedure

Natsumikan peel (1215.2 g) was cut into small pieces with scissors and air-dried at 50°C for 24 h in a mechanical air-dryer. Extraction was performed by placing the dried peel (412.4 g) in a 5-fold volume of methanol (Wako Pure Chemical Institute) at 40°C for 2 h. The resulting extract was filtered and concentrated under reduced pressure to evaporate the methanol, then lyophilized using a freeze-drying machine (Taitec, Saitama, Japan) to provide 14.2 g of dried natsumikan peel.

### Animals

All experiments and procedures were approved by the Chiba University Institutional Animal Care and Use Committee. Male ICR mice, 5 weeks of age, were obtained from Japan SLC Inc. (Hamamatsu, Japan) and housed under controlled conditions of light (07:00-19:00) and temperature (24°C) with food and water available *ad libitum*.

### Drug treatment regimens

Natsumikan extract (300 or 1,000 mg/kg) and silymarin (300 or 1,000 mg/kg) were dissolved in distilled water and administered orally once a day for 3 days. Mice were fasted for 24 h starting at 16 h before the final administration of natsumikan or silymarin but were given tap water *ad libitum*. APAP (300 mg/kg) was dissolved in distilled water and administered orally once, 2 h after the last administration of natsumikan or silymarin. Control animals were treated with vehicle [Figure 1].

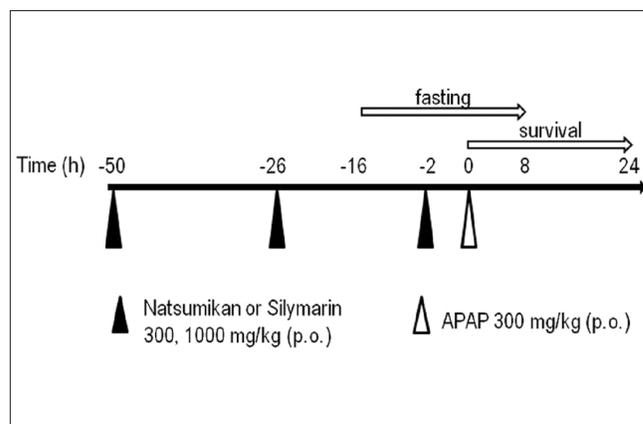
### Survival experiments

Mice were administered as mentioned above and received the final administration of natsumikan or silymarin 2 h before 300 mg/kg (lethal dose) of APAP intoxication [Figure 1]. Mortality was observed for 24 h after administration of APAP. Each group contained 6 mice.

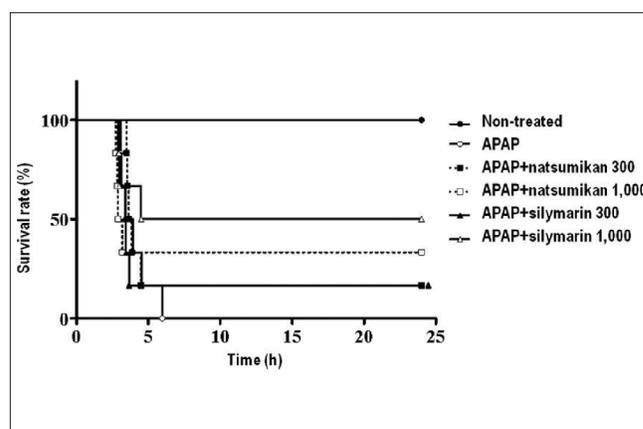
## RESULTS AND DISCUSSION

The aim of this study was to investigate the protective effect of natsumikan on APAP-induced hepatotoxicity. When mice were administered the lethal dose of APAP (300 mg/kg), all died within 6 h. However, in mice pre-treated with natsumikan extract (300 and 1,000 mg/kg) before APAP dosing, the survival rate was 16.7% and 33.3%, respectively, at 24 h after APAP. Similarly, silymarin (300 and 1,000 mg/kg) increased the survival rate to 16.7% and 50%, respectively, at 24 h [Figure 2].

Silymarin is known to exhibit a hepatoprotective effect through antioxidant and cell-regenerating functions,<sup>[12]</sup> and is often used as a positive control for studies of hepatic injury. Moreover, many types of herbal extracts that can prevent APAP-induced acute liver failure via mediation of antioxidant effects.<sup>[15-17]</sup> Recently, we



**Figure 1:** Schedule for the elicitation of APAP-induced hepatotoxicity showing administration of reagents



**Figure 2:** The effect of natsumikan treatment on APAP-induced mortality. Survival rate in control group treated with distilled water (●; n=6) and mice treated with APAP alone (○; n=6), or APAP plus natsumikan 300 mg/kg (■; n=6), natsumikan 1,000 mg/kg (□; n=6), silymarin 300 mg/kg (▲; n=6), or silymarin 1,000 mg/kg (△; n=6)

reported that the extract of immature natsumikan peel is effective for the treatment of chronic allergic contact dermatitis, and this effect might be mediated by its antioxidative properties.<sup>[18]</sup> Therefore, it can be assumed that the inhibitory effect of natsumikan on APAP-induced hepatotoxicity in the present study is mediated, at least in part, by its antioxidative properties. We hypothesized that the antioxidative elements of natsumikan may be hesperidin and naringin, which have been reported to exhibit antioxidative effects,<sup>[19,20]</sup> but further studies are required to confirm which component has the greatest protective effect.

High doses of APAP generate the highly reactive intermediate *N*-acetyl-*p*-benzoquinone imine (NAPQI) via cytochrome P450 (CYP) enzymes, which results in acute liver failure. In particular, CYP2E1 is an important isoform that generates NAPQI. Recently, it was reported that some herbal extracts attenuate APAP-induced acute liver failure through inhibition of CYP2E1.<sup>[21,22]</sup> Furthermore, it is possible that natsumikan inhibits APAP-induced acute liver failure via inhibition of CYP2E1 activity, but further studies are required to confirm this.

To our knowledge, this is the first study to show the protective effects of immature natsumikan peel extract on APAP-induced lethal hepatotoxicity in mice. If APAP is taken to reduce fever caused by illnesses such as the common cold or flu, prior ingestion of immature natsumikan peel as a dietary supplement may be useful for preventing unintentional overdosing and consequent APAP-induced acute liver failure.

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