Note

Suppression by *Hydrangeae Dulcis Folium* of D-Galactosamine-induced Liver Injury in *Vitro* and in *Vivo*

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*Hydrangeae Dulcis Folium*, the fermented and dried leaves of *Hydrangea macrophylla* SER. var. thunbergii MAKINO, suppressed D-galactosamine-induced liver injury by 85.2% when added to the diet at 1% and fed to rats for fifteen days. The hepatoprotective effect is more potent than that of a milk thistle extract and turmeric powder. Some fractionated extracts showed hepatoprotective activity in the D-galactosamine-induced *in vitro* liver injury model.

**Key words:** hepatocyte; hepatoprotective; liver injury; *Hydrangeae Dulcis Folium*; D-galactosamine

Liver injury is caused by such agents as viruses, chemicals, and alcohol. Some food products are known to protect against liver injury, although scientific evidence for their effect is not necessarily sufficient.

We have been examining various edible plants in a search for possible dietary supplements that would maintain a healthy liver. We describe here for the first time a simple and efficient process of obtaining an extract of *Hydrangeae Dulcis Folium* that had strong efficacy on D-galactosamine (GalN)-induced liver injury *in vivo* and *in vitro*.

*Hydrangeae Dulcis Folium* (amacha), the fermented and dried leaves of *Hydrangea macrophylla* SER. var. thunbergii MAKINO, is a saxifragaceous plant with anticoccidial,1) antifungal,2) anti-ulceric,3) anti-allergic,4) anti-hypercholesterolemic,3) antioxidant,5) and cholagogic activities.3) *Hydrangeae Dulcis Folium* was purchased from Shihira Shoten (Tokyo, Japan), and extracts were prepared twice with water or 60% ethanol for 24 hours to respectively obtain a water extract (WE) and ethanol extract (EE). Each suspension was then filtered through Miracloth (Calbiochem). The residue of *Hydrangeae Dulcis Folium* after water extraction was further extracted twice with 60% ethanol to obtain a water-ethanol extract (WEE). These three extracts (WE, EE and WEE) were vacuum dried. Each dried extract was added to a control diet at the expense of corn starch. The composition of the control diet was as follows (g/100 g): casein, 25.0; corn oil, 5.0; sucrose, 20.0; mineral mix (AIN-76), 3.5; vitamin mix (AIN-76), 1.0; choline bitartrate, 0.4; cellulose, 5.0; and corn starch, 40.1. Five-week-old male Wistar rats were fed on the control diet containing 1% of the respective extract for 15 days. On the fourteenth day, GalN was administered (350 mg/kg i.p.). Twenty-two hours after this GalN injection, blood was collected, and the serum alanine aminotransferase (ALT) activity was determined with Fuji Dri-Chem 3500 apparatus (Fuji Film). All animal experiments were approved by the Animal Ethical Committee of Kyowa Hakko Kogyo Co., Ltd. (Tsukuba, Japan). Statistical differences were determined by a one-way analysis of variance and subsequent Dunnett multiple-comparison test of the control versus other groups. *p* < 0.05 is considered significant. The results show that the serum ALT activity of the GalN-injected rats was higher (1034.1 ± 209.0) than that of the non-treated rats (6.2 ± 0.2). WE (1082.3 ± 153.2) and EE (766.0 ± 196.3) did not suppress the GalN-induced elevation of serum ALT. On the other hand, WEE inhibited the GalN-induced elevation of serum ALT by 85.2% (165.2 ± 88.3) (Fig. 1). The serum ALT activity of the rats fed on a diet containing 0.02, 0.07, 0.21, 0.7, or 2.1% of WEE was 1011.9 ± 173.8, 803.7 ± 161.1, 988.9 ± 149.1, 561.4 ± 205.7, and 239.8 ± 91.9 respectively. The minimum dose to cause an effect was between 0.7% and 2.1%.

Curcuma is the most popular dietary supplement in Japan for liver care, and milk thistle is used as a hepatoprotective agent in Germany. The serum ALT activities of rats fed on a diet containing a 1% milk...
Fig. 1. Hepatoprotective Activity of the *Hydrangea Dulcis Folium* Extracts.

Rats were fed on a diet containing 1% of either the water extract (WE), 60% ethanol extract (EE), or water-ethanol extract (WEE) of *Hydrangea Dulcis Folium* for 15 days. On the fourteenth day, GalN was administered at a dosage of 350 mg/kg to induce liver injury. Twenty-two hours after this GalN injection, blood was collected, and the serum GPT activity was determined. Each data value is presented as the mean ± SEM (n = 12). WEE: 60% ethanol extract of the residue obtained after water extraction of *Hydrangea Dulcis Folium*.

![Graph showing serum ALT activity for different treatments](image)

Fig. 2. Comparison of the Water-ethanol Extract *Hydrangea Dulcis Folium* with Milk Thistle Extract and Turmeric Powder.

Rats were fed on a diet containing 1% of the water-ethanol extract (WEE) of *Hydrangea Dulcis Folium*, milk thistle extract, or turmeric powder for 15 days. On the fourteenth day, GalN was administered at a dosage of 350 mg/kg to induce liver injury. Twenty-two hours after this GalN injection, blood was collected, and the serum GPT activity was determined. Each data value is presented as the mean ± SEM (n = 12).

![Graph showing serum ALT activity for different treatments](image)

Fig. 3. Inhibition of the GalN-Induced *In Vitro* Liver Injury by Fractions of the Water-ethanol Extract (WEE) of *Hydrangea Dulcis Folium*.

The water-ethanol extract of *Hydrangea Dulcis Folium* was applied to an HP-20 column and sequentially eluted with 60 ml of water, 33 ml methanol, 66 ml methanol, 100 ml methanol and 100 ml acetone. Each eluent, except for water, was divided into two fractions, the former and latter of 30 ml each. The obtained 9 fractions (Fr. 1–Fr. 9) were vacuum dried, and each was added to the medium at 20 μg/ml. After one hour, GalN was added to the medium at 20 mM. The survival rates 48 hours after the addition of GalN were measured by an MTT assay. Each data value is presented as the average (n = 2).

As a result, fraction 3 (hepatoprotective activity of 65.2%), fraction 4 (79.9%), and fraction 6 (45.8%) each showed more than 40% hepatoprotective activity (Fig. 3).
The results of the present study demonstrate that the water-ethanol extract (WEE) of *Hydrangeae Dulcis Folium* suppressed the GalN-induced increase of serum ALT activity. The extent of increase in the serum enzyme activity is known to be parallel to that of liver injury, so the results indicate that WEE of *Hydrangeae Dulcis Folium* may prevent GalN-induced liver injury in vivo. Since the mechanism for GalN-induced liver injury is known to share some similarities with that induced by a virus or alcohol,\(^7\) it can be presumed that *Hydrangeae Dulcis Folium* would protect against virus- or alcohol-induced liver injury. We have already confirmed that WEE of *Hydrangeae Dulcis Folium* inhibited ethanol and lipopolysaccharide-induced liver injury in rats.\(^9\)

Inhibition of tumor necrosis factor-\(\alpha\) is speculated to be involved in the action of WEE.

Water extracts of *Hydrangeae Dulcis Folium* have been used as a sweetener and a traditional Japanese crude drug. We have revealed in this work that not the water extract, but the water-ethanol extract (WEE) of *Hydrangeae Dulcis Folium* inhibited liver injury. This suggests that the active compounds are likely to be lipophilic. The active compounds are thought to be more concentrated in WEE than in EE, because, in the former, water-soluble compounds that would show no effect have been removed. The reason why the hepatoprotective activity of *Hydrangeae Dulcis Folium* has not previously been identified is probably that only the water extract of *Hydrangeae Dulcis Folium* has been used.

In the liver injury model we used here, although the milk thistle extract and turmeric powder weakly suppressed the increase of serum ALT activities, the activity of WEE of *Hydrangeae Dulcis Folium* was much more potent at the same dosage.

We have demonstrated that some fractions of WEE of *Hydrangeae Dulcis Folium* inhibited GalN-induced hepatocyte death in vitro. This suggests that several active compounds are involved in the WEE’s hepatoprotective activity and that they directly affected the hepatocytes to suppress cell death. A further study on the isolation and identification of the active compounds of *Hydrangeae Dulcis Folium* is now in progress.

**References**