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RESEARCH ARTICLE

Toxicological Study on MUNOPHIL, Water Extract of *Panax ginseng* and *Hericium erinaceum* in Rats

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Abstract
Objective: As data on the safety profile of *Panax ginseng* and *Hericium erinaceum* is lacking, the safety of these two compounds was examined in a series of toxicological studies.
Materials and Methods: MUNOPHIL, the water extract mixture of *Panax ginseng* and *Hericium erinaceum* was tested in an oral subchronic 28-day toxicity study in rats at doses of 1250, 2500 and 5000 mg/kg/day.
Results: In repeated dose toxicity studies, no mortality was observed when varying doses of the extracts were administered once daily for a period of 28 days. There were no significant differences in body weight, absolute and relative organ weights between controls and treated rats of both sexes. Hematological analysis showed no differences in most parameters examined. In the biochemistry parameter analysis, no significant change occurred. Pathologically, neither gross abnormalities nor histopathological changes were observed. Therefore, MUNOPHIL appears to be safe and non-toxic in these studies and a no-observed adverse effect level in rats was established at 5000 mg/kg/day.
Conclusion: The data could provide satisfactory preclinical evidence of safety to launch clinical trials on standardized formulation of plant extracts.

1. Introduction

MUNOPHIL is a water extract mixture of *Panax ginseng* and *Hericium erinaceum* for use as an agent for activating vital energy and improving immune function. The species *Panax ginseng* and *Hericium erinaceum* have been identified and many are known to have medicinal properties [1–4]. *Panax ginseng* is a widely used and respectable anticancer herb around the world [5–7] and studies performed in rats showed that an extract of *Hericium erinaceum* has immunomodulating functions such as activating/macrophages and natural killer (NK) cells [8–10].

Even though the safety of *Panax ginseng* has been substantiated with significant scientific research [11–13], no systematic non-clinical toxicity studies have yet been performed on *Hericium erinaceum*. Early animal studies, conducted in dogs, reported no adverse effect of ginseng on body weight or blood chemistry. In mice, the LD_{50} for ginseng ranges from 10 to 30 g/kg, with a lethal oral dose of purified

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ginseng as high as 5 g/kg body weight [14]. In a 2-year human study, 14 out of a total of 133 subjects were reported to experience side effects attributed to long-term exposure of ginseng when consumed at levels up to 15 g/day. Average intakes of ginseng were equivalent to consuming 6500 mg ginseng capsules daily and produced side effects that included hypertension, gastrointestinal disturbances, insomnia and nervousness [15].

Hericium erinaceum has a stimulatory effect on interleukin (IL)-1 production in macrophages via activation of transcription factors such as natural factor (NF)-κB. It also has an inductive effect on macrophage activation leading to inducible nitric oxide synthase (iNOS) expression and nitric oxide (NO) production through the activation of transcription factor NF-κB. Furthermore, *Hericium erinaceum* has an inductive effect on splenocyte-derived NK cell activation leading to cytolysis of Yac-1 cells [8–10]. Therefore, a large potential exists for the development of *Hericium erinaceum* as an immunomodulating drug for cancer patients; however its safety is not established. *Panax ginseng* and *Hericium erinaceum* contain components common to many herbs that are routinely consumed without reported adverse effects. Even so, starting human clinical trials using these herbs is not possible without evidence of safety. Because data on the safety profile of MUNOPHIL, the water soluble extracts of *Panax ginseng* and *Hericium erinaceum*, are insufficient to allow a human clinical trial, we decided to perform toxicological studies to evaluate the safety of MUNOPHIL, to build the safety evidence for possible future clinical trials.

2. Materials and Methods

2.1. Test material

Dried *Panax ginseng* and *Hericium erinaceum* were obtained from Dunsan Oriental Hospital (Daejeon, Korea). MUNOPHIL containing 50% water was extracted from a mixture of *Panax ginseng* and *Hericium erinaceum* according to over-the-counter Korean monographs by Samik Pharmaceutical Company and used in this experiment.

2.2. Animals and experiment

Eighty Sprague Dawley rats (CrjBgi:CD(SD)-IGS(Rat)) (aged 5 weeks, 40 male, 40 female) were obtained from Orientbio (Gyoenggi-do, Korea). The animals were housed in wire cages (500 × 300 × 200 mm). Five rats were reared in each cage and identification cards stating test number and animal number were attached to the cage. The animal room was maintained at a temperature of 20.1–23.1°C, 45.2–68.3% relative humidity and ambient light (200–300 Lux) controlled to produce 12 h light/dark cycle. The animals were fed with Purina Certified Rodent Meal sterilized with radiation (2.0 M rad) and UV sterilized water *ad libitum* during the study. Animals were acclimated to laboratory conditions for at least 1 week before use.

Repeated toxicity studies were conducted on four groups of rats (0 mg/kg control, 1250 mg/kg low dose, 2500 mg/kg medium dose and 5000 mg/kg high dose) for 28 days. While the extract was orally administered using gavage to test groups, distilled water was administered to control group. The rats were observed for mortality, signs of gross toxicity and behavioral changes at least once daily during this study. Body weights and food and water consumption were recorded weekly. Ophthalmological examination was conducted by observing the appearance of the eyes in the last week of this experiment. At the end of the experiment, all animals were sacrificed after blood collection under deep anesthesia with ether. After bleeding of residual blood, internal organs were removed; weighted and gross findings were recorded. All organs were fixed and individually stored for histopathological examination.

2.3. Hematology, biochemistry parameters and urine analysis

The animals were fasted overnight and blood was collected from the abdominal artery under anesthesia with ether. Two milliliters of blood was transferred into a CBC bottle (EDTA 3K, Sewon Medical) and analyzed using an automatic hematoanalyzer, or blood count machine (ADVIA120E, Bayer). Blood was then transferred into a Vacutainer® (sodium citrate 3.2%, USA) and centrifuged at 3000 rpm for 10 minutes. Plasma was isolated and used to determine aggregation time using a coagulometer (Coagrex-100s, Japan).

The hematology examinations (white blood cell (WBC), red blood cell (RBC), hemoglobin, hematocrit, mean corpuscular volumn (MCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC), prothrombin time (PT), activated partial thromboplastin time (APTT) and platelets) and biochemistry examinations (total protein, albumin, albumin/globulin (A/G) ratio, total bilirubin, aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP), creatinine, blood urine nitrogen (BUN), total cholesterol, triglycerides, glucose, calcium, inorganic phosphorus, creatinine kinase, sodium, potassium and chloride) were performed. Urine tests were performed in the last week using Bayer Diagnostics Multistix 10SG (Not. 5J06C, USA) REF 2300(03536597)

strip and urine analyzer (Clinitek 500, USA) for specific gravity, pH, leukocyte, nitrite, protein, glucose, ketone, urobilinogen, bilirubin and blood.

2.4. Histopathological examination

Internal organs were individually removed and fixed with 10% neutral formalin, but testes and epididymis were fixed with Bouin's solution. The organs of the high dosage and control groups were used to make histological slides and examined under the microscope.

2.5. Statistics

The body weight, feed and water consumption, hematological data, biochemistry data and organ weight were analyzed for homogeneity of variance using Levene's test. Tests of significance were performed using ANOVA analysis for homogeneous data and were reanalyzed with Scheffé test. All analysis was performed using SPSS program (Version 10.1).

3. Results

3.1. Survival and clinical observations

There were no mortalities. There were no ophthalmologic findings or clinical observations noted during the study that were considered to be of biological significance. Food and water consumption were not statistically significantly different among treated groups compared with the controls (data not shown).

3.2. Body weight and relative organ weights

Average overall body weight and body weight gain data indicated that the treated rats regardless of dose level were comparable to the controls. Absolute and relative organ weights of treated male and female rats indicated that there were no statistically significant differences among treated groups compared with the control (Table 1).

3.3. Hematology, biochemistry parameters and urine analysis

There were no significant differences between the control group and any treatment group in the urinalysis and hematological analysis (data not shown). Blood chemistry in males and females is shown in Table 2. Analysis of blood chemistry revealed that chloride levels of males in the low, middle and high dosage groups were increased significantly compared with the control, and those of females in the high dosage group were also significantly increased.

3.4. Histopathological examination

Diffuse micro lipid droplet deposits were observed in the liver of female and male administered groups as well as control. Focal lymphocyte aggregation was weakly observed in treatment groups in both males and females. In the kidneys of male control and administered groups, two cases of focal lymphocyte aggregation, one case of lymphocyte infiltration and cortical cystic degeneration considered to be congenital hypoplasia were observed. However, there were no considerable changes between control and treatment groups.

4. Discussion

The aim of the present study was to characterize the potential toxic effects of MUNOPHIL, the herbal formula of the water extract mixture of *Panax ginseng* and *Hericium erinaceum*. MUNOPHIL is the herbal drug developed for mitigating the side-effects of chemotherapy or radiation therapy, increasing immunological activity and enhancing anti-cancer drug effects during chemotherapy or radiation therapy.

Panax ginseng and *Hericium erinaceum* contain components common to many herbs routinely consumed without reported adverse effects, but human clinical trials using this herbal drug cannot proceed without evidence of safety. Because data on the safety profile of MUNOPHIL, incorporating the water soluble extracts of *Panax ginseng* and *Hericium erinaceum,* are insufficient to allow human clinical trials, we decided to perform toxicological studies in rats, allowing important information about the toxic effects of MUNOPHIL to be determined.

There are few reported cases of ginseng toxicity or descriptions of side effects attributed to either the quantity or quality of ginseng when taken at the recommended dosages. Early animal studies, conducted in dogs, reported no adverse effect of ginseng on body weight or blood chemistry. In mice, the LD_{50} for ginseng ranges from 10–30 g/kg, with a lethal oral dose of purified ginseng as high as 5 g/kg body weight [14]. In a 2-year human study, 14 out of a total of 133 subjects were reported to experience side effects attributed to long-term exposure of ginseng when consumed at levels up to 15 g/day. Average intakes of ginseng were equivalent to consuming 6500 mg ginseng capsules daily and produced side effects that included hypertension, gastrointestinal disturbances, insomnia and nervousness [15].

Table 1 Body weight and relative organ weights

Group/dose (mg/kg/day)	Male				Female			
	G1 (0)	G2 (1250)	G3 (2500)	G4 (5000)	G1 (0)	G2 (1250)	G3 (2500)	G4 (5000)
Body weight (g)	363.34±26.36	351.21±25.13	346.93±22.63	348.03±23.60	223.41±16.15	232.69±12.48	235.17±16.58	210.96±16.37
Liver (%)	3.11±0.23	3.09±0.18	3.08±0.20	3.08±0.21	2.90±0.10	3.06±0.17	3.05±0.14	2.94±0.14
Kidney (%)								
Left	0.37±0.03	0.39±0.04	0.38±0.03	0.40±0.04	0.40±0.04	0.42±0.04	0.40±0.04	0.43±0.05
Right	0.37±0.03	0.39±0.03	0.38±0.02	0.41±0.04	0.41±0.03	0.46±0.12	0.42±0.04	0.45±0.06
Spleen (%)	0.19±0.02	0.20±0.02	0.19±0.02	0.20±0.04	0.24±0.03	0.25±0.04	0.24±0.03	0.22±0.03
Adrenal gland (%)								
Left	0.0089±0.0015	0.0095±0.0015	0.0169±0.0257	0.0182±0.0301	0.0153±0.0021	0.0154±0.0018	0.0156±0.0017	0.0163±0.0038
Right	0.0086±0.0011	0.0170±0.0278	0.0486±0.1257	0.0088±0.0009	0.0141±0.0010	0.0151±0.0027	0.0140±0.0021	0.0330±0.0544
Testis/ovary (%)								
Left	0.43±0.03	0.45±0.04	0.442±0.025	0.43±0.04	0.03±0.00	0.03±0.01	0.026±0.005	0.03±0.01
Right	0.43±0.04	0.45±0.04	0.463±0.059	0.43±0.03	0.05±0.06	0.03±0.00	0.0028±0.004	0.03±0.01
Brain (%)	0.58±0.04	0.60±0.04	0.544±0.194	0.59±0.04	0.86±0.07	0.84±0.06	0.853±0.057	0.94±0.06
Pituitary gland (%)	0.0032±0.0008	0.0032±0.0005	0.0428±0.1257	0.0033±0.0008	0.0060±0.0005	0.0059±0.0016	0.0062±0.0006	0.0074±0.0015
Lung (%)	0.38±0.03	0.40±0.03	0.380±0.037	0.39±0.02	0.52±0.04	0.51±0.02	0.474±0.033	0.51±0.03
Heart (%)	0.30±0.02	0.30±0.02	0.288±0.049	0.29±0.02	0.34±0.03	0.34±0.03	0.329±0.022	0.34±0.02
Thymus (%)	0.16±0.03	0.15±0.03	0.142±0.063	0.15±0.03	0.24±0.05	0.25±0.04	0.245±0.052	0.25±0.04
Thyroid gland (%)								
Left	0.0024±0.0004	0.0026±0.0004	0.0027±0.0011	0.0025±0.0005	0.0037±0.0015	0.0034±0.0007	0.0032±0.0007	0.0040±0.0008
Right	0.0026±0.0004	0.0026±0.0006	0.0199±0.0053	0.0028±0.0007	0.0036±0.0010	0.0038±0.0005	0.0040±0.0010	0.0042±0.0007
Prostate gland/uterus (%)	0.14±0.03	0.14±0.03	0.144±0.024	0.16±0.05	0.24±0.07	0.25±0.08	0.21±0.06	0.24±0.04
Epididymis (%)								
Left	0.13±0.02	0.13±0.02	0.13±0.02	0.13±0.01	–	–	–	–
Right	0.13±0.02	0.13±0.01	0.13±0.01	0.13±0.01	–	–	–	–

Table 2 Blood chemistry values obtained from rats

Group/dose (mg/kg/day)	Male				Female			
	G1 (0)	G2 (1250)	G3 (2500)	G4 (5000)	G1 (0)	G2 (1250)	G3 (2500)	G4 (5000)
TP (g/dL)	5.75±0.20	5.73±0.18	5.94±0.15	5.79±0.28	6.26±0.11	6.43±0.39	6.31±0.53	6.19±0.21
ALB (g/dL)	2.37±0.09	2.39±0.09	2.44±0.11	2.44±0.10	2.75±0.08	2.79±0.21	2.76±0.18	2.77±0.12
A/G (g/dL)	0.70±0.05	0.72±0.04	0.71±0.06	0.74±0.05	0.8±0.05	0.77±0.07	0.79±0.06	0.83±0.05
T-BIL (mg/dL)	0.10±0.03	0.11±0.04	0.11±0.04	0.09±0.04	0.158±0.05	0.179±0.03	0.208±0.03	0.192±0.03
ALP (U/L)	371.50±64.42	405.10±83.71	414.10±50.31	370.50±72.96	288.6±40.44	248.7±46.52	275.4±53.88	237.4±37.37
AST (U/L)	74.90±5.69	83.40±8.60	80.00±8.31	82.60±9.67	81.7±12.54	78.9±8.91	82.3±10.75	90.3±18.93
ALT (U/L)	30.80±4.80	31.60±8.14	31.10±3.07	31.00±5.73	24±5.03	24.4±4.62	24.4±3.60	22.5±5.34
CREA (mg/dL)	0.47±0.05	0.45±0.05	0.50±0.00	0.49±0.03	0.53±0.05	0.51±0.03	0.58±0.06	0.52±0.06
BUN (mg/dL)	13.81±2.44	13.03±1.49	13.18±1.79	14.09±2.42	14.29±2.16	15.77±1.70	16.9±3.05	14.42±1.84
CHOL (mg/dL)	69.60±11.68	68.90±8.92	70.60±8.02	71.00±9.12	86.1±15.37	89.1±22.83	90.6±18.69	80.2±13.86
TG (mg/dL)	82.00±26.45	73.10±15.28	90.90±36.54	78.70±24.08	21.2±8.43	33.0±30.90	14.9±5.53	15.6±9.54
GLU (mg/dL)	159.30±20.84	156.10±18.02	163.50±21.23	162.80±15.92	148.8±15.02	141.7±12.71	143±13.22	130.1±15.95
CA (mg/dL)	10.75±0.27	10.62±0.27	11.01±0.30	10.86±0.21	11.08±0.28	11.24±0.40	11.33±0.60	10.88±0.29
IP (mg/dL)	7.83±0.42	8.06±0.39	7.78±0.33	7.91±0.62	7.95±0.49	8.03±0.50	8.36±0.59	8.04±0.50
CK (IU/L)	171.00±37.36	167.00±67.60	182.80±66.30	197.50±124.82	169.6±102.89	138.5±58.37	161.9±79.20	243±165.95
Na (mmol/L)	143.70±0.82	143.70±1.25	143.30±1.25	143.20±1.14	142.9±1.29	143.2±1.23	142.6±1.58	141.9±1.20
K (mmol/L)	4.03±0.24	3.95±0.27	3.97±0.30	4.05±0.25	4.113±0.26	3.98±0.38	4.033±0.28	4.039±0.24
Cl (mmol/L)	106.60±1.17	108.20*±0.92	108.00*±0.94	108.30*±1.06	107±1.25	107.9±1.79	108.7±1.89	109.3*±1.16

*Significantly different from values of control group at $p<0.05$ with Scheffé test.

The possibility of more serious adverse events is indicated in isolated case reports and data from spontaneous reporting schemes; however, causality is often difficult to determine from the evidence provided. Possible drug interactions have been reported between *Panax ginseng* and warfarin, phenelzine and alcohol. Collectively, these data suggest that *Panax ginseng* monopreparations are rarely associated with adverse events or drug interactions [11–13].

Hericium erinaceum has a stimulatory effect on IL-1 production in macrophages via activation of transcription factors such as NF-κB. It also has an inductive effect on the macrophage activation leading to iNOS expression and NO production through the activation of transcription factor NF-κB. Furthermore, *Hericium erinaceum* has an inductive effect on splenocyte-derived NK cell activation leading to cytolysis of Yac-1 cells [8–10]. This demonstrates that *Hericium erinaceum* could be developed as an immunomodulating drug for cancer patients but information regarding its safety in humans is not available.

MUNOPHIL was therefore subsequently tested in an oral subchronic 28-day toxicity study in rats at doses of 1250, 2500 and 5000 mg/kg/day. In repeated dose toxicity studies, no mortality was observed when varying doses of the extracts were administered per day for a period of 28 days. There were no significant differences in body weight, absolute and relative organ weights between controls and treated animals of both sexes. Hematological analysis showed no differences in most parameters examined. In the biochemistry parameter analysis, we observed a significantly higher value of chloride in males of the 1250, 2500 and 5000 mg/day group and females of the 5000 mg/day group compared with respective control groups. Chloride is derived from chlorine and is a chemical that our body needs for metabolism and for maintenance of acid-base balance. The amount of chloride in the blood is carefully controlled by the kidneys. High blood chloride levels may be due to the absorption of normal salt. These differences were not considered biologically relevant and were not considered treatment related because they did not occur with any consistent relationship to dose.

There were no significant differences between the control group and any treatment group in the urinalysis during the dosing period. Pathologically, neither gross abnormalities nor histopathological changes were observed.

From these results, oral administrations of MUNOPHIL appeared to be well tolerated by the rats. The extract seemed to have no discernable biologically significant toxic effect on the nervous system, respiratory system and other physiological functions of animals of both sex during single and repeated dose studies. The extracts had also inconsistent effects on body growth, organ weights and hematological and biochemistry parameters that failed to be supported by gross and histopathologic examinations of major organs.

The no-observed adverse effect level (NOAEL) for the 28-day study with MUNOPHIL was considered to be over 5000 mg/kg/day. This data suggests that adverse human health effects at lower levels of daily exposure would not be expected. Also, these findings could provide satisfactory preclinical evidence of safety to launch clinical trials on standardized formulation of plant extracts.

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