Comparative Studies of *Panax ginseng* and *Panax quinquefolium* on TCDD-induced Toxicity in Rats

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Abstract

One prominent characteristic of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) toxicity in rats is a reduction of body weight accompanied by an altered serum lipid profile such as hyperlipidemia. A single administration of TCDD (50 ug/kg) resulted in a decrease of body weight and increase of serum cholesterol in rats. TCDD-induced weight loss and serum cholesterol elevation was reduced in rats administered with water extract (100 mg/kg) or saponin fraction (40 mg/kg) of *Panax ginseng* C.A.Meyer. In contrast, the administration of *Panax quinquefolium* did not inhibit the TCDD-induced weight loss and serum cholesterol elevation. Histological examinations of liver and testis revealed the administration of saponin fraction of *Panax ginseng* attenuated the TCDD-induced hispathological lesions whereas the administration of saponin fraction of *Panax quinquefolium* did not. High performance liquid chromatographic analysis demonstrated high percentiles of ginsenoside Rg_3 and ginsenoside Rh_1 were evident in saponin fraction of *Panax ginseng*. Results indicate that the protective effects of *Panax ginseng*, not *Panax quinquefolium*, on the TCDD-induced toxicity might be resulted from different compositions of saponins in *Panax ginseng*.

Introduction

2,3,7,8-Tetrachlorodibenzo-*p*-dioxin (TCDD) is one of the most toxic halogenated polycyclic hydrocarbons among environmental pollutants. Weight loss associated with hypophagia is acommon characteristic of TCDD toxicity in experimental animals (1,2). In addition, hepatic lesions, thymic atrophy, immune suppression and testicular dysfunction have been reported (3,4).

Because of such diverse effects of TCDD, the search for biochemical mechanisms of its toxic action has been difficult. Poland proposed that TCDD first binds with a cytosolic receptor (aryl hydrocarbon receptor), is transferred into the nucleus and mediates the expression of a number of microsomal and cytosolic enzymes (5). Upon ligand binding, aryl hydrocarbon (AH) receptor enters the nucleus and dimerizes with the AH receptor nuclear translocator (ARNT) and binds to the DNA, resulting in an initiation of transcription from the AH receptor-responsive genes (6,7). Although toxicity of TCDD appears to be mediated by AH receptor, the exact mechanism has remained elusive.

As extensive studies have focused on the pathogenetic steps of the toxicity of TCDD, there are few informations on the protectants on the toxic action of TCDD. In such a situation, we have tried to determine whether ginseng administration to TCDD-exposed experimental animals inhibited the TCDD-induced weight loss and tissue damages. In the previous report, we have demonstrated that the *in vivo* administration of water extract of *Panax ginseng* C.A.Meyer lowered the TCDD-induced body weight losses and necrotic lesions of testis in the guinea pigs (8). In the present report, we aimed to reveal the differences between *Panax ginseng* C.A.Meyer and *Panax quinquefolium* on the protective effects on the toxic actions of TCDD in rats. Results indicate that the pharmacological activities as well as chemical compositions between *Panax ginseng* and *Panax quinquefolium* are different.

Materials and Methods

Preparation of saponin fraction from ginseng root

Roots of Panax ginseng C.A.Meyer and Panax quinquefolium were extracted with 10 volumes of distilled water at 85°C, concentrated under a reduced pressure and lyophilized to a dark brownish powder. Resulting powder was dissolved in water and subjected to adsorption chromatography using macroreticular resin (Diaion HP-20). The column was eluted with water, 25% ethyl alcohol (EtOH) and 100% EtOH consecutively. Saponin fraction was obtained from 100% EtOH eluate.

In vivo administration

Male Sprague Dawley rats (180-200 g) were maintained under controlled conditions of 23±1°C, 40-60% of relative humidity and 12 hr-light/dark cycle. Rats were allowed free access to

chow diet and drink tap water. Experimental groups consisted of normal control, TCDD only and TCDD plus ginseng samples. Ginseng samples, either water extract (100 mg/kg) or saponins (40 mg/kg), were given orally to rats from day one to to twenty-one. TCDD was dissolved in acetone/dimethylsulfoxide/ corn oil (9:1:790, v/v) and intraperitoneally administered to rats at a single dose of 50 ug/kg on the day seven. Numbers of rats in each experimental group were ten.

Serological analysis and histological examination

Blood was obtained at the day of sacrifice by cardiac puncture, allowd to clot and centrifuged to obtain serum. Total cholesterol in serum was determined using a diagnostic kit from Sigma. SGOT was determined using a diagnostic kit from ASAN (Korea). Liver and testis were fixed in buffered 10% formalin, embedded in paraffin and stained with Masson's trichrome.

HPLC analysis

Saponin fraction from *Panax ginseng* or *Panax quinquefolium* was dissolved in methanol and injected to HPLC system equipped with Lichrosorb NH_2 column (Merck, Germany). Mobile phase was accomplished in a linear gradient of solvent A [acetonitrile/water/isopropyl alcohol (80/5/15)] and solvent B [acetonitrile/water/isopropyl alcohol (70/30/15)]. Mixing ratio of solvent B was 10% at the start and increased to 100% in 45 minutes. Flow rate of mobile phase was 0.8 ml/min.

Results

Effects of ginseng administration on the TCDD-induced weight loss

Since a weight loss is most pronounced in experimental animals treated with TCDD, we first assessed whether the administration of ginseng inhibited the TCDD-induced weight loss in rats. At a dose of 50 ug/kg, TCDD exposure induced the weight loss of about ninety grams from the normal weight gains of naive rats. Administration of water extract of *Panax ginseng* (100 mg/kg) resulted in an attenuation of weight loss as compared with the rats treated with TCDD. However, the administration of water extract of *Panax quinquefolium* (100 mg/kg) did not reduce the TCDD-induced weight loss (Fig. 1a).

To reveal out which fraction from water extract is responsible for the attenuation of TCDD-induced weight loss, saponin fraction was prepared and administered to the TCDD-exposed rats.

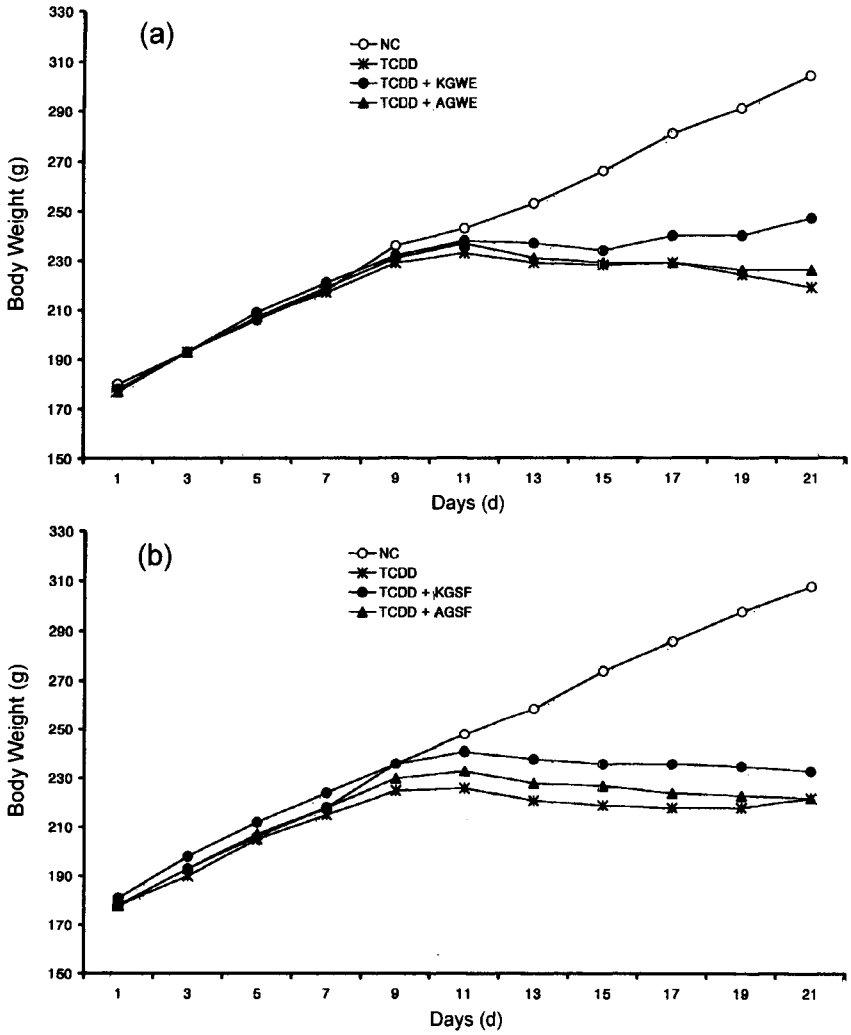

Fig. 1. Effect of ginseng administration on changes of body weights.
Water extract (100 mg/kg) (a) or saponin fraction (40 mg/kg) (b) have been daily administered to rats par oral from day one to twenty-one. TCDD (50 ug/kg) was injected intraperitoneally at the day seven for one time. NC represents normal control. KGWE and AGWE designate water extract of *Panax ginseng* and *Panax quinquefolium*, and KGSF and AGSF designate saponin fraction of *Panax ginseng* and *Panax quinquefolium* respectively.

Administration of saponin fraction (40 mg/kg) from *Panax ginseng* attenuated the TCDD-induced weight losses whereas saponin fraction from *Panax quinquefolium* (40 mg/kg) did not reduce the TCDD-induced weight losses (Fig. 1b).

Effects of gineng administration on the levels of serum cholesterol and SGOT

In the TCDD-exposed experimental animals, loss of body weight is mainly caused by a rapid loss of adipose tissue and, in a result, accompanied by serum hyperlipidemia, particularly hypertriglyceridemia or hypercholesterolemia. As shown in figure 2, exposure of TCDD to rats induced an increase of serum cholesterol. However, the elevation of serum cholesterol was attenuated in the rats treated with water extract (100 mg/kg) or saponin fraction (40 mg/kg) of *Panax ginseng* (Fig. 2a & 2b). In the rats treated with water extract or saponin fraction of *Panax*

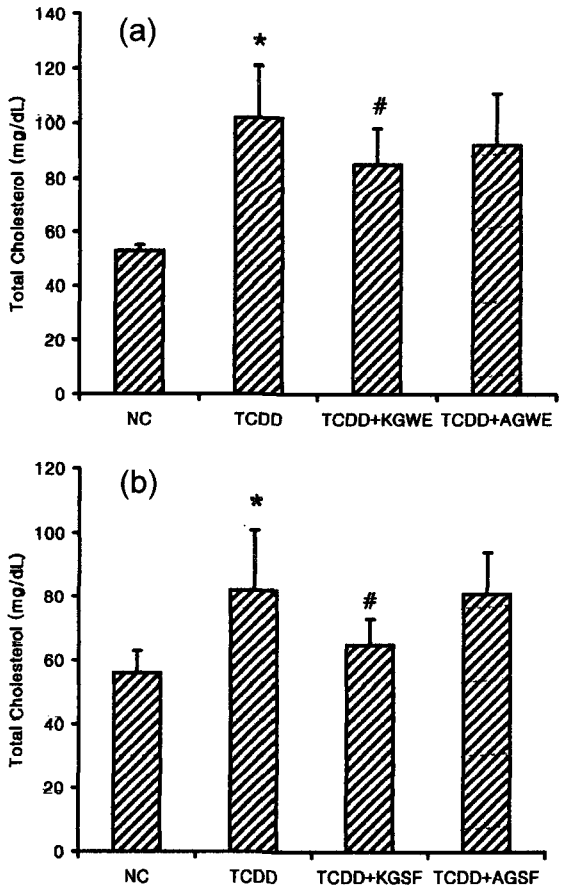

Fig. 2. Effect of ginseng administration on levels of serum cholesterol.
In vivo treatment of rats was same as in Fig. 1. Serum cholesterol was determined in rats administered with water extract (100 mg/kg) (a) and saponin fraction (40 mg/kg) (b). NC represents normal control. KGWE and AGWE designate water extract of *Panax ginseng* and *Panax quinquefolium*, and KGSF and AGSF designate saponin fraction of *Panax ginseng* and *Panax quinquefolium* respectively.

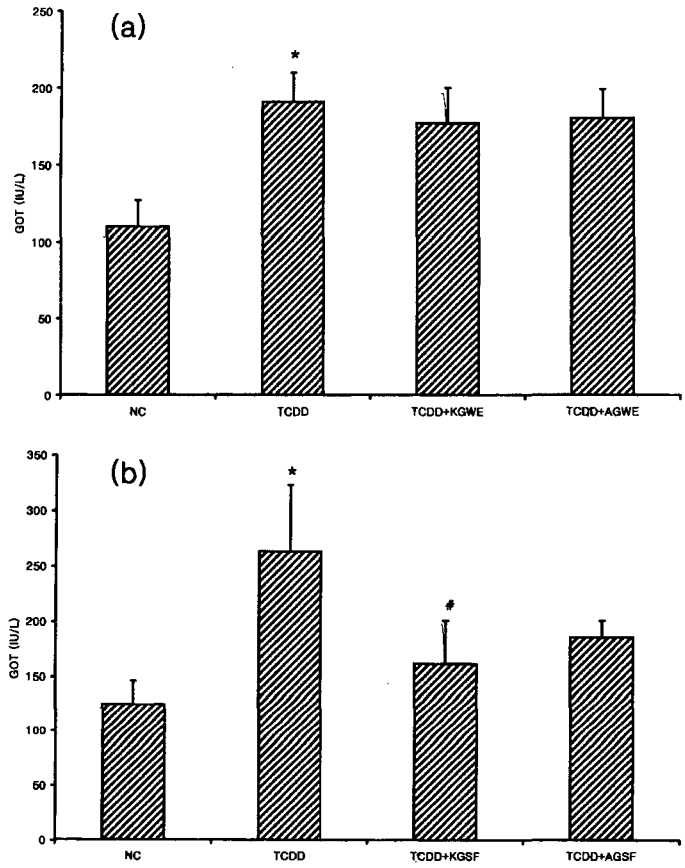

Fig. 3. Effect of ginseng administration on SGOT.
In vivo treatment of rats was same as in Fig. 1. SGOT was determined in rats administered with water extract (100 mg/kg) (a) and saponin fraction (40 mg/kg) (b) NC represents normal control. KGWE and AGWE designate water extract of *Panax ginseng* and *Panax quinquefolium*, and KGSF and AGSF designate saponin fraction of *Panax ginseng* and *Panax quinquefolium*, respectively.

quinquefolium, levels of serum cholesterol were as high as those of rats treated with TCDD.

Additionally, TCDD exposure resulted in an elevation of SGOT indicating that TCDD exposure induced a hepatic damage. Administration of saponin fraction of *Panax ginseng* (40 mg/kg) attenuated the elevation of SGOT though the administration of water extract did not attenuate successfully (Fig. 3a & 3b). Administration of water extract (100 mg/kg) or saponin fraction of *Panax quinquefolium* (40 mg/kg) did not reduce the TCDD-induced increases of SGOT.

Effects of ginseng saponin administration on the TCDD-induced hapatic damages

To demonstrate the protective effects of ginseng on the TCDD-induced liver damages, a small block of liver was fixed with formalin and stained with Masson's trichrome. A section of rat's liver demonstrated a classical lobular structure composed of a central vein and interconnecting plates of hepatocytes (Fig. 4a). Hepatocytes from TCDD-exposed rats exhibited a loss of lobular structure, diffuse fatty change, hyperplasia of Kuffer cells and severe variation of hepatocyte size (Fig. 4b). However, hepatocytes from the rats administered with saponin fraction of *Panax ginseng* (40 mg/kg) exhibited a well-constructed lobular structure similar to those of naive normal rats (Fig. 4c). In contrast, hepatocytes from the rats administered with saponin fraction of *Panax quinquefolium* (40 mg/kg) exhibited a dissipated lobular structure as similar as those of rats exposed to TCDD (Fig. 4d).

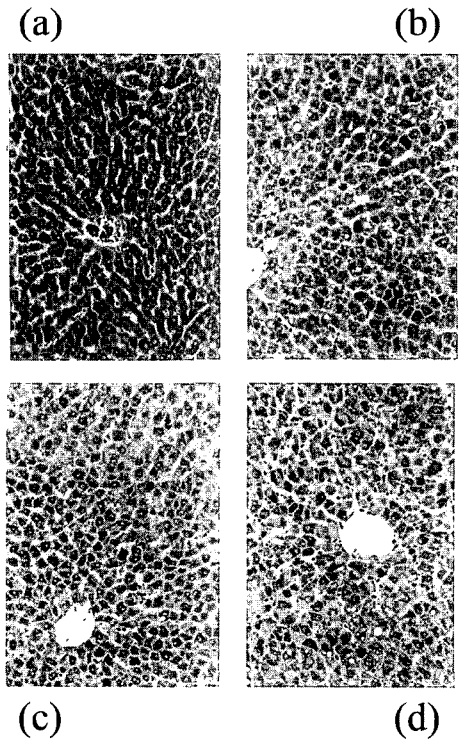

Fig. 4. Effect of saponin administration on the TCDD-induced hepatic lesions.
Liver was fixed in buffered 10% formalin and stained with Masson's trichrome. Livers from normal (a) TCDD-treated (b) TCDD plus saponin fraction of *Panax ginseng*-treated (c) TCDD plus saponin fraction of *Panax quinquefolium*-treated rats (d) were successively arranged. Magnification was 200

Effects of ginseng saponin administration on the testicular damages

Figure 5 shows sections of several seminiferous tubules which are lined with non-proliferating supporting cells, Sertoli cells and proliferating germ cells. From the periphery to the lumen of the tubule, spermatogonia, spermatocytes and immature and mature spermatids were successively arranged (Fig. 5a). Microscopically, the seminiferous tubules from the rats exposed to TCDD showed a marked destruction of regular arrangement in successive stages of cells in differentiation and a degeneration of Sertoli cells (Fig. 5b). However, the seminiferous tubules from the rats administered with saponin fraction of *Panax ginseng* (40 mg/kg) showed a mild recovery from the TCDD-induced destruction of spermatogenesis (Fig. 5c). Some tubules exhibited a regular arrangement of spermatogenetic cells in the rats administered with saponin fraction of *Panax ginseng* (40 mg/kg). Differently from the rats administered with saponin

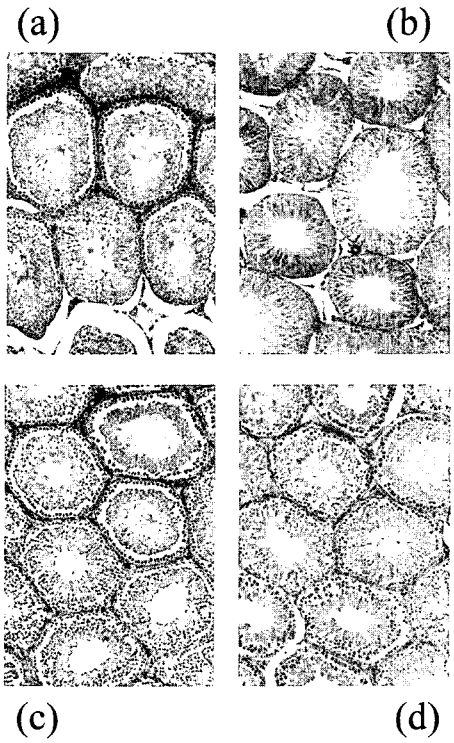

Fig. 5. Effect of saponin administration on the TCDD-induced testicular lesions.
Testis was fixed in buffered 10% formalin and stained with Masson's trichrome. Testes from normal (a) TCDD-treated (b) TCDD plus saponin fraction of *Panax ginseng*-treated (c) TCDD plus saponin fraction of *Panax quinquefolium*-treated rats (d) were successively arranged. Magnification was 100

fraction of *Panax ginseng*, rats administered with saponin fraction of *Panax quinquefolium* (40 mg/kg) exhibited a destruction of spermatogenesis similar to rats exposed to TCDD (Fig. 5d).

High performance liquid chromatographic (HPLC) analysis of saponin fractions from Panax ginseng and Panax quinquefolium

HPLC analysis of saponins from *Panax ginseng* and *Panax quinquefolium* revealed marked differences between the two. *Panax ginseng* contained a high percentile of Rg_3 which have been produced from heat-labile ginsenosides in the process of hot water extraction of ginseng roots (Fig. 6a). Ratio of Rg_3 to Rb_1 in *Panax ginseng* was about thirteen whereas less than one in *Panax quinquefolium*. Additionally, the percentile of ginsenoside Rh_1 was prominently high in *Panax ginseng* but trace in *Panax quinquefolium* (Fig. 6b). Thus, it is assumed that differential effects of ginseng species against TCDD-induced toxicity might be mediated by their differential compositions of saponins.

Discussions

A major purpose of the present work was to reveal the differences between *Panax ginseng* and *Panax quinquefolium* in pharmacological and chemical contexts. The results imply protective effects of *Panax ginseng* on the toxic action of TCDD resulted from saponins different from those of *Panax quinquefolium*.

Inasmuch as weight loss and hypophagia are most pronounced in rats treated with lethal doses of TCDD, the time courses of body weight loss were compared in rats administered with *Panax ginseng* and *Panax quinquefolium*. It was shown that rats administered with *Panax ginseng*, not with *Panax quinquefolium,* lost body weight at a smaller extent than rats exposed to TCDD. These results were in agreement to serum cholesterol levels; rats administered with *Panax ginseng* exhibited an increase of serum cholesterol at a lesser extent than rats administered with *Panax quinquefolium*.

Pathological changes in rats exposed to TCDD were observed consistently in liver and testis. Alterations associated with the effects of TCDD in liver were a diffuse fatty change, destruction of lobular structure and appearance of multinucleated hepatocytes. Evidence for the protection of *Panax ginseng* on the TCDD-induced hepatotoxicity was noted upon histochemical examination. Hepatocytes from rats administered with saponin fraction of *Panax ginseng*, not with saponin

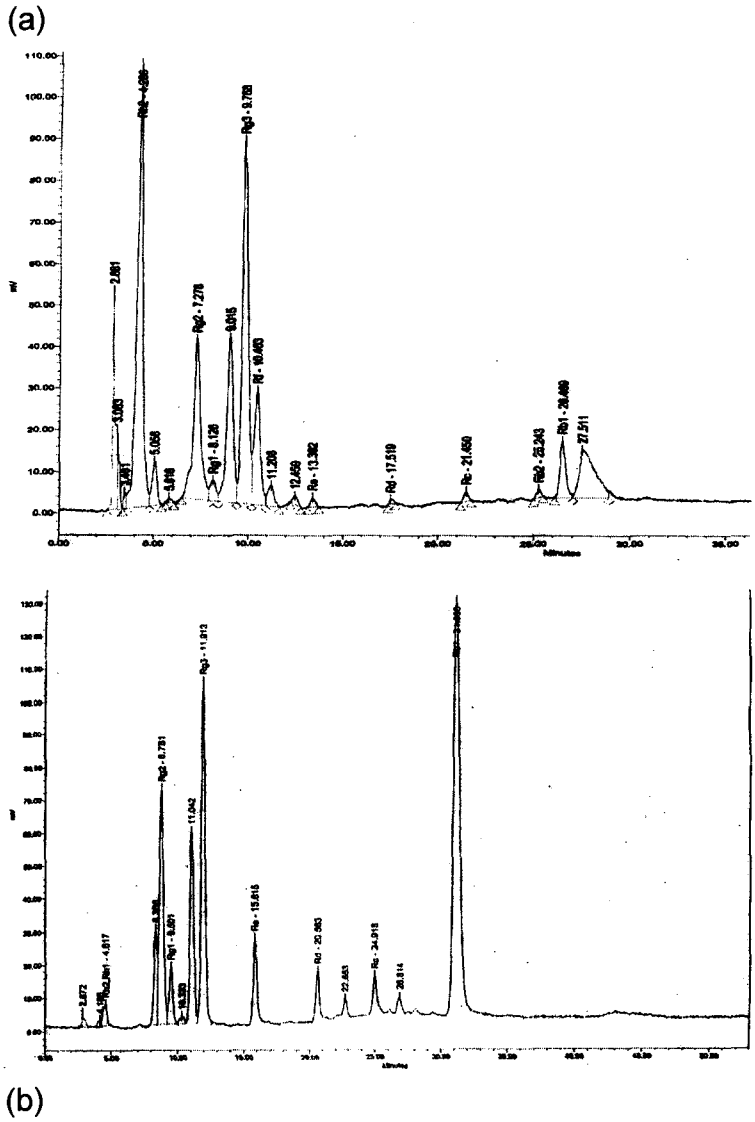

Fig. 6. HPLC analysis of saponins from *Panx ginseng* and *Panax quinquefolium*.
Saponin fractions of *Panx ginseng* (a) and *Panax quinquefolium* (b) were analyzed by HPLC equipped with Lichrosorb NH_2 column.

fraction of *Panax quinquefolium,* displayed an well-organized lobular structure similar to naive normal rats. In addition to liver, alterations associated with the effects of TCDD in testis were noted especially in spematogenetic activities. Within the testes of rats administered with saponin fraction of *Panax ginseng*, the TCDD-induced decrease of spermatogenesis and degeneration of

Sertoli cells were much attenuated whereas were not in the testes of rats administered with saponin fraction of *Panax quinquefolium*. This association between attenuation of weight loss and histochemical damage supports the protective effects of saponin fraction of *Panax ginseng* on the TCDD-induced toxicity in rats.

Pertinent to the discussion of difference between *Panax ginseng* and *Panax quinquefolium* is the composition of their saponins. High percentiles of ginsenoside Rg_3 and ginsenoside Rh_1 with low percentile of ginsenoside Rb_1 were prominent in *Panax ginseng*. In contrast, *Panax quinquefolium* contained high percentiles of ginsenoside Rb_1 and ginsenoside Rg_3. These compositions of saponins in *Panax ginseng* could therefore be responsible for the protective effects on the toxicity of TCDD. Given these results, it might be emphasized the present investigation proves out that *Panax ginseng* and *Panax quinquefolium* are different in their pharmacological and chemical contexts.

Acknowledgments

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