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Toxicity of *Panax* Genseng – An Herbal Medicine and Dietary Supplement

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ABSTRACT

Ginseng is one of the most popular herbal dietary supplements in the U.S. market, with five to six million persons using it even before the recent boom in the herbal supplement industry. Ginsana (G115 ginseng), a standardized extract of *Panax* ginseng (Asian ginseng, also called Chinese or Korean ginseng) controls half of this market. Numerous reports of adverse effects from products containing ginseng have been filed with the U.S. Food and Drug Administration (FDA). The literature also documents "ginseng abuse syndrome" among regular users. The chronic effects of ginseng are not well characterized. Because of its significant human exposure and little information on toxicity is available, *Panax* ginseng has been nominated by the U.S. National Institutes of Health (NIH) to the U.S. National Toxicology Program (NTP) for assessing its carcinogenic potential. In this review, we discuss the environmental occurrence, purported therapeutic effects, biological effects, and toxicity of ginseng, with focus on *Panax* ginseng. To demonstrate how NTP chronic tumorigenicity bioassays are conducted, the tumorigenicity bioassay of *Panax* ginseng is detailed described.

Key words: ginseng dietary supplement, NTP, chronic tumorigenicity bioassay

INTRODUCTION

Ginsengs are members of the genus *Panax* in the Araliaceae family. *Panax ginseng* (Asian ginseng, also called Chinese or Korean ginseng) and *Panax quinquefolius* (American and Canada ginseng) are the two most popular species; other ginsengs include *Panax japonicus* (Japanese ginseng), *Panax notoginseng* (Sanqui or Tienqi ginseng), *Panax elegantior* (Pearl ginseng), *Panax* pseudoginseng (Himalayan ginseng), and *Panax zingiberensis* (ginger ginseng)[(1)]. In this review, only Ginseng from *Panax ginseng* and *Panax quinquefolius* is considered.

Ginseng is a perennial aromatic herbal plant, reaching maturity after five years; and for medicinal purposes, it is usually harvested in its sixth year[(2,3)]. White root ginseng and red root ginseng are the most popular ginseng products. White root ginseng is prepared by drying the peeled roots in the sun; red root ginseng is the steam-dried roots without peeling. In general, ginseng roots are graded by size. Ginseng root with a larger number (size) is more valuable than that with a smaller number (size)[(1)].

Because ginseng is expensive, adulteration or substitution with cheaper products occurs[(1)]. For example, commercial ginsengs were found to be adulterated with phenylbutazone and aminopyrine[(4)]. A comprehensive program was initiated by the American Botanical Council to determine ginseng adulteration[(1,5)].

The standardized ginseng products are Ginsana (contains 4% ginsenosides) or G115 for *Panax ginseng* C.A. Meyer and CNT-2000 for *Panax quinquefolius*, with which the assessment of efficacy and safety standards for ginseng products can be pursued[(6)].

CHEMICAL COMPOSITION

There are many chemical components in ginseng root, including triterpene saponins, polyacetylenes, sesquiterpenes, polysaccharides, peptidoglycans, fatty acids, carbohydrates, and phenolic compounds[(2)]. The principal chemical constituents exhibiting purported pharmacological effects are triterpene saponins, which are secondary metabolites of ginseng. Triterpene saponins are named ginsenosides Rx, in which the "x" is designated following their mobility on thin-layer chromatography plates, with polarity decreasing from index "a" to "h". Ginsenosides are glycosides containing an aglycone (protopanaxadiol or protopanaxatriol) with a dammarane skeleton[(7)]. Ginsenosides are polar compounds, with the polarity a function of the number

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Journal of Food and Drug Analysis, Vol. 15, No. 4, 2007

of monosaccharide residues present in the sugar chain. There are 31 ginsenosides that have so far been isolated and identified from the roots of white and red ginseng. Ginsenosides can be categorized into three groups based upon their aglycone groups: protopanaxadiol-type ginsenosides, protopanaxatriol-type ginsenosides, and oleanolic acid-type saponins[2]. The types and quantities of ginsengosides are different among different ginseng species, the age of the plant, and the part of the plant extract. These variations account for the varying purported therapeutic effects of ginseng products. The more highly regarded ginseng products contain more ginsenosides of the protopanaxtriol-type.

Most of dammarane ginsenosides in white ginseng root are derivatives of 20(S)-protopanaxadiol and 20(S)-protopanaxatriol (Table 1). The majority of the ginsenosides isolated in white ginseng are also present in red ginseng. Some ginsenosides are characteristic saponins for red ginseng[2]. *Panax* ginseng and *Panax* quinquefolium are closely related chemically and taxonomically, with both containing ginseng saponin below 0.1 percent[8]. The major difference between *Panax ginseng* (Asian ginseng) and *Panax quinquefolius* (American and Canada ginseng) is that *Panax ginseng* contains ginsenoside Rf and *Panax quinquefolius* does not[9].

The structures of the common ginsenosides are shown in Figure 1 and the names (in abbreviation) of the principal ginsenosides are listed in Table 1.

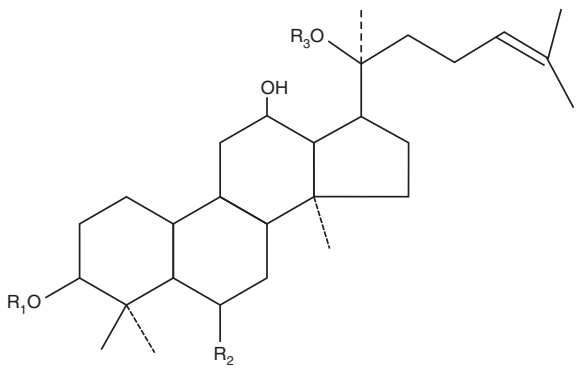

Figure 1. The 20(S)-protooanaxadiol and 20(S)-protopanaxatriol components in ginseng root.

Table 1. The structures of the common ginsenosides.

I. 20(S)-protopanaxadiols			
Ginsenoside	R1	R 2	R3
Rb1	glc-glc	H	glc-glc
Rb2	glc-glc	H	glc-ara(p)
Rc	glc-glc	H	glc-ara(f)
Rd	glc-glc	H	glc
II. 20(S)-protopanaxatriols			
Ginsenoside	R1	R2	R3
Re	H	-O-glc-rha	glc
Rf	H	-O-glc-glc	H
Rg1	H	-O-glc	glc
Rg2	H	-O-glc-rha	H
Rh1	H	-O-glc	H

glc = glucose; ara(p) = arabinose in pyranose form; ara(f) = arabanose in furanose form; rha = rhamnose. Sources: [2,11].

ENVIRONMENTAL OCCURRENCE

Panax ginseng species are produced in the Northern Hemisphere, from the eastern Himalayas through China and Japan to North America. *Panax quinquefolius* is found in eastern North America. Due to continuous harvest for therapeutic usage and health care over thousands of years, the natural grown (wild) *Panax* ginseng root has been exhausted in China for a long time. Naturally, wild ginseng has long been considered a threatened, rare, or endangered species[2].

American ginseng has been exported to foreign countries, mainly Asia, since eighteenth century. For commercial needs, cultivated American ginseng was developed in the twentieth century[10]. American ginseng is now cultivated in China, Korea, Japan, and North America.

DISPOSITION

After oral administration, ginsenosides are only slightly decomposed by gastric juice[12], but are metabolized by intestinal bacteria; with protopanxadiols (Rb1) probably being metabolized to 20-O-β-D-glucopyranosyl-20(S)-protopanaxadiol (compound K) in rat and human by intestinal anaerobes[12-16]. Prevotella oris strains of intestinal bacteria hydrolyzed protopanxadiols, and the resulting metabolites are absorbed from the intestine[17]. However, protopanxadiols (Rb1) are not well absorbed in the intestine[18]. After orally administering protopanxadiols to mice, the level of 20-O-β-D- glucopyranosyl-20(S)-protopanaxadiol gradually increased in the serum, reaching the peak at 8 hr. When 20-O-β-D-glucopyranosyl-20(S)-protopanaxadiol itself was orally administered to the mice, its level reached the highest in serum within 2 hr and decreased rapidly with time[13].

While ginsenoside Rg1, a 20(S)-protopanaxatriol, (Table 1) had a short half-life of 27 min after i.v. administration to minipigs, protopanaxadiol ginsenoside Rb1 had a much larger half-life (16 hr) in the B-phase[2]. When orally administered to the mice, ginsenoside Rg1 was quickly absorbed and metabolized; with 30% of ginsenoside Rg1 being absorbed in one hr. These findings are consistent with the results that mouse urine and feces contained high levels of metabolites, including ginsenoside Rh1 and 25-OH-Rh1, while only a small amount of

unchanged ginsenoside Rg1 was found[2].

Ginsenoside Rh2 underwent deglycosylation to produce protopanaxadiol in B16 melanoma cells *in vitro*, leding to growth inhibition of the B16 cells[19].

PURPORTED THERAPEUTIC EFFECTS AND BIOLOGICAL EFFECTS

Much literatures, including original research papers, reviews, and books, has reported the purported therapeutic effects and biological effects of ginseng in details. In the present review, these effects are described very briefly.

In China, *Panax ginseng* is regarded as "the all-healing man-herb", and in the Far East it is considered as something of a panacea. Ginseng is popularly used as an aphrodisiac and a stimulant[20]. It has been widely used as geriatric tonic in Asia and recently in many Western countries, purportedly for enhancing stamina and endurance both mentally and physically. The claimed therapeutic effects include anti-aging; beneficial effects on diabetes, radiation sickness, ulcers, cardioprotection, vasorelaxant, anemia, atherosclerosis, hypertension, and edema; improving central nervous system with effects on memory, learning and behavior; improving concentration and depression, and stress reduction[4,11,21].

Protopanaxadiol, a metabolite of ginsenoside Rh2, inhibited growth of B16 melanoma cells *in vitro* strongly than Rh2 did[19]. Administered orally to mice, ginsenosides Rb1, Rb2, Rg3 and Rc inhibited lung metastasis of B16-BL6 and colon 26 cells by inhibition of angiogenesis[13, 22,23]. Ginsenoside Rh2 was found to enhance antitumor activity and decrease cyclophosphamide-induced genotoxicity[24].

Panax ginseng has been reported to inhibit a chemical-induced carcinogen in rodents. Yun *et al*.[25] determined that mice receiving prolonged administration of Korean red ginseng inhibited or prevented carcinogenesis induced by 7,12-dimethylbenz[*a*]anthracene (DMBA), urethane, and aflatoxin B1. Korean red ginseng administered orally inhibited liver cancer induced by diethylnitrosamine (DEN) in Wistar rats[26]. When red ginseng was given together with urethane, there was a significant reduction in urethane-induced lung adenoma[27]. Gum *et al*.[28] determined that wild *Panax* ginseng C.A. Meyer efficaciously protected against benzo[*a*]pyrene-induced hepatotoxicity through inhibition of metabolic regulation of CYP1A1 and the enhancement of electrophilic detoxification. *Panax* ginseng extract, EFLA400, inhibited benzo[a]pyrene induced lung adenoma in Swiss albino mice[29]. *Panax* ginseng was found to be capable of improving survival rate and sperm quality to guinea pigs exposed to 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD)[30].

Ginseng total saponins were found useful for the prevention and therapy of the behavioral side effects induced by psychotropic agents[31]. Ginsenoside Rg[3], an active ingredients in *Panax* ginseng, induced neuroprotection against homocysteine-induced excitotoxicity in rat hippocampus. This protective effect is achieved via inhibition of homocysteine-mediated NMDA receptor activation[32].

Orally administered ginsenosides are metabolized to 20-O-beta-D-glucopyranosyl-20(S)-protopanaxadiol (compound K) by intestinal bacteria, which can protect tert-butyl hydroperoxide induced liver injury in mice[16]. Lee *et al*.[33] found that *Panax* ginseng exhibited a therapeutic capacity to reduce pathological and genotoxical damage in testes of rats exposed to 2,3,7,8-tetrachlorodibenzo-p-dioxin via reduction of CYP1A1 mRNA.

Ginsenosides (ginseng saponins) are the principal components responsible for the majority of purported pharmacological activities of ginseng[16]. 20-O-beta-d-glucopyranosyl-20(S)-protopanaxadiol is an intestinal bacterial metabolite of protopanaxadiol-type saponins, ginsenosides Rb1, Rb2, and Rc. It possesses antitumor effects, including inhibition of invasion, metastasis and angiogenesis, and induction of tumor cell apoptosis. This metabolite exerts anti-inflammatory effects by inhibiting TPA-induced COX-2 expression, expressing its antitumor-promoting effects on mouse skin carcinogenesis[16]. 20-O-(beta-D-glucopyranosyl)-20(S)-protopanaxadiol induced apoptosis in human hepatoblastoma HepG2 cells[34]. The very low toxicity in normal hepatocytes and high activity in hepatoblastoma HepG2 cells suggest that 20-O-(beta-D-glucopyranosyl)-20(S)-protopanaxadiol is a potential chemopreventive agent[34]. Ginseng extract exerted protective effects against apoptotic cell death induced by 2,2',5,5'-tetrachlorobiphenyl in neuronal SK-N-MC cells[35].

Ginsenosides Rb1 and Rg1 are effective therapeutic agents for neurological disorders, including spinal cord injuries[36]. *Panax* ginseng extract standardized with ginsenoside Rg3 protected effectively against acrylamide-induced neurotoxicity in rats[37].

Ginseng has been reported to have a positive effect on the immune system, stimulating the phagocytic function of the reticoloendothelial system leading to an increase in serum-specific antibodies and IgG content and also an increase in the relative percentages of protective B-lymphocytes[2]. Ginsenosides were reported to effectively reduce cisplatin-induced nephrotoxicity[7]. Ginsenosides induced differentiation of F9 teratocarcinoma stem cells and loss of tumorigenicity[38]. Bao *et al*.[39] determined that ginsenosides Rg3(S) and Rg5/Rk1 significantly reversed the memory dysfunction induced by ethanol or scopolamine, and exhibited neuroprotective effects. Ginsenoside Rb(1) possessed anti-inflammatory properties[40].

Van Kampen *et al*.[41] determined that oral administration of ginseng extract G115 exerted neuroprotective actions in two rodent models of Parkinson's disease.

Administration of ginsenoside-Rb2 to streptozotocin-induced diabetic rats reduced the level of blood glucose, producing an improvement in hyperglycemia. Rb2-treated animals also showed a significant decrease in activity of glucose-6-phosphatase, a significant rise of

glucokinase activity in the liver, and a moderate increase in glycogen content[42]. Ginseng seems to have a direct and an indirect action on oxidative damages. Evidence from *in vitro* studies showed that the pharmacological actions of ginseng are due to its antioxidant/nitric oxide (NO) stimulating properties[43,44].

Ginseng exhibits antioxidant/nitric oxide effects[43,44]. Ginseng activates the transcription of the superoxide dismutase gene[45]. The cardiovascular effects of ginseng root and individual ginsenosides have been studied. Many reports describe vasodilator actions, in some cases followed by vasoconstriction and increase in blood pressure[11].

GINSENG TOXICITY

I. *Human Data*

There are no epidemiological studies or case reports that are associated with exposure to ginseng and cancer risks in humans. When toxicity of ginseng was reported, the form, quantity, and quality of ginseng used were not specified. The diets, the use of other drugs, and the stress condition of the patients were also not known. Thus, toxicity of ginseng in humans is difficult to evaluate. Characteristic signs and symptoms of overexposure to ginseng have been termed ginseng abuse syndrome. Siegel[46] studied ginseng abuse syndrome examining 133 persons who took ginseng regularly for at least one month. Most subjects experienced central nerve system (CNS) excitation and arousal. Fourteen subjects who took *Panax* ginseng roots suffered from hypertension, nervousness, sleeplessness, skin eruptions, and morning diarrhea, among which, five had edema and ten became euphoric, restless, agitated, and insomniac. Ten taking high doses (15 g) felt depersonalization and confusion. The average daily dose of ginseng roots was 3 g for persons experiencing ginseng abuse syndrome. Ginseng abuse syndrome appeared periodically in the first 12 months of ginseng use but the syndrome was rarely reported in follow up examinations at 18 and 24 months[46].

A 28-year old woman who ingested a large quantity of ethanol-extracted ginseng developed a severe headache, nausea and vomiting, and chest tightness. It has also been reported that ginseng exerted estrogenic effects in women, causing vaginal bleeding in postmenopausal women[4]. Chronic administration of ginseng has been associated with the occurrence of vaginal bleeding, mastalgia, mental status changes and Stevens-Johnson syndrome[20]. A 44-year old woman taking ginseng-cotaining face cream developed an episode of postmenopausal bleeding[47]. A 39-year old man who long term ingested ginseng developed hypertension, dizziness, and inability to concentrate. He stopped taking ginseng, became normotensive within five days, and after three months his symptoms resolved[48].

The U.S. Food and Drug Administration's Special Nutritionals Adverse Event Monitoring System reported 114 illnesses or injuries associated with the use of ginseng-containing special nutritional products and dietary supplements on May 14, 1998. Thirteen deaths were reported. For 17 products that apparently contained only ginseng as an active ingredient, the following effects were reported: tonic-clonic seizure and two mild strokes; pruritus and jaundice; vomiting, nausea, diarrhea, and perspiration; dermatomyositis; coma; stomach pains; rash and searing pain; heart palpitations, sweating, and "felt like speeding"; scratched esophagus; nausea, vomiting, dizziness, and blurred vision; atrial fibrillation; and thrombocytopenia, necrotic tissue in bone marrow, followed by death; chest pain, feeling of constriction, heart pounding, anxious, and pale; headache, nausea, and vomiting; and abnormal uterine bleeding[49].

Hypertensive individuals who chronically take ginseng may have problems with control of blood pressure[50].

II. *Herb-Drug Interaction*

Herbal medicines, including herbal supplements, are often administered in combination with therapeutic drugs, both of which require metabolizing enzymes for metabolism, and very likely raise the potential of herb-drug interactions, causing an adverse nature[51-56]. Ginseng is one of the most popular geriatric dietary supplements. Gurley *et al.*[55] studied the effects of *Panax* ginseng on cytochrome P450 phenotypes in the elderly and determined that *Panax* ginseng significantly inhibited CYP2D6, but the magnitude of the effect did not appear to be clinically relevant.

Hu *et al.*[53] stated that upon herb-drug interactions, *Panax* ginseng reduced the blood concentrations of alcohol and warfarin and induced mania when used concomitantly with phenelzine.

III. *Animal Data*

(I) *Acute Studies*

The Registry of Toxic Effects of Chemical Substances (RTECS) listed several acute toxicity values for various ginseng products, which are shown in Table 2.

The work by[59,60] demonstrated that ginsenoside aglycones 20(S)-protopanaxadiol, 20(S)-protopanaxatriol, and

Table 2. LD_{50} (mg/kg) values for ginseng[57,58]

Compound	Species	Route	LD_{50} (mg/kg)
Panax ginseng	Rat	Oral	750
	Mouse	Oral	200
	Mouse	IP	54
Ginseng root extract	Mouse	IP	545
Ginsenoside No. 3	Mouse	IP	910
Ginseng, saponin extract	Mouse	IP	637

ginsenoside Rh2 induced cytotoxicity in intestinal Caco-2 cells. Ginsenoside Rd from *Panax notoginseng* was found cytotoxic towards HeLa cancer cells and induces apoptosis through down-regulating Bcl-2 expression, up-regulating Bax expression, lowering the mitochondrial transmembrane potential, and activating the caspase-3 pathway[61].

(II) *Subacute/Subchronic Studies*

Hess *et al*.[62] reported that male and female Sprague-Dawley rats fed a diet containing G115 with 15 mg/kg/d for 13 weeks did not develop histopatholgical changes. Beagle dogs fed diets containing 0, 1.5, 5, or 15 mg ginseng extract G115 per kg of body weight per day for 90 days also exhibited no toxicity was observed. Gross and microscopic examinations of major organs revealed no morphological or pathological effects. The highest dose, 15 mg/kg, is approximately twice the recommended dose for humans[63]. No toxic effects were found in rats following ingestion of ginseng extract at daily dose levels of 105-210 mg/kg for 25 weeks[64].

(III) *Chronic/Carcinogenicity Studies*

In a chronic study in LACa mice, there were three groups with 90 animals per group; one group consumed ginseng extract from 8 weeks of age throughout life; the second group received ginseng from 52 weeks onward; and the third group served as untreated controls. Ginseng extract was administered in drinking water at a dose of 8 mg/kg/day, corresponding to 40 mg of whole root/kg/day[65]. This study was not designed to examine carcinogenicity; therefore, histopathology was not performed and no attempt to define maximum tolerated dose was made. No significant differences in mean weights or survival were observed in mice consuming *Panax* ginseng, although increased behavioral responses to mild stress were observed. Ginseng administration did not alter the lifespan of the mice but their behavioral response to stress was exaggerated[65]. However, the dose level used was considered low.

(IV) *Genotoxicity Studies*

Chang *et al*.[66] reported that both the water extract of *Panax* quinquefolius roots and the *n*-butanol extract of ginsenosides were not mutagenic in Salmonella typhimurium strain TM677 with or without metabolic activation. When assayed in Bacillus subtilis strains H17Rec+ and M45Rec- and in *Salmonella typhimurium* strains TA98 and TA100 with or without PCB-induced rat liver S-9, *Panax* japonicum and *Panax* ginseng were not mutagenic[67]. However, the extract of *Panax* ginseng root exhibited inhibitory effects on DNA synthesis, measured by thymidine incorporation into V79 Chinese hamster lung cells[68].

While ginsenoside Rg1, an active component of ginseng, stimulated mitosis in the bulb and seedling root tip cells of Allium cepat, ginsenoside Rb1 inhibited mitosis in the cells[69].

The U.S. National Toxicology Program (NTP) is conducting a 2-year carcinogenicity study of *Panax* ginseng (Asian ginseng). A detailed description of this study is given in a following section.

(V) *Reproductive Toxicity Studies*

Chan *et al*.[70] studied embryotoxicity of ginsenoside Rc and Re in rat whole embryo culture and found that ginsenoside Re resulted in a significant lower median morphological score, a fewer number of somites, and a smaller yolk sac diameter and crown-rump length. In contrat, there was no significant embryotoxic effect by exposure to ginsenoside Rc. Liu *et al*.[71] employed whole embryo culture to explore the developmental toxicity of ginsenoside Rb1on mouse embryos. The results suggest that GRb1 exhibited teratogenic effect during the mouse organogenetic period. Ginsenoside GRg1 exerted embryotoxicity during both rat and mouse organogenetic period, with the effect higher in rats than mice[72].

(VI) *Effect on Cytochrome P450 Enzymes and Herb-Drug Interactions*

Lee *et al*.[73] studied the *in vivo* effects of *Panax* ginseng extracts on the cytochrome P450 enzymes in the liver of guinea pig treated with 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD). It was found that *Panax* ginseng extracts may act as an inhibitor of CYP1A, but not of CYP2B.

REGULATORY STATUS

In the United, no standards or guidelines have been set by NIOSH or OSHA for occupational exposure to or workplace maximum allowable levels of ginseng. Ginseng is not on the American Conference of Governmental Industrial Hygienists (ACGIH) list of compounds for which recommendations for a Threshold Limit Value (TLV) or Biological Exposure Index (BEI) are made.

International trade in American ginseng is regulated under the provisions of the Convention on International Trade in Endangered Species (CITES), which regulates trade through permit requirements for imports, exports, and re-exports of listed species. Harvest and commerce are regulated and restricted both jointly and separately by state agencies, the US Fish and Wildlife Service, and the United States Department of Agriculture[74].

Since 1994, dietary supplements have been regulated under the Dietary Supplement Health and Education Act (DSHEA). The DSHEA requires no proof of safety for dietary supplements on the market prior to October 15, 1994. Labeling requirements for such supplements allow warnings and dosage recommendations as well as

Journal of Food and Drug Analysis, Vol. 15, No. 4, 2007

substantiated "structure or function" claims. All claims must prominently note that they have not been evaluated by the FDA, and they must bear the statement "This product is not intended to diagnose, treat, cure, or prevent any disease"[75].

Ginseng is more closely regulated in Europe. The German government's Commission E requests *Panax* ginseng products containing at least 1.5 percent ginsenosides, calculated as ginsenoside Rg1. The products must also be labeled for use as a tonic for invigoration and fortification[76].

TOXICOLOGY EVALUATION BY THE NATIONAL TOXICOLOGY PROGRAM (NTP)

The NTP is the U.S. government program established to evaluate substances for health-related effects, generally using rodent models for study and protocols specifically designed to characterize fully the toxic potential and address specific deficiencies in the toxicology database for the chemical. The studies generally provide toxicological information on effects on hematological and clinical chemistry, immunotoxicity, neurotoxicity, reproductive toxicity, genotoxicity, and carcinogenicity of the chemical. In addition, the studies also attempt to identify the active metabolite and its mode of action. The data generated are used in the hazard identification step of the risk assessment process. The NTP study data are used by the U.S. Federal and State Regulatory Agencies in considering the need for regulation of specific chemicals to protect human health.

Ginseng was presented to the NIH Chemical Study Working Group as part of a review of botanicals used as dietary supplements. Worldwide, ginseng production is a $3 billion industry. Asia is the largest market. Ginseng is also a popular herbal remedy in the United States, with five to six million persons using it even before the recent boom in the herbal supplement industry. Although ginseng root is commonly used, a standardized ginseng extract, Ginsana (G115 ginseng), with annual sales of over $40 million, is the most popular encapsulated form.

Numerous reports of adverse effects from products containing ginseng have been filed with the U.S. Food and Drug Administration (FDA). The literature also documents "ginseng abuse syndrome" among regular users. The chronic effects of ginseng are not well characterized; studies of some components suggest anticarcinogenic activity. The rationale for nomination of ginseng for study was because of 1) Significant human exposure. Ginseng is one of the most popular herbal supplements in the U.S. market; Ginsana (G115 ginseng) is a standardized extract that controls half of this market, 2) Little information on toxicity is available. The active ingredients may be dammaranes; RB1 ginsenoside is a commercially available dammarane found in ginseng, and 3) The possibility that these compounds may have anticarcinogenic activity should also be considered.

In general, the effects of ginseng reported in the literature are mostly derived from observation of the use of genseng extracts, not individual ginsenosides. Consumers also use extracts sold on the market. Since Ginsana (G115 ginseng) is a standardized product containing 4% extract and has more than 50% of the market, the use of Ginsana (G115 ginseng) in the NTP studies was recommended. The study of ginseng by the NTP is on-going. The protocol outline of the NTP pre-chronic and chronic toxicity evaluation of ginseng will be described in detail after the following Perspectives section.

PERSPECTIVES

Since the U.S. Congress passed the Dietary Supplement Health and Education Act (DSHEA) in 1994, herbal products represent the fastest growing segment of the VMH (Vitamin, Mineral supplements, and Herbal products) industry. According to DSHEA, a dietary supplement is considered unsafe only if it presents a significant or unreasonable risk of illness or injury under conditions of use recommended or suggested in labeling, or if no conditions of use are suggested or recommended in the labeling, under ordinary conditions of use. Thus, to ensure consumer health protection, the quality and safety of herbal plants and herbal dietary supplements have to be determined. To date, safety of herbal plants and herbal dietary supplements is a major concern but the subject has been focused primarily on external contaminations such as microbial toxins and environmental pollutants of metals and pesticide residues. Other safety issues, such as undesirable side-effects and product stability are not addressed adequately, and toxicological data on the identification of genotoxic and tumorigenic ingredients in many herbal plants and herbal dietary supplements that are commercially available in the market are also lacking. As exemplified with the study of *Panax* ginseng in this review, currently, the United States National Toxicology Program (NTP) is conducting long term research projects to determine the toxicity and tumorigenicity of a number of dietary supplements and active ingredients nominated by the U.S. FDA and U.S. National Institutes of Health. An organized effort with international participation on the subject should be actively pursued to ensure the safety of commercial herbal plants and their derived products, such as herbal dietary supplements.

PROTOCOL OUTLINE OF THE NTP PRECHRONIC AND CHRONIC TOXICITY EVALUATION OF GINSENG

Title: Chronic gavage toxicity/carcinogenicity study of ginseng in Fischer 344 rats (Project No. G004512-O)
CAS Number: 50647-08-0

Objective: To characterize the toxicity of ginseng in Fischer 344 rats and B6C3F1 mice.

A. 14-Day Repeated Dose Toxicity Study

Purpose: This study shall provide a basis for determining doses for the 13-week subchronic toxicity study.

Treatment:

The product G115 extract (Ginsana), which was used in the present study, was purchased from PlusPharma, Inc. (Vista, CA). It is an extract of Panax ginseng and contains 4% ginsenosides or triterpene saponins.

After a ten to fourteen day quarantine period, animals shall be assigned at random to treatment and control groups. At each of five concentrations (0.125, 0.25, 0.5, 1.0, 2.0 g/kg) plus a vehicle control group, five animals per sex per species shall receive the subject chemical in methylcellulose via gavage. The schedule will be twelve dose days, not including weekends or holidays with at least two consecutive dose days before the terminal sacrifice day. Dose days missed due to holidays are to be made up before sacrifice. All mice shall be housed individually and rats shall be housed five per cage.

Test Groups	Animals		Sexes		Species		Dose Levels		Total
Treatment	5	×	2	×	2	×	5	=	100
Untreated/vehicle Controls	5	×	2	×	2	×	1	=	20
Total									120

Observations:

Animals shall be weighed individually on day one on test, after seven days, and at sacrifice. The animals shall be observed two times daily, once in the early morning and once in the late afternoon at least 6 hr apart (before 10:00 AM and after 2:00 PM), including holidays and weekends, for moribundity and death. Observations shall be made twice daily for clinical signs of pharmacologic and toxicologic effects of the chemical. Clinical signs shall be recorded daily by animal number and made a part of the study report. Organ weights shall be determined for all animals surviving until the end of the study. The organs to be weighed are: liver, thymus, right kidney, right testicle, heart, and lungs. Organs shall be weighed to the nearest 10 mg except for testis and thymus which shall be weighed to the nearest 1.0 mg.

Necropsy and Histopathologic Evaluation:

A complete necropsy shall be performed on all treated and control animals that either die or are sacrificed, and all tissues specified in the SOW (Section IV.B.) shall be saved in formalin. This shall be done for all animals in all groups.

Histopathologic evaluation shall be done on 4 tissues (brain, liver, lung, kidney) and those organs/tissues showing gross evidence of lesions. The male and female reproductive organs shall also be evaluated if abnormal.

B. 13-Week (90-day) Subchronic Toxicity Study

Purpose: In addition to obtaining toxicological data, this study will also help to determine the doses for each strain and species to be used in the chronic two year toxicity study.

Treatment:

After a ten to fourteen day quarantine period, animals shall be assigned at random to treatment and control groups. At each of five concentrations plus a vehicle control group, ten animals per sex per species shall receive the subject chemical in methylcellulose via gavage. The schedule shall be five times per week, weekdays only and exclusive of holidays, with all animals remaining on the treatment regimen until the day (within 24 hr) of sacrifice. All mice shall be housed individually and rats shall be housed five per cage.

Test Groups	Animals		Sexes		Species		Dose Levels		Total
Treatment	10	×	2	×	2	×	5	=	200
Untreated/vehicle Controls	10	×	2	×	2	×	1	=	40
*Special rats for clinical lab studies	10	×	2	×	1	×	5	=	100
*Special controls for clinical lab studies	10	×	2	×	1	×	1	=	20
Total									360

* Special rats are to be treated for 21 ± 2 days for use in conducting clinical lab studies at days 4 ± 1 and 21 ± 2, after which they are to be sacrificed by exsanguination (see special studies for serum hormone determinations) and no tissues saved. Body weights and clinical observations will not be measured for the additional rats treated for clinical lab studies. Clinical lab studies and serum hormone determinations shall also be conducted on core study rats at terminal sacrifice. Hematology measurements shall be made on mice at terminal sacrifice.

Observations:

Animals shall be weighed individually on day one on test, after seven days and at weekly periods thereafter. The animals shall be observed twice daily, once in the early morning and once in the late afternoon, at least 6 hr apart (before 10:00 AM and after 2:00 PM), including holidays and weekends for signs of moribundity and death. Signs of toxicity noticed during these routine checks shall be recorded. Formal clinical observations shall be performed and recorded weekly. An animal survival report shall be submitted on the 1st and 15th day of each month during the course of the study.

Organ weights shall be determined from all animals surviving until the end of the study. Those organs to be weighed are: Liver, thymus, right kidney, right testis, heart, and lungs. Organs shall be weighed to the near-

Journal of Food and Drug Analysis, Vol. 15, No. 4, 2007

est 10.0 mg except for testis and thymus which shall be weighed to the nearest 1.0 mg.

Necropsy and Histopathologic Evaluation:

A complete necropsy shall be performed on all treated and control animals that either die or are sacrificed, and all tissues as listed in Section IV.B.of the SOW shall be saved in formalin. All tissues required for complete histopathology as listed in this protocol shall then be trimmed, embedded, sectioned and stained with hematoxylin and eosin for possible histopathologic evaluation. This shall be done for all animals in all groups.

A complete histopathologic evaluation inclusive of gross lesions shall be done on all control animals, all animals in the highest dose group with at least 60% survivors at the time of sacrifice, plus all animals in higher dose groups inclusive of early deaths and survivors. Chemical-related lesions (target organs) shall be identified, and these organs plus gross lesions shall be examined in all lower doses. The pathologist shall evaluate only those tissues designated as target tissues and gross lesions in lower dose levels. However, a complete histopathologic evaluation shall be performed on all natural death/moribund sacrifice animals in lower dose groups.

LIST OF TISSUES FOR COMPLETE HISTOPATHOLOGIC EVALUATION FOR THE 13-WEEK (SUBCHRONIC) STUDIES

Adrenal glands
Brain (3 sections including frontal cortex and basal ganglia, parietal cortex and thalamus, and cerebellum and pons)
Clitoral glands
Esophagus
Eyes (if grossly abnormal)
Femur, including diaphysis with marrow cavity and epiphysis (femoral condyle with epiphyseal cartilage plate, articular cartilage and articular surface)
Gallbladder (mouse)
Gross lesions
Heart and aorta
Intestine, large (cecum, colon, rectum)
Intestine, small (duodenum, jejunum, ileum)
Kidneys
Liver (2 sections including left lateral lobe and median lobe)
Lungs and mainstem bronchi
Lymph nodes - mandibular and mesenteric
Mammary gland with adjacent skin
Muscle, thigh (if neuromuscular signs were present)
Nasal cavity and nasal turbinates (3 sections)
Ovaries
Pancreas
Parathyroid glands
Pituitary gland
Preputial glands
Prostate
Salivary glands
Seminal vesicle
Spinal cord and sciatic nerve (if neurologic signs were present)
Spleen
Stomach (forestomach and glandular)
Testes with epididymus
Thymus
Thyroid glands
Tissue masses and regional lymph nodes
Trachea
Urinary bladder
Uterus

Specific Toxicologic Parameters to be Evaluated in the 13-Week Subchronic Study

(1) Clinical Laboratory Studies

Additional rats shall be treated, both sexes, all dose groups, for collections at days 4 ± 1 and 21 ± 2. Blood shall be collected from the retroorbital sinus under CO_2 anesthesia at days 4 ± 1 & 21 ± 2 from the special study rats and these animals shall be sacrificed after the collection for day 21. The core study rats shall be bled for the terminal collection. Core study mice shall be bled at terminal sacrifice for hematology determinations.

All male and female animals shall be treated the same number of days before collection of samples and all animals of a sex shall be bled on the same day. The period between the start of the study and the collection of the first sample at day 4 ± 1 shall be continuous, not interrupted by a weekend without treatment.

(i) Hematology -
Erythrocyte count
Mean corpuscular volume
Hemoglobin
Packed cell volume
Mean corpuscular hemoglobin
Mean corpuscular hemoglobin concentration
Erythrocyte morphologic assessment
Leukocyte count
Leukocyte differential
Reticulocyte count
Platelet count and morphologic assessment

(ii) Clinical chemistry to include -
Sorbitol dehydrogenase (SDH)
Alkaline Phosphatase (ALP)
Creatine Kinase (CK)
Creatinine
Total Protein
Albumin
Urea Nitrogen (BUN)
Total Bile Acids
Alanine Aminotransferase (ALT)
Glucose
Cholesterol

Triglyceride

(iii) Serum corticosterone (blood to be drawn early in the morning)

The results of all automated measurements for clinical pathology (unaudited data) shall be reported to the NTP within seven calendar days after sample collection, for the early time points and within 21 days for measurements conducted at terminal sacrifice.

(2) Blood Smears for Micronuclei

Two unstained blood smears shall be prepared from mice at termination of the 13-week study for use by the NTP in micronuclei determinations (procedures are described in the SOW).

(3) SMVCE

(4) Special Studies

(i) Optional neuroreproductive, and developmental toxicity studies

(ii) Optional special staining for brain tissues (Note: brain tissue shall be removed and fixed immediately after sacrifice before examining and removing other organs)

C. Chronic Toxicity/Carcinogenicity Study

Objective: To determine the chronic toxicity and carcinogenicity of ginseng administered by govage to Fischer 344 rats and B6C3F1 mice.

Treatment:

After a ten to fourteen day quarantine period, rats and mice shall be assigned at random to treatment and control groups. Female rats and female mice shall be housed in groups of five animals per cage, and male rats shall be housed in groups of three per cage. Male mice shall be individually housed.

Rats and mice shall receive the test chemical in methylcellulose for 104 weeks via gavage at 3 doses plus controls. Treatment shall be five days a week, excluding weekends and holidays.

	Animals		Sexes		Species		Dose Levels		Total
Treatment	50	×	2	×	2	×	3	=	600
Controls	50	×	2	×	2	×	1	=	200
Sentinel Animals	15	×	2	×	2			=	60
Total									860

Observations:

Individual animal body weight for test and control group animals shall be recorded on day one on test, and at 4 week intervals thereafter. If life threatening tumors develop, a significant number of deaths occur, or a significant effect on body weight is observed, the weighing frequency may be increased to every two weeks upon approval by the NTP Project Officer. It is estimated that animals will be weighed every two weeks for the final thirteen weeks of the chronic study.

Animals shall be observed two times daily, once in the early morning and once in the late afternoon at least six hours apart (before 10:00 am and after 2:00 pm including holidays and weekends) for morbidity and mortality.

Each animal shall be formally examined for clinical signs of toxicity at four week intervals and these observations shall be recorded. Signs of toxicity detected at times other than the formal four week observations shall be noted and recorded.

Necropsy and Pathology:

Necropsy Examination: All animals from all treated and control groups which die or are sacrificed during and at the end of the experiment receive a complete necropsy examination. All tissues from all animals shall be preserved in formalin as specified in the NTP Specifications. All tissues required for complete histopathologic examination shall be trimmed, embedded, sectioned and stained with hematoxylin and eosin for histopathologic examination.

Histopathology: All animals which die (or are sacrificed in a moribund condition) are subjected to a complete necropsy and slides of all tissues required for complete histopathologic evaluation shall be prepared and evaluated. PEIS Individual Animal Necropsy Records for these animals shall be submitted to the NTP Project Officer on the 1st and 15th day of each month. As a routine, the complete histopathologic evaluation of these tissues will be conducted at the end of the study. However, if histopathology evaluation on early death/sac animals is requested earlier by the NTP, it shall be completed within 30 days of notification.

Animals that are exposed for 104 weeks shall be sacrificed without a recovery period and shall be given a complete necropsy and histopathologic evaluation. All tissues required for complete histopathology shall be evaluated in all dose groups and controls.

LIST OF TISSUES FOR COMPLETE HISTOPATHOLOGIC EVALUATION IN CHRONIC STUDIES

Adrenal glands

Brain (3 sections including frontal cortex and basal ganglia, parietal cortex and thalamus, and cerebellum and pons)

Clitoral glands

Esophagus

Eyes (if grossly abnormal)

Femur, including diaphysis with marrow cavity and epiphysis (femoral condyle with epiphyseal cartilage plate, articular cartilage and articular surface)

Gallbladder (mouse)

Gross lesions

Heart and aorta
Intestine, large (cecum, colon, rectum)
Intestine, small (duodenum, jejunum, ileum)
Kidneys
Liver (2 sections including left lateral lobe and median lobe)
Lungs and mainstem bronchi
Lymph nodes - mandibular and mesenteric
- inguinal, gluteal, internal iliac (chronic studies only, if lesion observed, not merely discoloration)

Mammary gland with adjacent skin
Muscle, thigh (only if neuromuscular signs were present)
Nasal cavity and nasal turbinates (3 sections)
Ovaries
Pancreas
Parathyroid glands
Pituitary gland
Preputial glands
Prostate
Salivary glands
Seminal vesicle
Spinal cord and sciatic nerve (if neurologic signs were present)
Spleen
Stomach (forestomach and glandular)
Testes with epididymus
Thymus
Thyroid glands
Tissue masses and regional lymph nodes
Trachea
Urinary bladder
Uterus

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